BIOCHEMICAL STUDIES ON THE PROTECTIVE EFFECT OF TAURINE ON EXPERIMENTALLY INDUCED MYOCARDIAL INFARCTION IN RATS

THESIS

Submitted to

COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

In partial fulfillment for the degree of

DOCTOR OF PHILOSOPHY

In

BIOCHEMISTRY

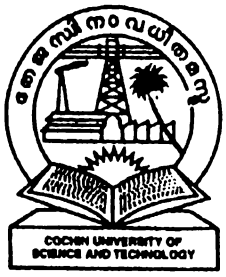
By

SHINY. K. S

BIOCHEMISTRY AND NUTRITION DIVISION

CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY

COCHIN-682029, INDIA



DEPARTMENT OF MARINE BIOLOGY, MICROBIOLOGY AND BIOCHEMISTRY

COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY

COCHIN-682016, INDIA

FEBRUARY 2007

CERTIFICATE

This is to certify that this thesis is an authentic record of the research work carried by Mrs. Shiny. K.S, under my supervision and guidance in the Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Cochin- 682 029 in partial fulfillment of the requirements for the degree of Doctor of Philosophy and that no part of this work thereof has been submitted for any other degree.

Cochin- 682 029
26th February 2007

Dr. R. Anandan

CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY
(Indian Council of Agricultural Research)
WILLINGDON ISLAND, MATSYAPURI P. O.
COCHIN - 682 029.

DECLARATION

I, Shiny. K. S, do hereby declare that the thesis entitled, "Biochemical Studies on the Protective Effect of Taurine on Experimentally Induced Myocardial infarction in Rats" is a genuine record of research work done by me under the guidance of Dr. R. Anandan, Scientist (Senior Scale), Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Cochin- 682 029, and no part of this work has previously formed the basis for the award of any degree, diploma, associate-ship, fellowship or other similar title of any university or institution.

Cochin- 682 029

26th February 2007

Shiny. K. S

ACKNOWLEDGEMENT

I would like to express my deep sense of gratitude to my guide **Dr. R. Anandan, Scientist (Senior Scale),** Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Cochin-682 029, for his inspiring guidance, affectionate treatment, constant support and valuable suggestions during the course of the study.

I am grateful to **Dr. K. Devadasan, Director,** Central Institute of Fisheries Technology, Cochin- 682 029, for providing me the opportunity, the prospect, the encouragement and the interest shown in this study.

I remain thankful to **Dr. P.G. Viswanathan Nair, Head,** Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Cochin- 682 029, for his guidance and support.

I would like to express my gratitude to **Dr. K.C. Radhakrishnan, Head,** Department of Marine Biology Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology for his valuable comments and inspiration.

The encouragement extended by **Dr. T.V. Sankar, Senior Scientist, Dr. Suseela Mathew, Scientist (Selection Grade)** and **Mrs. K.K. Asha, Scientist**, Biochemistry and Nutrition Division, Central Institute of Fisheries Technology are gratefully acknowledged.

I remain thankful to **Mr. B. Ganesan**, for his valuable support and guidance throughout the course of the study. I am very much thankful to **Dr. G Usharani, Mrs. P.A. Jaya, Ms. N. Lekha, Mrs. G. Ramani, Mrs. Shyla, Mr. T. Mathai, Mr. P.A. Sivan, Mr. M.N. Sreedharan, Mr. P.K. Raghu** and **Mr. Gopalakrishnan** for their technical assistance and help rendered throughout my work.

I am thankful to **Dr. Babu Philip, Professor**, Department of Marine Biology Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, **Dr. K. Ashok Kumar, Senior Scientist**, Quality Assurance and Management Division CIFT and **Dr. P.T. Mathew, Principal Scientist**, Fish Processing Division, CIFT for their valuable comments and guidance. I am thankful to **Dr.C. S Vijayalakshmi,** Department of Pathology, Madras Medical College for her validation and

comments in histopathological studies. I hereby acknowledge the help rendered by **Vijaya hospital**, Kadavanthra, Cochin for analyzing some of the clinical parameters using diagnostic kits.

I acknowledge the help provided by the CIFT library staffs, **Mr. Devasya, Mrs Silaja, Mr. Radhakrishnan** and **Mr. Bhaskaran** for my reference collection.

I wish to share my deepest feeling of gratitude to my colleagues **Dhanya, Hari, Sabeena, Siva** and **Mukund** for their sincere support, love and care during the course of my work. My sincere thanks are also due to **Mrs. Kayalvizhi Anandan** for her love and care.

I am very much thankful to my colleagues **Santhosh**, **Martin**, **Sini, Sindhu** and **Swapna** for their encouragement and enthusiasm.

I take this opportunity to acknowledge all my friends especially Sindhu, Smitha, Gayathri, Bindhu, Neema and Syam for their love and moral support.

I owe everything achieved to my loving Achan, Amma, Madhu, Ajith and his family for their affection, enthusiasm and care. I take this moment to reminisce all my family members especially my Ammavan and family and well-wishers for their blessings and prayers.

Not but the least, I extend my heartfelt gratitude for all those good people whom I might have missed unknowingly but has helped me any time, any way during my thesis work.

Above all I thank **"God"**, the Almighty without whose blessings this would never have been completed successfully.

Shiny. K. S

CONTENTS

LIST OF PLATES

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ACE	-	Angiotensin converting enzyme
ADP	-	Adenosine -5-diphosphate
ALT	-	Alanine aminotransferase
AMI	-	Acute myocardial infarction
ANSA	-	Aminoaphthosulfonic acid
APS	-	Ammonium per sulphate
AST	-	Aspartate aminotransferase
ATP	-	Adenosine triphosphate
$Ba(OH)_2$	-	Barium hydroxide
BF_3	-	Boron trifluoride
BSA	-	Bovine serum albumin
Ca^{2+}	-	Calcium ion
Cal	-	Calories
cAMP	-	Cyclic adenosine monophosphate
CAT	-	Catalase
CDNB	-	1-Choloro-2, 4-dinitrobenzene
CHF	-	Congestive heart failure
CHM	-	Choloroform, Heptane, Methanol
CPCSEA	-	Control and supervision of experiments on Animals
CPK	-	Creatine phosphokinase
CSAD	-	Cysteine sulfinic acid decarboxylase
cTnT	-	Cardiac-specific Troponin T
^0C	-	Degree Celsius
CV	-	Cardiovascular
DDC	-	Diethyldithiocarbomate
DHA	-	Docosahexaenoic acid
dl	-	Decilitre
DNPH	-	2,4 Dinitrophenyl hydrazine
DTNB	-	5,5;-Dithiobis(2-nitrobenzoic acid)
ECG	-	Electrocardiogram
EDTA	-	Ethelene diamine tetraacetic acid
EPA	-	Eicosapentaenoic acid

ER	-	Entoplasmic reticulum
FAME	-	Fatty acid methyl ester
$FeCl_3$	-	Ferric Chloride
FFA	-	Free fatty acid
Fig	-	Figure
g	-	Grams
GPx	-	Gluthathione peroxidase
GSH	-	Reduced gluthathinoe
GST	-	Gluthathione-S-tranferase
h	-	Hours
H_2O_2	-	Hydrogen peroxide
H_2SO_4	-	Sulphuric acid
H_3BO_3	-	Boric acid
HCl	-	Hydrochloric acid
HDL	-	High density lipoprotein
HPLC	-	High performance liquid chromatography
i.p	-	Intra peritoneal
IAEC	-	Institutional Animal Ethics Committee
IDL	-	Intermediate density lipoprotein
IU	-	International Unit
K^+	-	Potassium ion
KCl	-	Potassium chloride
Kg	-	Kilogram
KOH	-	Potassium hydroxide
LDH	-	Lactate dehydrogenase
LDL	-	Low density lipoprotein
LOO	-	Lipid peroxy radical
LPO	-	Lipid peroxides
LV	-	Left ventricle
M	-	Molar
MDA	-	Malondialdehyde
mg	-	Milligram
Mg^{2+}	-	Magnesium ion
MI	-	Myocardial infarction

min	-	Minutes
ml	-	Millilitre
mM	-	Millimolar
N	-	Normal
Na^+	-	Sodium ion
Na_2CO_3	-	Sodium carbonate
Na_2SO_4	-	Sodium sulphate
NaCl	-	Sodium chloride
NAD+	-	Nicotinamide adenine dinuclcotide
NADH	-	Reduced nicotibnamide adenine dinucleotide
NADP	-	Nicotinamide adenine dinucleotide phosphate
$NaHCO_3$	-	Sodium bi carbonate
NaN_3	-	Sodium azide
NaOH	-	Sodium hydroxide
nm	-	Nanometer
OD	-	Optical density
OH	-	Hydroxyl radical
OPA	-	O-Phthalaldehyde
PE	-	Petroleum ether
PEG	-	Polyethylene glycol
Pi	-	Inorganic phosphorus
PKA	-	Protein kinase A
PL	-	Phospholipids
PUFA	-	Poly unsaturated fatty acids
ROS	-	Reactive oxygen species
rpm	-	Revolutions per minute
SD	-	Standard deviation
SDS-PAGE	-	Sodium dodecile sulphate-polyacrylamide gel electrophoresis
SOD	-	Superoxide dismutase
TBA	-	Thiobarbituric acid
TCA cycle	-	Tri carboxylic acid cycle
TCA	-	Trichloroacetic acid
TEMED	-	N,N,N^1,N^1 -Tetra ethyl methylene diamine

TEP	-	Tetraethoxy propane
TG	-	Triglyceride
UV	-	Ultra violet
v/v	-	Volume/Volume
VLDL	-	Very low-density lipoprotein
w/v	-	Weight/Volume
WHO	-	World health organization
β-AR	-	Beta adrenergic receptor
μ moles	-	Micromoles
μg	-	Microgram

Introduction

1. INTRODUCTION

Despite improved clinical care, heightened public awareness, and widespread use of health innovations, myocardial infarction remains a leading cause of death all over the world. It is estimated that by the year A. D. 2020, up to three quarters of deaths in developing countries would result from non-communicable diseases and in that myocardial infarction will top the list of killers (Gupta & Gupta, 1998). With changing life style in developing countries like India, particularly in urban areas, myocardial infarction is making an increasingly important contribution to mortality statistics of such countries (Farvin *et al.*, 2004). In India, the number of patients being hospitalized for myocardial infarction is on the increase over the past 35 years, more strikingly among male patients. It is predicted that by the year 2020, India will have the highest incidence of myocardial infarction in the world (Krishnaswami, 1998). There are an estimated 45 million patients of coronary heart disease in India. This increased prevalence of myocardial infarction is contributed largely to adoption of "western" life-style and its accompanying risk factors such as smoking, high fat diet, obesity and lack of exercises.

Myocardial infarction (MI) is the medical term for heart attack. "Myocardia" refers to the heart muscle, "Infarction" means an irreversible injury to a portion of the heart tissue resulting from lack of oxygen and blood supply, which occurs 98% of the time from a process called atherosclerosis (commonly called "hardening of the arteries") in coronary vessels (Ye *et al.*, 1997). Myocardial infarction and the resultant abnormalities in cardiac function are well recognized and it is a complex phenomenon affecting the mechanical, electrical, structural and biochemical properties of the heart. Earlier it was felt that most heart attacks were caused from the slow closure of artery, now it is clear that this process

can occur even in minor blockages where there is rupture of cholesterol plaque. This in turn causes blood clotting within the artery, blocking the blood flow.

Extensive research is being carried out to understand the major factors responsible for myocardial infarction. The relationship between lipid levels and myocardial infarction has been studied in detail and it has contributed enormously to the literature. Higher cholesterol level especially of low-density lipoprotein (LDL) cholesterol is a recognized potent risk factor for heart attack (Griffin *et al.*, 1994). Reports suggest that hypertriglyceridemia also contribute to myocardial dysfunction regardless of cholesterol levels (Fredrickson, 1969; Ryder *et al.*, 1984). In addition to it, low HDL cholesterol confers great risk compared to high serum triglycerides (Castelli, 1988). The lipid abnormalities seen in myocardial infarction appear to correlate with changes in cellular and cell membrane functions. The rise in the intracellular calcium efflux, an inducer of phospholipase A_2, which degrades membrane phospholipids, is also designated as a destructive factor involved in the myocardial damage (Zhang *et al.*, 1995). A considerable body of clinical and experimental evidence is now emerging which suggests that reactive oxygen-derived radicals play an important role in the pathogenesis of acute myocardial infarction (Kukreja & Hess, 1992). Also reports indicate that reduction in free radical scavengers and altered myocardial antioxidant status worsens myocardial injury.

Despite this complexity, impressive recent progress has been achieved in advancing our understanding and appreciation of the cellular processes and mechanistic bases underlying cardiac dysfunction associated with myocardial infarction and most importantly applying this knowledge to therapeutic interventions (Karmazyn, 1996). As myocardial injury is irreversible in nature, most of the drugs available are effective in the prevention of spreading or dispersal of necrotic damage to the adjacent cells. Drugs

available for the treatment of myocardial infarction includes thrombolytic agents, anti-platelet agents, the anti-coagulants, vasodilators, ACE (angiotensin converting enzyme) inhibitors, β-blocking agents and blood-thinning agents. But all these drugs are having their own adverse effects and limitations. Hence, it is important to search for drugs capable of protecting myocardial cells from necrotic damage especially by strengthening the cardiac cell membrane.

Early in this century, Thomas A. Edison predicted "the doctor of the future will give no medicine, but will interest his patients in the care of the human frame, in diet, and in the cause and prevention of disease." In the years ahead physicians and patients alike have embraced Mr. Edison's prediction and looked to natural sources for healing and wellness. Employing natural substances including vitamins, minerals, trace elements, amino acids, fatty acids, and phytonutrients (substances derived from plant sources) in optimal supplemental quantities can produce efficacious therapeutic results.

Much information has been disseminated in the past two decades regarding nutrition and cardiovascular diseases, mainly myocardial infarction. There are numerous inter-connections between nutrients and biochemical pathways, which are involved in the prevention of myocardial infarction and its treatment. Ensuring more efficient functioning of the biochemical pathways by promoting proper diet and or supplementation can have a significant positive impact on this multi-factorial disease process. The major abnormalities noticed in myocardial infarction are lipidaemia, peroxidation and loss of plasma membrane integrity. Hence the drug should possess antilipidemic, antiperoxidative and membrane stabilizing properties. Also, it should be devoid of any adverse side effects. So it is better to be a biological molecule. If that molecule possesses

all the desired properties and also involved in the biochemical pathways related to cardiovascular function, it could be of significance.

A number of scientists have been investigating the connection between micronutrients such as vitamins, minerals, amino acids, flavanoids, coenzymes, and myocardial infarction. For example, vitamin E is a biological molecule possessing antioxidant (Amann *et al.*, 1999) and membrane stabilizing (Mukherjee *et al.*, 1997) properties but it is not directly involved in any of the metabolic pathways related to myocardial infarction. Though carotenoids have been found to be effective in counteracting free radical generation in myocardial infarction condition it is not directly involved in the myocardial function (Konovalova *et al.*, 1989). L-Arginine and L-lysine are found to be effective in preventing myocardial damage and ensures normal myocardial function through nitrous oxide metabolism, but their membrane stabilizing capability is so far not clear (Ebenezar *et al.*, 2003[b]). Aspartate and glutamate have been shown to improve cardiac recovery after hypoxia or ischemia under normothemic conditions. Although these carboxylic amino acids have been reported to mediate the recovery of left ventricular pressure and contractile function of the myocardium, they are poor free radical scavengers in nature. Grape seed proanthocyanidine extract has been reported to attenuate oxidative stress and to improve cell survival and permit recovery of contractile function in myocardium, but it is not involved in any of the biochemical pathways of myocardium (Bagchi *et al.*, 2000). The pineal gland hormone, melatonin has been proved to provide protection for myocardium by its antioxidant and membrane stabilizing properties. Since it is involved in regulating the biological rhythm, a hormonal imbalance is often observed upon administration of melatonin (Acikel *et al.*, 2003).

The consumption of diets rich in seafood is associated with a reduced risk of vascular diseases and certain cancers. The marine polyunsaturated fatty acids (PUFA) have been reported to exert cardioprotective effects through prostaglandin metabolism (Nair *et al.*, 1997). PUFA are well known for its peroxidative properties, which is highly deleterious to the stabilization of membrane. Reports by Farvin *et al.* (2004) suggest that the cardio protective effect of squalene, an antioxidant isoprenoid derived from shark liver oil is ascribable to its membrane stabilizing property and antioxidant nature. However, at lower supplementation rate it may lead to excess synthesis of cholesterol.

Taurine (2-aminoethanesulfonic acid), a non-protein sulfur containing amino acid, is the most abundant free amino acid and has been shown to play several essential roles in the human body (Lombardini., 1996). It is widely distributed in very high concentrations in brain, heart, kidney, lens and reproductive organs (Huxtable, 1992). Some sea foods are rich in taurine. It is involved in various important biological and physiological functions, which include cell membrane stabilization (Heller-Stilb *et al.*, 2002), antioxidation (Atmaca, 2004), detoxification (Birdsall, 1998), osmoregulation (Timbrell *et al.*, 1995), neuromodulation and brain (Renteria *et al.*, 2004) and retinal development (Wright *et al.*, 1986). Taurine makes up more than 50% of the total free amino acid pool in the mammalian heart (Lombardini, 1996). Earlier studies (Warskulat *et al.*, 2004) demonstrated that pathology develops in the myocardium if the animal is depleted of taurine stores either through a taurine deficient diet or use of taurine transport antagonists. Pion *et al.* (1987) were the first to explain the role of dietary taurine deficiency associated with a dilated cardiomyopathy observed in experimental animals. Other studies by Keith *et al.* (2001) and Lake (1994) have explored the relationship between taurine deficiency and cardiac contractility, loss of cardiac myofibrils, and arrhythmogenesis. Though there is considerable evidence concerning the pharmacological significance of taurine in

maintaining the integrity of organism, the protective effect of taurine in experimentally induced myocardial infarction condition in rats have not explored in detail.

Intraperitoneal administration of isoproterenol [L-β-(3, 4-dihydroxyphenyl)-α-isopropyl amino ethanol hydrochloride], a β- adrenergic agonist, produces acute irreversible myocardial injury in rats that morphologically resembles myocardial infarction of human beings (Ravichandran *et al.*, 1990). It induces myocardial necrosis by a multiple step mechanism (Chagoya de Sanchez *et al.*, 1997). Peroxidation of endogenous lipids has been shown to be a major factor in the cardio toxic action of isoproterenol (Chattopadhyay *et al.*, 2003). Isoproterenol-induced myocardial infarction is generally attributed to the formation of the highly reactive hydroxyl radical ($OH^{\bullet}$), stimulator of lipid peroxidation and source for the destruction and damage to cell membranes (Farvin *et al.*, 2004). Alterations in tissue defense systems including chemical scavengers or antioxidant molecules and the antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase have been reported in isoproterenol-induced myocardial infarction (Saravanan & Prakash, 2004).

In the present study, an attempt has been made to assess the preventive effects of taurine against isoproterenol-induced myocardial infarction in rats, an experimental animal model for myocardial infarction of human beings.

The main objectives of the work are

- To study the cardio protective effects of taurine in experimentally induced myocardial infarction by assaying the levels of serum diagnostic marker enzymes, troponin T, homocysteine, protein, glycoproteins and apolipoproteins.

- To evaluate the antilipidemic effect of taurine against isoproterenol-induced myocardial infarction in rats by determining the levels of lipid components.
- To study the antilipid peroxidative effect of taurine on tissue antioxidant defense system in isoproterenol-induced myocardial infarction in rats.
- To determine the membrane stabilizing action of taurine by assaying the activities of lysosomal enzymes, membrane-bound ATPases and mineral status.
- To study the effect of taurine on mitochondrial function in experimentally induced myocardial infarction by assaying the activities of TCA cycle enzymes and respiratory marker enzymes.
- To investigate the electrophoretic pattern of serum proteins.
- To study the effect of taurine on amino acid composition and fatty acid profile in experimentally induced myocardial infarction in rats.
- To study the histopathological pattern to confirm the protective action of taurine against isoproterenol-induced myocardial infarction in rats.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Myocardial Infarction

The term "myocardial infarction" focuses on the heart muscle, which is called the myocardium, and the changes that occur in it due to the sudden deprivation of circulating blood. The word "infarction" comes from the Latin "infarcire" meaning "to plug up or cram." It refers to the clogging of the artery, which is frequently initiated by cholesterol piling up on the inner wall of the blood vessels that distribute blood to the heart muscle.

Coronary arteries are blood vessels that supply the heart muscle with blood and oxygen. Coronary atherosclerosis (or coronary artery disease) refers to the atherosclerosis that causes hardening and narrowing of the coronary arteries. Blockage of a coronary artery deprives the heart muscle of blood and oxygen, causing injury to the heart muscle. Diseases caused by the reduced blood supply to the heart muscle from coronary atherosclerosis are called coronary heart diseases (CHD). Coronary heart diseases include heart attacks, sudden unexpected death, chest pain (angina), abnormal heart rhythms and heart failure due to weakening of the heart muscle.

Myocardial infarction results from the blockage of artery due to atherosclerosis, a gradual process in which plaques (collections) of cholesterol are deposited in the walls of arteries. Cholesterol plaques cause hardening of the arterial walls and narrowing of the inner channel (lumen) of the artery. Plaque rupture with subsequent exposure of the basement membrane results in platelet aggregation, thrombus formation, fibrin accumulation, hemorrhage into the plaque and varying degrees of vasospasm. This can result in partial or complete occlusion of the vessel and subsequent myocardial ischemia

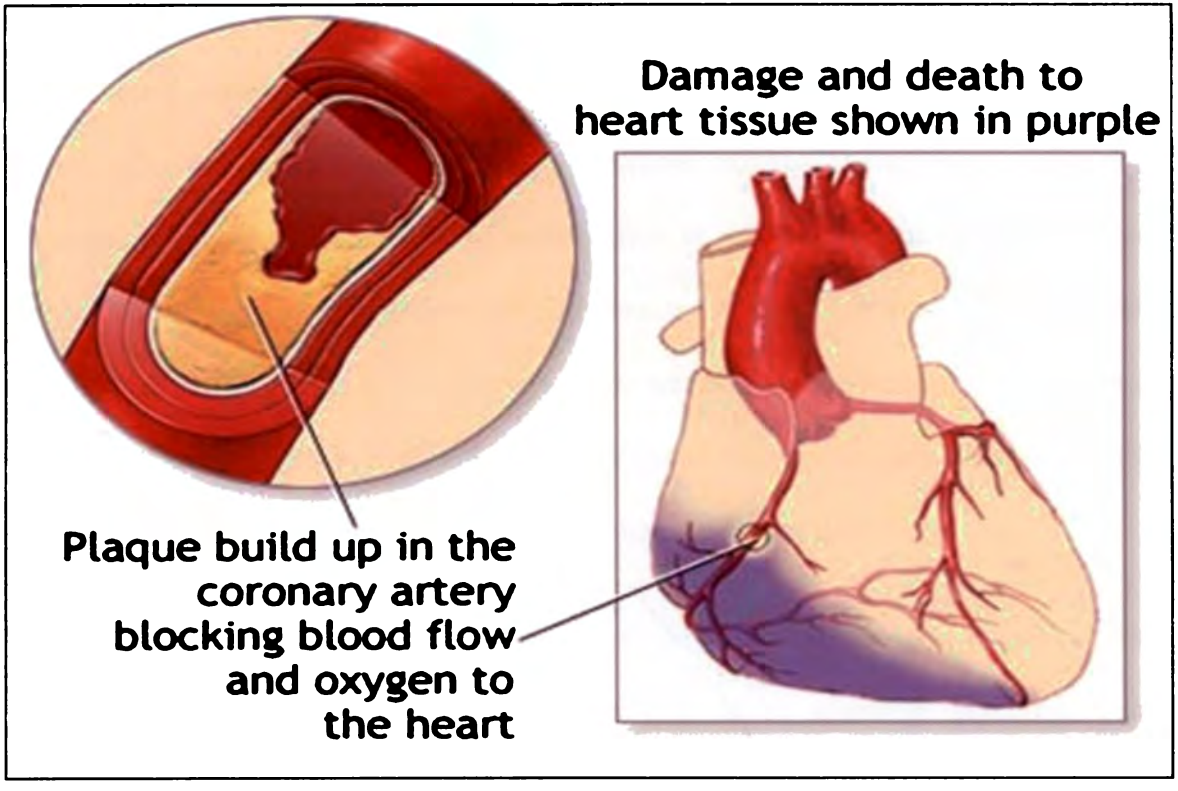


Fig 2.1.1 Myocardial Infarction

resulting in an acute reduction of blood supply to a portion of the myocardium. Arteries that are narrowed by atherosclerosis cannot deliver enough blood to maintain normal function of the parts of the body they supply. The initial events occur within the few seconds or minutes after total coronary artery occlusion and are associated with reversible changes. Total occlusion of the vessel for more than 4-6 hours results in irreversible myocardial necrosis, but reperfusion within this period can salvage the myocardium and reduce morbidity and mortality.

The severity of myocardial infarction is dependent on three factors: the level of the occlusion in the coronary artery, the length of time of the occlusion, and the presence or absence of collateral circulation. Generally, the more proximal the coronary occlusion, the more extensive is the amount of myocardium at risk of necrosis. The larger the myocardial infarction, the greater is the chance of death due to a mechanical complication or pump failure. The longer the time period of vessel occlusion, the greater the chances of irreversible myocardial damage distal to the occlusion. The extent of myocardial cell death defines the magnitude of the myocardial infarction.

Myocardial infarction is characterized by a reduced production of energy stores (ATP molecules), as the myocyte shifts from aerobic to anaerobic glycolysis and increased glycogenolysis. Enzymes that participate in the breakdown of glycogen such as the phosphorylases are putatively released during this time. In order to conserve energy, there is impairment or failure of the ATP-dependent ion membrane pumps resulting in the release of intracellular electrolytes such as potassium and phosphate. Concomitant to energy deficits is the inability of the heart to remove waste products. This leads to accumulation and release of metabolites such as lactate and adenosine. Low molecular weight proteins may be able to pass through reversibly injured but

reparable membranes. If the affected artery becomes potent during the early time intervals either spontaneously or by pharmacologic (thrombolytic therapy) or surgical (angioplasty or bypass) means, the jeopardized myocytes can fully recover.

Prolonged or permanent occlusion, however, leads to the onset of irreversible damage. The hallmark of irreversible damage is disruption of cellular membranes and release of macromolecules such as enzymes and large molecular weight proteins. The release of mitochondrial proteins in particular, is indicative of cell death and tissue necrosis. Cardiac enzymes and proteins have the advantage of organ specificity, and essentially are only released during irreversible damage. However, they cannot directly pass to the vasculature, and must travel through slow lymphatic drainage. Therefore there is a delay before they appear in the blood. In addition, proteins with low molecular weight will appear in blood sooner than large proteins and enzymes. The size of the protein and its distribution within the cell dictates the appearance rate. Small intracellular proteins (e.g., myoglobin and fatty acid binding protein) appear first, while large proteins (e.g., CK and LDH) and those that are part of the contractile apparatus (e.g., troponin) have a delayed appearance. Strategies for development of early acute myocardial infarction markers should be focused on proteins that are specific to the heart.

Myocardial infarction can be subcategorized on the basis of anatomic, morphologic, and diagnostic clinical information. From an anatomic or morphologic standpoint, the two types of myocardial infarction are transmural and nontransmural. A transmural myocardial infarction is characterized by ischemic necrosis of the full thickness of the affected muscle segments, extending from the endocardium through the myocardium to the epicardium. A nontransmural myocardial infarction is defined as an area of ischemic

necrosis that does not extend through the full thickness of myocardial wall segments. In a nontransmural myocardial infarction, the area of ischemic necrosis is limited to either the endocardium or the endocardium and myocardium. It is the endocardial and subendocardial zones of the myocardial wall segment that are the least perfused regions of the heart and are most vulnerable to conditions of ischemia. If a large amount of heart muscle dies, the ability of the heart to pump blood to the rest of the body is diminished, and this can result in heart failure. The body retains fluid, and organs (for example, the kidneys) begin to fail.

2.1.1 Symptoms

Everyone will experience different symptoms with each heart attack. Heart attacks frequently occur from 4:00 A.M. to 10:00 A.M due to higher adrenaline amounts released from the adrenal glands during the morning hours (Willich *et al.*, 1992; Brezinski *et al.*, 1988) and include the following symptoms - a sensation in the chest that may be felt as choking, numbness, squeezing or pressure. Chest pain behind the sternum is a major symptom of heart attack (Manfredini *et al.*, 2003). But in many cases the pain may be subtle or even completely absent (called a "silent heart attack"), especially in the elderly and diabetics (Jalal *et al.*, 1999). Often, the pain radiates from chest to arms or shoulder, neck, teeth, or jaw, abdomen or back, lasts longer than 20 min. Not fully relieved by rest or nitrioglycerine, both of which can clear pain from angina, the pain can be intense and severe or quite subtle and confusing. Other symptoms either alone or along with chest pain include shortness of breath, cough, lightheadedness, dizziness, fainting, nausea or vomiting sweating, which may be profuse, feeling of "impending doom", anxiety, pallor (paleness) and restlessness.

2.1.2 Risk factors

2.1.2.1 Smoking

Prolonged exposure to cigarette smoke, either active or passive, increases the risk of dying from a heart attack or complications arising from atherosclerosis by three to fivefold. Much of the ill-omened health effects related to smoking occur due to an increase in free-radical activity. Unfortunately, as the population of free radicals increases, vitamin C (a powerful antioxidant) decreases in the smoker. The following reactions define the hardship cigarette smoking imposes upon the cardiovascular system, increased heart rate (one cigarette can increase the heart rate 20-25 beats a minute) and disrupted circulation to the legs and feet. It takes 6 h for the circulation to return to normal after just one cigarette.

Data published in the Journal of the American Medical Association (JAMA), indicate that the critical phase of cardiovascular disease is significantly accelerated in smokers. The critical phase is marked by 60% coverage of arterial surfaces with atheromatous materials. Although the ages were hypothetically assigned, a smoker with normal blood pressure and cholesterol levels reaches the critical phase 10 years earlier than the nonsmoker and 20 years earlier if the smoker is also hypertensive (Grundy, 1986).

2.1.2.2 Obesity

Excessive body weight is a risk factor in so many diseases that obesity itself is now regarded as a disease. A troublesome weight problem is no longer just an annoyance but a significant risk for heart disease, both independently and in association with other risk factors such as diabetes, hypertension and dyslipidemia (Rao *et al.*, 2001). The pattern of the fat distribution is another important prognosticator of host vulnerability. Overeating in

the absence of obesity poses a cardiac risk, as well. Reports from patients indicated that unusually heavy meals were often consumed during a 26h period preceding a myocardial infarction (Lopez-Jimenez *et al.*, 2000). Other factors increasing cardiovascular risk, such as excessive fibrinogen, elevated C-reactive protein, and insulin resistance, often shares common denominator obesity. During the American Heart Association's 71st Scientific Session (in 1998), the guidelines for assessing the risks imposed by obesity (as measured by Body Mass Index) were reported. This study was based on data from the Framingham Heart Study (Kagan *et al.*, 1962), Third National Health and Nutrition Examination Survey (Thompson *et al.*, 1998).

2.1.2.3 Diabetes

The degenerative process that accompanies diabetes significantly affects the heart. Atherosclerosis tends to develop early, progress rapidly, and be more virulent in the diabetic. Data released from the Framingham Study showed a 2.4-fold increase in congestive heart failure in diabetic men and a 5.1-fold increase in diabetic women over the course of the 18-year study (Fein *et al.*, 1994). Diabetics are particularly susceptible to silent myocardial infarctions, that is, an asymptomatic attack that interrupts the blood flow to coronary arteries. More than 80% of people with diabetes die as a consequence of cardiovascular diseases, especially heart attacks (Whitney *et al.*, 1998). High homocysteine levels also play a significant role in diabetes-induced cardiovascular disease.

In fact, hyperhomocysteinemia is considered a reliable predictor of mortality among diabetic patients. The symptoms of hypoglycemia can mimic a heart attack, that is, dizziness, fatigue, sweating, shakiness, lightheadedness, palpitations, and in some cases, unconsciousness. Normal brain function requires 6 g of glucose an hour, which can be

delivered only if arterial blood contains over 50 mg/dl of glucose (Pike & Brown, 1984). Although hypoglycemia is not a heart attack, the stress imposed upon the heart can be significant. Chronic hyperglycemia causes monocytes and adhesion molecules to bind to vessel walls. In turn, cholesterol and other lipids are more easily deposited. Lipids become disorganized, with more of the LDL cholesterol and less of the beneficial HDL cholesterol appearing in the bloodstream (Garg & Grundy, 1990).

2.1.2.4 Hypercholesterolemia

It is established that high cholesterol levels account for about 10-15% of ischemic strokes. When levels of HDL (high density lipoproteins, also known as good cholesterol) are elevated, cardiovascular disease is reduced. The HDL_2 sub fraction is even more correlated with cardiac protection and longevity than total HDL cholesterol (Sardesai, 1998). Typically, low triglyceride/LDL levels and high HDL levels place an individual in a better position cardiovascularly. Elevated triglyceride levels usually modulate when less food is consumed, particularly foods causing a rise in blood sugar levels. Too much cholesterol is not good, but too little may not be good either. The American Heart Association announced in 1999 (at the annual Stroke Conference) that people with cholesterol levels less than 180 mg/dl doubled their risk of hemorrhagic stroke compared to those with cholesterol levels of 230 mg/dL, however, the risk of a stroke escalated as cholesterol levels exceeded 230 mg/dl. The National Cholesterol Education Program announced that cholesterol levels of approximately 200 mg/dL appear ideal for stroke prevention (Castelli, 1988).

2.1.2.5 Homocysteine

Homocysteine is a sulfur containing non-essential amino acid produced by the demethylation of the essential amino acid methionine. Because of an increasing

awareness of the risks imposed by newer risk factors, homocysteine is being factored into the genetic equation. Hyperhomocysteinemia may arise from genetic defects of enzymes involved in homocysteine degradation and remethylation. With a gene frequency between one in 70 and one in 200, elevated blood levels of homocysteine may be more common than previously thought (Berwanger *et al.*, 1995). Canadian researchers estimate the inherited amino acid disorder (homocysteinemia) is present in approximately 20% of coronary artery disease patients (Superko, 1995). There are multiple mechanisms involved in the pathogenesis of hyperhomocysteinemia, including not only heterozygosity, but dietary factors as well (Kardaras *et al.*, 1995).

2.1.2.6 Stress

More than one-quarter of a million heart episodes occur annually, that is, palpitations, angina, arrhythmias, and heart attack as a result of a stressful experience. During periods of mental or emotional arousal, a silent ischemic attack (a decreased supply of oxygenated blood) can occur. Unlike an angina attack, which is usually prompted by physical exertion, more than three-fourths of silent ischemic attacks are caused by mental arousal. There is also a definite link between the hardening of the carotid artery and higher levels of stress (Barnett *et al.*, 1997). A recent study of 2800 men and women over 55 years of age showed that even minor depression can increase cardiac mortality 60%, while major depression may actually triple the rate of cardiac-related deaths (Penninx *et al.*, 2001). When an ailing heart is struggling to keep pace with circulatory demands it is forced to deal with an emotional provocation. It is reported that an individual who is prone to anger is about 3 times more likely to have a heart attack or sudden cardiac death than someone who is the least prone to anger (Williams *et al.*, 2000). Higher levels of homocysteine are associated with feelings of aggression and rage in both men and women

(Stoney *et al.*, 2000). Under stress, the sympathetic nervous system is alerted and the release of adrenaline increases, ultimately, one's breathing, heartbeat, and blood pressure also increase.

2.1.2.7 Gender

Studies have demonstrated that heart disease is the number one killer for both men and women (Kagan *et al.*, 1962; Kannel *et al.*, 1998). In both men and women, coronary heart disease has exceeded that of other cardiovascular illnesses, such as stroke or congestive heart failure. While coronary events occurred twice as often in men, with advancing age the incidence of heart disease in women approaches that seen in men (Swahn, 1998). Premenopausal women appear to be somewhat protected from atherosclerosis due to the presence of estrogen, which lowers LDL cholesterol and raises HDL cholesterol, reducing the risk (Wenger, 2003). Menopause appears to be the interval associated with a significant rise in coronary events, as well as a shift to more serious manifestations of the disease.

2.1.2.8 Heredity

The risk is higher if there is a family history of heart diseases and people with such a history should therefore be made aware of the risk of developing heart diseases. Geneticists are looking for mutated genes that may be expressing themselves as contributors to coronary artery disease. For example, 50% of suppressed HDL cholesterol can be linked to genetic factors. A gene (ABC1), when mutated, appears responsible for increasing the risk of heart disease by lowering levels of HDL cholesterol. It is reported that people with defects in ABC1 have just as much risk for heart disease because of too little HDL as individuals with high levels of LDL cholesterol (Marcil *et al.*, 1999).

2.1.2.9 Sedentary lifestyle

Scientists believe that a properly planned exercise program may be the single greatest preventive measure against cardiovascular disease. However, it is extremely important that the individual and the activity be properly matched. Exercise reduces blood pressure and heart rate by influencing sympathetic neural and hormonal activity. As epinephrine (adrenaline) and nor-epinephrine levels are decreased, one's blood pressure and heart rate subsequently decrease (Katona *et al.*, 1982; Duncan *et al.*, 1985; Smith *et al.*, 1989). A regular exercise program reduces the risk of stroke, not only by lowering blood pressure, but also by increasing peripheral circulation and oxygen delivery. C-reactive protein, another of the newer risk factors for cardiovascular disease, also appears lowered by exercise (Szymanski *et al.*, 1994; Ford, 2002). Excessive fibrinogen, a risk factor for cardiovascular disease, is impacted by exercise. Exercise of moderate intensity increases fibrinolytic activity by increasing tissue plasminogen activators, which break down fibrinogen, decreasing the risk of blood clot formation.

2.1.2.10 Newer risk factors

In the last 25 years, the incidence of coronary fatalities has decreased 33%. This is due largely to avoiding the traditional risk factors. An auxiliary list of newer predictive factors may significantly increase the numbers benefiting from 21st century diagnostics and treatment (Ridker, 1999). Those with high levels of fibrinogen were more than twice as likely to die of a heart attack, the risk of a stroke increases as well (Wilhelmsen *et al.*, 1984; Packard *et al.*, 2000). Lipoprotein (a) modulates fibrinolysis, inhibits plasminogen binding to fibrin, and may also inhibit t-Pa, a clot-dissolving substance produced naturally by cells in the walls of blood vessels. The end result is a greater risk of blood clot formation, and thus heart attack and stroke (Loscalzo *et al.*, 1990; Ridker, 2000; Caplice

et al., 2001). Homocysteine is regarded as more dangerous than cholesterol because homocysteine damages the artery and then oxidizes cholesterol before cholesterol infiltrates the vessel (Braverman, 2003). It is now widely recognized by scientists as the single greatest biochemical risk factor for heart disease, estimating that homocysteine may be a participant in 90% of cardiovascular problems. Syndrome X represents clusters of symptoms and includes an inability to fully metabolize carbohydrates, hypertriglyceridemia, reduced HDL levels, smaller and denser LDL particles, increased blood pressure, visceral adiposity, disrupted coagulation factors, insulin resistance, hyperinsulinemia, and often, increased levels of uric acid, a forerunner to heart disease (Reaven, 2000; Fang *et al.*, 2000). C - reactive protein appears intricately involved in the inflammatory process, thus proving to be a potential target for the treatment of atherosclerosis (Pasceri *et al.*, 2000; Alvaro-Gonzalez *et al.*, 2002).

2.1.3 Signs and tests

Physical examination may show rapid pulse, crackles in the lungs, a heart murmur, or other abnormal sounds. Blood pressure may be normal, high or low. The following tests may reveal a heart attack and the extent of heart damage:

Cardiac enzymes are muscle proteins that are released into the blood circulation by dying heart muscles when their surrounding membranes dissolve. Such enzymes include creatine kinase (CK), special subforms of CK, and troponin (Collinson *et al.*, 2003). The following tests may show the by-products of heart damage and factors indicating a high risk for heart attack,

- Troponin T
- Creatine kinase
- Diagnostic marker enzymes

- Lipid profile
- Homocysteine
- Electrocardiogram (ECG) single or repeated over several hours changes (Kennon *et al.*, 2003)
- Echocardiography
- Coronary angiography
- Nuclear ventriculography (MUGA or RNV)

2.1.4 Treatment

The goals of treatment are to stop the progression of the heart attack, to reduce the demands on the heart so that it can heal, and to prevent complications. The immediate goal of treatment is to quickly open the blocked artery and restore blood flow to the heart muscle, a process called "reperfusion". Delay in establishing reperfusion can result in irreversible death to the heart muscle cells and reduced pumping force of the remaining heart muscle (Gersh, 2003; Janousek, 2003). An intravenous line will be inserted to administer medications and fluids. A urinary catheter may be inserted to closely monitor fluid status. Oxygen is usually given, even if blood oxygen levels are normal. This makes oxygen readily available to the tissues of the body and reduces the workload of the heart. Nitrates such as nitroglycerin are given for pain and to reduce the oxygen requirements of the heart. Morphine or morphine derivatives are potent painkillers that may also be given for a heart attack.

If the ECG recorded during chest pain shows a change called "ST-segment elevation," clot-dissolving (thrombolytic, blood thinning medications) therapy may be initiated as an IV infusion of streptokinase or tissue plasminogen activator. Blood clots are a major factor in heart attacks. Anti-clotting agents that inhibit or break up blood clots are used at

every stage of heart disease. They are generally either anti-platelet agents or anticoagulants. It will be followed by an IV infusion of heparin as a blood-thinning agent to prevent blood clots and to maintain an open artery during the initial 24-72 hours (Neri Serneri *et al.*, 1989). Taken orally, warfarin may be prescribed to prevent further clot development.

Thrombolytic therapy is not appropriate for people who have had a major surgery, organ biopsy or major trauma within the past 6 weeks, recent neurosurgery, head trauma within the past month, history of gastrointestinal bleeding, brain tumor, stroke within the past 6 months and current severely elevated high blood pressure. Significant bleeding can complicate use of thrombolytic therapy. A cornerstone of therapy for a heart attack is antiplatelet medication. One antiplatelet agent widely used is aspirin. Aspirin alone has been reported to reduce risk of death from heart attack or stroke by 25% to 50% and to cut risk of non-fatal heart attacks by 34 % (Buerke & Rupprecht, 2000). Two other important antiplatelet medications are ticlopidine (Ticlid) and clopidogrel (Plavix). Other medications include β-blockers, ACE Inhibitors and calcium channel blockers.

β-blockers reduce the oxygen demand of the heart by slowing the heart rate and lowering pressure in the arteries. They are now well known for reducing deaths from heart disease by reducing the workload of the heart. They include propranolol (Inderal), carvedilol (Coreg), bisoprolol (Zebeta), acebutolol (Sectral), atenolol (Tenormin), labetalol (Normodyne, Trandate), metoprolol (Lopressor. Toprol-XL) and esmolol (Brevibloc) (Gottlieb & McCarter, 2001). A number of agents are available for lowering cholesterol and other dangerous fat molecules (lipids). They include statins, fibrates and niacin. Statins may have significant benefits for heart patients. ACE Inhibitors includes

(Khattar, 2003; Bauersachs & Fraccarollo, 2003) ramipril, lisinopril, enalapril, or captopril and calcium channel blockers also serves to prevent heart failure.

2.1.5 Surgery and other procedures

Emergency coronary angioplasty may be required to open blocked coronary arteries. This procedure may be used instead of thrombolytic therapy or in cases where thrombolytics should not be used. Often the re-opening of the coronary artery after angioplasty is ensured by implantation of a small device called a stent. Emergency coronary artery bypass surgery may be required in some cases. The different types of laboratory tests (biochemical, immunological and coagulative) now available, should soon allow improvement in the diagnosis and therapy of ischemic coronary diseases.

2.1.6 Prevention

To prevent a heart attack:

- Control blood pressure
- Control total cholesterol levels.
- Stop smoking
- Eat a low fat diet rich in fruits and vegetables and low in animal fat.
- Control diabetes
- Lose weight if overweight.
- Exercise daily or several times a week by walking and other exercises to improve heart fitness. (Consult your health care provider first.)

After a heart attack, follow-up care is important to reduce the risk of having a second heart attack. Often, a cardiac rehabilitation program is recommended to return to a "normal" lifestyle. Follow the exercise, diet, and medication regimen prescribed by the doctor.

2.2 Taurine

Taurine is one of the most common sulfur-containing amino acids found in nature. This non-protein amino acid is present in high concentration in most of the tissues, amounting to about 50-60% of the total free amino acid pool. Tiedemann & Gmelin were the first to isolate taurine from ox bile in 1827, where it was found in high concentration (Huxtable, 1992). Demarcay, in 1838 gave the name taurine to a similar crystalline material obtained from ox bile. The bovine connection (Latin name "bos taurus") clearly explains the descriptive name, "Taurine". However, the name of taurine was credited by Demarcay to Gmelin. In the succeeding years, intensive analytical work produced a vast quantity of information on the distribution of taurine in animal organs.

Taurine is a conditionally essential amino acid involved in a large number of metabolic processes. Its function in the body has been underestimated for a long time. In recent years, it has become clear that taurine is a very important amino acid in the visual pathways, the brain, nervous system and cardiac functions. It is a conjugator of bile acids and hence performs key functions in cholesterol metabolism (Gaull *et al.*, 1985). Basically, its function is to facilitate the passage of sodium, potassium, calcium and magnesium ions into and out of cells, and to stabilize the structural and functional integrity of the cell membranes (Satoh, 1998). It is involved in detoxification of xenobiotics and is also very essential for efficient fat absorption and solubilization (Loria *et al.*, 1997). The requirement of this free amino acid is absolutely indispensable in prenatal and infant development (Chesney *et al.*, 1998). Though absence of taurine does not results in immediate deficiency and disease, long-term deprivation can cause a multitude of health problems. One is not stumbling into the abyss of teleology in thinking

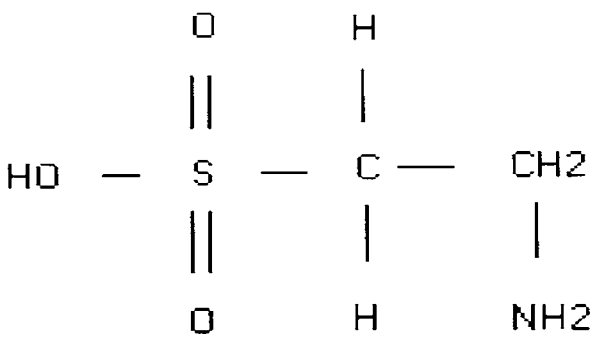


Fig. 2.2.1 Structure of Taurine

that a compound conserved so strongly and present in such high amounts is exhibiting functions that are advantageous to the life forms containing it.

2.2.1 Distribution of taurine

Taurine is a phylogenetically ancient compound with a disjunctive distribution in the biosphere. It is present in high concentration in algae and in animal kingdom, including insects and arthropods. It is generally absent or present in traces in the bacterial and plant kingdoms. In many animals, including mammals, it is one of the most abundant low-molecular-weight organic constituents. A 70-kg human contains up to 70 g of taurine. Taurine is found in greater concentrations in all animal products. Meat, poultry, eggs, dairy products, and fish are good sources of taurine. **Table: 2.2.1** shows the level of taurine content present in some seafood (ZhaoXi-he, 1994). In plant kingdom, taurine occurs in traces, averaging ~0.01 μmol/g fresh wt of green tissue. This is <1% of the content of the most abundant free amino acids (Huxtable, 1992).

2.2.2 Structure of taurine

The structure of taurine was well established by Redtenbacher (1846). Taurine (2-aminoethane sulphonic acid) is a small organic molecule consisting of hydrogen (H), nitrogen (N), carbon (C), sulfur (S) and oxygen (O) **(Fig: 2.2.1)**. It is structurally different from most of the biological amino acids in following ways;

i. It is a sulfonic acid rather than a carboxylic acid

ii. It is a β-amino acid rather than an α-amino acid

iii. It does not have a chiral center and

iv. It does not have an L- or D-configuration.

Table: 2.2.1 Taurine content in sea food

Sea Food	Taurine content
Conch (Strombus gigas)	850
Ink fish	672
Blood Clam	617
Clam	496
Shellfish	332
Crab	278
Prawn	143
Sole	256
Crucian carp	205
Silver carp	90
Hairtail fish	56
Yellow croaker	88
Eel	91

Values are mg/100g edible portion (Zhao Xi-he, 1994).

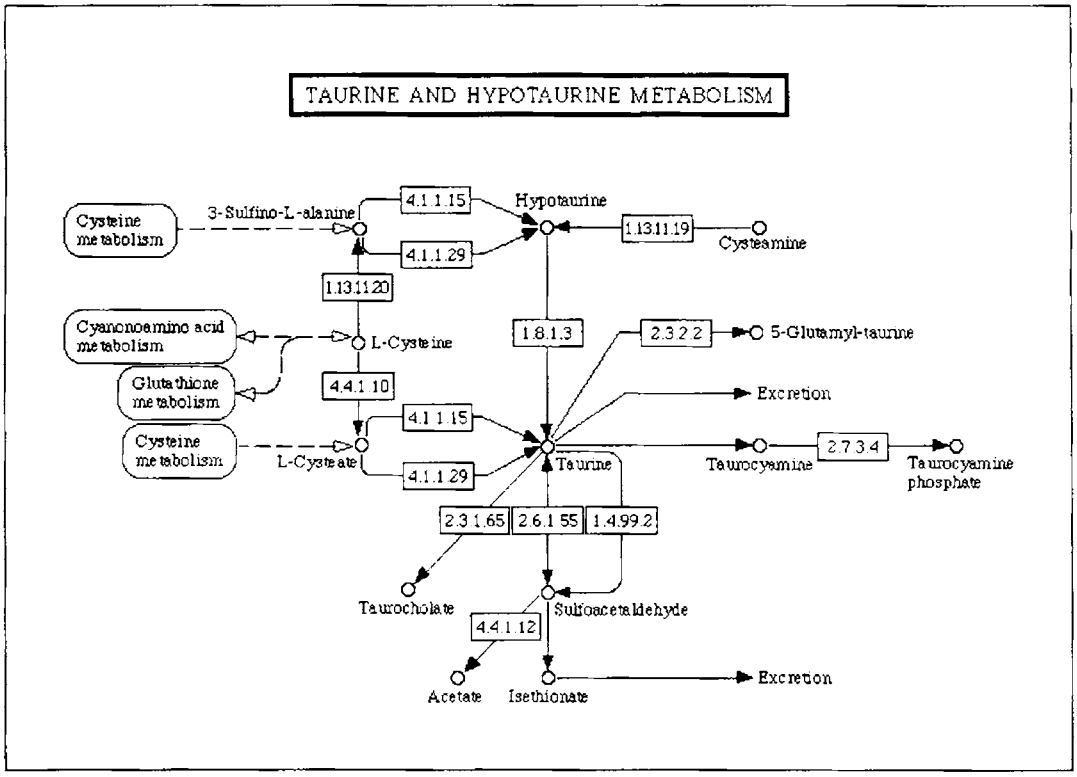
2.2.3 Pharmacokinetics

Though taurine can be synthesized from methionine and cysteine in living beings, it is usually derived from food sources. The average intake of taurine varies widely from 40 to 400 mg per day. Following ingestion, taurine is absorbed from the small intestine, via the β-amino acid or taurine transport system, which is sodium and chloride dependent carrier system located in the apical membrane of intestinal mucosa. Intestinal health is a major factor in the ability to absorb and provide taurine in sufficient amounts for the multitude of biological processes in which it is involved. The absorbed taurine is transported to the liver via the portal circulation, where much of it forms conjugates with bile acids. Taurochoate, the bile salt conjugate of taurine and cholic acid, is the principal conjugate formed by the action of the enzyme choloyl-CoA N-acyltransferase. The taurine conjugates are excreted through the biliary route. Taurine that is not conjugated in the liver is distributed from the systemic circulation to various tissues in the body. It is not usually completely reabsorbed by the kidneys and some fraction is excreted in the urine (Hayes & Sturman, 1981). Reports by Barada *et al.* (1997) indicated that the intestinal capacity to absorb taurine decreased with aging.

2.2.4 Taurine metabolism

The body synthesizes taurine from the amino acids methionine and cysteine. Vitamin B-6 (pyridoxal-5' phosphate) is a key cofactor in this process. From cysteine, hypotaurine (an intermediary product in the metabolic process) is produced. Its turnover into taurine occurs rapidly thereafter. Taurine synthesis is regulated by the enzyme cysteine sulfinic acid decarboxylase (CSAD). CSAD is activated under conditions that promote protein phosphorylation (a specific step in the conversion of amino acids into protein). Membrane changes initiated via glutamate or potassium promote CSAD activity, which implies an

Fig. 2.2.2 Metabolism of Taurine



www.genome.jp/kegg/pathway.html

1. EC 4.1.1.15 :glutamate decarboxylase
2. EC 4.1.1.29 :L-cysteinesulfinic acid decarboxylase (CSAD)
3. EC 1.13.11.19 :cysteamine oxygenase
4. EC 1.13.11.20 :cysteine dioxygenase
5. EC 1.8.1.3 :hypotaurine dehydrogenase
6. EC 2.3.2.2 :gamma-glutamyltransferase
7. EC 4.4.1.10 :cysteine lyase
8. EC 2.7.3.4 :taurocyamine kinase
9. EC 2.3.1.65 :glycine N-choloyltransferase
10. EC 2.6.1.55 :taurine-2-oxoglutarate transaminase
11. EC 1.4.99.2 :taurine dehydrogenase
12. EC 4.4.1.12 :sulfoacetaldehyde lyase

anti-excitotoxic role of taurine. There is evidence that taurine is converted in to five other compounds in biological systems. It is converted either to inorganic sulfate and carbamyltaurine or to taurocyamine and phosphotaurocyamine. It can also be converted in to isothionic acid, or conjugated with bile acid which is the major route. Three different types of reactions, transamination, oxidation and oxygenation initiate taurine degradation. In the first two cases, sulfoacetaldehyde is an intermediate, whereas in the third case, taurine is converted to sulfite and aminoacetaldehyde **(Fig: 2.2.2)**.

2.2.5 Properties of taurine

2.2.5.1 Physico-chemical properties

Molecular formula	$C_2H_7NO_3S$
Molecular weight	125.15
Physical state	Tetragonal needle shaped crystals.
Colour	Colorless
Odor	Odorless
Solubility	Soluble in water (10.48 g/100ml at 25 °C) and insoluble in absolute alcohol.
Melting point	328 °C (decomp.)
pH (0.5M in water, 25 °C)	4.5-6
Optical rotation	Nil

2.2.5.2 Physiological properties

(i) Antilipidemic effect of taurine

Taurine has been reported to attenuate the elevation in total and LDL cholesterol levels in people consuming high fat/ high cholesterol diet. Recently, physicians have

adopted 2:1 as the ideal ratio of total cholesterol to HDL and prescribed taurine to reach that magical lipids ratio (Mizushima *et al.*, 1996). Reports by Murakami *et al.* (1999) indicate that the administration of taurine lowers serum LDL and VLDL by 44% and elevates HDL by 25% in experimental mice. Taurine conjugates have been reported to suppress very low-density lipoprotein (VLDL) secretion. VLDLs are produced through a series of reactions of proteins with aldehydes. Researchers have demonstrated that taurine exhibits a high reactivity with aldehydes, thus it acts to inhibit protein modification to LDL. With regard to HDL, taurine enhances serum HDL concentration in a dose-dependent manner. High fat diets produce hypercholesterolemia (elevated cholesterol), atherosclerosis, and accumulation of lipids on the aortic valve of the heart. The taurine supplementation to the cholesterol-free diet has also been reported to produce 43% reduction in hepatic triglycerides content (Park & Lee, 1998). The anitlipidemic and cholesterol-lowering action of taurine may lie in its ability to promote the degradation of potentially detrimental cholesterol to relatively harmless bile acids.

(ii) Antidiabetic effect of taurine

In both forms of diabetes, insulin dependent (Type 1) and non-insulin dependent (Type 2), taurine exerts a multitude of beneficial actions. Supplementation of taurine has been reported to prevent the triggering of platelet aggregation in Type 1 diabetes, a major risk factor of cardiovascular diseases (Franconi *et al.*, 1995). Administration of taurine has also been found to normalize the adverse blood lipid profile associated with the diabetic condition. Reports (You & Chang, 1998[a]) indicate that administration of taurine can counteract the elevated plasma triglycerides and LDL cholesterol in diabetics with no adverse effect on serum glucose levels. This effect is largely due to a correction of vascular endothelial vasodialation characteristic of the diabetic state (Kamata *et al.*,

1996). In Type 2 diabetics, the impaired glycemic control is largely due to peripheral insulin resistance, hepatic insulin resistance, and a failure of β-cell function. Recently, taurine as well as the more established natural agents, has found a role in correcting the metabolic anomalies in vascular smooth muscle produced by Type 2 diabetes (McCarty, 1997).

In models of diabetic mice, researchers found that taurine supplementation yields specific beneficial effects on levels of malondialdehyde (MDA), a marker of lipid peroxidation resulting from free radical damage. Dietary supplementation of taurine is able to correct abnormal elevations of MDA and thus to prevent depletion of glutathione in diabetics (Lim *et al.*, 1998). Diabetes is notable for its impact on eyesight and to produce a generalized decline in the content of free amino acids in the retina and retinal pigment epithelium of the eye. Taurine supplementation has been reported to reduce the deleterious action of diabetes on amino acid transport systems, which results in alteration of the cellular amino acid balance. It has also been found as an effective method for correcting the antioxidative imbalances that produce diabetic cataracts (Obrosova *et al.*, 1999).

Taurine supplementation has also been reported to prevent diabetic nephropathy, which is the leading cause of end-stage renal disease in over half of Type 1 diabetics. Reports by Trachtman *et al.* (1995) indicated that taurine supplementation is able to exert beneficial changes in the kidneys of diabetic rats, specifically reducing proteinuria and albuminuria by nearly half. This effect is attributed to the ability of taurine to decrease lipid peroxidation and reduce accumulation of advanced glycosylation end products within the kidney. Nandhini *et al.* (2004) have reported that taurine can be used as a therapeutic supplement for the prevention of diabetic pathology.

(iii) Anticancer properties of taurine

Taurine has been found to inhibit tumors and extend the survival period of tumor induced experimental mice. Zhang *et al.* (1997) observed that, tumor cell membrane fluidity was much improved with taurine treatment. The calcium homeostatic mechanism is one of the critical features involved in the anti-cancer functions of taurine (Finnegan *et al.*, 1998). Also the oral administration of taurine has been found to reduce the degree of membrane damage and to activate glutathione antioxidant system in rats exposed to carcinogens. Reports by You and Chang, (1998[b]) suggested that taurine inhibited hepatocarcinogen-induced lipid peroxidation by operating the tissue antioxidant defense status at a higher level in the liver of experimental animals. Taurolidine, a derivative of taurine produced a significant reduction in the growth of both tumor cells and intraperitoneal tumors (Jacobi *et al.*, 1997). Taurine level in serum and other tissues is a useful marker for providing valuable prognostic information in cancer patients (Scioscia *et al.*, 1998; Vecer *et al.*, 1998).

(iv) Taurine and detoxification

Taurine and to a lesser extent glycine are the major amino acids associated with the removal of toxic chemicals and metabolites from the body (Birdsall, 1998). Low taurine levels are important in chemically sensitive individuals, particularly to chemicals such as chlorine, chlorite (bleach), aldehydes, alcohols, petroleum solvents and ammonia. It has also been found that reactive unstable aldehyde compounds are formed in states of taurine deficiency (Kozumbo *et al.*, 1992). Reports by Timbrell *et al.* (1995) indicate that taurine deficient individuals are more prone to xenobiotics-induced tissue damage. In the liver, taurine administration has been reported to inhibit the toxic effects of high fructose feeding, alcohol, acetaminophen, and thioacetamide in rats (Dogru *et al.*, 2001). In

kidneys, taurine has been proved to protect against cisplatin toxicity and to prevent salt feeding induced renal damage in salt-sensitive rats (Saad *et al.*, 2002). Taurine also protected against ulcers caused by monochloramine, a toxin associated with *Helicobacter pylori* infection (Kodama *et al.*, 2000). Supplementation of taurine has been found to inhibit intestinal endotoxin translocation and subsequent hepatic injury (Roth *et al.*, 1997; Wang. 1995). Accumulation of heavy metals exhibits a variety of toxic effects, and taurine reduces the damage caused by excess levels of cadmium, copper, and lead in rats (Hwang *et al.*, 1998; Hwang *et al.*, 2001; Gurer *et al.*, 2001). Taurine also reduced the toxic effect of oxidized fish oil in rats (Hwang *et al.*, 2000).

(v) Antioxidant effect of taurine

Free radicals are particularly detrimental to tissues which contain high concentration of lipids (fat molecules) like LDL, which free radical atoms readily attack. Physicians now regularly recommend the use of antioxidant vitamins (A, beta-carotene, C and E) and the mineral selenium as counter-measures to free radicals. Recently, the value of taurine as a potent antioxidant has been discovered. If present in sufficient concentration, taurine protect against oxidative cellular damage (Aruoma *et al.*, 1988). Reports indicate that taurine administration effectively counter the stimulation induced by excitatory agents in cerebella neurons. Taurine modulates cell viability, which is an important alternate protective mechanism to offer protection against free radical damage. The antioxidant properties of taurine are also seen in its ability to inhibit neutrophil burst and subsequent oxidative stress, which results in reperfusion injury to heart tissue (Raschke *et al.*, 1995). Taurine prevents the inactivation of superoxide dismutase by H_2O_2 (Pecci *et al.*, 2000). It also acts as an antioxidant by preventing changes in the levels of non-enzymatic free radical scavengers (Tadros *et al.*, 2005).

(vi) Role of taurine on cellular tonicity

Tonicity (synonymous with osmolarity) is a term that describes the status of cell fluid volume in relation to its external medium. Taurine plays an important role in maintaining the delicate balance of tonicity in animal cells (Pasantes-Morales *et al.*, 1998). Cells demonstrate an ability to change their concentration of taurine, in response to how plumped or shrunken in volume they become. Taurine, as an important amino acid osmolyte, helps to regulate osmolarity without causing additional perturbations of cellular tonicity. When cells are hypo-osmotic during hyponatremia, they would normally swell and could lyse if the hyponatremic state continued. Taurine is thus extruded to help prevent such severe osmolar changes. In hypernatremia, cells are usually shrunken or "crenated" and have a reduced fluid volume, taurine uptake is thus increased to help regulate osmolarity and avert severe osmolar changes associated with possible cell death (Trachtman *et al.*, 1990). Under hyper osmolar conditions, net taurine production increases in cells so that they may maintain high intracellular levels. The pathway for taurine efflux also serves to conduct ions (potassium and chloride) from the cells as well. When researchers cultured astrocyte cells under hypo-osmolar conditions, the cells lost 88% of their taurine contents (in addition the amino acids alanine and aspartate) while restoring their normal volume (Olson, 1999). This study suggests that loss of taurine is a major factor in the process of volume regulation.

(vii) Cytoprotective effect of taurine

Taurine is able to resist the action of free radical damage that would otherwise be caused by radioactive substances (Song *et al.*, 1998[a]). Its suppression of transcription counters the production of collagen and elevations of hydroxyproline in pulmonary tissue, serving to protect against radiation-related pulmonary injury. Taurine has been found to

attenuate apoptosis due to oxidative stress and endothelial cell necrosis is halted with similar efficacy (Wang *et al.*, 1996). It is also found that taurine is effective in inhibiting liver fibrosis in experimental model (Refik Mas *et al.*, 2004). Reports by Hilgier *et al.* (1999) indicated that taurine's role in cell volume regulation and neuro-protection may be particularly valuable in those suffering from hepatic encephalopathy. Supplementation of taurine ameliorates ischemic damage of the liver by increasing its biliary, serum and liver calcium concentrations (Ono *et al.*, 1998). Taurine supplementation has benefit as an adjunct therapy in fatty liver associated with simple obesity (Obinata *et al.*, 1996). The protective action of taurine against cyclosporineA-induced hepatotoxicity suggested that taurine may find clinical application against a variety of toxins during occurrence of cellular damage as a consequence of reactive oxygen species (Hagar *et al.*, 2004). These functions are due, in large part, to its antioxidant activity and regulation of intracellular calcium flux, which has great implications for the therapeutic value of taurine (Wang *et al.*, 1996).

(viii) Anti-inflammatory effect of taurine

In inflammatory disease, plasma taurine becomes depleted, signifying a greater demand by the body in this state. Taurine prevents the tissue damage resulting from inflammation. The mechanism involves taurine monochloramine, a product formed through a series of reactions based in the leukocytes (white blood cells) (Nagl *et al.*, 1998). In a dose-dependent manner, taurine monochloramine inhibits the production of substances that promote inflammation, such as nitric oxide, prostaglandin PGE2, and tumor necrosis factor. Thus, taurine itself counters the inflammatory response by reducing the expression of nitric oxide synthase and cyclooxygenase-2 (COX-2), not unlike the role of the new COX-2 specific inhibitor drugs celecoxib and rofecoxib (Barua

et al., 2001). Taurine monochloramine reduces the toxicity of free radical oxidants, serving to decrease the production of tissue-damaging inflammatory substances and regulate the function of neutrophils to promote their protective effect (Nakamori *et al.*, 1990). Taurine also works cooperatively with the cysteine pool to lessen the depressive impact of tumor necrosis factor on cells of the lung. Son *et al.* (1998) reported that taurine ameliorates inflammatory bowel disease by increasing the ability of the colon to defend against oxidative damage. By inhibiting neutrophil (white blood cell) activation and lipid peroxidation, taurine prevents the adhesion of neutrophils to the gastric lining (Son *et al.*, 1996). Taurochenodeoxycholic acid (TCDCA) is reported to attenuate raise in the hydrophobicity of the bile and to reduce the intestinal inflammation (Uchida *et al.*, 1997). Combined treatment of taurolin with vitamin E has been shown to effectively decrease oxidative stress during peritonitis, an inflammation of the membrane lining in the abdominal cavity (Konukoglu *et al.*, 1999). Magnesium taurate may become a valuable drug to reduce migraine incidents (McCarty, 1996).

(ix) Protective effect taurine on pulmonary function

The depletion of taurine is particularly harmful to pulmonary tissue. Alveolar macrophages reside on the surface of lung alveoli. Their main purpose is to ingest inhaled particular matter to dispose of it. Alveolar macrophages become more susceptible to reactive forms of oxygen when deprived of the antioxidant protective capacity that taurine provide (Giri &Wang, 1992). Taurine is useful as an adjunct therapy for the management of cystic fibrosis patients experiencing complications of fat malabsorption and essential fatty acid deficiency because taurine supplementation provides a more healthy profile of triglyceride absorption and fatty acid composition of chylomicrons (Belli *et al.*, 1987; Smith *et al.*, 1991). Hence taurine is a novel and valuable aid for cystic fibrosis treatment

(Colombo *et al.*, 1996). Both taurine and niacin are reported to down-regulate genetic actions that express lung fibrosis (Giri *et al.*, 1994). Taurine and niacin completely or partially, ameliorated pulmonary fibrosis of a chemical origin (Gurujeyalakshmi *et al.*, 1998; Wang *et al.*, 1992). Taurine exerted a protective antioxidant effect in the prevention of damage from acute ozone exposure, especially in the bronchioles (Gordon *et al.*, 1998).

(x) Taurine in kidney function

Taurine is necessary for the proper functioning of the kidney as an organic osmolyte. Absence of taurine resulted in diminished renal function such that the process of excretion of unwanted substances from the blood is grossly impaired. Depending on the concentration of the final urine emerging from the kidney, the medulla area will deliberately modify its own tonicity. When the fluid in medulla is hypertonic, its cells accumulate taurine and similar osmolytes, thus exerting a conservatory effect (Trachtman *et al.*, 1992). Thus, the kidney exercises self-preservation by modulating the volume of its cells to promote its functions. This osmotic response results from an increased activity of specific sodium-coupled transporters. Supplementation of taurine prevented glomerular hypertrophy, diminished glomerulosclerosis, tubulointerstitial fibrosis and diabetic nephropathy by reducing renal oxidant injury possibly through its antioxidant effects (Chiba *et al.*, 2002; Sener *et al.*, 2005).

(xi) Taurine and membrane stabilization

Taurine's ability to stabilize cell membranes may be attributed to several events. Taurine has been shown to regulate osmotic pressure in the cell, maintain homeostasis of intracellular ions, inhibit phosphorylation of membrane proteins, and prevent lipid peroxidation. As an osmotic regulator, it has been suggested that taurine, along with

glutamic acid, is instrumental in the transport of metabolically-generated water from the brain (Van Gelder, 1990). Taurine acts as an antioxidizing agent and a membrane stabilizer to maintain the functions of membrane-bound protein enzymes. It is of particular value to the preservation of erythrocytes. Nandhini & Anuradha, (2003) have reported that taurine lowers glucose-induced lipid peroxidation, protein glycosylation and Na^+/K^+ and Ca^{2+} -ATPase activities in red blood cells. It is suggested that the effect of taurine on lipid peroxidation could be due to its antiacidotic action as well as to its membrane stabilizing activity.

(xii) Taurine in fetal development

Taurine is critical during fetal development to produce normal fetal β-cell function (Cherif *et al.*, 1998). According to Dhillon *et al.* (1998), the decrease in taurine levels in whole blood is speculated to assist with the maturation and efficiency of auditory synapses in full-term infants (Tyson *et al.*, 1989). Reduction in the activity of placental taurine transporters resulted in low plasma taurine concentrations, with subsequently compromised availability of the amino acid for cellular processes (Norbreg *et al.*, 1998). Taurine is now added to many infant formulas to provide improved nourishment because of its ability to improve fat absorption in pre-term infants and in children with cystic fibrosis and its beneficial effects on auditory response development (Gaull, 1989).

(xiii) Effect of taurine on alcoholism

Some of the most novel applications for taurine result from studies of its role in alcoholism. Taurine can either promote or repress the reward effects associated with alcohol consumption, the delineating factor being the amount of alcohol consumed. In a study designed to assess the preference of rats to alcohol coupled with an odor stimulus, the group that did not receive supplemental taurine became conditioned for either a

significant aversion for the stimulus (prompted by high doses of alcohol) or no reaction (prompted by lower doses). When ethanol in a 2.0 g/kg dose was given to rats pretreated with oral taurine, they responded with a reduced aversion for a particular odor stimulus when paired with alcohol consumption. This may provide important insight into the modulation of the reward effect of alcohol (Quertemont *et al.*, 1998). Previous studies had found that while taurine does not interact with alcohol to produce an effect on alcohol consumption, its major metabolite taurocholic acid is responsible for the metabolic conversion of alcohol (Spanagel & Zieglgansbereger, 1997). Alcohol-induced fatty liver was prevented in animals receiving taurine supplementation. The protective effect of taurine is attributed to the potential of taurine conjugated bile acids (particularly taurocholic acid) to inhibit adverse enzymatic functions associated with alcohol consumption (Kerai *et al.*, 1998).

(xiv) Taurine in vision

In the healthy eye, taurine is found in very high concentration. Nutritional factors including taurine are now recognized as important factors in the reversal of retinitis pigmentosa (Allen & Lowry, 1998). Fletcher & Kalloniatis (1997) suggest that amino acid neurochemistry may have an underlying metabolic role in the onset and progress of retinitis pigmentosa. A combination of taurine with vitamin E, vitamin C, and alpha lipoic acid has been shown to protect against radiation-associated protein leakage and may become important for the prevention of damage to the vision of people involved in radiation associated occupations (Bantseev *et al.*, 1997). Taurine is important for the regeneration of damaged cells in the retina. Taurine activity implies a critical and changing role in the development of vision (Nag *et al.*, 1998).

(xv) Anti-aging properties of taurine

Dawson *et al.* (1999) reported that the impairment in spatial learning ability of older rats was correlated to the reduction in taurine in the striatum of the brain and decline in taurine levels of the spleen, kidney, eye, cerebellum, and serum are associated with age in rats. Taurine administrated to experimental animals has been able to increase the level of acetylcholine in the brain, which is abnormally low in Alzheimers disease (Tomaszewski *et al.*, 1982; Csernansky *et al.*, 1996). Taurine, along with magnesium, may target the channels of energy metabolism to reduce the risk of Alzheimer's (McCarty *et al.*, 1998). Taurine also improved the mechanical threshold for contraction, shifting it toward the normal value (Pierno *et al.*, 1998). The observed age related decline in taurine in L-cysteinesulfinic acid decarboxylase and cysteine dioxygenase activities in F344 rat hepatic tissue suggests that the observed decrease in tissue taurine levels might be associated with a reduction in taurine biosynthesis (Eppler & Dawson, 1999).

(xvi) Taurine and dermatological disorders

Psoriasis of a chronic, plaque-type nature has been correlated to marked depression of neutrophil taurine levels. Stapleton *et al.* (1996) and Yamaguchi *et al.* (1998) have demonstrated that the taurine conjugated bile acid taurine ursodeoxycholic acid exerted a growth suppressive effect on keratinocytes, and thus its presence may be of importance in skin conditions. Previous reports (Degim *et al.*, 2002; Farriol *et al.*, 2002) indicate that administration of taurine improves recovery from burn-injured skin and a topical taurine gel accelerates wound healing in mice. As an osmolyte, taurine helps maintain hydration in the epidermis when it is exposed to a dry environment (Janeke *et al.*, 2003).

(xvii) Antimicrobial effect of taurine

The taurine derivative N-chlorotaurine is a weak oxidant produced by leukocytes in response to bacterial and fungal exposures and it destroys pathogens incurred as a result of inflammatory reactions (Nagl *et al.*, 1998). This may become an important addition to the list of substances that are useful as antiviral agents. By virtue of its detergent activity, taurolithocholic acid 3-sulfate demonstrates excellent anti-pathogen activity against chlamydia, herpes simplex (types 1 and 2), gonorrhea, and human immunodeficiency virus. It is also less cytotoxic than other agents used. Taurolithocholic acid 3-sulfate may be a valuable topical microbiocidal agent against sexually transmitted diseases (Herold *et al.*, 1999).The amino acid taurine, down regulates polymorphonuclear neutrophil cell death and preserves function in the urine, suggesting taurine as a therapuetic option for urinary tract infection (Condron *et al.*, 2004). Taurolin, a derivative of taurine is a potent chemotherapeutic agent that mobilizes anti-microbial activity against bacteria, yeast, and mycetes (fungi). Jurewitsch *et al.* (1998) reported that low daily doses of taurine instilled in conjunction with the parenteral infusion has been shown to successfully reduce catheter-related bloodstream infections.

(xviii) Other properties

Nitric oxide and nitric oxide generating compounds (L-arginine, potassium, anti-oxidants and fish oil) promote N-methyl-D-aspartate activity and releases taurine (Albrecht, 1998). Taurine modification of the polyurethane heart valves, improves their durability by promoting and prolonging the flexing capacity over long-term usage (Bernacca *et al.*, 1998). The taurine-conjugated bile salt taurochlorate, exerting a detergent-like activity, markedly inhibits occlusive action encountered in enteral feeding (Yeoh *et al.*, 1996). A hormone called glutataurine was discovered in the parathyroid

gland of rats. Feuer *et al.* (1983) found that this peptide had highly selective action on adrenal hormones, which are involved in the body's response to stress. Glutataurine has vitamin A-like effects and it antagonizes cortisone and thyroxine and increases the development of the thymus. Lampson *et al.* (1983) have found that taurine increases some of the effects of insulin.

2.3 Isoproterenol

2.3.1 Chemistry

Chemically, isoproterenol is an L-β-(3,4-dihydroxyphenyl)-α-isopropyl amino ethanol hydrochloride with a molecular formula of $C_{11}H_{17}NO_3$.HCl. Its molecular weight is 247.7. The hydrochloride salt of isoproterenol is a white to off-white crystalline powder of melting point 170-171 ^{o}C. It is soluble in water and ethanol **(Fig: 2.3.1)**.

2.3.2 Mechanism of action and biological effects of isoproterenol

Isoproterenol is a synthetic β-adrenergic agonist and have been used for the induction of myocardial infarction. The pathophysiological changes associated with myocardial infarction induced by isoproterenol mimics to a greater extent with those occurring in humans (Ravichandran *et al.*, 1990). Isoproterenol is a β- adrenergic receptor agonist that increases cytosolic cAMP. In the case of β-adrenergic agonist action, the circulating hormones or drug is the first "messenger", interacting with β-adrenergic receptor on the external surface of the target cells (Rendon & Lopez, 2001). The drug hormone receptor complex activates the enzyme adenyl cyclase on the internal surface of the plasma membrane of the target cells. This accelerates the intracellular formation of cyclic adenosine monophosphate (cyclic AMP), the second "messenger" which then stimulates

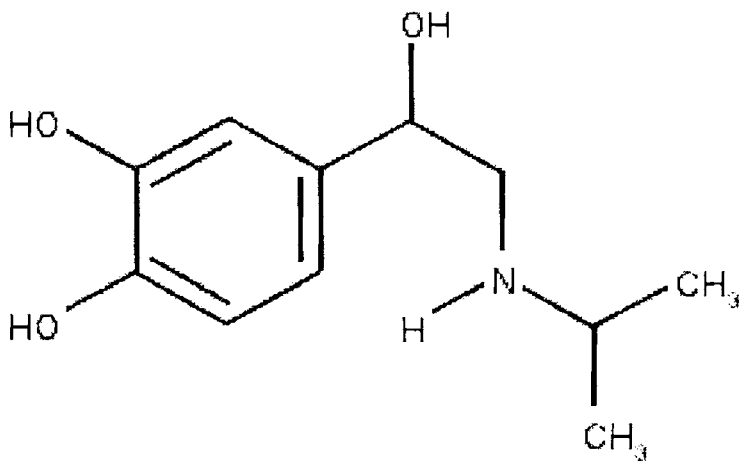


Fig.2.3.1 Structure of Isoproterenol

or inhibits various metabolic or physiological processes (Robison *et al.*, 1968; Motulsky & Insel, 1982).

Isoproterenol induces MAP (mitogen activated protein) kinase activation and cardiomyocyte hypertrophy through two different G proteins, Gs and Gi. cAMP-dependent protein kinase A (PKA) activation through Gs phosphorylates β-AR, leading to the coupling of the receptor from Gs to Gi. The activation of MAP kinase through Gβr, Sre family tyrosine kinase leads to the formation of She-Grb2-Sos complex, Ras and Raf-I kinase (Yamazaki & Yazaki, 2000). Thus isoproterenol increase the activities of Raf-I kinase and MAP kinase, which accelerate phenylalanine incorporation into proteins (Yamazaki *et al.*, 1997), leading to cardiomyocyte hypertrophy (Zou *et al.*, 1999).

It has been shown that isoproterenol induces myocardial cell injury similar to that reported for myocardial infarction, myocardial ischemia, cardiac stress and Chagasic cardiacmyopathy (Rona *et al.*, 1983; Cebelin & Hirsch, 1980). Several studies have investigated the molecular and cellular mechanism of isoproterenol-induced cell injury of the myocardium (Chagoya De Sanchez *et al.*, 1997; Curti *et al.*, 1990; Capozza *et al.*, 1992; Rendon & Lopez, 2000; Kondo *et al.*, 1987). Among these, the investigation by Chagoya De Sanchez *et al.* (1997) establishes a long-term, integrated model of isoproterenol-induced myocardial cell damage encompassing structural, biochemical and physiological aspects.

The enhanced adenylate cyclase activity as result of isoproterenol induction increases cAMP formation, which in turn would have lead to the higher lipid accumulation in the myocardium (Subhash *et al.*, 1978). Isoproterenol is reported to increase lipolysis (Mohan & Bloom, 1999) and this may play a role in isoproterenol-induced myocardial necrosis. Hypertriglyceridemia and high levels of ester cholesterol in serum and heart tissue are the

major factors responsible for the altered cardiovascular functions during isoproterenol-induced myocardial infarction (Freedmann *et al.*, 1988). Accelerated degradation of membrane phospholipids by phospholipase and lysophospholipase has also been proposed to be related to membrane dysfunction and irreversible ischemic injury (Farber & Young, 1981). The administration of isoproterenol increases the activities of myocardial cholesterol ester synthetase and triglyceride lipase with simultaneous decline in the activities of cholesterol ester hydrolase and lipoprotein lipase.

Accumulation of ester cholesterol occurs when the rate of esterification by cholesterol ester synthetase exceeds the rate of hydrolysis, which in turn results in myocardial membrane damage. Peroxidation of endogenous lipid is a major factor in the cytotoxic action of isoproterenol (Namikawa *et al.*, 1992). A growing body of evidence is emerging which suggests that reactive oxygen-derived free radicals play a crucial role in the pathogenesis of isoproterenol-induced myocardial infarction (Nirmala & Puvanakrishnan, 1994).

2.3.3 Ultrastructural features in isoproterenol-induced myocardial infarction

Histopathological studies of the heart show that, isoproterenol results in extensive necrosis extending from the apex to the left ventricle and into the portion of right ventricle and the severity is proportional to its dosage. Myocardial infarction induced by isoproterenol results in the loss of normal convergence of the cardiac cell plates to the central veins. Neighboring myocytes shows nuclear and cytoplasmic variations in size and shape. Cells with swollen or even "ballooned "cytoplasms are frequent. Various sizes of necrotic foci of cardiac cells appear, and infiltration of mesenchymal cells to these areas is seen (Fukuda, 2002). Most of the myocytes are eosinophilic in their cytoplasm and the nuclear chromatins appear to be irregularly arranged and endoplasmic reticulum

particularly clumped (Meyer *et al.*, 2001). Periportal fields show enlargement and show inflammatory infiltration (Cortinovis *et al.*, 1993). The rough surfaced endoplasmic reticulum is dilated and detached ribosomes are seen (Takahama & Barka, 1967). The nucleolus appears to be fragmented and dispersed (Alliende & Esponda, 1988). Ballooned cardiac cells and free acidophilic bodies resembling "multivesicular bodies" frequently appear. Moreover, in a few cells, concentric whorls of endoplasmic reticulum appear and numerous vesicles of smooth-surfaced endoplasmic reticulum containing small lipid droplets within their cisternae and U-shaped forms of mitochondria are frequently seen (Lewczuk & Przybylska-Gornowicz, 1997).

2.3.4 Structural and functional changes induced by isoproterenol in heart

Myocardial infarction induced by isoproterenol has been reported to show many metabolic and morphologic aberrations in the heart of experimental animals. It induces myocardial necrosis by a multiple step mechanism (Wexler, 1973; Ravichandran *et al.*, 1991). Isoproterenol has been reported to cause oxidative stress in the myocardium, which results in infarct like necrosis of heart muscle (Nirmala & Puvanakrishanan, 1994).

Administration of isoproterenol induces a significant elevation of the ST-segment elevation with an enhancement in ventricular wall motion (Yamamoto & Katori, 1995). It also elevates mean heart rate with an imbalance in autonomic regulation of cardiac automaticity, which accounts for the 27% incidence of arrhythmias in rats (Rote & Connor, 1992). A progressive enlargement of the LV cavity that is out of proportion to mass, similar to that observed in discrete myocardial infarction also commonly occurs in isoproterenol-induced myocardial necrosis (Teerlink *et al.*, 1994). A drastic decrease in left ventricular pressure and shortening of the arterio-ventricular interval also occur (Chagoya De Sanchez *et al.*, 1997).

Injection of isoproterenol stimulates the development of left ventricular hypertrophy (LVH) with an increase in relative left ventricle weight, LV protein content and LV beta-myosin heavy chain levels. The severity of isoproterenol-induced myocardial fibrosis is in correlation with a high LV ACE activity and ACE mRNA levels, which in turn lead to the parallel development of LVH (Ocaranza *et al.*, 2002). It also increases heart rate by 60% and lowers the blood pressure, resulting possibly in a functional ischemia (Chagoya de Sanchez *et al.*, 1997). The enhancement in heart rate is achieved by shortening the action potential duration (Dorian *et al.*, 2002). There is some functional alterations also induced in the isolated mitochondria such as decrease in oxygen consumption, respiratory quotient, ATP synthesis, protein synthesis and, membrane potential. In isoproterenol-induced myocardial infarction, the energy imbalance is reflected by a decrease in energy charge and in the creatine phosphate/creatine ratio.

2.3.5 Metabolic changes during isoproterenol-induced myocardial infarction

The isoproterenol-induced alterations in experimental animals includes increase in heart weight, marked electrocardiographic changes, increase in the level of serum marker enzymes and lipid peroxides and decrease in the levels of antioxidants (Manikandan *et al.*, 2002). Isoproterenol administration produces a marked increase in CPK, LDH, phospholipase and significant decrease in cardiac glycogen, ATP, creatine phosphate and phospholipid level (Kaul & Kapoor, 1989).

Injection of isoproterenol administration causes a dose dependant increase of lysosomal enzyme activity in vitro and in vivo (Macickova *et al.*, 1999). The increase in the serum lysosomal hydrolase activities in isoproterenol treated rats is mainly due to the decreased stability of the membranes, which is usually reflected by the lowered activity of cathepsins D in mitochondrial and microsomal fractions (Nirmala & Puvanakrinan,

1996a). During isoproterenol-induced myocardial infarction the cytoplasm of cardiac myocytes becomes more acidic due to lactate accumulation. The infiltration of inflammatory cells at the infarct regions and altered lysosomal fragility are responsible for the increased activity of these enzymes (Ravichandran *et al.*, 1991).

A considerable body of clinical and experimental evidence now exists suggesting the involvement of free radical mediated oxidative process in the pathogenesis of isoproterenol-induced myocardial infarction (Nirmala & Puvanakrishnan, 1996[b]). Alterations in tissue defense systems including chemical scavengers or antioxidant molecules and the enzymes catalase, superoxide dismutase, and glutathione peroxidase have been reported in isoproterenol-induced myocardial infarction (Sathish *et al.*, 2003[b]; Sasikumar & Devi, 2000). The administration of isoproterenol produces necrotic lesions in the myocardium and increases lipid peroxidation in the cardiac tissue, which plays a significant part in the pathogenesis of myocardial infarction (Noronha-Dutra *et al.*, 1985; Singal *et al.*, 1982 &1983). A significant depletion of cardiac glutathione (GSH) has been reported in isoproterenol-induced myocardial infarction in rats (Nirmala & Puvanakrishnan, 1996[a]; Hagar, 2002). Depletion of GSH is known to result in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased GSH consumption and increase the susceptibility of the myocardial cells to reactive oxygen metabolites (Meister, 1988). GSH and GSH-dependent antioxidant enzyme systems are directly related to the pathogenic mechanism of isoproterenol-induced myocardial infarction (Remiao *et al.*, 2000).

There is an early degradation of collagen immediately occurring after isoproterenol-induced myocardial infarction (Ravichandran & Puvanakrishnan, 1993). Ornithine decarboxylase (ODC) is an initial rate-limiting enzyme in the synthesis polyamines

(putrescine, spermidine and spermine). They play a role in cell growth and differentiation. Isoproterenol induces an increase in ODC activity and putrescine and spermidine level in the heart. These polyamines are one of the intracellular factors that contribute to cardiac injury (Tipnis *et al.*, 2000). Elevation in the level of metallothionein production is induced in heart cells during experimentally induced myocardial condition for the protection of myocardial cells from injury (Namikawa *et al.*, 1993). The cardiotoxic effect of isoproterenol is associated with calcium overload. Injection of isoproterenol leads to an increase in Ca and water content and to a reduction in the levels of Zn, Cu and Mg in heart cells (Namikawa *et al.*, 1991 & 1993; Brembilla *et al.*, 1993).

2.3.6 Cardioprotective agents and isoproterenol-induced myocardial infarction

Studies of Remla *et al.* (1991) on the effect of coconut oil and safflower oil on lipids in isoproterenol-induced myocardial infarction indicates that safflower oil exerts better protection than coconut oil by reducing the levels of cholesterol and triglycerides in heart and aorta. AO-8, a poly herbal formulation has been reported to prevent isoproterenol-induced myocardial infarction in experimental animals by counteracting the isoproterenol-induced free radical formation by its antioxidant property and membrane stabilizing action (Mitra *et al.*, 1999). α-Tocopherol intake has been reported to be cardioprotective against experimentally induced myocardial infarction by inhibiting lipid peroxidation and by maintaining the tissue antioxidant status normal levels (Ithayarasi *et al.*, 1996).

Reports by Manjula *et al.* (1992) have shown that, aspirin treatment counteracts the effects of isoproterenol on lipid peroxide formation and associated enzymes changes in serum and heart. Earlier the administration of potassium channel opener cromakalim has been reported to have less myocardial degenerative changes on histopathological

examinations when compared with those treated with isoproterenol alone (Aghi *et al.*, 1992). Nirmala & Puvanakrishnan (1996[b]) have studied the protective effect of curcumin against isoproterenol-induced myocardial infarction. The curcumin administration reduces the myocardial damage caused by isoproterenol by maintaining the levels of lysosomal enzymes at near normal. Hashimoto & Ogawa (1981) have stated that premeditation of sulfinpyrazone and propranolol reduce cardiac necrosis and hypertrophy induced by isoproterenol, but aspirin did not have such cardioprotective effects.

Studies by Djandjighian *et al.* (2000) on the hemodynamic and anti adrenergic effects of dronedarone and amiodarone in animals with a healed myocardial infarction indicate that both dronedarone and amiodarone significantly reduces the exercise-induced tachycardia and at the highest dose, decreases the isoproterenol-induced tachycardia. The administration of Ca^{2+} sensitizer levosimendan has been reported to exert dose- and time-dependent positive inotropic and lusitropic effects on the post-ischemic myocardium, lending support to the hypothesis that Ca^{2+} desensitization of myofibrils is involved in myocardial stunning (Kristof *et al.*, 1999).

Karthekeyan *et al.* (2003) have shown that, chronic administration of alcoholic extract of *Terminalia arjuna* prevents the isoproterenol-induced myocardial ischemic reperfusion injury by a dose dependent modulating effect on the antioxidant milieu of the heart. It is reported to enhance myocardial endogenous antioxidants without producing any cytotoxic effects. Dantrolene, a blocker of sarcoplasmic reticulum Ca^{2+}release channel has a significant effect in the protection of heart against myocardial infarction induced by isoproterenol on the rat myocardium (Acikel *et al.*, 2005). Vimal & Devaki (2004) have reported that oral treatment of marmesinin exerts protective action against isoproterenol-induced myocardial injury. It inhibits the release of enzymes from nuclear, mitochondrial,

lysosomal and microsomal fractions which could be due to the stabilizing effect of marmisinin on the membrane.

Pretreatment with an ethanolic extract of calotropis procera significantly reduced the elevated marker enzyme levels in serum and heart homogenate in isoproterenol-induced myocardial infarction (Ahmed *et al.*, 2004). Garlic oil is reported to produce a marked reversal of the metabolic changes related to myocardial infarction induced by isoproterenol, by modulating the lipid peroxidation and enhancing antioxidant and detoxifying enzyme systems (Saravanan & Prakash, 2004). Reports by Sathish *et al.* (2003[a] & 2003[c]) have shown that the pretreatment with nicorandil and amlodipine could preserve lysosomal integrity and hence established the cardioprotective effect of the combination against isoproterenol-induced myocardial infarction. Yogeetha *et al.* (2006) have reported the protective effect of ferulic acid and ascorbic acid on lysosomal hydrolases and membrane-bound phosphatases during isoproterenol-induced myocardial necrosis in rats. Farvin *et al.* (2006) have shown that cardioprotective effect of squalene is probably related to an inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties. Kumar & Anandan (2007) have reported that treatment with glutamine exerted protective action against isoproterenol-induced myocardial injury by decreasing lipid peroxidation and enhancing antioxidant status.

Materials and Methods

3. MATERIALS AND METHODS

3.1 Chemicals

Taurine, epinephrine, tetraethoxypropane, amino acid standards and isoproterenol were obtained from M/s. Sigma Chemical Company, St. Louis. MO, USA. All the other chemicals used were of analytical grade.

3.2 Animals

Wistar strain male albino rats, weighing 100-120 g were used. They were housed individually in polypropylene cages under standard environmental conditions and allowed free access to food and water. The experiment was carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee.

3.3 Induction of myocardial infarction

The myocardial infarction was induced in experimental rats by injecting isoproterenol (11mg/100g body weight/day), i.p. for 2 days (Anandan *et al.*, 2003).

3.4 Experimental protocol

Seven days after acclimatization, the rats were divided into four groups of 6 rats each and housed individually in polypropylene cages. Group I rats served as the control and were intraperitoneally injected with physiological saline for 15 days. Group II animals were intraperitoneally (i.p.) injected with taurine (100mg kg^{-1} body wt day^{-1}, dissolved in physiological saline) for 15 days. Group III rats injected with physiological saline for 15 days and administrated isoproterenol [11mg (dissolved in physiological saline) $100g^{-1}$

body wt day^{-1} , i.p.] for 2 days for the induction of myocardial infarction. Group IV animals were injected with taurine at the above dosage for 15 days and then injected with isoproterenol [11mg $100g^{-1}$ body wt day^{-1} , i. p] for 2 days.

At the end of the experimental period, i.e., 24 h after last injection of isoproterenol, the experimental animals were sacrificed, and blood was collected with and without anticoagulant for the separation of plasma and serum respectively. The heart tissue was excised immediately and washed with chilled isotonic saline. One portion of tissue was fixed in 10% buffered formalin for histopathological observations. Some amount of tissue was used for amino acid and fatty acid compositional analysis. Accurately weighed heart tissue was homogenized in ice-cold 0.1 M Tris-HCl buffer, pH 7.2 and centrifuged. The plasma, serum and tissue homogenate were used for various biochemical analyses.

3.5 Histopathological studies

The slices of the heart were fixed in 10% neutral buffered formaldehyde. Fixing prevent autolysis and putrification of tissues. Then they were dipped in different concentration of alcohol in ascending order and finally in absolute alcohol (10 min each) for removing water. They were then kept in methyl benzoate until it sank and dipped in benzene for removing alcohol. The tissues were then infiltered with molten paraffin (60-70 °C) for 1 h and 15 min. A boat was made filled with molten paraffin and the tissues were placed in it. The paraffin was then cooled until it hardened, enclosing the tissue.

Using a rotary microtome, section of 4 to 5μ paraffin infiltered tissues were made. The tissues were de-paraffinised with xylene and treated with 100%, 90% and 70% alcohol (10 min each) for removing undesirable pigment and other materials. The sections were then stained with haematoxylin and counter stained with eosin and dehydrated with

70%, 90% and 100% alcohol for 10 min each. The sections were mounted using dibutylpthalate in xylene and examined under microscope.

3.6 Diagnostic markers

3.6.1 Assay of alanine aminotransferase (EC 2.6.1.2)

The activity of alanine aminotransferase (ALT) was determined by the method of Mohur & Cook (1957).

Reagents

1. Substrate: (0.1 M phosphate buffer, pH 7.4, 0.2 M DL-alanine, 2.0 mM 2-oxoglutarate): 1.5 g dipotassium hydrogen phosphate, 0.2 g potassium dihydrogen phosphate, 30 mg 2-oxoglutaric acid and 1.78 g DL-alanine were dissolved in distilled water. The pH was adjusted to 7.4 with 1 N NaOH and made up to 100 ml.
2. 2, 4-dinitro phenyl hydrazine (DNPH) reagent: Dissolved 200 mg of DNPH in 85 ml of concentrated hydrochloric acid and made up to a liter with distilled water.
3. Sodium hydroxide: 0.4 N
4. Standard pyruvic acid: 11.01 mg of sodium pyruvate was dissolved in 100 ml of distilled water and this was prepared freshly for the calibration curve.

Procedure

To 1.0 ml of the buffered substrate, 0.1 ml sample was added and incubated at 37 °C for 30 min. The reaction was arrested by adding 1.0 ml of DNPH and left aside for 20 min at room temperature. Color developed by the addition of 10 ml of 0.4 N NaOH was read at 540nm in a Shimadzu UV-1601 spectrophotometer against the reagent blank.

The enzyme activity was expressed as μ mol pyruvate liberated /h/l (plasma).

3.6.2 Assay of aspartate aminotransferase (EC 2.6.1.1)

The activity of aspartate aminotransferase (AST) was assayed by the method of Mohur & Cook (1957).

Reagents

1. Phosphate buffer: 0.15 M, pH 7.5.
2. Substrate: 300 mg of L-aspartic acid and 50 mg of α-ketoglutaric acid were dissolved in 20-30 ml of the phosphate buffer and added 10% sodium hydroxide to bring the pH to 7.5 and was made up to 100 ml with phosphate buffer.
3. 2, 4-dinitro phenyl hydrazine (DNPH) reagent: Dissolved 200 mg of DNPH in 85 ml of concentrated hydrochloric acid and made up to a liter with distilled water.
4. Sodium hydroxide: 0.4 N
5. Standard pyruvic acid: 11.01 mg of sodium pyruvate was dissolved in 100 ml of distilled water and this was prepared freshly for the calibration curve.

Procedure

To 1.0 ml of the buffered substrate, 0.1 ml of the sample was added and incubated for one hour at 37 ^{o}C. Then 1.0 ml of DNPH reagent was added and left for 20 min. At the end of incubation, 10 ml of 0.4 N NaOH was added and the colour developed was read at 540nm in a Shimadzu UV-1601 spectrophotometer after 10 min. The standards were also treated similarly.

The enzyme activity was expressed as μ mol pyruvate liberated /h/l (plasma).

3.6.3 Assay of lactate dehydrogenase (EC 1.1.1.27)

The lactate dehydrogenase (LDH) activity was assayed according to the method of King (1965) with slight modification. The amount of pyruvate formed in the forward reaction was measured colorimetrically.

Reagents

1. Substrate : 2.76 g of lithium lactate was dissolved in 125 ml of glycine buffer containing 75 ml of 0.1N sodium hydroxide to adjust the pH 10. This was prepared just prior to use.
2. 0.1 M Glycine buffer : 7.5 g of glycine and 5.85 g of sodium hydroxide were dissolved in one liter of distilled water.
3. Sodium hydroxide : 0.4N
4. NAD^+ : 5.0 mg was dissolved in 1.0 ml of distilled water. This was prepared just before use.
5. 2, 4-dinitro phenyl hydrazine (DNPH) reagent: Dissolved 200 mg of DNPH in 85 ml of concentrated hydrochloric acid and made up to a liter with distilled water.
6. Standard pyruvate solution 11.01 mg of sodium pyruvate was dissolved in 100 ml of distilled water and this was prepared freshly for the calibration curve.

Procedure

To 1.0 ml of the buffered substrate, 0.1 ml of the sample was added and the tubes were incubated at 37 °C for 15 min. After adding 0.2 ml of NAD^+ solution, the incubation was continued for 30 min and then 1.0 ml of DNPH reagent was added. And the tubes

were incubated at 37 °C for 15 min. Then 7.0 ml of 0.4 N NaOH was added and the colour developed was read at 540 nm in a Shimadzu UV- 1601 spectrophotometer against the reagent blank. Suitable aliquots of the standards were also treated in the same manner.

The enzyme activity was expressed as μ mol pyruvate liberated /h/l (plasma).

3.6.4 Assay of creatine phosphokinase (EC 2.7.3.2)

The assay of creatine phosphokinase (CPK) was done by the method of Okinaka *et al.* (1961) with slight modifications.

Reagents

1. Tris buffer : 100 mM, pH 7.4
2. Magnesium acetate solution : 25 mM
3. Creatine phosphate : 12 mM
4. Adenosine -5-diphosphate (ADP) : 4 mM
5. Cysteine : 150 mM
6. p- Chloromercuric benzoate (PCB) : 30 mM
7. Zinc sulphate solution : 50 g Zinc sulphate in 1 liter of distilled water
8. Barium hydroxide 150mM : 10 g $Ba(OH)_2$ was boiled in 100 ml distilled water for few min, cooled and filtered. Took 5.0 ml zinc sulphate solution in a conical flask and added 20 ml distilled water and titrated with barium hydroxide using phenolphthalein as indicator until a light rose colour end point. Adjusted the volume of barium hydroxide to 5 ml.
9. Alkali solution 60 g NaOH and 128g anhydrous sodium carbonate were dissolved in 1 liter of distilled water.

10. α-Naphthol solution : 160 mg of α-naphthol was dissolved in 10 ml of alkali solution. Freshly prepared before use. Filtered if not clear.
11. Diacetyl stock solution : 1.0 ml of diacetyl first was dissolved in small quantity of methanol and made the volume to 100 ml with distilled water. Kept in a brown bottle at 4 °C
12. Working solution of diacetyl : Diluted the stock solution into 1 to 20 with distilled water. Prepared freshly before use.
13. Standard creatine solution (6 mM) : 89.5 mg Creatine hydrate was dissolved in 100 ml distilled water. 1.0 ml of the solution was made up to 5.0 ml with distilled water.

Procedure

0.2 ml of the sample was added to the test tubes containing 0.2 ml manganese solution, 0.1ml cysteine, and 0.25 ml creatine phosphate, mixed and incubated at 37 °C for 5 min. Added 0.25 ml ADP and incubated 37 °C for 30 min. After incubation 0.5 ml of PCB, 0.5 ml $Ba(OH)_2$, 0.5 ml zinc sulphate solution were added and mixed well after each addition, centrifuged at 4000 rpm for 5 min. 1.0 ml of the supernatant was taken and added 2.5 ml of α-naphthol, 0.5 ml of diluted diacetyl solution and 6.0 ml of distilled water. Incubated 37 °C for 1 hr, shaken intermittently in 15 min. Read the OD at 520nm using a Shimadzu-UV-1601 spectrophotometer. Standard and blank were also treated in the same way.

The enzyme activity was expressed as μ mol creatine liberated /h/l (plasma).

3.6.5 Assay of alkaline phosphatase (EC 3.1.3.1)

Alkaline phosphatase was assayed by the method of King (1965) using disodium phenyl phosphate as the substrate.

Reagents

1. Carbonate- bicarbonate buffer : 0.1M pH 10.0
2. Substrate : 0.01 M disodium phenyl phosphate solution.
3. Folin's phenol reagent : This was diluted 1:2 with double distilled water before use.
4. Sodium carbonate : 15%
5. Magnesium chloride : 0.1 M
6. Standard phenol solution : A solution of distilled crystalline phenol in water, containing 50 μg/ ml was prepared.

Procedure

The incubation mixture contained the following components in a final volume of 3.0 ml - 1.5 ml of carbonate- bicarbonate buffer, 1.0 ml of substrate and 0.1 ml of magnesium chloride and requisite amount of the enzyme source (0.2 ml serum). The reaction mixture was incubated at 37 °C for 15 min. The reaction was terminated by the addition of 1.0 ml of Folin's phenol reagent. If turbidity appeared, the tubes were centrifuged. Controls without enzyme sources were also incubated and the enzyme source was added after the addition of Folin's phenol reagent. The 1.0 ml of 15% sodium carbonate solution was added and incubated for a further 10 min at 37 °C. The blue colour developed was read at 640 nm using a Shimadzu-UV-1601 spectrophotometer against a blank. The standards were also treated similarly.

The activity of the enzyme is expressed as μmol phenol liberated/ h/l (plasma); μ mol phenol liberated/ mg protein (tissue).

3.6.6 Assay of acid phosphatase (EC 3.1.3.2)

Acid phosphatase was assayed by the method of King (1965) using disodium phenyl phosphate as the substrate.

Reagents

1. Citrate buffer : 0.1 M pH 4.9
2. Substrate : 0.01 M Disodium phenyl phosphate solution.
3. Folin's phenol reagent : This was diluted 1:2 with double distilled water before use.
4. Sodium carbonate : 15%
5. Standard phenol solution : A solution of distilled crystalline phenol in water, containing 50μg /ml was prepared.

Procedure

The incubation mixture contained the following components in a final volume of 3.0 ml. 1.5 ml of citrate buffer, 1.0 ml of substrate, 0.3ml of distilled water and requisite amount of the enzyme source (0.2 ml serum). The reaction mixture was incubated at 37 °C for 15 min. The reaction was terminated by the addition of 1.0 ml of Folin's phenol reagent. If turbidity appeared, the tubes were centrifuged. Controls without enzyme sources were also incubated and the enzyme source was added after the addition of Folin's phenol reagent. 1.0 ml of 15% sodium carbonate solution was added and incubated for a further 10 min at 37 °C. The blue colour developed was read at 640 nm using a Shimadzu-UV-1601 spectrophotometer against a blank. The standards were also treated similarly.

The activity of the enzyme is expressed as μmoles of phenol liberated/ h/l (plasma); μmol phenol liberated/ mg protein (tissue).

3.6.7 Estimation of Troponin T

Troponin was estimated by Immunoassay. Electrochemiluminescence immunoassay "ECLIA" on Roche Elecsys 1010/2010 and Modular Analytics E170 (Elecsys module) immunoassay analyzers was used.

3.6.8 Estimation of Homocysteine

Homocysteine Microtiter Plate Assay package (Diazyme Laboratories) was used for the analysis. Homocysteine Microtiter Plate Assay is an EIA- like assay for the determination of tHcy in blood.

3.7 Protein, Amino acid and Glycoprotein Components.

3.7.1 Estimation of protein

The protein content in the sample was estimated by the method of Lowry *et al.* (1951)

Reagents

1. Alkaline copper reagent
    i. Solution A: 2% sodium carbonate in 0.1 N sodium hydroxide solution.
    ii. Solution B: 0.5% Copper sulfate in water.
    iii. Solution C: 1% sodium potassium tartarate in water.
2. 50 ml of solution A was mixed with 0.5 ml of solution B and 1 ml of solution C just before use.
3. Folin's phenol reagent: Diluted 1:2 with double distilled water before use.

4. Standard bovine serum albumin (BSA): dissolved 100 mg of BSA in 100 ml of distilled water in a standard flask.10 ml of the stock was diluted to 100ml to get a working standard of 100 μg/ml.

Procedure

Pipetted out 0.1ml of sample and standard BSA in the range of 20-100 μg into test tubes and the total volume was made up to 1.0 ml with distilled water. The blank contained 1.0 ml of distilled water. Exactly 4.5 ml of alkaline copper reagent was added to all the tubes and left at room temperature for 10 min after which was added 0.5 ml of Folin's phenol reagent. The blue colour developed was read after 20 min at 640 nm against the reagent blank, in a Shimadzu-UV-1601 spectrophotometer.

The protein values are expressed as mg/dl (plasma); mg /g (tissue).

3.7.2 Electrophoretic separation of plasma proteins

Plasma proteins were separated by SDS-PAGE technique as described by Laemmli (1970).

It is based on the principle that, in the presence of 10% SDS and 2- mercaptoethanol, proteins dissociate into their sub units and bind large quantities of the detergent which mask the charge of the proteins and giving a constant charge to mass ratio. So that the proteins move according to their molecular weight in an electric field. In this discontinuous buffer system, the separating (resolving gels) and stacking gels are kept in the Tris-glycine electrode buffer. During electrophoresis, the leading ion is chloride while the trailing ion is glycine. In this experiment, 7.5% gel concentration is used for the effective separation.

Reagents

1. Tris-HCl : 0.5 M, pH 6.8
2. Tris HCl : 1.5 M, pH 8.8
3. SDS : 10%
4. Ammonium per sulphate (APS) : 10%
5. Acrylamide/Bis (2.67 % C premix) : 30 %
6. Sample buffer :

Distilled water	: 3.8 ml
Tris-HCl 0.5 M, pH 6.8	: 1 ml
Glycerol	: 0.8 ml
10%SDS	: 1.6 ml
2-Mercapto ethanol	: 0.4 ml.
1% Bromophenol blue	: 0.4 ml.

7. Electrode buffer :

Tris base	: 9 g
Glycine	: 43.2 g
SDS	: 3 g

These reagents are dissolved in 600 ml of distilled water.

Working solution: Dilute 100 ml from stock to 500 ml with distilled water.

1. Separating gel (7.5%)

Distilled water	: 4.05 ml
Tris-HCl 1.5M	: 2.5 ml
10%SDS	: 100 µl

Acrylamide	: 3.3 ml
TEMED	: 10 µl
APS 10%	: 50 µl

2. Stacking gel (4%)

Distilled water	: 6.1 ml
Tris-HCl 0.5M	: 2.5 ml
10%SDS	: 100 µl
Acrylamide	: 1.33 ml
TEMED	: 10 µl
APS 10%	: 50 µl

Procedure

Taken 0.1 ml of the suitably diluted plasma into a microfuge tube and added 0.1 ml of the sample buffer, heated in a boiling water bath for 4 min, cooled and kept at 4 °C in refrigerator.

The separating gel was prepared without TEMED and APS. Evacuated for 15 min to remove air bubbles. Added TEMED and APS with intermittent shaking after each addition immediately transferred the solution to the electrophoretic apparatus (BIORAD-Mini-PROTEAN II cell). Added a little water on the top of the gel to level it and kept for 45 min. Prepared stacking gel in the same way. Kept the comb over the apparatus, tilted it to 45°, poured the gel slowly, and pressed the comb slowly and evenly. Kept for 45 min. and marked the wells. After removing the comb, the whole apparatus was transferred to the sandwich clamp assembly in to the inner cooling core. Rinsed the apparatus and wells with electrode buffer and filled the inner chamber of the apparatus completely and the

outer chamber to the optimum level. Injected 10 µl of the sample into the wells. The electrode lid was placed at proper position and connections were established. The power of 200V was supplied. Electrophoresis was carried out for 45 min approximately until the dye reaches the bottom. Subsequently, the gel was removed and is placed in a big petridish containing the stain, Coomassive blue. Kept for 30 min, and transferred the gel into 7% acetic acid for destaining. 7% acetic acid was changed intermittently till the gel got completely destained.

3.7.3 Extraction of gycoconjugates

100 mg of the tissue was weighed and the lipids were extracted using chloroform - methanol method by homogenization in a Potter- Elvehjem homogenizer with a Teflon pestle. The extraction was repeated thrice with fresh aliquots of the solvent mixture. The lipid extract was filtered through a fat free Whatmann No: 41filter paper into a separating funnel. The defatted tissue in the filter paper was dried and used for the estimation of hexose and sialic acid.

3.7.3.1 Estimation of Hexose

Hexose was estimated by the method of Niebes (1972).

Reagents

1. Sulphuric acid: Water mixture : (3:2,v/v)
2. 800 mg of orcinol dissolved in 50 ml of 1 N H_2SO_4
3. Orcinol-sulphuric acid mixture : 1.0 ml of reagent (2) was mixed with 7.5 ml of reagent (1). This mixture was prepared fresh at the time of assay.

4. Standard hexose : Equal quantities of galactose and mannose were dissolved in water to give a concentration of 100μg/ml.

Procedure

An aliquot of the delipidised sample was treated with 1.0 ml of 0.1 N NaOH. Blank contained 1.0 ml of 0.1 N NaOH .The tubes were cooled by placing in an ice-bath and 8.5 ml of orcinol-sulphuric acid mixture was added slowly and mixed well. The tubes were stoppered, incubated at 80 °C for 15 min in a water bath. Cooled and the color was allowed to develop in the dark for 25 min. The intensity was measured at 540 nm using a Shimadzu-UV-1601 spectrophotometer. Standard solutions containing 20 to 100 μg were treated similarly and hexose concentration was estimated.

The concentration of hexose was expressed as mg/g heart tissue.

3.7.3.2 Estimation of Sialic acid.

Sialic acid was estimated by the method of Warren (1979).

Reagents

1. Sodium metaarsenite : A 10% solution was prepared in 0.5 M sodium sulphate in 0.1 N H_2SO_4.
2. Sodium metaperiodate : 0.2 M solution in 9 M phosphoric acid.
3. Thiobarbituric acid reagent : 0.6% solution was prepared in 0.5 M sodium sulphate
4. Acidified butanol : 5 ml of con HCl in 95 ml of n-butanol.

5. Standard sialic acid : 10 mg of N-acetyl neuraminic acid was dissolved in 100 ml of distilled water.

6. Working standard : 1ml of the stock was diluted to 10 ml using distilled water.

Procedure

An aliquot of the delipidised sample was hydrolyzed with 0.1 N H_2SO_4 at 80 °C for 1 hour. 0.2 ml of the hydrolysate was mixed with 0.1ml of meta periodate and the solution was kept at room temperature for 20 min. 1.0 ml of sodium meta arsenite was added and shaken well so that the yellow brown colour disappeared. 3.0 ml of thiobarbituric acid reagent was added and heated in a boiling water bath for 15 min. After cooling, 4.0 ml of acidified butanol was added, shaken well and the colour was extracted into butanol phase. The butanol phase was transferred to another set of tubes and the colour intensity was measured at 530 nm using a Shimadzu-UV-1601 spectrophotometer. The blank containing 0.2 ml of 0.1 N H_2SO_4 and standard sialic acid solutions were treated similarly.

Sialic acid is expressed as mg/g tissue.

3.7.4 Free amino acids

Total amino acids and free amino acids in the serum and heart tissue were determined as per the procedure of Ishida *et al.* (1981).

Reagents

1. 10% : TCA

2. HCl : 6 N

3. HCl : 0.05 M

4. Buffer A : Dissolved tri sodium citrate (58.8 g) in 2l of double distilled water, add 210 ml ethanol of 99.5%, and adjust the pH to 3.2 by adding 60% perchloric acid and make up to 3 l using double distilled water.

5. Buffer B : Dissolved tri sodium citrate, 58.8 g and boric acid, 12.4 g in double distilled water, adjust the pH to 10 by adding 4 N NaOH, and make up the volume to 1l using double distilled water.

6. Phthaladehyde (OPA) Buffer : Dissolved 122.1 g of Na_2CO_3, 40.7 g of H_3BO_3 and 56.4 g of K_2SO_4 in double distilled water and make up the volume to 3L.

7. Phthalaldehyde solution (OPA) : Dissolved 400 mg OPA, 7 ml ethanol, 1 ml of 2-Mercaptoethanol and 2 ml of 30% w/v Brij-35 in 500 ml OPA buffer.

8. Sodium hypochlorite solution : 4% w/v Sodium hypochlorite in OPA buffer. ie., 0.3 ml Sodium hypochlorite in100 ml OPA buffer.

Procedure

Preparation of Trichloroacetic acid extract:

Weighed the sample accurately (100mg) and extracted with 10% trichloroaceticacid by grinding in mortar. Filtered the content quantitatively through Whatman filter paper No: 1 and made up the filtrate to 1 ml. Used the TCA extract to measure free amino acids. Pipetted out about 1.0 ml TCA extract accurately and bring the pH to below 3.0 with NaOH and made up to definite volume with buffer.

Filtered the sample thus prepared again through a membrane filter of 0.45 μm and injected 20 μl of this to an amino acid analyzer (HPLC- LC 10 AS) equipped with cation exchange column packed with a strongly acidic cation exchange resin i.e., styrene di vinyl

benzene co polymer with sulphonic group. The column used was Na type i.e., ISC- 07/S 1504 Na having a length of 19 cm and diameter 5mm.

The instrument was equipped with Shimadzu FL 6A fluorescence detector and Shimadzu CR 6A Chrompac recorder. The mobile phase of the system consists of two buffers, Buffer A and buffer B. A gradient system can be followed for the effective separation of amino acids. The oven temperature can be maintained at 60 °C. The total run was programmed for 60 min. The amino acid analysis can be done with non-switching flow method and fluorescence detection after post–column derivatization with o-phthalaldehyde. In the case of proline and hydroxyl proline, imino group is converted to amino group with hypochlorite.

Run an amino acid standard (Sigma chemical Co., St. Lousis, USA) also to calculate the concentration of amino acids in the sample. Calibration of equipment using standards needs to be done before the start of analysis. The amount of each free amino acid is expressed as percentage of total amino acids.

3.8 Lipids

3.8.1 Extraction of total lipids

The total lipid content of the tissues was estimated by the method of Folch *et al.* (1957).

Reagents

1. Chloroform-Methanol mixture (2:1 v/v)

Procedure

A weighed amount of the tissue was subjected to lipid extraction using chloroform-methanol mixture (2:1). The extraction was repeated twice with fresh aliquot of

chloroform-methanol mixture. The lipid extracts were transferred to a separating funnel and added 20% of water into it and left overnight. Next day the lipid extracts were drained through filter paper containing anhydrous sodium sulphate and was collected in round bottom flask and was evaporated to dryness in a flash evaporator. The lipid in the round bottom flask was made up to 10 ml by using chloroform. From this 1.0 ml was taken into a pre-weighed vial and allowed to dry in warm temperature to constant weight and total lipid content were calculated from the difference in weight. Sample made up to 10 ml was used for the estimation of various lipid components viz., cholesterol, triglycerides, free fatty acids and phospholipids after evaporating the solvent in air at room temperature.

3.8.1.1 Estimation of total cholesterol

The total cholesterol present in plasma and heart was estimated according to method of Parekh & Jung (1970) with slight modifications.

Reagents

1. $FeCl_3$ stock solution : 10 g $FeCl_3$ in 100 ml acetic acid.
2. $FeCl_3$ - H_2SO_4 reagent : 2.0 ml of $FeCl_3$ stock solution was diluted to 200 ml with conc. H_2SO_4.
3. 33% KOH (w / v) : 10 g of KOH was dissolved in 20 ml distilled water.
4. Alcoholic KOH solution : 6.0 ml of 33% KOH was made up to 100 ml with distilled ethanol. This solution is prepared fresh before use.
5. Standard cholesterol solution (stock) : 1mg /ml in chloroform.

6. Working standard : 1.0 ml of the stock was diluted to 10 ml with chloroform. Concentration 100 μg/ml.

Procedure

1.0 ml of the lipid sample was taken into a 25 ml glass stoppered tube and evaporated off the chloroform. Added 5ml of freshly prepared alcoholic KOH solution. The tubes were shaken well and incubated in a water bath at 37 °C for 55 min. After cooling to room temperature, added 10 ml of petroleum ether and inverted the tubes once to mix the contents. Then added 5.0 ml of distilled water and shaken the tubes vigorously for 1 min. Take 0.5-2 ml aliquots from the supernatant (petroleum ether) into test tubes. Evaporated the petroleum ether extract under nitrogen. To each of the sample as well as the standard tubes including the blank, added 3.0 ml of glacial acetic acid followed by 0.1ml -distilled water. Mixed the tubes thoroughly and added 2 ml of the $FeCl_3$ - H_2SO_4 reagent to the sides of the test tubes. A brown ring was formed at the interface, tap the bottom of the tubes well to effect mixing and a light colour appeared which changed to an immense purple colour, which was measured in a Shimadzu-UV spectrophotometer-1601 at 560nm.

The amount of total cholesterol was expressed as mg/dl (plasma); mg/g (heart tissue).

3.8.1.2 Estimation of triglycerides

The level of triglycerides in plasma and heart were determined by the method of Rice (1970) with slight modifications.

Reagents

1. Activated silicic acid.
2. Saponification reagent : 5.0 g of potassium hydroxide was dissolved in 60 ml distilled water and 4.0 ml isopropanol.

3. Sodium metaperiodate reagent : To 77 g of anhydrous ammonium acetate in 700 ml distilled water, added 60 ml glacial acetic acid and 650 mg of sodium metaperiodate and was dissolved and diluted to 1 litre with distilled water.

4. Acetyl acetone reagent : To 0.75 ml of acetyl acetone, 20 ml of isopropanol was added and mixed well.

5. Stock solution : 200 mg of tripalmitin was dissolved in 25 ml chloroform.

6. Working standard : 1.0 ml of the stock solution was diluted to 10ml with chloroform. Concentration 800 µg/ml.

Procedure

0.2 ml of the lipid sample was taken into a test tube and evaporated off the chloroform, added 4.0 ml isopropanol. It was mixed well and added 0.4 g of activated silicic acid, shaken in a vortex mixer for 15 min and centrifuged at 4000 rpm for 5 min. To 2.0 ml of the supernatant and standards ranging from 20-100 µg made up to 2.0 ml with isopropanol, 0.6 ml of saponifying reagent was added and incubated at 60-70 °C for 15 min. After cooling, 1.0 ml sodium metaperiodate solution was added and mixed. To this, 5ml acetyl acetone was added, mixed and incubated at 50 °C for 30 min. After cooling, the colour developed was read at 405nm in a Shimadzu-UV-1601 spectrophotometer.

The value of triglyceride was expressed as mg/dl (plasma); mg/g (heart tissue).

3.8.1.3 Estimation of free fatty acids

Free fatty acid in the sample was estimated by the modified method of Horn & Menahan (1981) with colour reagent of Itaya (1977).

Reagents

1. Activated silicic acid.
2. Chloroform, heptane, methanol (CHM) : It was prepared by mixing chloroform, heptane and methanol in the ratio of 200: 150: 7(v/v).
3. Copper-triethanolamine solution : 50 ml of 0.1 M copper nitrate and 50 ml of 2 M triethanolamine were mixed with 33 g of sodium chloride. The pH of the solution was adjusted exactly to 8.1.
4. Diethyldithiocarbomate (DDC) solution : 0.1% DDC in butanol was prepared freshly.
5. Standard stock : A solution containing 2 mg per ml of palmitic acid was prepared in CHM solvent.
6. Working standard : The stock was diluted to 1:10 in CHM solvent to give a concentration of 200 μg per ml.

Procedure

To 0.1 ml of the lipid sample, 6.0 ml of CHM solvent and 200 mg of silicic acid were added. The mixture was shaken well, centrifuged at 4000 rpm for 5 min and 3.0 ml of the supernatant was taken. Standard solution in the range of 25-100 μg were taken and made up to 3.0 ml with CHM solvent. The blank contained 3.0 ml of CHM solvent. To all these tubes, 2.0 ml of copper triethanolamine solution was added and then mixed on a mechanical shaker for 10 min. The tubes were centrifuged at 4000 rpm for 5 min. 2.0 ml

of the supernatant was taken, 1.0 ml of DDC solution was added and shaken well. The colour intensity was read immediately at 430 nm in a Shimadzu-UV-1601 spectrophotometer.

Values were expressed as mg/dl (plasma); mg/g (tissue).

3.8.1.4 Estimation of phospholipids

Phospholipid content of the sample was estimated by the method of Fiske & Subbarow (1925) as inorganic phosphorus liberated after Bartlette's perchloric acid digestion (1959).

Reagents

1. Ammonium molybdate reagent : 2.5 g of ammonium molybdate was dissolved in 100 ml of water.
2. Aminonaptho sulfonic acid (ANSA) : 0.5 g of 1,2,4 aminonapthosulfonic acid was dissolved in 195 ml of 15% sodium metabisulfite and 50 ml of 20% sodium sulfite was added for complete solubilisation. The solution was filtered and stored in a brown bottle.
3. Perchloric acid
4. Stock standard solution 35.1 mg of potassium dihydrogen phosphate was accurately weighed, dissolved and made upto 100 ml with double distilled water to give a final concentration of 80 μg phosphorus per ml.
5. Working standard : 1 ml of the stock was diluted to 10 ml to give a conc. of 8 μg phosphorus per ml.

Procedure

0.1 ml of the lipid sample was taken into a test tube and evaporated off chloroform. Added 0.5 ml of perchloric acid, and kept for digestion in a sand bath till the colour of the solution becomes clear. The solution was made up to 3.0 ml with double distilled water, and 1.0 ml of aliquot was taken. The tubes were made up to 4.0 ml with double distilled water. To all the tubes, 0.5 ml of ammonium molybdate reagent was added. After 10 min, added 0.5 ml of ANSA to all tubes. Aliquots of the standards and blank were carried through the same procedure. The blue colour developed was read after 20 min, at 620nm in a Shimadzu-UV-1601 spectrophotometer.

The phospholipid content of plasma was expressed as mg /dl (serum); mg/gm (tissue).

3.8.2 Lipoprotein fractionation

Addition of heparin-manganous chloride to plasma caused the precipitation of VLDL and LDL. The supernatant represented the HDL fraction. To another aliquot of plasma, addition of sodium dodecyl sulphate resulted in aggregation of VLDL. The cholesterol content of each fraction was carried out in the following manner.

Total plasma cholesterol – (HDL+LDL) cholesterol = VLDL cholesterol

3.8.2.1 Estimation of high density lipoprotein fraction

Total HDL was separated by the method of Burstein & Scholnick (1972).

Reagents

Heparin-Manganous chloride reagent: 3.167 gm of manganous chloride was added to 1.0 ml of heparin containing 20,000 units/ml. This was made up to 8.0 ml with water.

Procedure

0.1 ml of plasma was added to 9 µl of heparin-manganous chloride reagent and mixed well. The solution was allowed to stand at 4 °C for 30 min. The supernatant represented HDL fraction. Aliquots were taken from HDL fraction for the estimation of cholesterol.

3.8.2.2 Estimation of low density lipoproteins

This differential analysis was made by the method of Brustein & Scholink (1972) using sodium dodecyl sulphate.

Reagent

Sodium dodecyl sulphate : 10% in 0.15 M sodium chloride pH 9.0

Procedure

To 0.1 ml of plasma, 75 µl of sodium dodecyl sulphate solution was added, which was taken in a poly carbonate centrifuge tube. The contents were swirled briefly and packed for 2 h in a water bath at 35 °C. The contents were centrifuged in a refrigerated centrifuge at 10,000g for 30 min. VLDL got aggregated as a pellicle at the top. The supernatant was a mixture containing HDL and LDL cholesterol was estimated in 0.05 ml aliquot of the supernatant as described above.

3.8.2.3 Estimation of Lipoprotein (a)

Lipoprotein (a) was determined by using immunoturbidimetric test kit from DiaSys Diagnostic Systems GmbH, Germany and Photometric measurement of antigen antibody reaction.

Reagents

1. Tris buffer : 80 mM, pH 7.5
2. Antiserum N-Morpholine ethanesulfonic acid : 4 mM

Procedure

7.5 µl of sample and 7.5µl of distilled water were taken and added 250 µl of Tris buffer. The mixture was incubated at 37 °C for 3-5 min. The absorbance of the mixture was measured at 340 nm (A1). To the above mixture 50 µl of antiserum was added and incubated for 5 min at 37 °C and read at 340 nm (A2). Standards were also treated in same manner and the amount of Lp (a) present in the sample was calculated by comparing the sample and standard.

$$\Delta A = [(A2\text{-}A1)\ \text{Sample}] - [(A2\text{-}A1)\ \text{Blank}]$$

The values were expressed as mg/dl.

3.8.2.4 Estimation of apolipoprotein AI

Apolipoprotein AI was determined by using immunoturbidimetric test kit from DiaSys Diagnostic Systems GmbH, Germany and Photometric measurement of antigen antibody reaction.

Reagents

1. Tris buffer : 100 mM pH 7.5
2. Antiserum : Antihuman apolipoprotein AI antibody

Procedure

The estimation of apolipoprotein AI was carried out in a sophisticated semi auto analyzer by the addition of the following reaction mixtures. 250 µl of Tris buffer was

taken in a test tube and add 50 µl of antilipoprotein antibody. The reaction was started by the addition of 3 µl of sample. The mixture was read at 560 nm in a Shimadzu-UV-1601. Standards also treated in same manner and used for the calibaration setup.

The values are expressed as mg/dl

3.8.2.5 Estimation of apolipoprotein B

Apolipoprotein B was determined by using immunoturbidimetric test kit from DiaSys Diagnostic Systems GmbH, Germany and Photometric measurement of antigen antibody reaction.

Reagents

1. Tris buffer : 100 mM pH 7.5

2. Antiserum : Anti human apolipoprotein B antibody

Procedure

The estimation of apolipoprotein B was carried out in a sophisticated semi auto analyzer by the addition of the following reaction mixtures. 250 µl of Tris buffer was taken in a test tube and add 50 µl of antilipoprotein antibody. The reaction was started by the addition of 3 µl of sample. The mixture was read at 560 nm in a Shimadzu-UV-1601. Standards also treated in same manner and used for the calibration setup.

The values are expressed as mg/dl

3.8.3 Analysis of fatty acid composition (FAME)

Fatty acids were analyzed according to the method of AOAC (1975). Lipid content of the tissues was estimated by the method of Folch *et al.* (1957). Methyl esters of fatty acids from animal and vegetable origin having 8-24 atoms are separated and detected by

gas chromatography. Method is not applicable to epoxy, oxidized or polymerized fatty acids (Metcalfe *et al.*, 1966).

Reagents

1. Boron trifluoride reagent
2. Methanolic sodium hydroxide solution
3. Petroleum ether
4. Sodium sulphate

Procedure

Added sample (lipid of known weight) to a flask followed by 6 ml methanolic NaOH and boiling chip. Attach condenser, and reflux until fat globules disappear (usually 5-10 min). Add 6-7 ml BF_3 solution from bulb or automatic pipette through condenser and continue boiling for 2 min. Remove heat, then condenser, and add 15 ml saturated NaCl solution. Stopper flask and shake vigorously 15 s while solution is still tepid. Transfer aqueous phase to 250 ml separator. Extract with two 30 ml portions of petroleum ether (b.p 60-80 ^{o}C). Wash combined extracts with 20 ml portions H_2O, dry over anhydrous Na_2SO_4, filter and evaporate solvent under stream of nitrogen on steam bath.

Methyl esters of the fatty acid thus obtained were separated by gas liquid chromatography (Varian CP 3800. U.S.A) equipped with a capillary column (Elite 225, 30m long and 0.25mm diameter) and a flame ionization detector in the presence of hydrogen and air. The carrier gas was nitrogen and the flow rate was 0.5ml/min the chromatograph temperature started at 150 ^{o}C and was increased 4 ^{o}C/min until a temperature of 250 ^{o}C was obtained. Fatty acids separated were identified by the comparison of retention times with those obtained by the separation of a mixture of

standard fatty acids. Measurement of peak areas and data processing were carried out by Star WS software package. Individual fatty acids were expressed as weight percentage of total fatty acids.

3.9 Lipid peroxidation and tissue antioxidant status

3.9.1 Estimation of lipid peroxides (LPO) in plasma

Serum lipid peroxide content was estimated by the method of Yagi (1976).

Reagents

1. Sulphuric acid : 0.085 N
2. Phosphotungstic acid : 10%
3. TBA reagent : Mixture of equal volumes of 0.67 % TBA aqueous solution and glacial acetic acid.
4. n-Butanol

Procedure

0.1 ml of serum was mixed with 4.0 ml of 0.085 N sulphuric acid and shake gently. To this 0.5 ml of phosphotungtic acid was added and stirred well. The contents were centrifuged for 10 min. The supernatant was discarded and the pellet was suspended in 2 ml 0.085 N sulphuric acid and 0.3 ml of 10% phosphotungtic acid. The mixture was centrifuged for 10 min. The pellet obtained was suspended in 4.0 ml of distilled water and 1.0ml of TBA reagent. The tubes were kept in a boiling water bath for one hour. After cooling 5.0 ml of n-butanol was added to each tube and the color obtained in butanol phase was read at 532 nm in a Schimadzu-UV-1601 spectrophotometer.

The serum lipid peroxide content was expressed as nmoles of malondialdehyde/ml.

3.9.2 Estimation of lipid peroxides in tissue (LPO)

Lipid peroxides content was determined by thiobarbituric acid reaction as described by Ohkawa *et al.* (1979).

Reagents

1. Acetic acid 20% : 20 ml of glacial acetic acid dissolved in 100 ml distilled water.
2. Thiobarbituric acid : 0.8% in 20% acetic acid.
3. Sodium dodecyl sulphate : 8.1%
4. Standard : 41.66 mg of Tetraethoxy propane (TEP) dissolved in 100 ml distilled water. 1.0 ml of above was made up to 100 ml with distilled water.

Procedure

To 0.2 ml of sample, 1.5 ml of 20 % acetic acid, 0.2 ml of SDS and 1.5 ml of TBA were added. The mixture was made up to 4.0 ml with distilled water and heated in a boiling water bath for one hr. After cooling the mixture was centrifuged at 3000 g for 10 min. Supernatant was taken and absorbance was read at 532 nm in a Shimadzu-UV-1601 spectrophotometer.

The lipid peroxides content was expressed as n mol malondialdehyde/mg protein.

3.9.3 Determination of total reduced glutathione (GSH)

The total reduced glutathione was determined by the method of Ellman (1959). The method is based on the reaction of reduced glutathione with 5, 5'-dithiosbis (2-nitrobenzoic acid) (DTNB) to give a compound that has absorbance at 412 nm.

Reagents

1. DTNB : 0.6 mM in 0.2 M Phosphate buffer pH 8.0
2. Phosphate buffer : 0.2 M, pH 8.0.
3. Trichloroacetic acid : 5%
4. Standard 61.4 mg of reduced glutathione was dissolved in 100 ml 0.02 M EDTA. 0.1 ml of this is made up to 10 ml with 0.02 M EDTA.
5. Working standard : 2.0 ml of the above was made up to 10 ml.

Procedure

0.5 ml of heart homogenates was precipitated with 0.1ml of 5 % TCA and made up the volume to 1.0 ml using distilled water. The contents were mixed well for complete precipitation of proteins and centrifuged at 4000 rpm for 15 min. To an aliquot of clear supernatant, 0.2 M phosphate buffer was added to make a final volume to 2.5 ml, added 50 µl of DTNB reagent just before reading. The absorbance was read at 412 nm against a blank containing TCA instead of sample, series of standards treated in a similar way were also run to determine the reduced glutathione content.

The amount of glutathione was expressed as nmol/g wet tissue.

3.9.4 Estimation of glutathione peroxidase (EC 1.11.1.9)

Glutathione peroxidase activity was determined by the non-enzymatic method of Paglia & Valentine *et al.* (1967).

Reagents

1. Phosphate buffer : 0.4 M pH 7.0
2. EDTA : 0.4 mM

3. GSH : 2 mM

4. NaN_3 : 10 mM

5. TCA : 10%

6. DTNB : 0.6 mM in 0.4 M phosphate buffer

7. H_2O_2 : 1 mM was prepared freshly from commercial 30%solution.

8. GSH standard : 61.4 mg GSH was dissolved in 100 ml distilled water. 1.0ml of this solution was made up to 10 ml with distilled water.

9. Working standard : 2 ml of the stock was made up to 10 ml with distilled water.

Procedure

0.2 ml of tissue homogenate was added to a mixture containing 0.2 ml of buffer, 0.2 ml of EDTA, 0.1 ml of sodium azide, mixed well and added 0.2 ml reduced glutathione and 0.1 ml of hydrogen peroxide. Incubated in a water bath at 37 °C for 10 min. At the end of incubation period, 0.5ml of 10%TCA was added and centrifuged at 10000 rpm for 5 min. 1.0 ml of the supernatant was taken into a separate test tube and added 0.1 ml DTNB. Immediately read the OD at 412 nm using a Shimadzu-UV-1601 spectrophotometer.

The enzyme activity was expressed as n mol glutathione oxidized/min/mg protein.

4.9.5 Assay of glutathione-S-transferase (EC 2.5.1.18)

Glutathione-S-tranferase activity was determined by the method of Habig *et al.* (1974).

Reagents

1. Phosphate buffer : 0.3 M, pH 6.5

2. 1-Chloro-2, 4-Dinitrobenzene (CDNB) : 30 mM

3. Reduced glutathione (GSH) : 30 mM.

Procedure

The reaction mixture containing 1.0 ml of buffer, 0.1 ml of CDNB and 0.1 ml of tissue homogenate was made up to 2.5 ml with water. The reaction mixture was pre-incubated at 37 °C for 5min. 0.1 ml of GSH was added and the change in the absorbance was measured at 340 nm in a Shimadzu-UV-1601 spectrophotometer, using UV PC software package for enzyme kinetics.

The enzyme activity was expressed as μ mol CDNB conjugate formed/min/mg protein.

3.9.6 Assay of catalase (EC 1.11.1.6)

Catalase was assayed according to the method of Takahara *et al.* (1960).

Reagents

1. Phosphate buffer : 50 mM, pH 7.0

2. Hydrogen peroxide : 30 mM solution in the above buffer.

Procedure

To 2.4 ml of the phosphate buffer, 0.1 ml of the enzyme source was added and the reaction was started by the addition of 1.0 ml of H_2O_2 solution. The decrease in absorbance was measured at 240nm using a Shimadzu-UV-1601 spectrophotometer, UV

PC Software package for enzyme kinetics. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen peroxide.

The enzyme activity was expressed as n moles of H_2O_2 decomposed per minute per mg protein.

3.9.7 Assay of superoxide dismutase (EC 1.15.1.1)

The superoxide dismutase was assayed according to the method of Misra & Fridovich (1972) based on the oxidation of epinephrine-adrenochrome transition by the enzyme.

Reagents

1. Carbonate-bicarbonate buffer : 0.1 M pH 10.0.
2. Epinephrine : 3 mM

Procedure

Taken 100 µl of sample into the cuvette and add 1.4 ml buffer and 0.5 ml epinephrine mixed well and immediately read the change in optical density at 480 nm using Shimadzu-UV-1601 spectrophotometer, UV PC Software package for enzyme kinetics.

One unit of SOD activity was the amount of protein required to give 50% inhibition of epinephrine auto oxidation.

3.10 Determination of the total, protein and non-protein sulfhydryl content

Total protein and non-protein sulphhydryl contents were estimated according to the method of Sedlak & Lindsay (1968). This method is based on the development of a yellow colour when DTNB is added to compounds contained sulfhydryl groups to form 2-nitro 5- mercaptobenzoic acid.

Procedure

Reagents	Blank(ml)	System(ml)
Total sulfhydryl content (TSH)		
Homogenate	_	0.5
Tris-HCl buffer (0.2M;pH 8.2) with 2mM EDTA	1.5	1.5
DTNB (99 mg/ 25 ml methanol)	0.1	0.1
Methanol	7.9	7.9
Distilled water	0.5	_

Vortexed and centrifuged at 3000 g for 10 min. Read at 412 nm. Standards (100 μ g GSH/ml) were also treated in a similar way.

Non-protein sulfhydryl content (NPSH)		
Homogenate	_	1.0
Distilled water	4.0	3.5
TCA (50%)	0.5	0.5
Centrifuged at 3000 g for 10 min. Both supernatant and pellet were saved.		
Supernatant	-	1.0
Tris-HCl buffer (0.4M;pH 8.0) with 2mM EDTA	2.0	2.0
DTNB (99 mg/ 25 ml methanol)	50μl	50μl

Read at 412 nm. Standards (100 μ g GSH/ml) were also treated in a similar way.

Protein sulfhydryl content (PSH)		
The above pellet	As such	As such
Tris-HCl buffer (0.2M;pH 8.2) with 2mM EDTA	1.5	1.5
DTNB (99 mg/ 25 ml methanol)	0.1	0.1
Methanol	8.4	8.4

Vortexed and centrifuged at 3000 g for 10 min. Read at 412 nm. Standards (100 μ g GSH/ml) were run in a similar pattern.

The total, protein and non- protein sulfhydryl contents are expressed as n mol/g wet tissue.

3.11 Membrane-bound ATPases

3.11.1 Estimation of inorganic phosphorus

Inorganic Phosphorus was estimated by the method of Fiske & Subbarow (1925). The method is based on the formation of phoshomolybdic acid by the reaction between a phosphate and molybdic acid and its subsequent reduction to a dark blue phosphomolybdic acid, the intensity of which is proportional to the phosphate ion concentration.

Reagents

1. Ammonium molybdate reagent : 2.5 g of ammonium molybdate was dissolved in 100 ml of 3 N sulphuric acid.
2. Amino naphthol sulphonic acid (ANSA) : 0.5 g of ANSA was dissolved in 195 ml of 15% sodium metabisulphite and 5.0 ml of 20% sodium sulphite was added for complete solubilization. The solution was filtered and stored in a brown bottle.
3. Standard Phosphorus 35.1 mg of potassium dihydrogen phosphate was accurately weighed, dissolved and made up to 100 ml with distilled water.
4. Working standard : 1.0 ml of the stock was diluted to 5.0 ml using distilled water.

Procedure

To suitable aliquots of the supernatant, 1.0 ml of ammonium molybdate reagent was added. 0.4 ml of ANSA was added after 10 min incubation at room temperature.

Standards and blank were also treated in the above manner. The blue colour developed was read after 20 min at 640 nm in a Shimadzu-UV-1601 Spectrophotometer.

The values were expressed as μg /mg protein.

3.11.2 Assay of Na^+/K^+-dependent ATPase (EC 3.6.3.9)

Na^+/K^+-dependent ATPase activity was measured from the amount of Pi released according to the method of Bonting (1970).

Reagents

1. Tris buffer : 184 mM, pH 7
2. Magnesium Sulphate : 50 mM
3. Potassium Chloride : 50 mM
4. Sodium Chloride : 600 mM
5. EDTA : 1.0 mM
6. ATP : 40 mM
7. TCA : 10%

Procedure

One ml of Tris-buffer and 0.2 ml each of the above assay reagents were mixed together. So the assay medium, in the final volume of 2.0 ml contained, 92 mM Tris-buffer, 5 mM magnesium sulphate, 60 mM sodium chloride, 5 mM potassium chloride, 0.1 mM EDTA and 4.0 mM ATP. After 10 min equilibration at 37 °C in an incubator, reaction was started by the addition of 0.2 ml of the enzyme solution. The assay medium was incubated for 30 min and at the end of the incubation period. The reaction was

stopped by the addition of 2 volumes of ice cold 10% TCA. The phosphorus (Pi) liberated was estimated by the method of Fiske & Subbarow (1925).

The enzyme activity was expressed as μ mol Pi liberated/min/ mg protein

3.11.3 Assay of Mg^{2+}-dependent ATPase (EC 3.6.3.1)

Mg^{2+}-ATPase was assayed according to the method described by Ohinishi *et al.* (1982)

Reagents

1. Tris- HCl buffer : 0.375 M, pH- 7.6
2. Magnesium chloride : 0.205 M
3. ATP : 0.01 M

Procedure

Buffer 0.1ml, 0.1 ml of ATP, magnesium chloride 0.1ml and distilled water 0.1ml were taken in test tubes. 0.1ml of enzyme preparation was added. The tubes were then incubated at 37 °C for 15 min. The reaction was stopped by the addition of 1.0ml of 10% TCA. 0.1 ml of enzyme was added to the control tubes. The phosphorus (Pi) liberated was estimated by the method of Fiske& Subbarow.

The enzyme activity was expressed as μ mol Pi liberated/min/ mg protein.

3.11.4 Assay of Ca^{2+}-dependent ATPase (EC 3.6.3.8)

Ca^{2+} dependent ATPase was assayed by the method of Hjerten & Pan (1983).

Reagents

1. Tris-HCl buffer : 0.125 M, pH 8.0
2. Calcium chloride : 0.05 M
3. ATP : 0.01 M
4. TCA : 10%

Procedure

Tris-HCl buffer 0.1 ml, calcium chloride 0.1 ml, ATP solution 0.1 ml and distilled water 0.1 ml were taken in test tubes. 0.1 ml of enzyme preparation was added and the tubes were incubated at 37 °C for 15 min. The reaction was arrested by the addition of 1.0 ml of 10% TCA to the incubation mixture. 0.1 ml enzyme source was added to the control tubes. The contents were centrifuged at 4000 rpm for 5 min. The supernatant was used for the estimation of inorganic phosphorous.

The enzyme activity was expressed as μ mol Pi liberated liberated/min/ mg protein.

3.12 Estimation of minerals using Atomic Absorption Spectrophotometer

Minerals were estimated according to the method of the AOAC (1980).

Reagents

1. Nitric acid
2. Perchloric acid
3. 1& 2 in 9:4
4. Stock solution of sodium, potassium and calcium were prepared by diluting concentrated solution of 1000 mg/l (Merck).

Procedure

Samples size of 1g of heart tissue and 1 ml serum were used for the experiment. To the sample containing flask, 7ml of nitric acid and perchloric acid (9:4) mixture was added, covered with a watch glass and left at room temperature over night. The sample was then digested using a microwave digester (Milestone ETHOS PLUS lab station Closed Vessel Microwave Digestion System). The completely digested samples were allowed to cool at room temperature, filtered (glass wool) carefully transferred into a clean 50 ml volumetric standard flask and then diluted to the mark with ultra pure water (Milli Q, Millpore). The digested samples were analyzed using Varian Spectra-220 AA, Atomic Absorption Spectrophotometer equipped with a deuterium back ground corrector for the determination of minerals viz sodium, potassium and calcium.

3.13 Estimation of ATP content

ATP content was estimated by the method of Ryder (1985) using Shimadzu LC 10 AT vp, HPLC System.

Reagents

1. Perchloric acid : 0.6 M

2. KOH : 2 M

3. KOH : 1 M

4. Mobile phase : Phosphate buffer solution prepared by mixing 0.04 M KH_2PO_4 (5.4436 g/l) and 0.06 M K_2HPO_4 (10.4508 g/l) in 1:1 proportion and is filtered through 0.45 μm filter paper.

Procedure

A 0.5 g portion of the heart tissue was homogenised with 2.5 ml of chilled 0.6 M perchloric acid and centrifuged at 10,000 rpm at 4 °C for 20 min. The supernatant was

neutralised to pH 6.8 using 1 M and 2 M KOH (visualized by formation of KCl precipitate). Measured the total volume. After standing at 0 °C for 30 min, it was filtered through a syringe filter of pore size 0.45µm and was analysed by Shimadzu-LC AT vp, HPLC, using Photo Diode Array detector.

50 µl of the sample was injected in to the HPLC. The method utilized a simple reverse phase (C_{18}RP), Hypersil C18, Column (4.6mm I.Dx250mm). Phosphate buffer (0.04 M KH_2PO_4 and 0.6 M K_2HPO_4 in 1:1proportion) was used as mobile phase at a flow rate of 1.5 ml/min. The peaks obtained from the sample were identified by comparing with the peak of chromatogram of the mixed standard solutions. The quantification of each nucleotide breakdown products was done by comparing the peak area of the samples with peak area of the standard corresponding to the sample.

The ATP content was expressed as n mol/g wet tissue.

3.14 Isolation of heart mitochondrial and lysosomal fractions

Mitochondrial and lysosomal fractions of the heart tissue were isolated by the method of Plummer (1998).

Reagents

Tris HCl : 50 mM, pH 7.4 containing 0.25 M Sucrose and 1mM EDTA.

Procedure

Immediately after sacrifice, the heart was removed and all the blood vessels and connective tissues were trimmed off. Wash the tissue free of blood in ice-cold sucrose,

neutralised to pH 6.8 using 1 M and 2 M KOH (visualized by formation of KCl precipitate). Measured the total volume. After standing at 0 °C for 30 min, it was filtered through a syringe filter of pore size 0.45μm and was analysed by Shimadzu-LC AT vp, HPLC, using Photo Diode Array detector.

50 μl of the sample was injected in to the HPLC. The method utilized a simple reverse phase (C_{18}RP), Hypersil C18, Column (4.6mm I.Dx250mm). Phosphate buffer (0.04 M KH_2PO_4 and 0.6 M K_2HPO_4 in 1:1proportion) was used as mobile phase at a flow rate of 1.5 ml/min. The peaks obtained from the sample were identified by comparing with the peak of chromatogram of the mixed standard solutions. The quantification of each nucleotide breakdown products was done by comparing the peak area of the samples with peak area of the standard corresponding to the sample.

The ATP content was expressed as n mol/g wet tissue.

3.14 Isolation of heart mitochondrial and lysosomal fractions

Mitochondrial and lysosomal fractions of the heart tissue were isolated by the method of Plummer (1998).

Reagents

Tris HCl : 50 mM, pH 7.4 containing 0.25 M Sucrose and 1mM EDTA.

Procedure

Immediately after sacrifice, the heart was removed and all the blood vessels and connective tissues were trimmed off. Wash the tissue free of blood in ice-cold sucrose,

lightly blot and place in a tared beaker to weigh. Cut the heart in to small fragments and homogenize in buffer containing 0.25 M sucrose and 1mM EDTA. Centrifuge the suspension in a refrigerated centrifuge.

The homogenate was centrifuged at 2000 rpm for 10 min. The supernatant was transferred in to test tubes. The pellet was dissolved in sucrose buffer. Centrifuged for 10 min at 2000 rpm. The supernatants were pooled and centrifuged for 10 min at 7400 rpm, the pellet collected represents mitochondrial fraction. The supernatant was again centrifuged for 10 min at 11400 rpm and the pellet was collected (lysosomal fraction). Each fraction should be resuspended in sucrose and the washings combined with the supernatants. This has the advantage of producing purer fractions. Carefully resuspend the mitochondrial pellet in about 2ml of sucrose, and lysosomal pellet in 1ml of sucrose and used as the enzyme source and store on ice until required.

3.14.1 Mitochondrial and respiratory marker enzymes

3.14.1.1 Assay of Isocitrate dehydrogenase (EC 1.1.1.42)

The enzyme activity was assayed according to the method of Bell & Baron (1960).

Reagents

1. Tris-HCl buffer : 0. 1 M, pH 7.5
2. Substrate : 0. 1 M trisodium DL-Isocitrate in 0.9% saline
3. Manganous chloride : 0. 015 M in 0.9% saline
4. NADP : 0. 001 M in 0.9% saline
5. EDTA : 5%
6. Sodium hydroxide : 0. 4 N
7. 2,4 dinitrophenyl hydrazine (DNPH) : 0. 001 M in 1N HCl

8. Standard Solution : 15 mg of α-ketoglutarate (potassium salt) in 50 ml of buffer.

Procedure

Buffer, 0.4ml was taken in a test tube and 0.2 ml of substrate, 0.3 ml of manganous chloride and 0.2ml of the mitochondrial suspension were added. A control tube was also prepared simultaneously, 0.2 ml 0f co-enzyme solution was added to the test tube and 0.2 ml of saline was added to the control tubes. After mixing well, both the tubes were incubated for 60min, 1.0 ml of DNPH was added to both the tubes, followed by 0.5 ml of EDTA. The tubes were kept at room temperature for 20 min and 10ml of 0.4 N NaOH was added to the tubes. A blank was run simultaneously. The colour intensity was measured at 390 nm in a Shimadzu- UV-1601 spectrometer. A standard curve was prepared using α-ketoglutarate.

The activity of isocitrate dehydrogenase is expressed as μ mol α-ketoglutarate liberated/ mg protein.

3.14.1.2 Assay of Succinate dehydrogenase (EC 1.3.99.1)

This enzyme activity was estimated accordingly to the method of Slater & Bonner (1952). The rate of reduction of potassium ferricyanide was measured in the presence of sufficient potassium cyanide to inhibit cytochrome oxidase by following the rate of decrease in the optical density at 420 nm.

Reagents

1. Phosphate buffer : 0.3 M pH 7.6
2. Sodium salt of ethylene diaminetetra acetate (EDTA) : 0.03 M solution.

3. Potassium cyanide : 0.03 M solution.

4. Sodium Succinate : 0.4 M solution.

5. Bovine serum albumin : 3% solution

6. Potassium ferricyanide : 0.075 M solution

Procedure

In a spectrophotometric cuvette, 1.0 ml of phosphate buffer, 0.1 ml of EDTA, 0.1 ml of bovine serum albumin, 0.3 ml of sodium succinate, 0.2 ml of potassium ferricyanide and 0.1 ml of potassium cyanide were added and the total volume was made up to 2.8 ml with double distilled water. The reaction was started by the addition of 0.2 ml of mitochondrial suspension. Changes in optical density at 420 nm were recorded in a Shimadzu- UV-1601 spectrophotometer using UV PC Software package for enzyme kinetics.

The activity of succinate dehydrogenase is expressed as per μ mol succinate oxidized/ mg protein.

3.14.1.3 Assay of Malate dehydrogenase (EC 1.1.37)

This enzyme activity was assayed by the method of Mehler *et al.* (1948). The activity determination is based on the measurement of the rate of oxidation of NADH in the presence of the enzyme and excess of oxaloacetate.

Reagents

1. Tris – HCl : 0.25 M, pH 7.4

2. NADH : 0.015 M

3. Oxaloacetate : 0.0076 M, pH 7.4

Procedure

To 0.3 ml of buffer 0.1 ml of NADH and 0.1 ml of oxaloacetate were added and the total volume was made to 2.9 ml with water. The reaction was started by adding 0.1 ml of mitochondrial suspension. The change in optical density was measured at 340 nm in a Shimadzu- UV-1601 spectrophotometer using UV PC Software package for enzyme kinetics.

The activity of malate dehydrogenase was expressed as μ mol NADH oxidized/mg of protein.

3.14.1.4 Assay of NADH dehydrogenase (EC 1.6.99.3)

The activity of NADH dehydrogenase was assayed according to the method of Minakami *et al.* (1962).

Reagents

1. Phosphate buffer : 0.1 M, pH 7.4.
2. NADH : 0.1% solution
3. Potassium ferricyanide : 0.03 M solution

Procedure

The reaction mixture contained 1.0ml of phosphate buffer, 0.1 ml of potassium ferricyanide, 0.1 ml of NADH and 1.6 ml of distilled water in a total volume of 3.0 ml. The temperature was brought to 30 ^{o}C and NADH was added just before the addition of the sample. A suitable aliquot of mitochondrial solution was added and change in absorbance was measured at 420 nm in a Shimadzu-UV-1601 spectrophotometer, using

UV PC Software package for enzyme kinetics. A control containing all the reagents except NADH was also treated similarly.

The activity of NADH-dehydrogenase is expressed as per μ mol NADH oxidized/mg of protein.

3.14.1.5 Assay of α-ketoglutarate dehydrogenase (EC 1.2.4.2)

This enzyme activity was estimated according to the method of Reed & Mukherjee (1969). It is based on the calorimetric determination of ferrocyanide produced by the decarboxylation of α-ketoglutarate with ferricyanide as electron acceptor.

Reagents

1. Phosphate buffer : 0.1 M, pH 6.0
2. Thiamine pyrophosphate : 0.002 M
3. Magnesium sulphate : 0.003 M
4. α-ketoglutarate(Pottassium salt) : 0.05 M
5. Potasium ferricyanide : 0.25 M
6. TCA : 0.25 M
7. SDS (Dupanol) : 4%
8. Ferric ammonium sulphate – dupanol reagent : To 1.7 g of ferric ammonium sulphate was added 10 ml of water and filtered. To this filtrate a solution of 1.5 g of dupanol in 20 ml of water was added. Then 27 ml of 85% phosphoric acid was added and diluted to 140 ml with water.
9. Standard potassium ferrocyanide : 0.01%

Procedure

To 0.15 ml of phosphate buffer 0.1ml each of thiamine pyrophosphate, magnesium sulphate, and potassium ferrocyanide was added. The total volume made up to 1.2ml with water. Exactly 0.2 ml of the mitochondrial suspension was added to the test. A control was prepared simultaneously without the addition of mitochondria. The tubes were incubated at 30 °C for 30 min. After this period, 1.0 ml of 10%TCA was added to the control tubes. The aliquots of the supernatant after centrifugation were pipetted out into test tubes for the colour reaction. To these tubes, 0.1ml of potassium ferricyanide was added and the volume was made up to 2.4 ml with water. 1 ml of 4% dupanol and 0.5ml of ferric ammonium sulphate dupanol reagent were added and the tubes were incubated at 25 °C for 30 min. The colour intensity was measured at 540 nm in a shimadzu UV-1601 spectrophotometer. A standard potassium ferrocyanide solution in the range of 10 µg to 50 µg was run simultaneously.

The activity of α-ketoglutarate dehydrogenase is expressed as n mol potassium ferrocyanide liberated/ mg protein.

3.14.2 Mitochondrial lipid peroxidation and antioxidant status

3.14.2.1 Determination of lipid peroxides

The heart mitochondrial lipid peroxide content was determined by the thiobarbituric acid reaction as described by Ohkawa *et al.* (1979) [Section 3.10.2].

3.14.2.2 Assay of superoxide dismutase

The mitochondrial superoxide dismutase activity was assayed by the method of Misra & Fridovich (1972). One unit of SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation [Section 3.10.7].

3.14.2.3Assay of catalase

The mitochondrial catalase activity was assayed by the method of Takahara, (1960). The enzyme activity was expressed as μ mol H_2O_2 consumed/min/mg of protein [Section 3.10.6].

3.14.2.4 Determination of mitochondrial GSH

GSH content in heart mitochondria was determined according to the method of Ellman (1959). The amount of glutathione is expressed as n mol/g tissue [Section 3.10.3].

3.14.2.5 Assay of glutathione peroxidase

The mitochondrial glutathione peroxidase activity was assayed by the method of Habig *et al.* (1974). The enzyme activity is expressed as nmol glutathione oxidized/min/mg protein. [Section 3.10.4]

3.14.2.6 Assay of glutathione-S-transferase

The mitochondrial glutathione-S-transferase activity was assayed by the method of Pagila & Valentine (1967). The enzyme activity is expressed as n mol CDNB conjugate formed/min/protein [Section 3.10.5].

3.14.3 Lysosomal marker enzymes

3.14.3.1 Assay of β- glucosidase (EC 3.2.1.21)

β- glucosidase was assayed according to the method of Conchie *et al.* (1967) based on the principle that β- glucosidase acts on P- nitrophenyl- β- D-glucopyranoside and liberate P-nitrophenol, which was measured at 410nm in alkaline pH.

Reagents

1. Substrate : 10 mM, p-nitro phenyl β-D-glucopyranoside in buffer
2. Phosphate buffer : 0.1 M Citrate-0.2 M phosphate, pH 4.5
3. Glycine-NaOH buffer : 0. 4 M pH-10.4
4. Standard : 6 mM, p- nitrophenol
5. Working standard : 1 ml diluted to 10 ml using distilled water.

Procedure

To 0.5ml of substrate and 0.3ml of citrate buffer in a test tube, 0.2ml of the enzyme solution was added, shaken gently and incubated at 37 °C for 1h. 3ml of glycine –NaOH buffer was added for reaction termination. Mixed and read at 410 nm using shimadzu UV-1601 spectrophotometer.

The activity of β- glucosidase is expressed as μ mol p- nitro phenol liberated/mg protein.

3.14.3.2 Assay of β- galactosidase(EC 3.2.1.23)

β- galactosidase was assayed according to the method of Conchie *et al.* (1967) based on the principle that β- galactosidase acts on p- nitrophenyl- β- D-glucopyranoside and liberate P-nitrophenol, which was measured at 410 nm in alkaline pH.

Reagents

1. Substrate : 2 mM , p-nitro phenyl β-D-galactopyranoside in buffer
2. Phosphate buffer : 0.1M Citrate-0.2 M phosphate, pH 4.5
3. Glycine-NaOH buffer : 0.4 M pH-10.4

4. Standard : 6 mM, p- nitrophenol

5. Working standard : 1 ml diluted to 10 ml using distilled water

Procedure

To 0.5 ml of substrate and 0.3 ml of citrate buffer in a test tube, 0.2ml of the enzyme solution is added, shaken gently and incubated at 37 °C for 1h. 3ml of glycine –NaOH buffer is added for reaction termination. Mixed and read at 410nm.

The activity of β- galactosidase is expressed as μmol p- nitrophenol liberated/ mg protein.

3.14.3.3 Assay of acid phosphatase (EC 3.1.3.2)

Acid phosphatase was assayed by the method of King, (1965) using disodium phenyl phosphate as the substrate [Section 3.6.6].

3.15 Statistical Analysis

Results are expressed as mean ± SD. Multiple comparisons of the significant ANOVA were performed by Tukey's multiple comparison test. A p-value <0.05 was considered as statistically significant. All data were analyzed with the aid of statistical package program SPSS 10.0 for Windows.

Results and Discussion

4. RESULTS AND DISCUSSION

One of the most attractive approaches to disease prevention involves the use of natural antioxidants to protect tissue against ischemic injury. Taurine, the major intracellular free β-amino acid, is well known to be an endogenous antioxidant molecule and a membrane-stabilizing agent. The focus of the current study is to evaluate the effects of taurine for its cardioprotective property in isoproterenol-induced myocardial infarction in rats by virtue of its membrane-stabilizing, antioxidant and antilipidemic properties.

4.1 Effect of taurine on diagnostic markers of myocardial infarction

Cardiac biomarkers are protein components of cell structures released into the blood stream when myocardial injury occurs and they can be measured in the systemic circulation. Elevation of cardiac markers in plasma is one of the criteria being used for the diagnosis of acute myocardial infarction. The optimal and ideal cardiac marker should be present in high concentration in myocardium and should be absent from non-myocardial tissues. It should be rapidly released into the blood stream at the time of myocardial injury and there should be a direct relation between the plasma level of the marker and the extent of myocardial injury. The marker should persist in plasma for a sufficient length of time to provide a convenient diagnosis time, and the measurement of the marker should be easy, inexpensive and rapid. In this regard, the plasma diagnostic marker enzymes are of particular interest because of their catalytic activity and tissue specificity. Troponin and homocysteine are the other newly emerged diagnostic markers, which are widely used because of their high sensitivity, specificity and reliability.

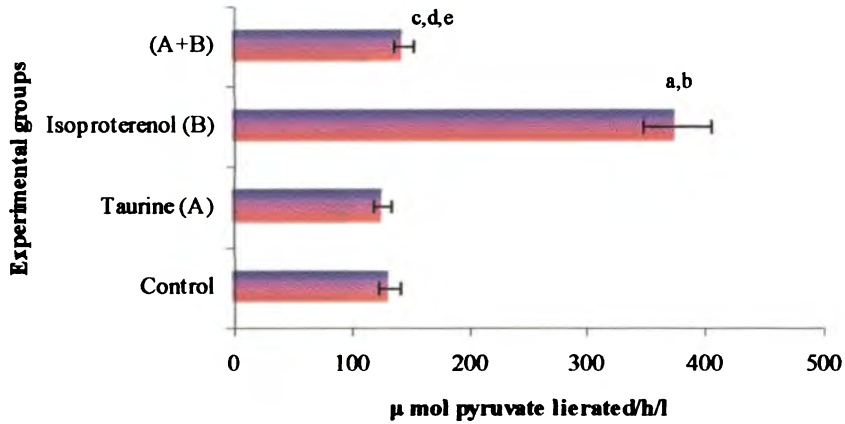


Fig 4.1.1 Level of aspartate amino transferase in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group I control animals

[e] $p<0.05$ significantly different compared with Group II taurine-administered animals

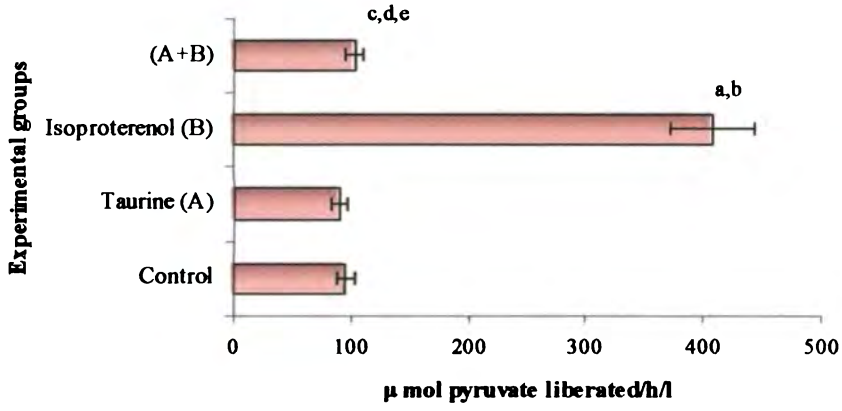


Fig 4.1.2 Level of alanine amino transferase in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group I control animals

[e] $p<0.05$ significantly different compared with Group II taurine-administered animals.

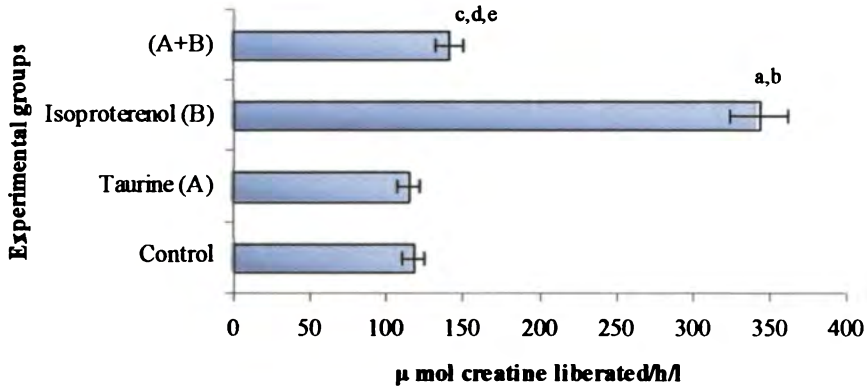


Fig 4.1.3 Level of creatine phosphokinase in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days.

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group I control animals

[e] $p<0.05$ significantly different compared with Group II taurine-administered animals.

4.1.1 Diagnostic marker enzymes

There was a significant ($p< 0.001$) increase noticed in the levels of diagnostic marker enzymes [AST, ALT, LDH, CPK, ACP and ALP] in plasma of Group III isoproterenol-administrated rats compared to Group I normal control animals **(Fig 4.1.1- 4.1.6)**, which is indicative of the severity of isoproterenol-induced necrotic damage to the myocardial membrane. This observation is in accordance with earlier studies (Farvin *et al.*, 2004; Suchalatha & Shyamala Devi, 2004), which indicated that of all the macromolecules leaking from the damaged cardiac tissue, these enzymes because of their tissue specificity and catalytic activity are the best markers of myocardial infarction. The use of myocardial specific enzymes as an index of acute myocardial infarction is equally reliable both in experimental and clinical investigations (Wexler, 1978). Earlier reports by Manjula *et al.* (1992) have shown that the amount of marker enzymes present in plasma is directly proportional to the number of necrotic cells present in the cardiac tissue. The release of these marker enzymes reflects a non-specific aberration in the structural and functional integrity of myocardial membrane in response to isoproterenol.

In the present study, prior administration of taurine resulted in a significant ($p< 0.001$) reduction in the levels of these marker enzymes in plasma of Group IV rats compared with Group III isoproterenol-administrated rats, indicating the cytoprotective action of taurine (Endo *et al.*, 2002; Schaffer *et al.*, 2003). It probably did so by its membrane stabilizing action (Redmond *et al.*, 1998). Timbrell *et al.* (1995) reported that taurine exerted membrane stabilization against carbon tetrachloride, hydrazine and 1, 4-naphthoquinone-induced necrotic damage by modulation of calcium levels and osmoregulation. Reports by Balkan *et al.* (2005) indicated that pretreatment with taurine and betaine significantly ameliorated the lipopolysaccharide-induced necrotic release of

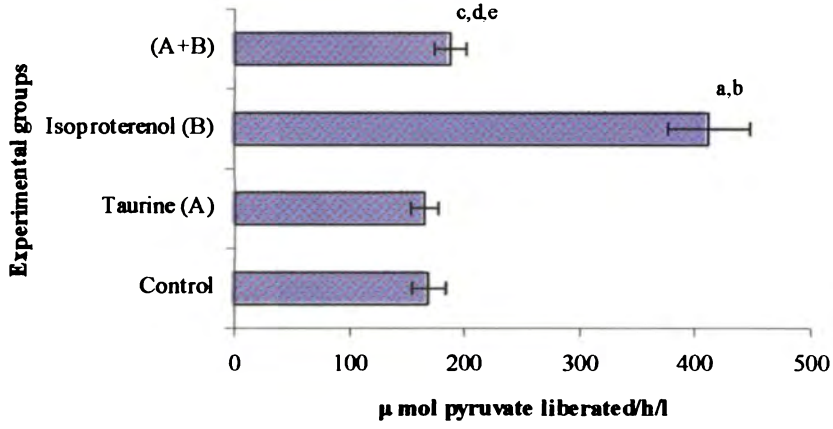


Fig 4.1.4 Level of lactate dehydrogenase in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days.

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group I control animals

[e] $p<0.05$ significantly different compared with Group II taurine-administered animals.

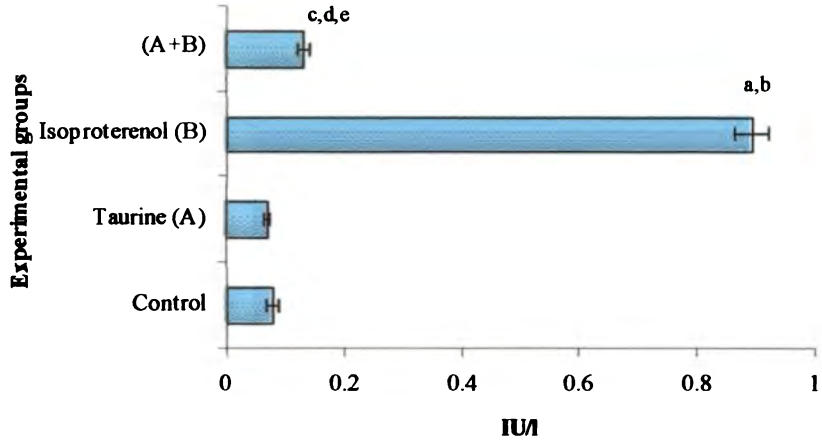


Fig 4.1.5 Level of acid phosphatase in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days.

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.01$ significantly different compared with Group II control animals

[e] $p<0.05$ significantly different compared with Group I control animals

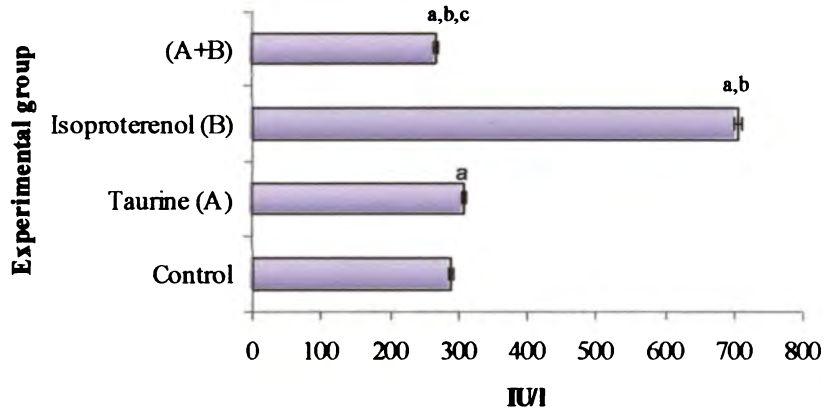


Fig 4.1.6 Level of alkaline phosphatase in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days.

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

577.1 : 547.456
SHI

diagnostic marker enzymes into the blood stream from hepatic tissue by maintaining the tissue antioxidant defense system at near normal status. Pushpakiran *et al.* (2004) also observed that simultaneous administration of taurine along with ethanol prevented the leakage of marker enzymes into circulation by its bioprotective effect.

4.1.2 Troponin T

Troponins are proteins of the troponin regulatory complex involved in cardiac contractility with high myocardial tissue specificity that overcomes the fundamental limitations of other available biomarkers of myocardial necrosis. In the present study, there was a significant ($p< 0.001$) increase in the level of troponin T in plasma of Group III isoproterenol-administrated rats compared to Group I normal control animals **(Fig 4.1.7)**. This is in accordance with the earlier reported studies (Padma *et al.*, 2006[a]; Acikel *et al.*, 2003). Reports by O'Brien *et al.* (1997) have shown that troponin T is not only a sensitive but also a specific biomarker for detection of cardiac injury in laboratory animals. Because of its high sensitivity and specificity to cardiac tissue, cardiac troponin has emerged as the preferred diagnostic tool and standard for assessing myocardial necrosis. They are the most cardiac specific currently available biochemical markers for the diagnosis of myocardial injury (Jaffe *et al.*, 2000).

In the present study, prior administration of taurine significantly ($p< 0.001$) decreased the isoproterenol-induced release of troponin T from myocardium into the blood stream in Group IV animals, demonstrating its protective action on the cell membrane. It probably did so by maintaining the delicate balance of tonicity in cells of the myocardium. Taurine concentration is a major factor involved in the processes of cell volume regulation (Trachtman *et al.*, 1990). Cell volume affects the most basic processes of cell function and as such it exerts an important role in the onset, severity, and outcome of myocardial

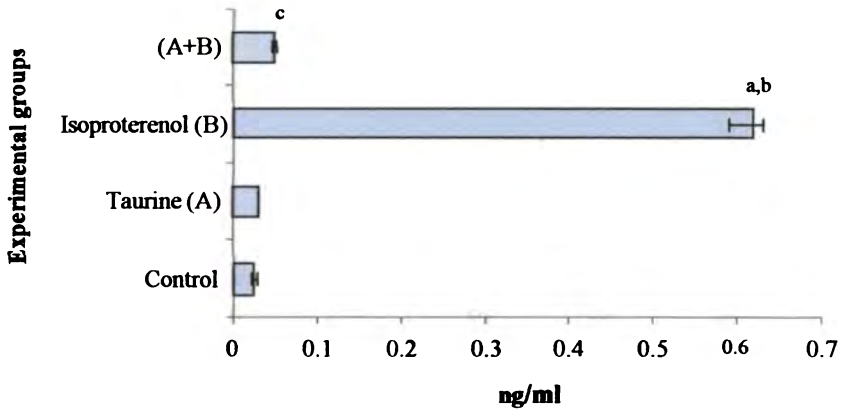


Fig 4.1.7 Level of troponin T in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days.

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

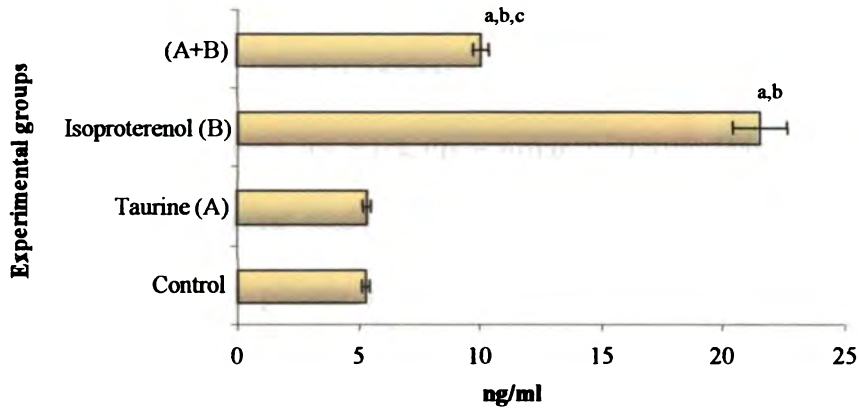


Fig 4.1.8 Level of homocysteine in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days.

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

infarction. Earlier reported studies (Trachtman *et al.*, 1992; Pasantes-Morales *et al.*, 1998) indicated that taurine administration was capable of averting severe osmolar changes associated with possible cell death. Reports by Song *et al.* (1998[a]) showed that supplementation of taurine exerted membrane protective action against radiation-induced pulmonary fibrosis. Administration of taurine has been reported to inhibit the production of tumor necrosis factor-alpha, malondialdehyde, cardiac troponin T, angiotensin II and high calicumion level of myocardiocytes in severely burned rats (Wan & Li, 2005).

4.1.3 Homocysteine

Homocysteine is a non-essential, thiol containing and potentially cytotoxic 4-carbon α-amino acid formed during methionine metabolism through the demethylation of methionine (Christensen *et al.*, 1991). Long-term elevation of homocysteine has been identified as independent risk factor for acute myocardial infarction (Mayer *et al.*, 1996; Stampfer *et al.*, 1992). In the present study, a significant ($p< 0.001$) elevation in the level of homocysteine was noted in plasma of Group III isoproterenol-administered rats compared to Group I control animals **(Fig 4.1.8)**. This is in accordance with earlier reported studies (Senaratne *et al.*, 2000; Hagar, 2002), which indicated that even mild hyperhomocysteinemia was associated with an increased risk of cardiovascular diseases independently of classical risk factors.

Homocysteine has been reported to induce atherosclerosis either by impairing coronary microvascular dilator function (Tawakol *et al.*, 2002), or by stimulating smooth muscle proliferation (Tang *et al.*, 1998) and platelet activation and thrombogenesis (Rodgers & Kane, 1986). Both *in vivo* and *in vitro* studies suggest that homocysteine is a potent inducer of inflammatory processes in endothelial cells at the level of gene expression (Roth *et al.*, 2001; Shai *et al.*, 2004). Elevated level of homocysteine has also

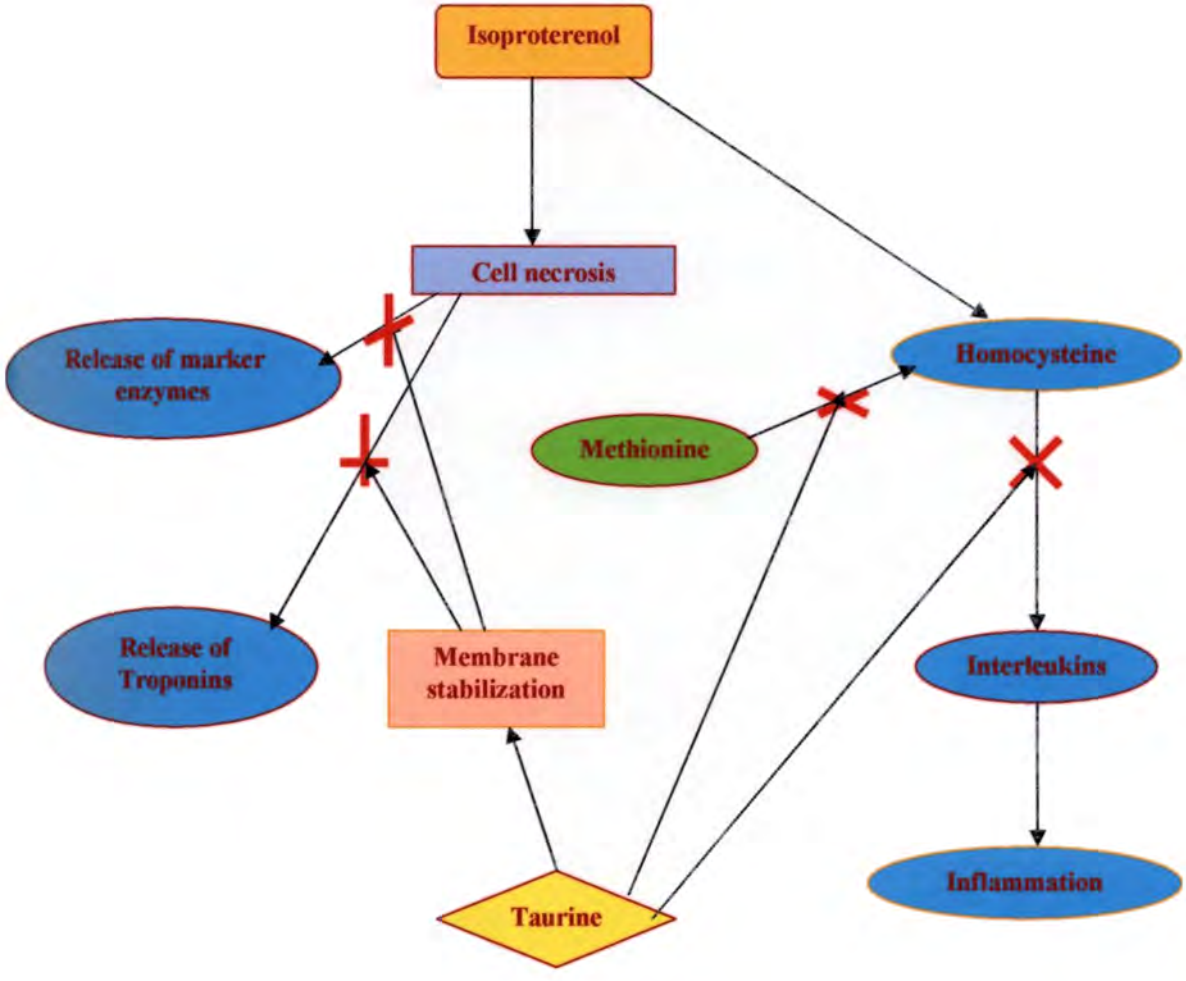


Fig. 4.1.9 Effect of taurine on diagnostic markers of myocardial infarction

been reported to be associated with increased interleukin production in monocytes and endothelial dysfunction (Van Aken *et al.*, 2000). It is also involved in up regulation of vascular cell adhesion molecules (Silverman *et al.*, 2002) and promotion of vascular smooth muscle cell growth (Woo *et al.*, 1997; Tsai *et al.*, 1994).

In the present study, it is observed that prior administration of taurine significantly ($p< 0.001$) reduced the level of homocysteine in plasma of Group IV rats compared to Group III myocardial infarction induced rats. It probably did so either by inhibiting the production of monocyte or macrophage-derived interleukins which triggers firm adhesion of rolling monocytes to vascular endothelium, a necessary prelude to the initiation of atherosclerosis (Geng *et al.*, 2005; Ding *et al.*, 1996), or by regulating the formation of homocysteine from methionine. Reports by Chang *et al.* (2004[a]) indicated that taurine and homocysteine had opposite effects in myocardial mitochondria with regard to Ca^{2+}ATPase activity and the generation of hydrogen peroxide and superoxide anions. Earlier Minor *et al.* (1996) observed that taurine reduced experimental liver injury after cold ischemic preservation and a period of rewarming prior to reperfusion by modulating vascular endothelial function. Investigations by Casey *et al.* (2006) also indicated that taurine effectively attenuated acute hyperglycaemia-induced endothelial cell apoptosis, leucocyte-endothelial cell interactions and cardiac dysfunction in experimental animals.

4.2 Histopathological observations

Plates 4.2.1-4.2.4 show the histology of heart tissue of normal and experimental groups of rats. Microscopical examination of heart tissue sections of Group I normal control animals revealed normal myofibrillar architecture with straiations, branched appearance and continuity with adjacent myofibrils **(Plate 4.2.1)**. But a number of significant histopathological alterations were noticed in the heart tissue sections of

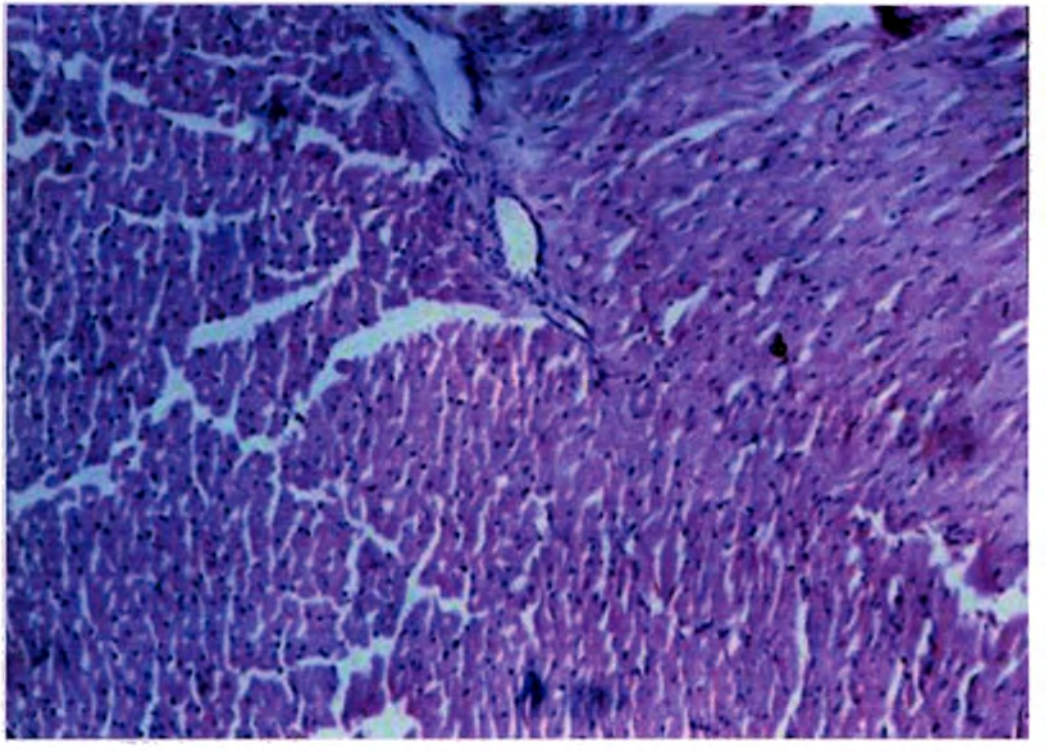


Plate 4.2.1 The architecture of normal cardiac tissue in control rat (Group I)

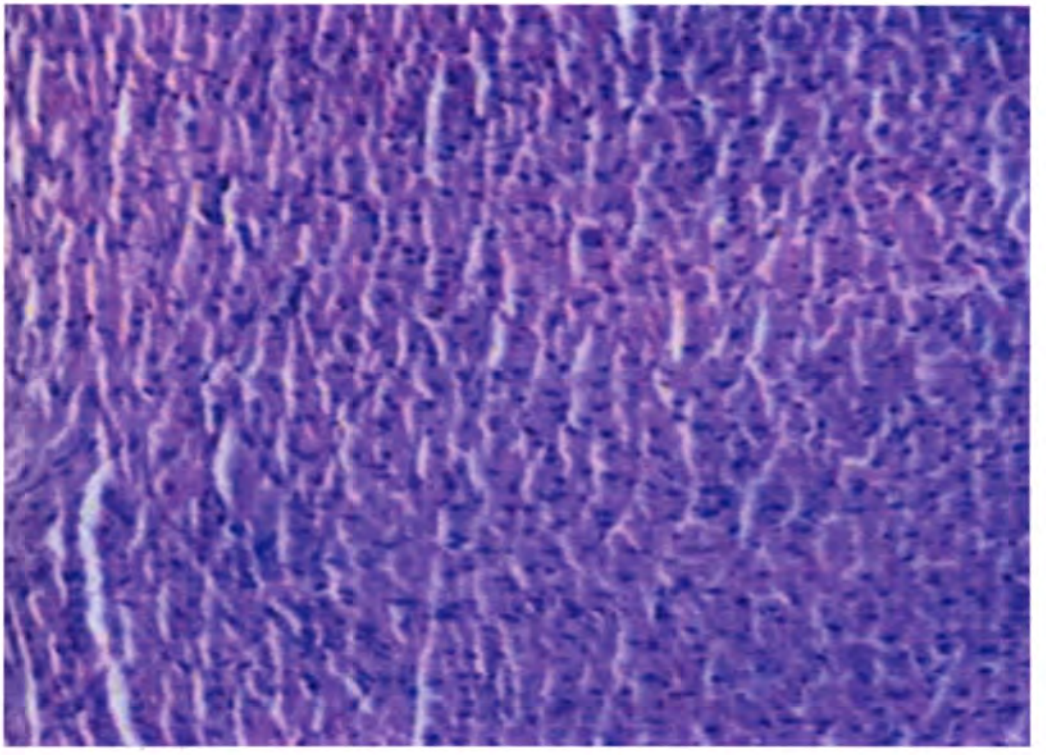


Plate 4.2.2 The cardiac tissue in rat pre-treated with taurine indicating no significant changes in architecture in comparison to the normal condition (Group II)

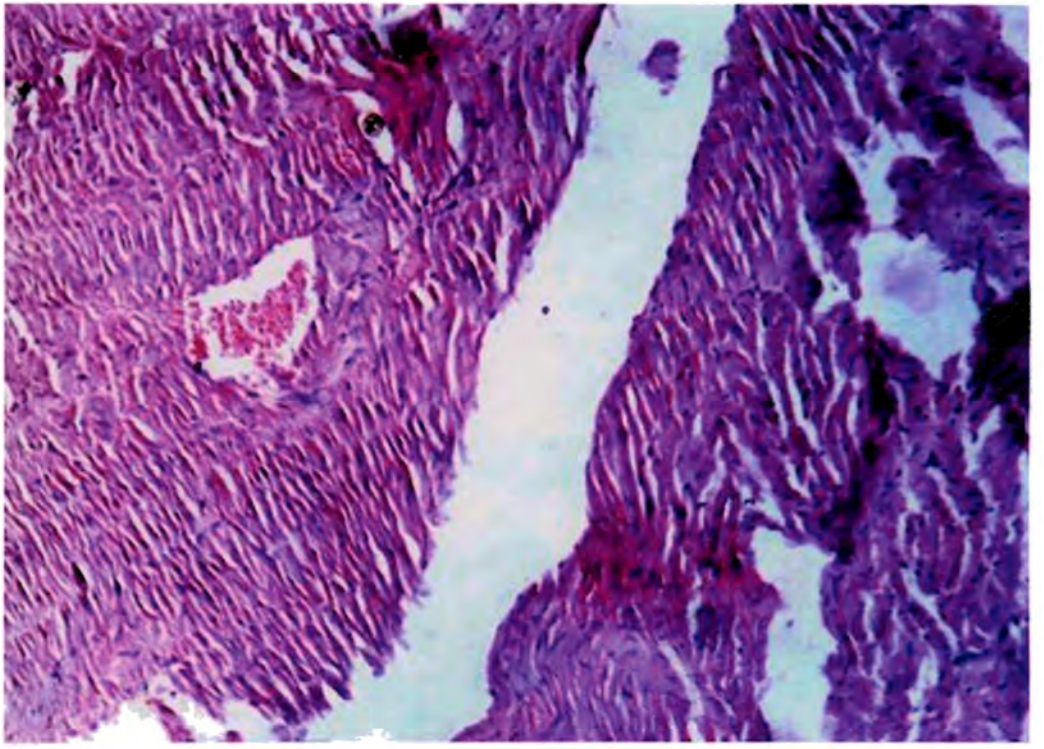


Plate 4.2.3 The architecture of cardiac tissue in the myocardial stress induced rat showing rupture of cardiac muscle fibers with inflammatory cells (Group III)

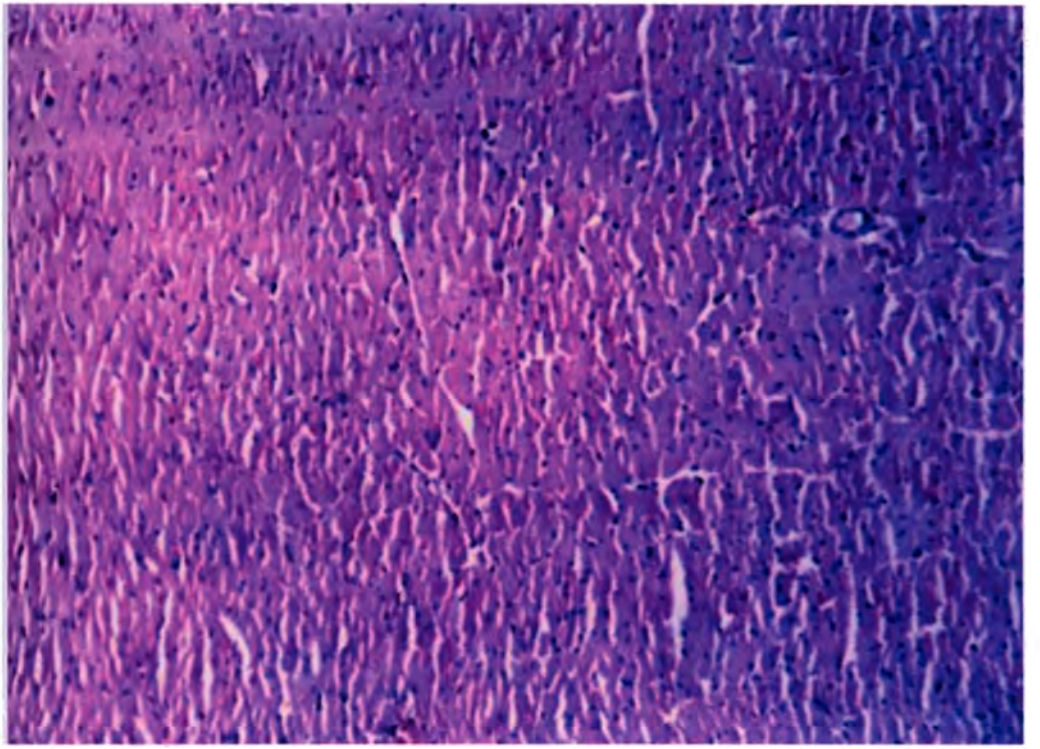


Plate 4.2.4 The architecture of cardiac tissue in rat pre-treated with taurine prior to induction of myocardial stress by isoproterenol, which shows no rupture of cardiac muscle and no inflammatory cell (Group IV)

Group III isoproterenol-injected rats as compared to the normal cardiac architecture of Group I control animals **(Plate 4.2.3)**. Administration of isoproterenol-induced pathological alterations in the myocardial tissue of Group III animals such as mild to diffused cloudy swelling, focal vacuolar degeneration and occasional pericentral infiltration of round cells, hyperemia and sinusoidal distension. Also in some cases occurrence of cellular hyperplasia, central necrosis and fibroplasias in portal areas were noted. This pathological aberration is probably related to a decline in oxygen supply with paramount rise in wall-stress. These observations concur with previous reported studies (Benjamin *et al.*, 1989; Jalil *et al.*, 1989).

Histology of the heart tissue sections of Group IV taurine-administered rats **(Plate 4.2.4)** showed normal architecture of myofibrillar striations, branched appearance and continuity with adjacent myofibrils compared to the altered cardiac architecture of Group III isoproterenol-injected animals. The morphology of cardiac muscle fibers was well preserved and found to be comparable to that of normal control rats, indicating the cytoprotective action of taurine. Previous reports by Zeybek *et al.* (2006) showed that supplementation of taurine prevented water avoidance stress-induced morphological degeneration changes in gastrointestinal tract and liver by its potent free radical scavenging ability. Reports by Hagar *et al.* (2006) indicated that supplementation of taurine attenuated hypertension and renal dysfunction and ameliorated nephritic morphological changes induced by cyclosporine-A in rats. Previously Tadros *et al.* (2005) reported that taurine significantly ameliorated the morphometrical and histopathological aberrations in an experimental animal model of Huntington's disease due to its antioxidant effect and γ-amino butyric acid agonistic action. Histopathological studies by Cetiner *et al.* (2005) showed that taurine protected against methotrexate-induced oxidant organ injury and inhibited leukocyte apoptosis in experimental rats. In the present study, the

histological examinations of the heart tissue of normal rats receiving taurine (Group II) alone did not show any significant changes when compared with that of normal control rats, showing that it dose not *per se* have any adverse effects **(Plate 4.2.2)**. The histopathological observations carried out in the heart tissue of control and experimental groups of rats confirmed the cytoprotective action of taurine in experimentally induced myocardial infarction condition.

4.3 Effect of taurine on protein metabolism

Protein synthesis and protein degradation are highly regulated cellular processes essential for cell viability. Alteration in steady state protein metabolism is an important factor in regulating cellular homeostasis in response to oxidative damage in myocardial infarction condition. In the present investigation, there was a significant ($p< 0.01$) increase in the levels of protein and glycoprotein components in plasma with a concomitant decline in their levels in heart tissue of Group III isoproterenol-administered rats compared to Group I normal control animal **(Fig 4.3.1, 4.3.2)**. This is in accordance with the earlier reported study (Dudnakova *et al.*, 2002), which indicated that the increase observed in the protein content in plasma of isoproterenol-administered rats is, at least in part, due to leakage of enzymes and protein-bound components from the damaged myocardium into the systemic circulation.

Reduced incorporation of amino acids into tissue proteins may be responsible to a significant extent for decline noticed in the level of protein synthesis in isoproterenol-induced myocardial infarction condition. Hexose and sialic acid are incorporated into polypeptide chain while they are still attached to ribosomes (Marshall *et al.*, 1991; Robinson *et al.*, 1995). The reduction in the hexose and sialic acid content observed in heart tissue of isoproterenol-induced myocardial infarction might be due to inhibition of

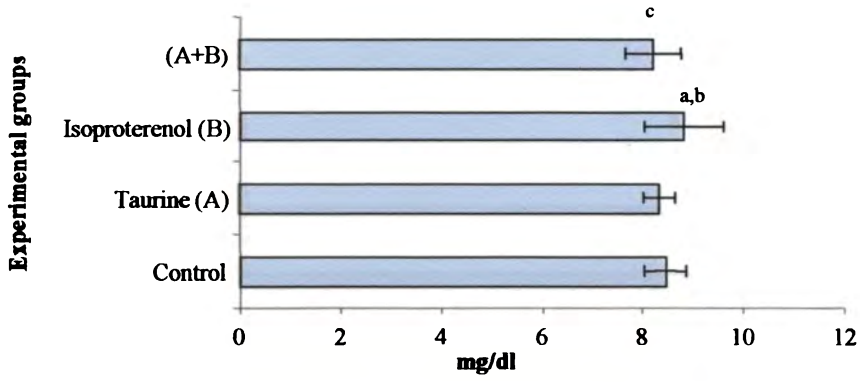


Fig 4.3.1 Level of protein in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days.

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.01$ significantly different compared with Group I control animals

[b] $p<0.01$ significantly different compared with Group II taurine-administered animals

[c] $p<0.01$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

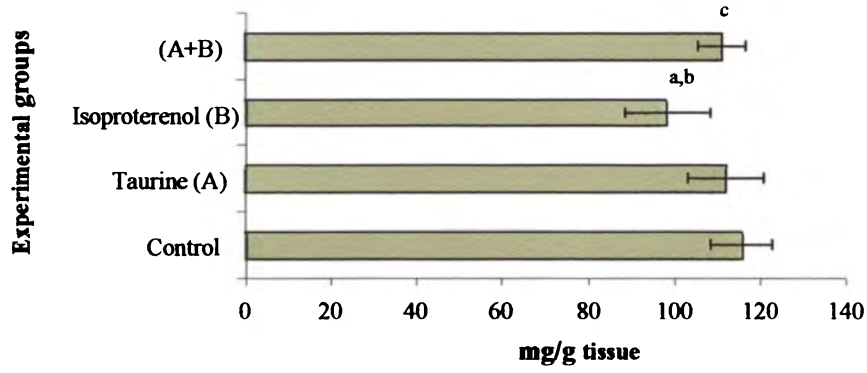


Fig 4.3.2 Level of protein in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days.

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.01$ significantly different compared with Group I control animals

[b] $p<0.01$ significantly different compared with Group II taurine-administered animals

[c] $p<0.01$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

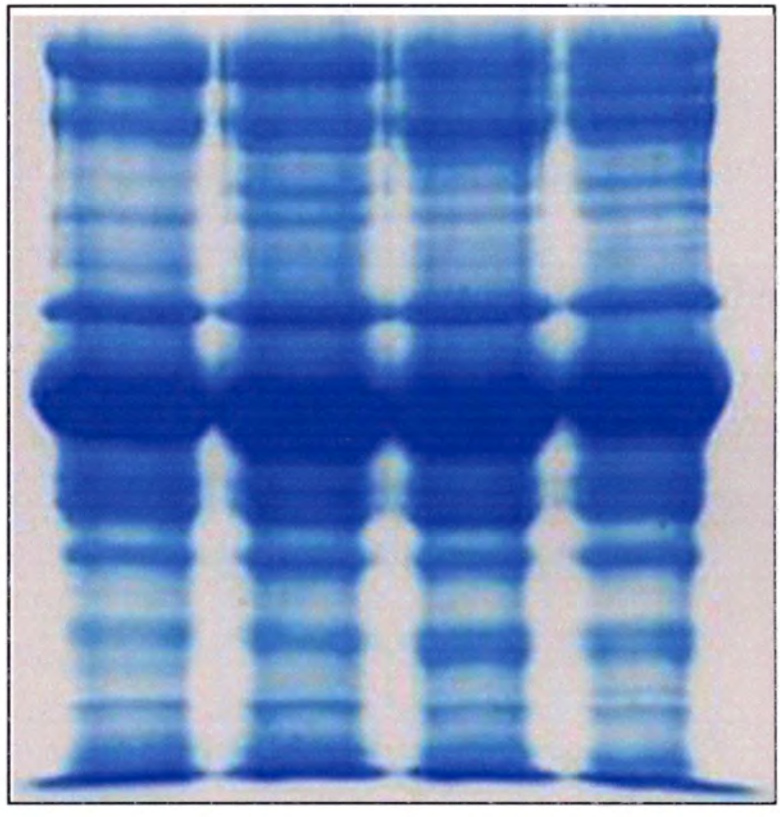


Plate 4.3.1 Electrophoretic Pattern of plasma proteins in normal and experimental groups of rats

Lane 1: Group I
Lane 2: Group II
Lane 3: Group III
Lane 4: Group IV

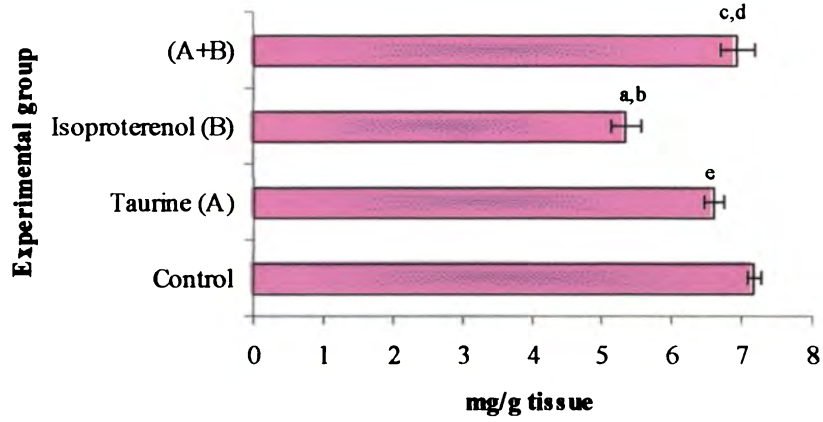


Fig 4.3.3 Levels of hexose in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days.

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.01$ significantly different compared with Group II taurine-administered animals

[e] $p<0.05$ significantly different compared with Group I control animals

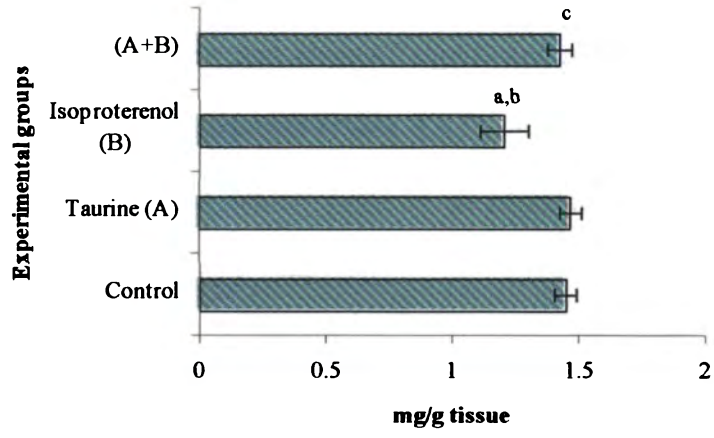


Fig 4.3.4 Level of sialic acid in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days.

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.01$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

glycoprotein synthesis **(Fig 4.3.3, 4.3.4)**. Oxidation of protein is a common phenomenon mediated by highly reactive agents in myocardial infarction condition and oxidized proteins are in turn capable of inducing oxidative stress, a potential mediator of the pathogenesis. This protein oxidation might also be a possible reason for the decline noted in the protein and glycoprotein levels in the heart tissue of Group III rats. Reactive oxygen species generated during isoproterenol-induced myocardial infarction may oxidize amino acid side chains and the protein backbone, leading to protein-protein cross-linking and protein fragmentation. As a consequence of these modifications, signaling proteins may not function properly, which in turn lead to organ malfunction and even cell death (Berlett & Stadtman, 1997), as observed in the present study. The SDS-polyacyrlamide gel electrophoretic separation of plasma proteins also confirmed the alterations in protein metabolism and showed significant reduction in the amount of proteins especially, the albumin fraction **(Plate 4.3.1)**. But a slight enhancement in the globulin content was also noticed, which might be an adoptive mechanism to counteract the isoproterenol-induced myocardial infarction.

In the present study, prior administration of taurine significantly ($p< 0.01$) ameliorated the isoproterenol-induced adverse effects on protein content and glycoprotein components in plasma and heart tissue of Group IV rats compared to Group III rats. It probably did so either by inhibiting the disaggregation of polyribosomes or by attenuating the isoproterenol-induced oxidation of myocardial proteins. Tabassum *et al.* (2006) reported that supplementation of taurine protected the structural and functional integrity of the cell membranes by counteracting the reactive oxygen species mediated lipid peroxidation and protein carbonyl formation. Investigations by Waterfield *et al.* (1998) showed that reduction in protein synthesis was correlated with increased urinary and serum levels of taurine. Reports by Vendemiale *et al.* (1998) indicated that taurine

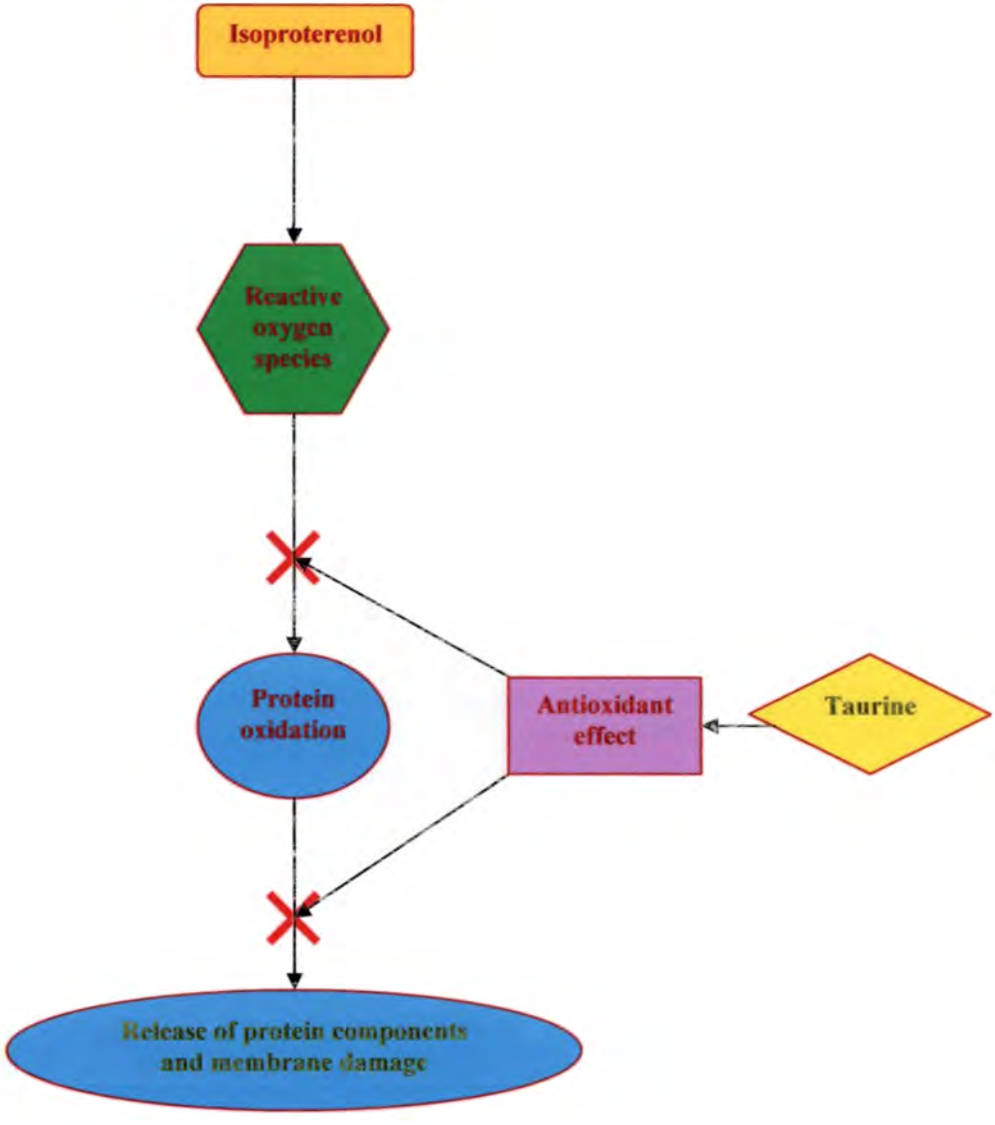


Fig 4.3.5 Effect of taurine on protein metabolism

administration protected proteins and lipids from ethanol-induced oxidative damage by its antiperoxidative property.

4.4 Effect of taurine on free amino acids

In the present study, significant alterations in the levels of free amino acids (taurine, aspartate, glutamate and arginine) were observed in heart tissue of Group III myocardial infarcted rats compared to Group I control rats. This is in agreement with earlier reports by Sulieman *et al.* (1997), which showed that myocardial ischemic arrest induced by cold crystalloid cardioplegic solution decreased intracellular concentrations of glutamate, ATP and aspartate in the hearts of patients undergoing coronary artery surgery. In sepsis, virtually all amino acid levels were decreased by 10-30%, whereas cystine and phenylalanine were significantly elevated (Vente *et al.*, 1989).

4.4.1 Taurine

Taurine is the principal intracellular free amino acid present in the tissues of animal species. In the present study, there was a significant ($p< 0.001$) decrease in myocardial taurine content in Group III myocardial infarction induced rats compared to Group I normal rats **(Fig 4.4.1)**. This is in agreement with earlier reports by Stapleton *et al.* (1996), which showed that both plasma and intracellular taurine were decreased in certain pathologic conditions. Pion *et al.* (1987) reported that myocardial concentrations of taurine were directly related to its plasma concentrations and low concentrations were found to be associated with myocardial failure in cats.

The rats pretreated with taurine showed significant ($p< 0.001$) increase in cardiac taurine content in Group IV rats as compared to Group III rats. This is in agreement with an earlier study (Chen *et al.*, 2004), which indicated that taurine supplementation resulted

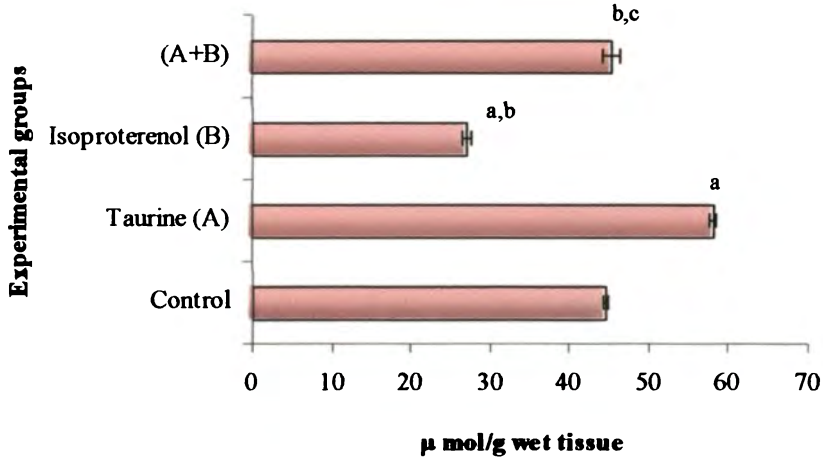


Fig 4.4.1 Level of taurine in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

in increased taurine content both in serum and liver of experimental animals. Takahashi *et al.* (2003) reported that both myocytes and non-myocytes expressed an identical taurine transporter with a Michaelis-Menten constant of 20-21 μM and that a higher taurine content in myocytes may be associated with a higher V[max]. Hence, it is possible that supplementation of taurine may preserve the cellular taurine content for other biological processes through the modulation of taurine transporters, as observed in the present study. The biological processes in which taurine involved are cell membrane stabilization (Heller-Stilb *et al.*, 2001), antioxidation (Atmaca, 2004), detoxification (Birdsall 1998), osmoregulation (Timbrell *et al.*, 1995), neuromodulation and brain (Renteria *et al.*, 2004) and retinal development (Wright *et al.*, 1986). Studies by Oudit *et al.* (2004) showed that treatment with taurine reduced iron-mediated myocardial oxidative stress and preserved cardiovascular function and improved survival rate in iron-overloaded mice.

4.4.2 Aspartate

Aspartate is one of the metabolites of the tricarboxylic acid cycle and is involved in energy production. The significant ($p< 0.001$) decline observed in the level of aspartate in heart tissue of isoproterenol-administered Group III rats compared to Group I control rats **(Fig 4.4.1)** might be due to its loss into extracellular space from the infarcted myocardium. Level of aspartate has been reported to decrease in human hearts subjected to cardioplegic arrest followed by reoxygenation (Pisarenko *et al.*, 1988 & 1995). These findings suggested that the myocardial levels of amino acids were closely associated with its energy state following ischemia and thus may affect the recovery of cardiac contractility. Although such decrease in this amino acid concentration has been primarily attributed to metabolism, they might also partly due to loss into extracellular space. Ischemia resulted in a large increase in perfusate levels of glutamate, aspartate, glycine,

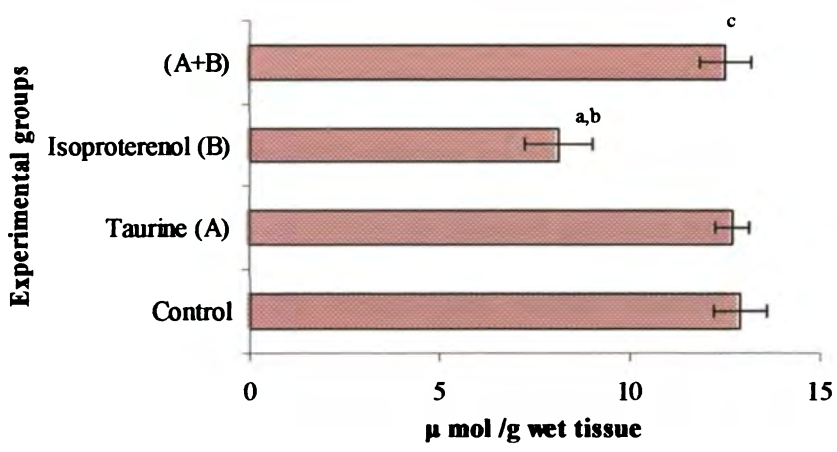


Fig 4.4.2 Level of aspartate in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

phosphoethanolamine, serine, alanine, taurine and glutamine in an experimental model to study the release of amino acids during ischemia, anoxia and hypoglycemia (Song *et al.*, 1996; Song *et al.*, 1998[b]; Li & Jefferson, 1977). Buyukuysal (2004) reported that ischemia-induced increases in glutamate, taurine and GABA outputs from rat striatal slices were accompanied with a similar amount of decline in their tissue levels.

Prior treatment with taurine prevented the isoproterenol-induced decrease in aspartate level in Group IV rats compared to Group III rats. This may be due to its involvement in cell volume regulation. Taurine is reported to regulate osmolarity without causing additional perturbations of cellular tonicity (Trachtman *et al.*, 1990). Taurine, betaine, and inositol were recently identified as osmolytes in cells associated with cell volume regulation and cell function (Wettstein & Haussinger, 2000). Molchanova *et al.* (2006 & 2007) reported that taurine attenuated aspartate release by regulation of mitochondrial Ca^{2+}sequestration by activation of a chloride channel and also suppressed the synaptic release of aspartate evoked by the voltage-gated sodium channel opener veratridine. Taurine supplementation reduced reoxygenation injury in cold and warm ischemia in rat liver (Wettstein & Haussinger, 2000).

4.4.3 Glutamate

Glutamate is involved in several classical biochemical pathways including tricarboxylic acid cycle, ammonia detoxification and gluconeogenesis and plays an important role as a chemical transmitter of excitatory signals. In the present study, there was a significant ($p< 0.001$) decrease noted in the level of glutamate in heart tissue of Group III rats compared to Group I control rats **(Fig 4.4.2)**. This is in line with earlier reports (Li & Jefferson, 1977; Pisarenko *et al.*, 1990; Dohovics *et al.*, 2003), which showed that β-adrenergic agonist, isoproterenol administration increased the loss of glutamate,

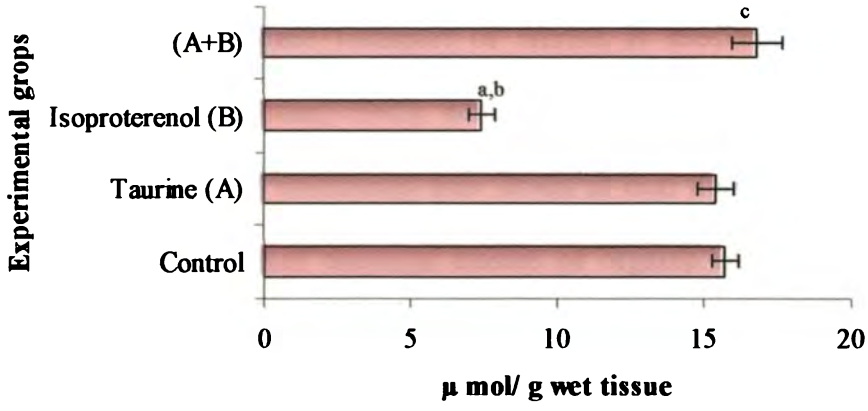


Fig 4.4.3 Level of glutamate in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

aspartate, serine and isoleucine from the pool of free amino acid in perfusate and muscle. The reversal of Na^+ dependant transporters during isoproterenol-induced ischemia might have also contributed to amino acid efflux accompanied by a similar amount of decline in their tissue levels (Buyukuysal, 2004). Loss of the plasma membrane integrity as a result of enhanced phospholipase A_2 activity by isoproterenol may also allow amino acids to diffuse down their concentration gradients into the extracellular space (Backstrom *et al.*, 2003).

In the present study, the prior treatment with taurine ameliorated the isoproterenol-induced decrease in glutamate level in Group IV rats compared to Group III rats. Neutral and acidic amino acids are taken up into cells by Na^+ dependant co-transport systems (Collarini & Oxender, 1987). Since transport of taurine is mediated through Na^+ dependant symport system (Suleiman, 1992), administration of taurine facilitated the transportation of amino acids and maintained their levels at near normal. Goldstein & Davis (1994) reported that different chemical classes of organic osmolytes shared a common volume-sensitive transporter. Reports by Dawson *et al.* (2002) showed that taurine supplementation significantly increased plasma glutamate levels in exercised rats. Molchanova *et al.* (2006) reported that taurine reduced glutamate release under ischemic conditions by affecting the depolarization-evoked component. Taurine supplementation attenuated glutamate excitotoxicity through both the enhancement of mitochondrial function and the regulation of intracellular (cytoplasmic and intra-mitochondrial) calcium homeostasis (El Idrissi, 2006). El Idrissi & Trenkner (2004) reported that taurine counteracted glutamate-induced mitochondrial damage and cell death as a result of its involvement in calcium regulation.

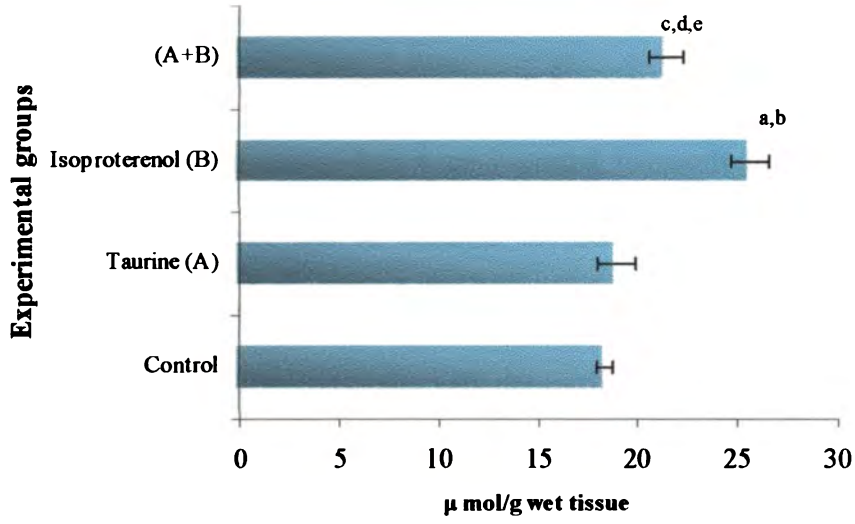


Fig 4.4.4 Level of arginine in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.01$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.01$ significantly different compared with Group I control animals

[e] $p<0.05$ significantly different compared with Group II taurine-administered animals

4.4.4 Arginine

Arginine is a conditionally essential amino acid involved in various metabolic functions in the body. It is the only substrate required for synthesis of nitric oxide (NO) by the action of the enzyme NO synthase (NOS) (Morris, 1992). In the present study, a significant ($p< 0.001$) elevation in the level of arginine was observed in heart tissue of Group III isoproterenol-administered rats as compared to that of Group I normal rats **(Fig 4.4.2)**. This is in accordance with an earlier study (Gustafsson & Brunton, 2000) which indicated that iNOS (inducible nitric oxide synthase) mediated nitrative stress played a pivotal role in isoproterenol-induced myocardial apoptosis and cellular injury Isoproterenol-mediated β-AR stimulation has been reported to result in a phenotypic upregulation of iNOS in the heart and to enhance the release of pro-inflammatory mediators, which trigger increased NO production through arginine metabolism (Hu, *et al.*, 2006). Though NO is short-lived and relatively unreactive radical, it combines with superoxide to form potent oxidant -ONOO- (peroxynitrite), which plays a significant role in iNOS-mediated postischemic cells damage (Arstall *et al.*, 1999). Under pathological conditions, where iNOS expression is increased and NO production is increased, significant amount of peroxynitrite is formed (Xia *et al.*, 1996).

In the present study, prior administration of taurine maintained the cardiac arginine content at near normalcy in Group IV rats compared to Group III rats. It probably did so by blocking the availability of NO radicals by inhibiting iNOS activity (Gurujeyalakshmi *et al.*, 2000). Studies by Barua *et al.* (2001) showd that taurine chloramine inhibited inducible nitric oxide synthase and tumor necrosis factor-α gene expression in activated alveolar macrophages, leading to a decrease in the production of peroxynitrite radicals. Eby & Halcomb (2006) also reported a hypothesis for nitric oxide stabilization of the

sinus node and the elimination of cardiac arrhythmias using oral taurine and L-arginine with case histories.

4.5 Effect of taurine on lipid metabolism

Lipids play an important role in cardiovascular disease, not only by way of hyperlipidemia and the development of atherosclerosis leading to myocardial infarction, but also by modifying the composition, structure and stability of cellular membranes. Hypercholesterolemia, high concentration of low-density lipoprotein cholesterol, hypertriglyceridemia and low high-density lipoprotein are accepted as independent risk factors for atherosclerotic cardiovascular disease and mortality (Wood *et al.*, 1998; Gotto *et al.*, 2000). Gudbjarnason *et al.* (1968) observed increased lipid synthesis in infarcted tissue when compared to non-infarcted tissue. Significant changes in the fatty acid composition of serum triglycerides, cholesterol ester and phospholipids were also reported in acute myocardial infarction condition (Padma *et al.*, 2006[a]). During myocardial infarction, the cardiac cells become fibrotic leading to excess accumulation of fat, as observed in the present study. The pathogenesis is multifactorial, reflecting complex biosynthetic, enzymatic and catabolic derangement in lipoprotein metabolism.

4.5.1 Cholesterol, triglycerides and free fatty acids

High levels of circulatory cholesterol and its accumulation are well associated with cardiovascular damage (Joan *et al.*, 1984). High levels of LDL-cholesterol and VLDL-cholesterol have shown a positive correlation with myocardial infarction, where as high levels of HDL-cholesterol have a negative correlation (Buring *et al.*, 1992). Plasma concentration of atherogenic LDL-cholesterol is regulated by the production of rate of VLDL and the utilization of LDL-cholesterol by LDL receptors. The protective role of HDL-cholesterol is attributed to its involvement in reverse cholesterol transport, its antioxidant and

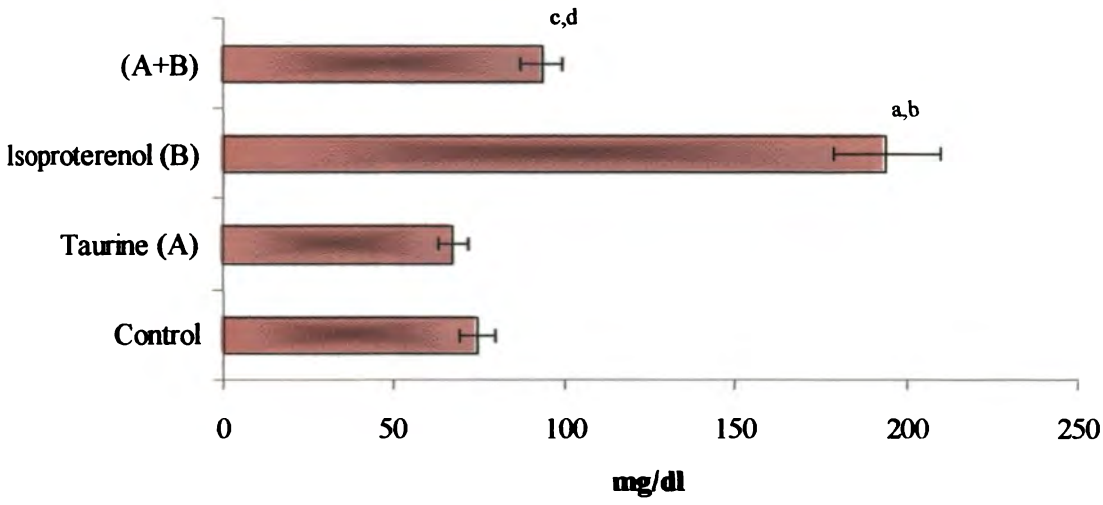


Fig 4.5.1 Level of total cholesterol in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.01$ significantly different compared with Group II taurine-administered animals

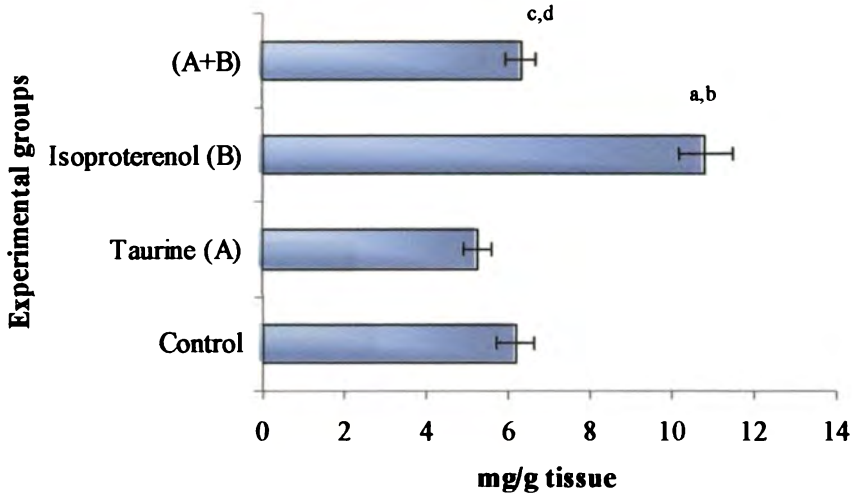


Fig 4.5.2 Level of total cholesterol in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.01$ significantly different compared with II taurine-administered animals

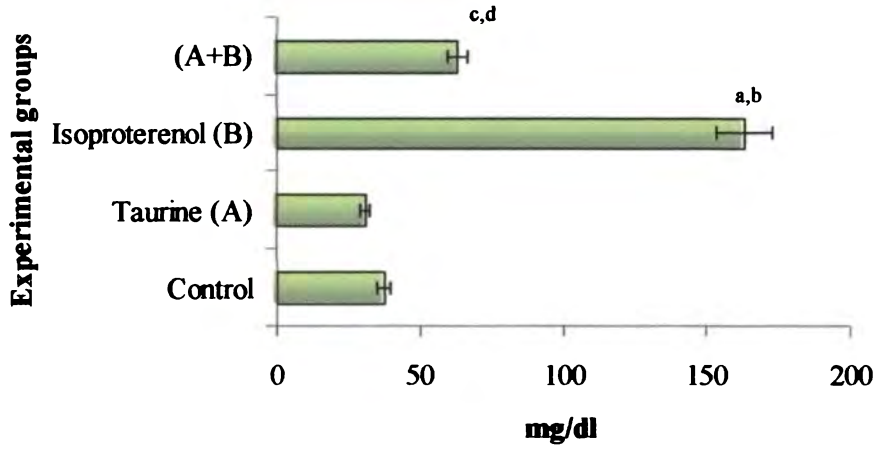


Fig 4.5.3 Level of LDL-cholesterol in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.01$ significantly different compared with Group II taurine-administered animals

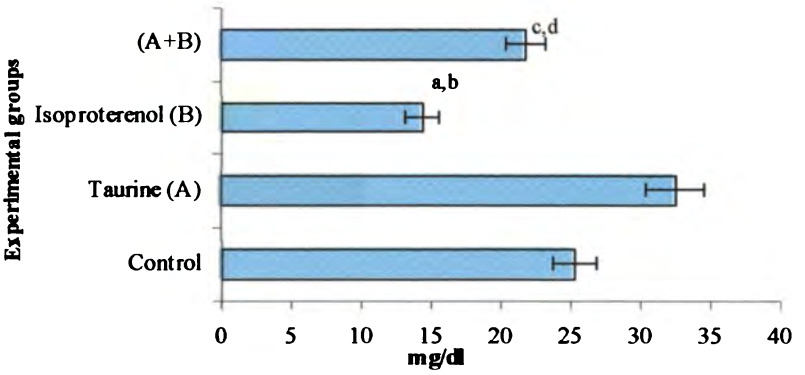


Fig 4.5.4 Level of HDL-cholesterol in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

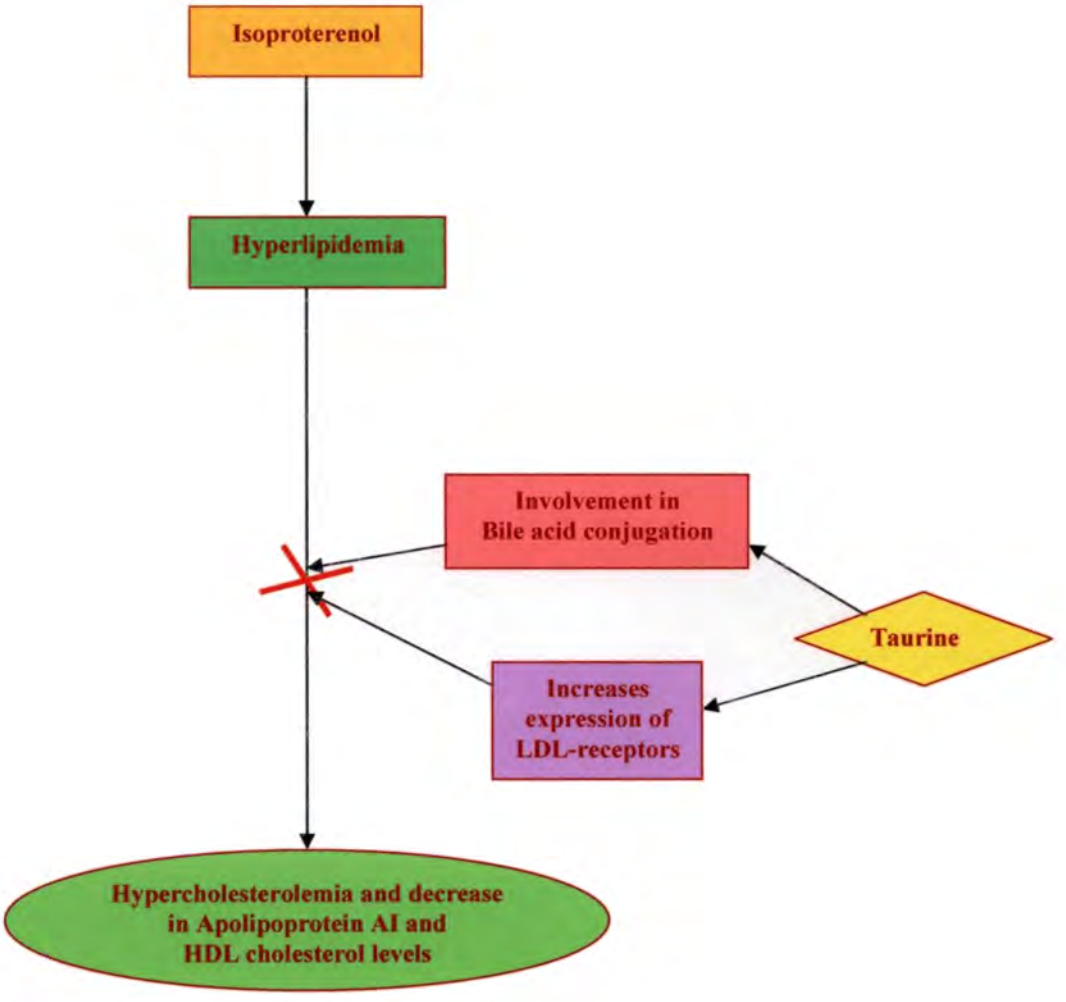
Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.001$ significantly different compared with Group II taurine-administered animals



4.5.18 Effect of taurine on cholesterol metabolism

anti-thrombotic properties (Barter *et al.*, 2003). In the present study, there was a significant ($p< 0.001$) elevation noticed in the levels of cholesterol, triglycerides and free fatty acids in plasma and heart tissue of Group III rats as compared to Group I control rats, which is an indication of severity of isoproterenol-induced hyperlipidemic condition **(Fig 4.5.1- 4.5.8)**.

The level of LDL cholesterol was significantly ($p< 0.001$) higher in Group III myocardial infarction induced rats, whereas HDL cholesterol levels were significantly lower compared to Group I animals. There was an increase in the mobilization of LDL-cholesterol from the blood into the myocardial membranes, resulting in abnormal cholesterol deposition in the myocardium. These findings are in accordance with earlier reported studies (Sreepriya *et al.*, 1998; Sangeetha & Quine, 2006), which showed that the free fatty acids liberated from adipose tissue enters into the myocardium, and the process is proportional to the free fatty acid concentration in the coronary sinus. Though heart utilize free fatty acids for its energy requirements, the excess free fatty acid may be used for the synthesis of triglycerides which ultimately leads to hypertriglyceridemia condition, as observed in the present study.

In the present study, prior treatment with taurine significantly ($p< 0.001$) prevented the isoproterenol-induced elevation in total cholesterol, triglycerides and free fatty acids in plasma and heart tissue of Group IV rats as compared to that of Group III rats. It also maintained the level of LDL-cholesterol and HDL-cholesterol in plasma at a concentration comparable to that of Group I rats. Earlier reports (Chen *et al.*, 2004) indicated that taurine supplementation was effective in lowering plasma total cholesterol, VLDL cholesterol and LDL cholesterol levels in experimental animals. In the present study, a slight reduction in the level of total cholesterol and LDL-cholesterol were also observed in Group II taurine-administrated normal rats, establishing the anticholesterolemic property of taurine. The hypolipidemic property of taurine has already been reported in high-fat diet fed experimental

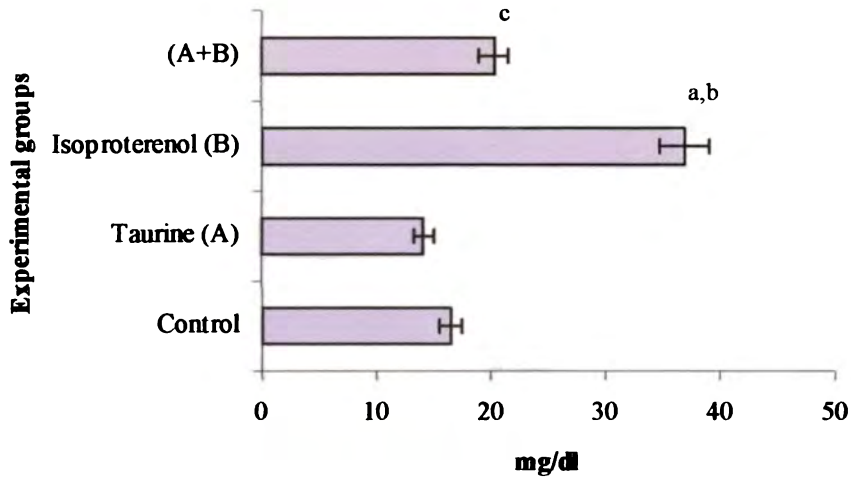


Fig 4.5.5 Level of free fatty acids in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

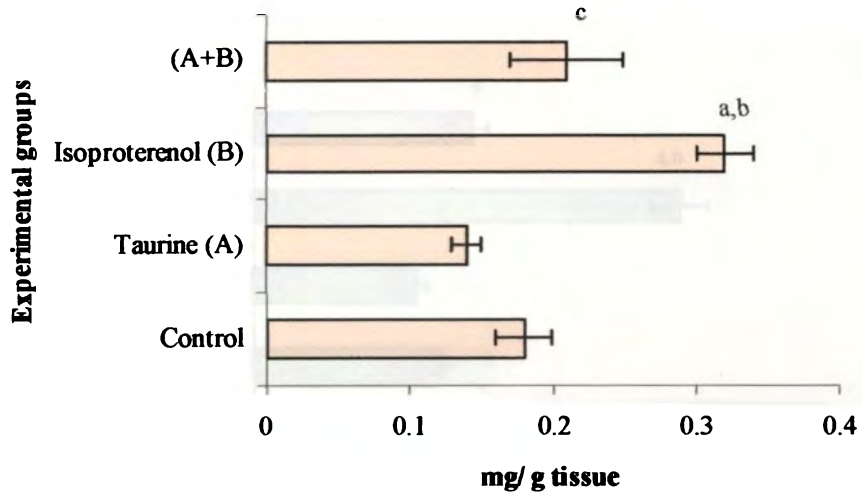


Fig 4.5.6 Level of free fatty acids in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

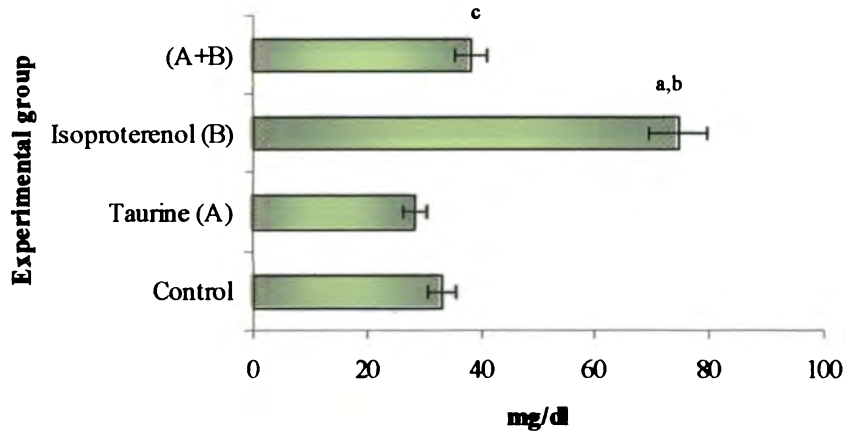


Fig 4.5.7 Level of triglycerides in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

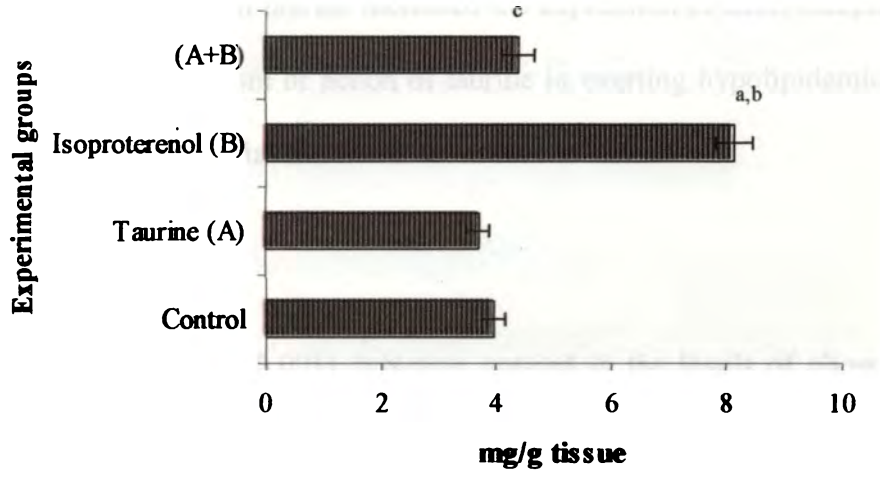


Fig 4.5.8 Level of triglycerides in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

animals (Murakami *et al.*, 1999). The cardioprotective effect of taurine is related to its ability to inhibit the increased accumulation of lipids both in systemic circulation and in myocardium by its antilipidemic property. This is probably achieved by its involvement in bile acid conjugation reactions. Nandhini *et al.* (2002) reported that taurine prevented the elevated levels of free fatty acids in rats fed high fructose-diet.

Previous reports (Lin *et al.*, 1996; Mochizuki *et al.*, 1998) indicated that taurine-bile acid conjugates suppressed VLDL secretion and enhanced plasma HDL concentration in a dose-dependent manner. LDL-receptors play an important role in the regulation of plasma LDL-cholesterol levels (Brown & Goldstein, 1986). Earlier reports by Stephan *et al.* (1987) indicated that supplementation of taurine increased the expression of LDL-receptors in cells. Hence, it might be the mechanism of action of taurine in exerting hypolipidemic activity in experimentally induced myocardial infarction condition.

4.5.2 Phospholipids

There was a significant ($p< 0.001$) depletion noticed in the levels of phospholipids in heart tissue of Group III animals compared of Group I rats **(Fig 4.5.9 & 4.5.10)**. This is in line with earlier reported studies (Kaul & Kapoor, 1989; Kumar *et al.*, 2001; Sreepriya *et al.*, 1998), which indicated that ischemic injury related alterations in lipid composition of myocardial tissue appeared to occur due to destruction of myocardial membrane lipid bi-layer. Hence, the significant elevation noticed in the levels of free fatty acids in plasma and heart tissue of isoproterenol-induced rats might be due to enhanced breakdown of membrane phospholipids both in adipose tissue and myocardium by the lipolytic action of phospholipase A_2 (Van Bilsen *et al.*, 1989; Nalbone *et al.*, 1990), which could be very likely the biochemical basis for the irreversible cell injury and ischemia. Previous studies (Mohan & Bloom, 1999; Vimal & Devaki, 2004) suggested that high lipid accumulation and

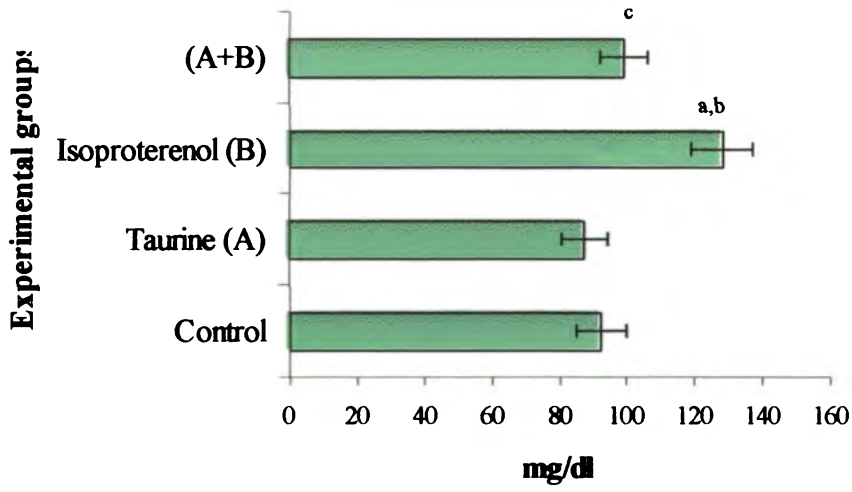


Fig 4.5.9 Level of phospholipids in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

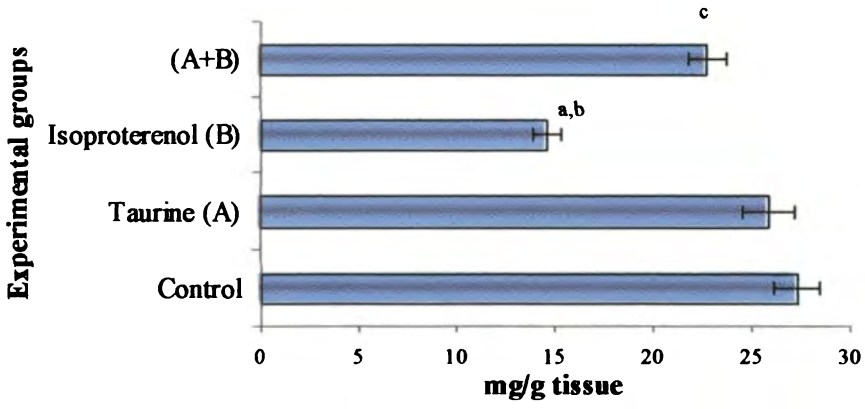


Fig 4.5.10 Level of phospholipids in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

increased lipid peroxidation in the myocardium might be the key events that determine isoproterenol-induced myocardial infarction. Further support for this conclusion comes from reports by Cao *et al.* (1996) which have shown that heart has less antioxidant protection than the liver, lung, or kidneys and therefore it may provide conditions conducive to free radical mediated necrotic damage, as observed in the present study.

The results of the current investigation showed that prior administration of taurine significantly ($p< 0.001$) prevented the isoproterenol-induced degradation of membrane phospholipids in heart tissue of Group IV rats compared to Group III rats, establishing its membrane stabilizing effect. It probably did so by decreasing isoproterenol-induced calcium overload in the myocardium. Previous reported studies (Kramer *et al.*, 1981; Schaffer *et al.*, 1995) indicated that taurine modulated the calcium-mediated cell death by its antioxidant and membrane stabilizing properties. It is possible that lipid peroxides and the spontaneous oxidation products of isoproterenol (Yates *et al.*, 1981) by their action on the sarcolemma may cause leakiness and contribute to a second phase of calcium accumulation (Dhalla *et al.*, 1996). This presumption is further supported by studies carried out in cultured cardiomyocytes in which inhibition of fatty acid accumulation by phospholipase inhibitors protected the cell membranes from calcium overload and morphological change (Jones *et al.*, 1989). Furthermore, the protective effect of phospholipase inhibitors apart from blocking calcium influx may also be due to their antioxidant activity and altered myocardial utilization of fuel from fatty acids to carbohydrates (Freedman *et al.*, 1991; Jenkins *et al.*, 1992). Hence, it is postulated that like wise taurine may also protect myocardial cell membrane from necrotic damage by its membrane-stabilizing action and antioxidant property.

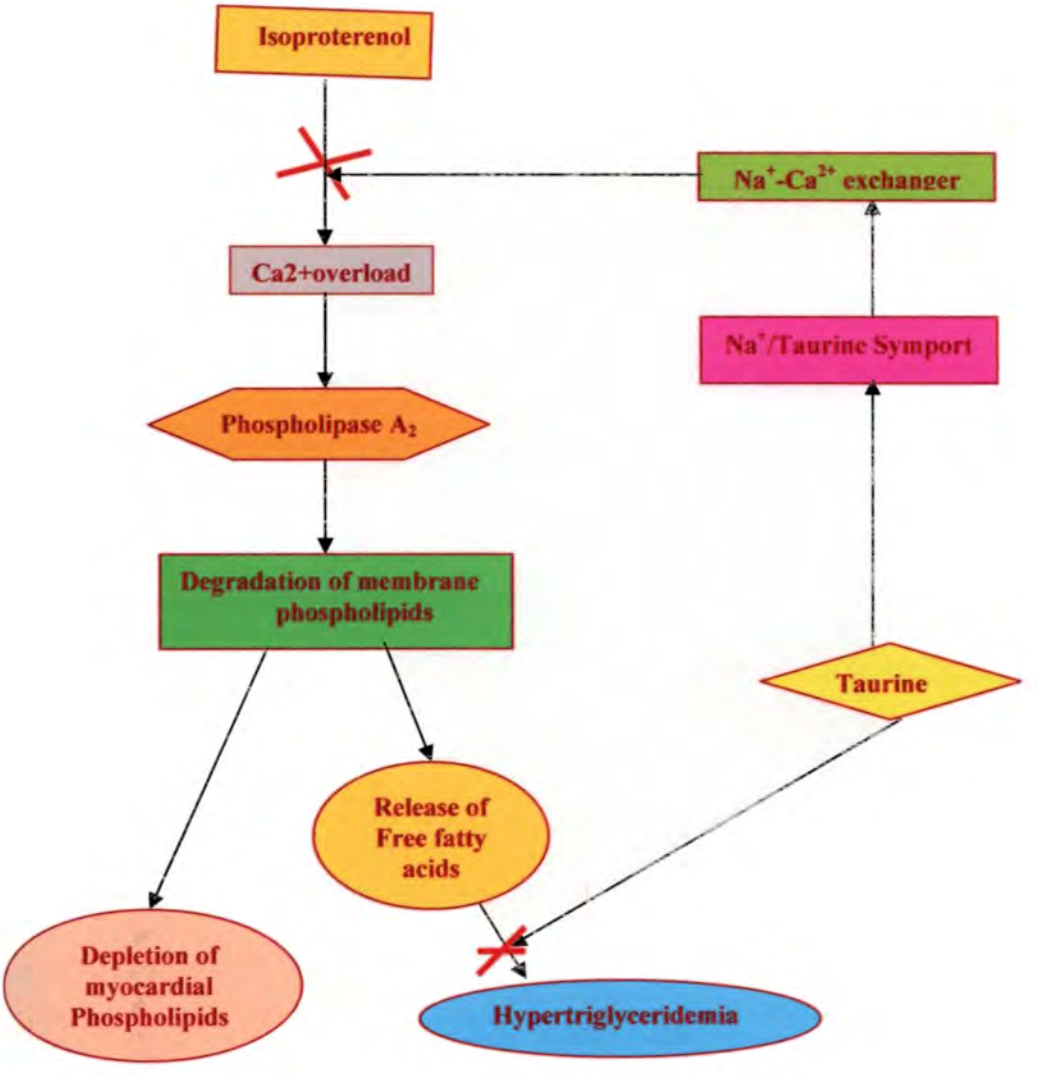


Fig. 4.5.17 Effect of taurine on lipid metabolism

4.5.3 Lipoprotein (a), apolipoprotein-B and apolipoprotein AI

Lipoprotein metabolism plays a pivotal role in atherogenesis leading to myocardial infarction (Bobkova *et al.*, 2003). Lipoprotein (a) is a complex of apolipoprotein (a) and low density lipoprotein is one of the most powerful and most prevalent independent non-modifiable risk factor for coronary artery disease (Luthra *et al.*, 1999). Apolipoprotein (a) is a glycosylated protein that is attached to apolipoprotein B-100 of low density lipoprotein and is reported to act as a competitive inhibitor of tissue type plasminogen activator there by inhibiting fibrinolysis (Angles-Cano *et al.*, 2001). This in turn contributes to blood clot formation, damaging coronary arteries (Hopkins *et al.*, 1998). In the present study, the levels of lipoprotein (a) and apolipoprotein-B increased significantly ($p< 0.001$) with a concomitant decrease in apolipoprotein AI in plasma of Group III isoproterenol-injected rats compared to Group I control rats **(Fig 4.5.11-4.5.13)**. This is in agreement with a previous reported study (Sandkamp *et al.*, 1990), which indicated that high plasma levels of Lp (a) was an independent risk factor for myocardial infarction. Apolipoprotein-B is the protein on the surface of each of the atherogenic particles, low density lipoprotein, intermediate density lipoprotein, and very low density lipoprotein remnants and lipoprotein (a), which exists as two isoforms B-48 and B-100. Over 90% of low density lipoprotein particle is composed of apolipoprotein B. Apolipoprotein AI is the primary protein constituent of high density lipoprotein and serves the function of preventing the deposition of cholesterol loaded macrophages on the arterial cell wall as foam cells. This in turn inhibits atherosclerotic lesion formations and myocardial infarction.

Pretreatment with taurine maintained the levels of these lipoproteins in Group IV animals at near normal compared to Group III isoproterenol-injected animals. Since

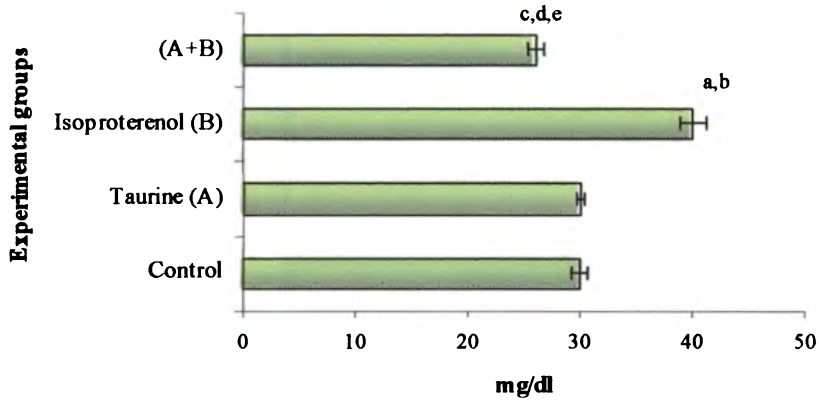


Fig 4.5.11 Level of lipoprotein (a) in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.01$ significantly different compared with Group I control animals

[e] $p<0.01$ significantly different compared with Group II taurine-administered animals

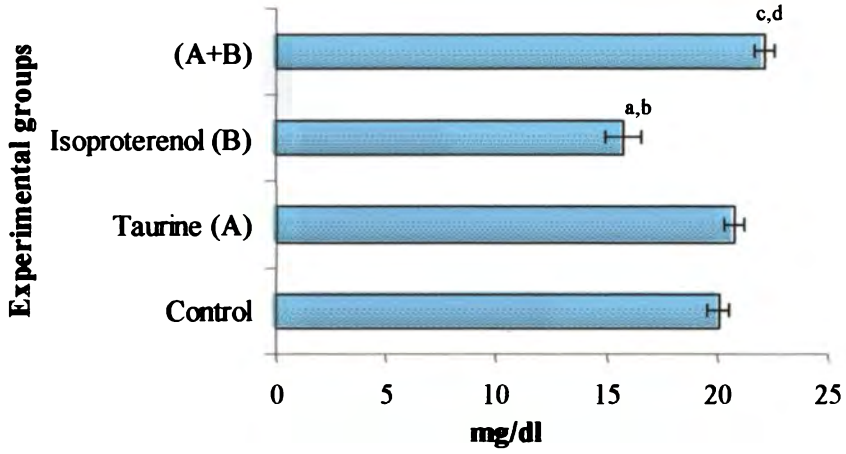


Fig 4.5.12 Level of apolipoprotein-AI in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group I control animals

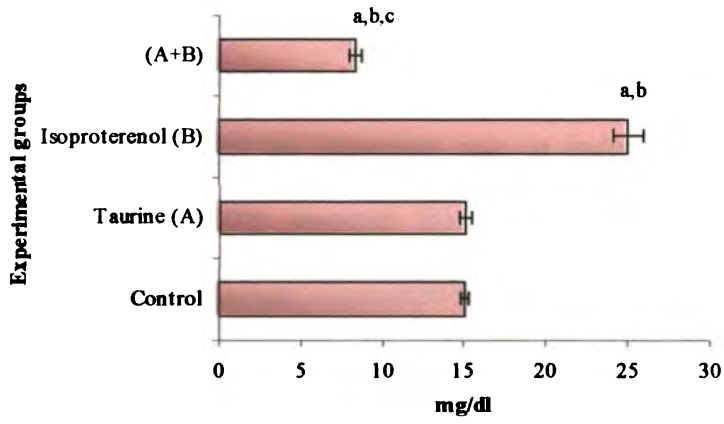


Fig 4.5.13 Level of apolipoprotein-B in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

apolipoprotein AI is associated with HDL and apolipoprotein-B is associated with LDL, the level of apolipoprotein AI might have increased with subsequent reduction in lipoprotein (a) and apolipoprotein-B in taurine treated-groups. Previously, Frank *et al.* (1995) reported that taurine interfered with the first step of lipoprotein (a) assembly, leading to a reduction in plasma concentration of lipoprotein (a). Reports by Field *et al.* (1994) and Elzinga *et al.* (2003) showed that taurocholic acid inhibited the secretion of apo-B in primary rat, mouse and human hepatocytes. Experimental studies by Kishida *et al.* (2003) and Yany *et al.* (2002) indicated that supplementation of taurine significantly lowered the plasma apolipoprotein-B levels and increased apolipoprotein AI levels in rats.

4.5.4 Fatty acid composition

Alterations in fatty acid composition of membrane phospholipids and consequent changes in membrane properties play an important role in induction of myocardial infarction. Pepe & McLennan (2002) reported that cardiac membrane fatty acid composition modulated myocardial oxygen consumption and post ischemic recovery of contractile function. **Table: 4.5.1** depicts the myocardial fatty acid composition of normal and experimental groups of rats. In the present study, significant alterations were observed in the composition of fatty acids in heart tissue of isoproterenol-administered Group III rats compared to Group I control rats. There was a slight increase noticed in the levels of saturated and monounsaturated fatty acids (C14:0, C16:0, C18:0 & C16:1, C18:1) in heart tissue of isoproterenol-administrated Group III rats compared to Group I control rats **(Fig. 4.5.14)**. This is in line with reports by Al Makdessi *et al.* (1987), which indicated modifications in the distribution of fatty acids evidenced by significant changes of monounsaturated or saturated and of 16:1 cis/16:1 trans ratios in arterial free fatty acids and tissue triglycerides in isoproterenol-administrated experimental animals.

Table: 4.5.1 Levels of fatty acids in heart tissue of control and experimental groups of rats

Fatty acids (%)	Control	Taurine (A)	Isoproterenol (B)	(A+B)
C14:0	3.02±0.20	3.53± 0.29	4.42± 0.35 [a,b]	3.12± 0.25[c]
C16:0	15.5±1.57	15.1± 1.3	17.2± 1.8 [a,b]	15.9± 1.8 [c]
C18:0	21.8±1.95	21.4± 1.9	25.1± 2.15 [a,b]	22.1± 2.08 [c]
C16:1	3.24± 0.27	3.15± 0.26	3.67± 0.31 [a,b]	3.28± 0.27 [c]
C18:1	12.7±1.15	11.9± 0.81	13.8± 1.3 [a,b]	12.5± 1.15 [c]
C18:2	18.1±1.35	18.3± 1.31	14.9± 1.11 [a,b]	17.6± 1.35 [c]
C20:3	0.72±0.052	0.75±0.055	0.68±0.051 [a,b]	0.72±0.052 [c]
C20:4	11.9± 0.9	12.3±0.96	9.31± 0.68 [a,b]	12.1± 0.9 [c]
C20:5	0.26±0.017	0.28± 0.02	0.21± 0.013 [a,b]	0.26±0.017 [c]
C22:6	11.8±0.85	12.3± 0.85	9.85± 0.62 [a,b]	11.7± 0.75 [c]

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's Multiple comparison test.

[a] $p<0.05$ significantly different compared with Group I control animals

[b] $p<0.05$ significantly different compared with Group II taurine-administered animals

[c] $p<0.05$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

Polyunsaturated fatty acids, major components of membrane phospholipids, play a key role in membrane functions (Al Makdessi *et al.*, 1994). There was a significant ($p< 0.05$) decline observed in the polyunsaturated fatty acid content (C18:2, C20:3, C20:4, C20:5, C22:6) in isoproterenol-administrated Group III rats compared to Group I control rats **(Fig. 4.5.14)**. A significant ($p< 0.05$) alteration was noticed in the composition of total n6 and n3 polyunsaturated fatty acids in isoproterenol-injected Group III rats compared to Group I control rats **(Fig 4.5.15)**. A slight change in the ratio of n6/n3 was also observed in the isoproterenol-administrated rats compared to normal rats **(Fig 4.5.16)**. This is in line with earlier reports (Gudbjarnason, 1989; Padma *et al.*, 2006[a]), which indicated that administration of isoproterenol modified the fatty acid composition and the balance between n-6 and n-3 fatty acids in cellular phospholipid composition. Isoproterenol-induced free radicals probably attacked the membrane phospholipids in the ischemic myocardium, resulting in the oxidation of membrane phospholipids rich in polyunsaturated fatty acids. This is in agreement with earlier reports (Meerson *et al.*, 1993; Dumelin & Tappel, 1977), which indicated that persistent cellular oxidative stress lead to enhanced peroxidation of polyunsaturated fatty acids, macromolecular damage, disruption of signaling pathways and stimulation of myocardial injury.

Pretreatment with taurine significantly ($p< 0.05$) prevented the isoproterenol-induced aberrations in the fatty acid composition of heart tissue in Group IV rats as compared to Group III rats. There was a decrease in the saturated and monounsaturated fatty acid content and an increase in the polyunsaturated fatty acid content in heart tissue of Group IV rats compared to Group III rats. It probably did so by protecting the membrane phospholipids from the isoproterenol-induced free radical attack by virtue of its antioxidant property (Atmaca, 2004). This is in agreement with reports by Ebrahim & Sakthisekaran (1997), which indicated the effect of vitamin E and taurine treatment

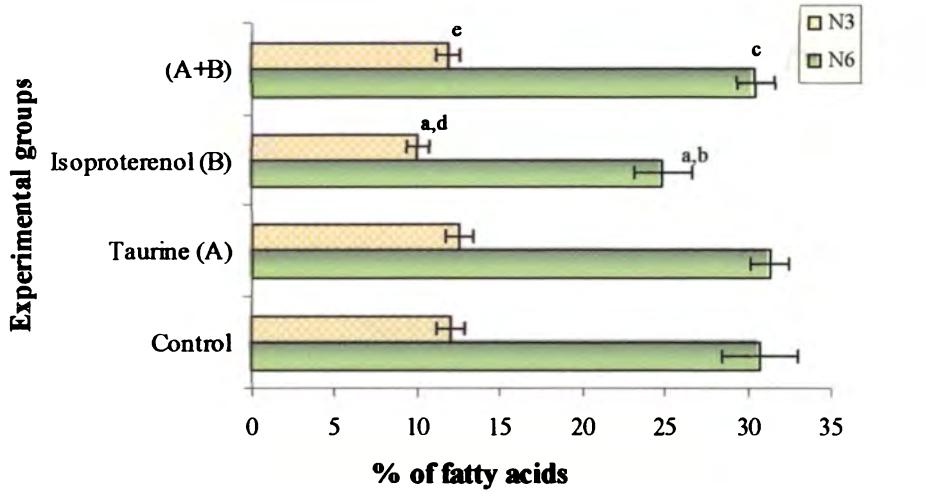


Fig 4.5.15 Levels of n6 and n3 polyunsaturated fatty acids in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.05$ significantly different compared with Group I control animals

[b] $p<0.01$ significantly different compared with Group II taurine-administered animals

[c] $p<0.01$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group II taurine-administered animals

[e] $p<0.05$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

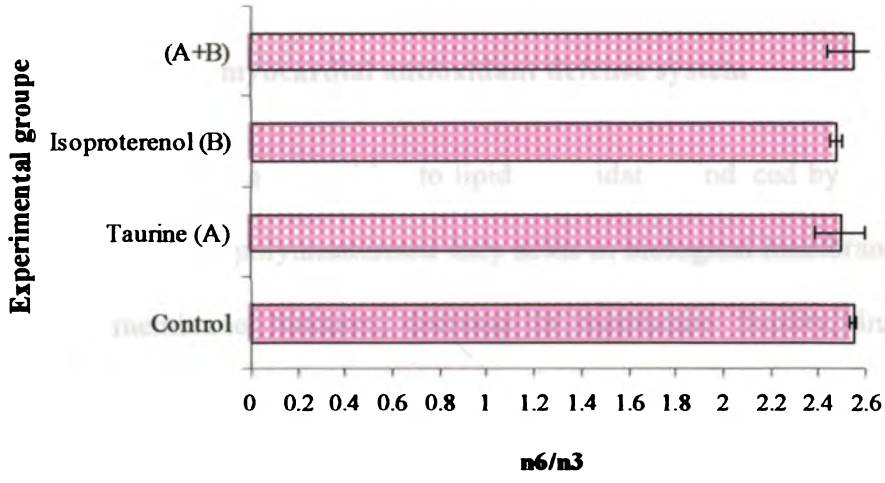


Fig 4.5.16 Ratio of n6 and n3 polyunsaturated fatty acids in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

protected membrane phospholipids by enhancing antioxidant defense status in perchloroethylene-induced cytotoxicity in mice. Mori & Beilin (2001) and Nageswari *et al.* (1999) reported that polyunsaturated fatty acids exerted anti-atherogenic effect and offerred significant protection against acute myocardial infarction in experimental animals. The increase observed in polyunsaturated fatty acid content in the heart tissue of Group IV rats might have also contributed to the protective effect of taurine. Hamaguchi *et al.* (1991) have reported that the cytoprotective effect of taurine could be due to its ability to alter polyunsaturated fatty acids in the mitochondrial cell membrane. Reports by Keys & Zimmerman (1999) indicated that taurine in combination with retinol prevented oxidative loss of polyunsaturated fatty acids in rod outer segment.

4.6 Effect of taurine on myocardial antioxidant defense system

Biological membranes are sensitive to lipid peroxidation induced by reactive oxygen species. The oxidation of polyunsaturated fatty acids in biological membranes may cause impairment of membrane function, decrease in membrane fluidity, inactivation of membrane receptors and enzymes, increase of non-specific permeability to ions and disruption of membrane structure. Peroxidation of endogenous lipids has been shown to be a major factor in the cardiotoxic action of isoproterenol (Kumar *et al.*, 2001; Chattopadhyay *et al.*, 2003). Isoproterenol-induced myocardial infarction is generally attributed to the formation of the highly reactive hydroxyl radical ($OH^{\cdot}$), stimulator of lipid peroxidation and source for the destruction and damage to cell membranes (Farvin *et al.*, 2004). Alterations in tissue defense systems including chemical scavengers or antioxidant molecules and the antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase, and glutathione-S-transferase have been reported in isoproterenol-induced myocardial infarction (Sharma *et al.*, 2001; Saravanan & Prakash, 2004; Padma *et al.*,

2006[b]). The crucial role of lipid peroxidation in the pathogenesis of myocardial injury can be illustrated by the use of antioxidants (Lieber *et al.*, 1994). Taurine has been shown to suppress lipid peroxidation in liver of carbon tetrachloride-intoxicated rats (Nakashima *et al.*, 1983) and in rabbit spermatozoa (Alvarez & Story, 1983). Reports by Wan & Li, (2005) indicated the antioxidant effects of taurine on myocardial injury in severely burned rats. Reports by Tabassum *et al.* (2006) showed that supplementation of taurine attenuated tamoxifen-induced hepatotoxicity in mice by its antioxidant property.

4.6.1 Lipid peroxidation

Lipid peroxidation *in vivo* has been identified as one of the basic deteriorative reaction in cellular mechanisms of the myocardial ischemia (Singal *et al.*, 1982; Rathore *et al.*, 1998). Lipid peroxidation of membranes is regulated by the availability of substrate in the form of polyunsaturated fatty acids, the availability of inducers such as free radicals and excited state molecules to initiate propagation, the antioxidant defense status of environment and the physical status of the membrane lipids.

In this investigation, the levels of lipid peroxides in plasma and heart tissue of Group III isoproterenol-administered rats were significantly ($p< 0.001$) higher compared with Group I control animals **(Fig 4.6.1 & 4.6.2)**. This was in agreement with the reports of Nirmala & Puvanakrishnan, (1996[b]), which indicated that a lack of antioxidant defense might lead to an increase in lipid peroxidation and subsequent deleterious effects on the myocardial membrane in isoproterenol-induced myocardial infarction condition. Synthetic catecholamine isoproterenol, by its property of generating free radicals during the course of its metabolism, alters redox homeostasis and causes damage to cell structure and function (Kukreja & Hess, 1992). In the present experiment, the generation of free radicals in the myocardium might have exceeded the ability of the free radical scavenging

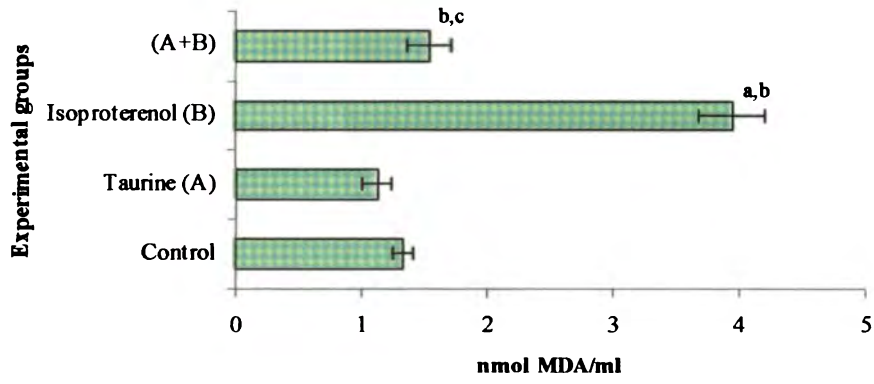


Fig 4.6.1 Level of lipid peroxides in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

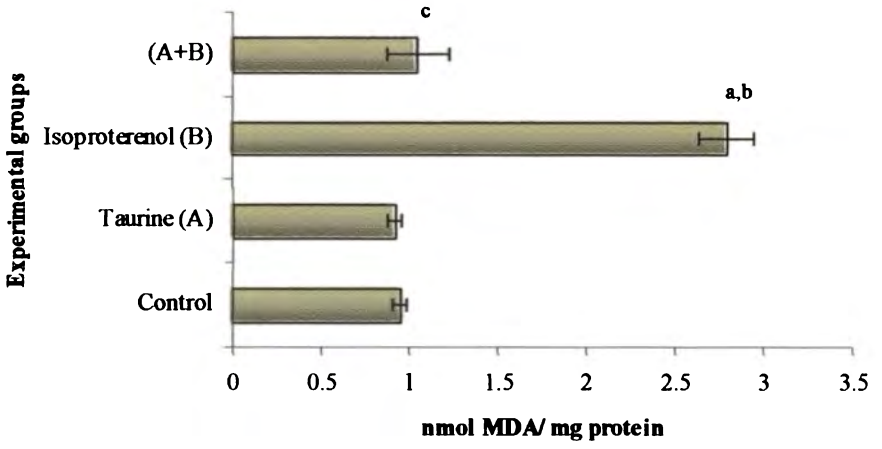


Fig 4.6.2 Level of lipid peroxides in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

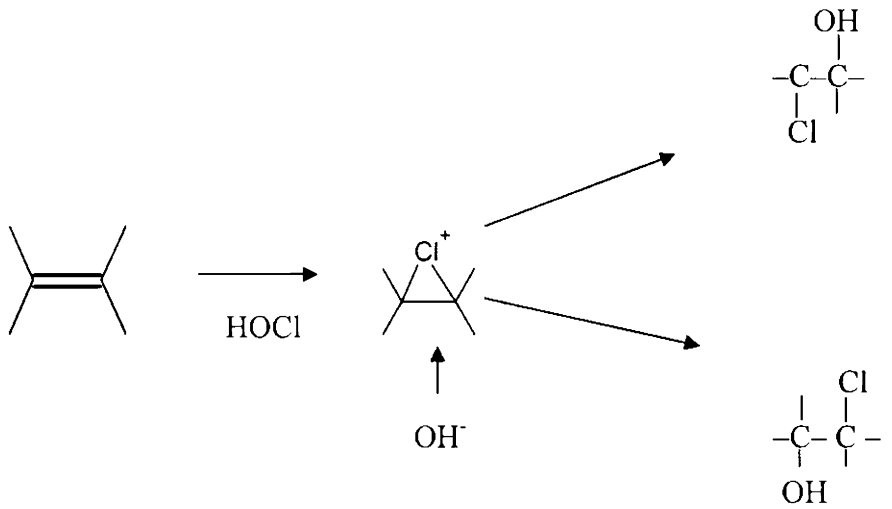
[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

enzymes to dismute isoproterenol-generated free radical formation resulting in myocyte lesions and reduction of scavengers.

Pathogenesis of myocardial injury is associated with the generation of reactive oxygen species. The polyunsaturated fatty acids that are abundant in myocardial membranes are easily susceptible to oxidative damage by free radicals, with consequent lipoperoxide formation, which may lead to the degeneration of membrane phospholipids (Chien *et al.*, 1978; Blasig *et al.*, 1984; Roig-Perez *et al.*, 2004). Hypochlorous acid is a potent oxidant produced by neutrophils, which are accumulated at the site of myocardial injury (Schaur *et al.*, 1998). Hypochlorous acid is able to modify a great variety of biomolecules by chlorination and/oxidation. Reports by Persad *et al.* (1999) showed that hypochlorous acid increased the isoproterenol-induced adenylate cyclase activity in a dose dependent manner. It forms chlorohydrin upon addition to double bonds in lipids (Winterbourn *et al.*, 1992). Chlorohydrins are polar than the parent fatty acids found in cell membrane and its formation cause disruption to membrane structure initiating lipid peroxidation and other free radical reactions, promoting destruction of protein-lipid complexes like biological membranes (Panasenko *et al.*, 1995).



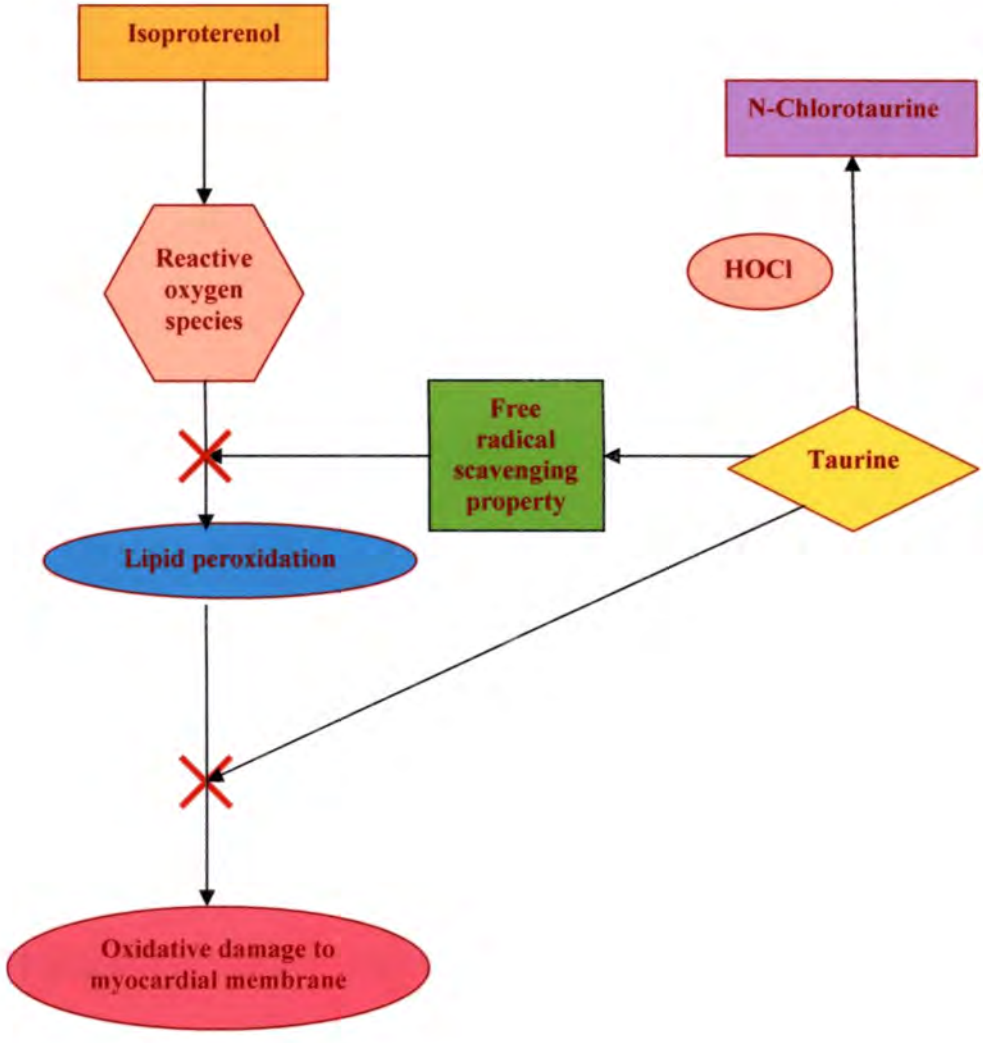


Fig 4.6.8 Effect of taurine on myocardial lipid peroxidation

In the present study, the Group IV rats pretreated with taurine showed a significant ($p< 0.001$) decrease in the level of lipid peroxidation in plasma and heart tissue compared to Group III isoproterenol-injected rats. This was probably achieved by means of its antioxidant nature (Rodriguez-Martinez, 2004) against lipid peroxidation induced by isoproterenol. The unpaired electron present in the hydroxyl radicals generated by isoproterenol might have been trapped and subsequently dismuted by taurine. Previously, Obrosova *et al.* (2001) reported that supplementation of taurine counteracted oxidative stress through the ascorbate system of antioxidant defenses in experimental diabetic nephropathy. Studies by Kerai *et al.* (1998) indicated the protective properties of taurine against hepatic steatosis and lipid peroxidation during chronic ethanol consumption in rats. Reports by Wright *et al.* (1985) indicated that taurine readily reacted with hypochlorous acid to form stable N-chlorotaurine.

$$\underset{\text{Taurine}}{NH_2\text{-}CH_2\text{-}CH_2\text{-}SO_3H} \xrightarrow{HOCl} \underset{\text{N-Chlorotaurine}}{ClNH\text{-}CH_2\text{-}CH_2\text{-}SO_3H} + H_2O$$

Nakamori *et al.* (1990) reported that the reaction of taurine with hypochlorous acid was an effective mechanism for the protection of biomembrane against oxidative stress. Studies by Patriarca *et al.* (2005) showed that supplementation with N-acetylcysteine and taurine attenuated oxidative stress in liver of streptozotocin-induced diabetic rats by their antioxidant property.

4.6.2 Reduced glutathione and antioxidant enzymes

Oxidative stress is the result of excessive production of oxygen species and/ depletion of intracellular antioxidant defenses leading to imbalance in the redox status of the cell.

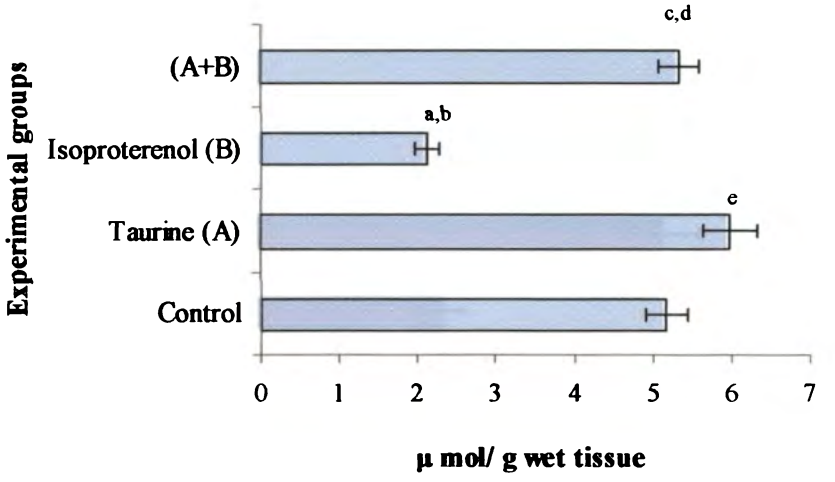


Fig 4.6.3 Level of reduced glutathione (GSH) in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group II taurine-administered animals

[e] $p<0.001$ significantly different compared with Group I control animals

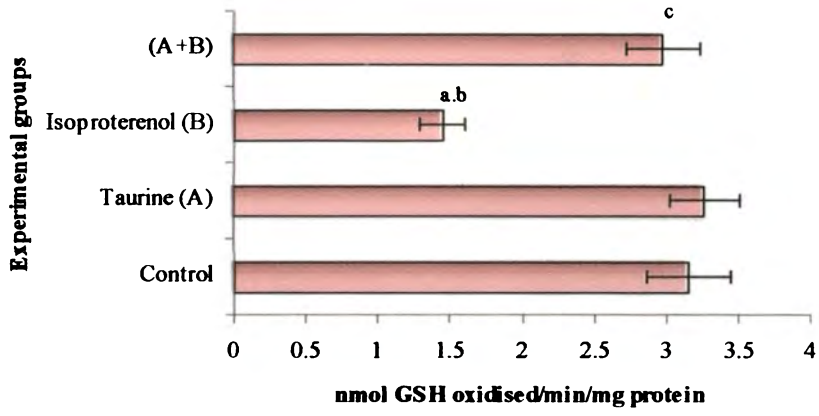


Fig 4.6.4 Activity of glutathione peroxidase (GPx) in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

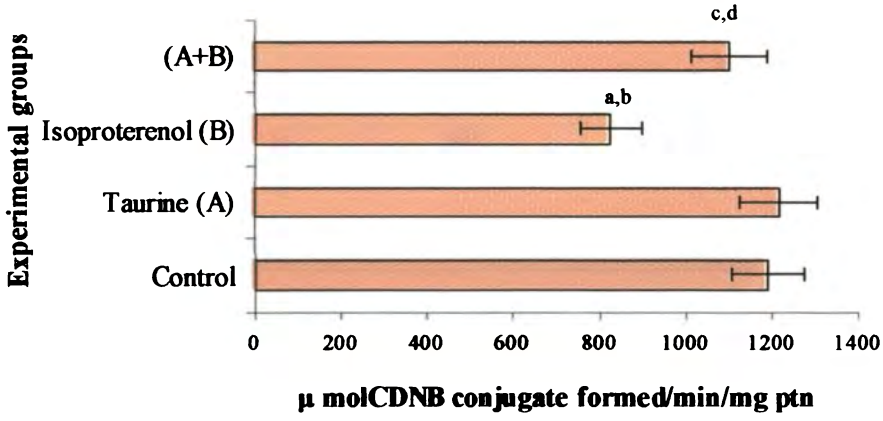


Fig 4.6.5 Activity of glutathione-S-transferase (GST) in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group II taurine-administered animals

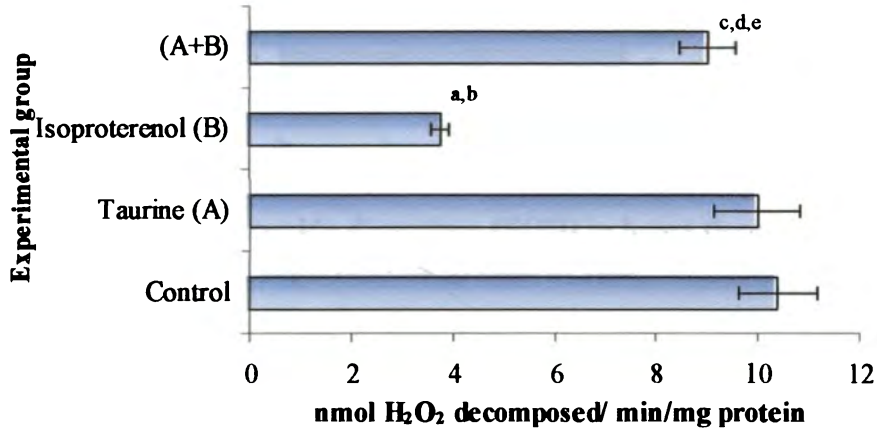


Fig 4.6.6 Activity of catalase (CAT) in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group I control animals

[e] $p<0.05$ significantly different compared with Group II taurine-administered animals

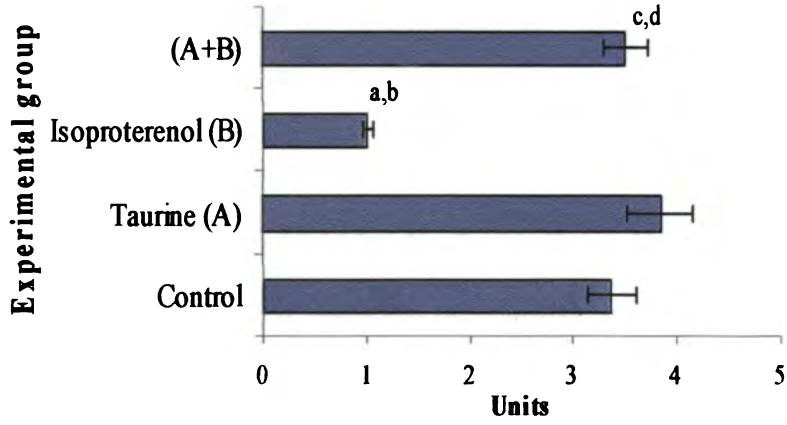


Fig 4.6.7 Activity of superoxide dismutase (SOD) in heart tissue of control and experimental groups of rats

Unit: One unit of the SOD activity is the amount of protein required to give 50% inhibition of adrenaline autoxidation.

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group II taurine-administered animals

Glutathione antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species (Meister, 1983). The cellular tripeptide GSH exerts protective antioxidant influence through a complex enzyme system including glutathione peroxidase (GPx) and glutathione-S-transferase (GST).

In this study, a significant ($p< 0.001$) reduction in the activity of glutathione-dependent antioxidant enzymes and antiperoxidative enzymes were observed in heart tissue of Group III isoproterenol-administered rats as compared with Group I control animals **(Fig 4.6.3-4.6.7)**. Also, the level of GSH was significantly ($p< 0.001$) reduced in isoproterenol-induced myocardial infarction. Glutathione exerts protective antioxidant functions by reaction with superoxide radicals and peroxy radicals, followed by the formation of oxidized glutathione and other disulphides. Decline in the activity of GPx in heart tissue of infarction-induced rats might have made the myocardial cells more sensitive to oxidative damage, leading to a change in the cell composition and function.

The significant ($p< 0.001$) decrease noted in the activity of GST, another scavenging enzyme involved in the removal of toxic metabolites by glutathione conjugation reactions, in the heart tissue of Group III myocardial infarction induced rats might have been due to the reduced availability of GSH. This was in accordance with previous reported studies (Sathish *et al.*, 2002; Farvin *et al.*, 2004), which indicated that GSH and GSH-dependent enzyme systems might be directly related to the pathogenic mechanism of isoproterenol-induced myocardial infarction. Significant ($p< 0.001$) reduction observed in the activity of antiperoxidative enzymes SOD and catalase in heart tissue of Group III rats might have led to the formation of O^{2-} and H_2O_2, which in turn formed hydroxyl radical ($OH^{\cdot}$) and brought about a number of reactions harmful to the myocardial cell membranes. Similar observations were reported by Kumar *et al.* (2007) and Gupta *et al.* (2004). $ONOO^{-}$ is a

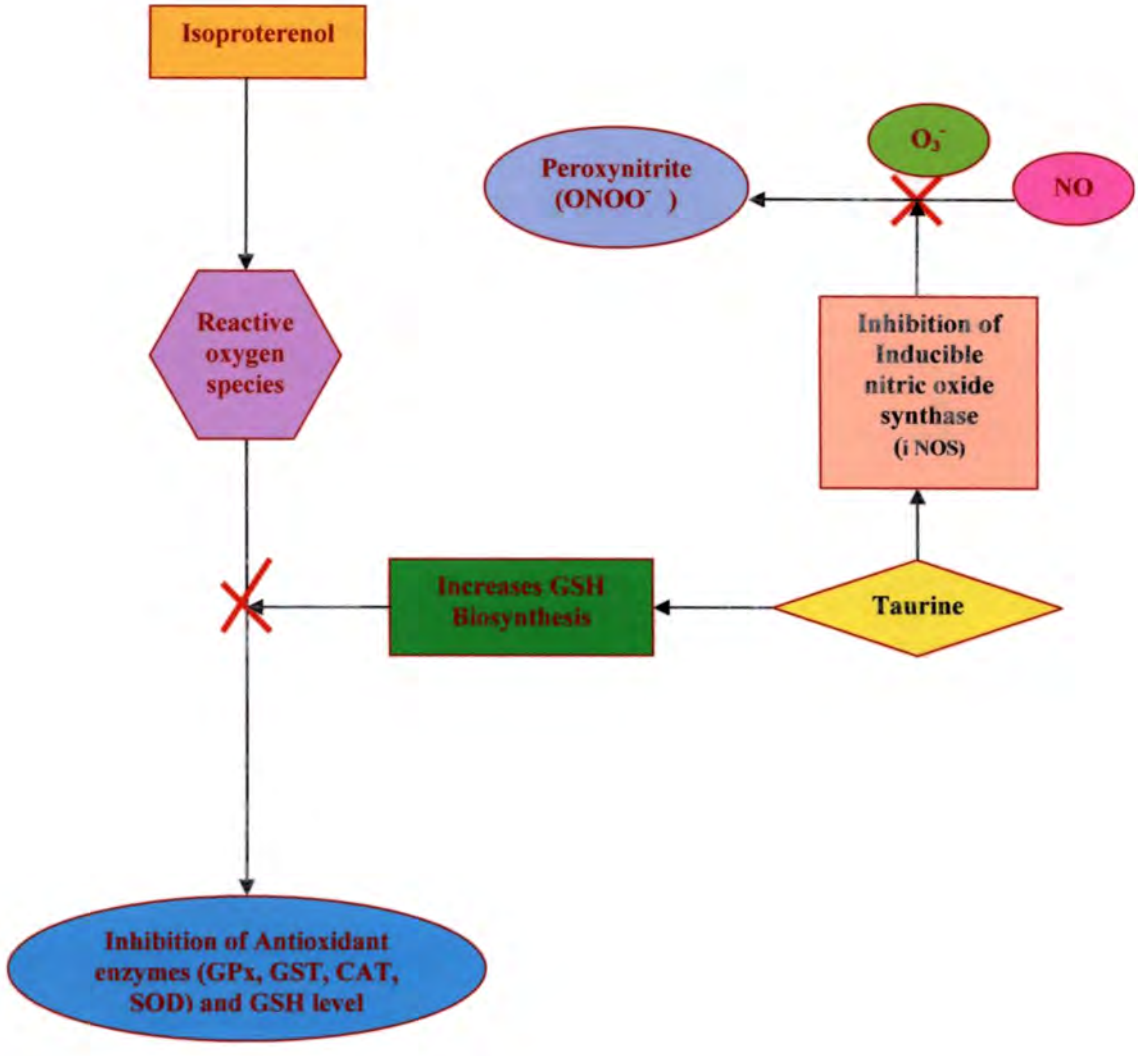


Fig 4.6.9 Effect of taurine on myocardial antioxidant defense system

powerful oxidant formed by the reaction of superoxide anion and nitric oxide. It causes oxidation of thiol groups, proteins and low molecular antioxidants and induce lipid peroxidation. It is also known to nitrate macromolecules to contribute to various pathophysiological conditions. The role of $ONOO^-$ in ischemia-reperfusion has been implicated to cause myocardial dysfunction and infarction (Vinten-Johansen, 2000; Sreepriya *et al.*, 1998). $ONOO^-$can inactivate the SOD via nitration of a specific tyrosine residue at its active site further promoting ROS availability (Yamakura *et al.*, 1998).

In the present study, prior oral administration of taurine significantly ($p< 0.001$) prevented the isoproterenol-induced depletion of reduced glutathione and maintained the activities of antioxidant enzymes in heart tissue of Group IV rats at near normalcy. Taurine might have protected the myocardium from the oxidative damage caused by $ONOO^-$ probably by either acting as an antioxidant or by inhibiting the production and availability of nitric oxide. This is in agreement with Barua *et al.* (2001). Kocak-Toker *et al.* (2005) reported that taurine acted as an antioxidant of ONOO- to decrease lipid peroxidation and restored plasma membrane Na^+, K^+-ATPase activity in liver. Tabassum *et al.* (2006) observed that the activities of antioxidant enzymes and glutathione-metabolizing enzymes were considerably stabilized in mice pretreated with taurine. A significant rise ($p< 0.001$) in the level of GSH was noticed in heart tissue in Group II taurine-administered rats compared to Group I control, indicating that tissue antioxidant status was operating at a higher rate in taurine-treated rats for the counteraction of lipid peroxides. This finding is in line with an earlier report (Tadros *et al.*, 2005), which indicated that taurine demonstrated antioxidant activity against oxidative stress induced by mycotoxin 3-nitropropionic acid administration, as evidenced by the reduced striatal malondialdehyde (MDA) and elevated striatal glutathione (GSH) levels. It is already reported that taurine administration affected the disposition of acetaminophen by enhancing its metabolism through the GSH-dependent

pathway (Lee *et al.*, 2004). The present results lead to the conclusion that a relation may exist between the protective effects of taurine and glutathione because both antioxidant molecules are derived from same precursor molecule, cysteine (Stipanuk *et al.*, 1992). Taurine is reported to increase the GSH content in rat liver and to improve the levels of SOD and CAT in perchloroethylene-induced cytotoxicity in mice (Ebrahim & Sakthisekaran, 1997; Hwang *et al.*, 2000). Studies by Hagar *et al.* (2006) indicated the protective potential of taurine against cyclosporine-A induced hypertension and nephrotoxicity and suggested a significant contribution of its antioxidant property to its beneficial effect. Therefore from the present study, it is clear that taurine significantly augmented the glutathione system in combating isoproterenol-induced myocardial necrosis in rats (Shiny *et al.*, 2005).

4.7 Effect of taurine on membrane stabilization

Cell membrane rupture by oxygen-derived free radicals is a systematic feature of myocardial injury. The generation of lipid peroxides and hydroperoxides by isoproterenol results in initiation of chain reactions that destabilizes myocardial membrane. Taurine is an endogenous antioxidant and a membrane-stabilizing intracellular free β-amino acid (Guz *et al.*, 2006). There is substantial evidence to suggest that taurine may protect myocardial membrane from isoproterenol-induced free radical mediated necrotic damage (Banks *et al.*, 1991; Huxtable, 1992).

4.7.1 Sulfhydryl content

Thiols are crucial targets for oxidation in cells. These reducing agents are an indirect measure of oxidative damage. The oxidation of sulfhydryl groups in proteins may affect their functional properties. Formation of protein disulfides, mixed disulfides with glutathione or sulfenic acids can result in changes in enzymatic activity, conformation or

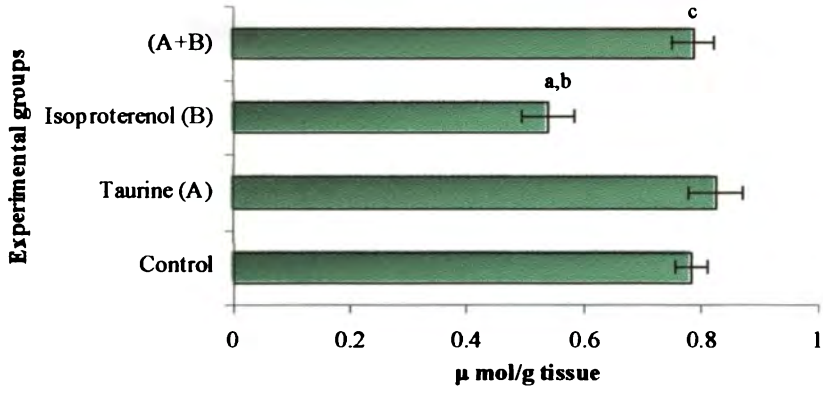


Fig 4.7.1 Level of total sulfhydryl content (TSH) in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

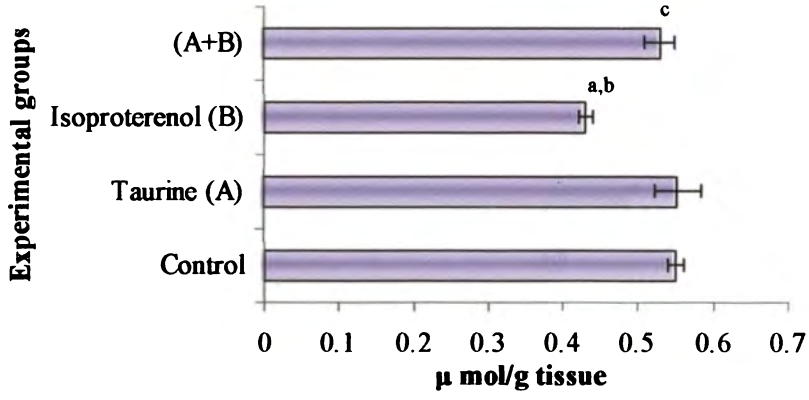


Fig 4.7.2 Level of non-protein bound sulfhydryl content (NPSH) in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.01$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

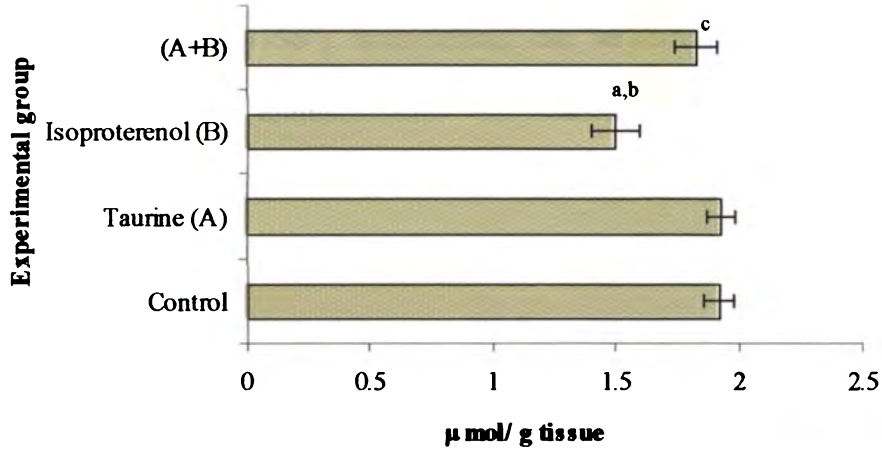


Fig 4.7.3 Level of protein bound sulfhydryl content (PSH) in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.01$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

affinity towards other molecules. Such changes contribute to the cell damage caused by oxidative stress. Modulation of thiol redox state also provides a sensitive mechanism for regulation of metabolic processes. Lower amounts of thiols are indicative of an increase in oxidative stress and cellular damage (Soszynski & Bartosz, 1997; Peskin & Winterbourn, 2001). In the present study, administration of isoproterenol significantly ($p< 0.001$) reduced the total sulfhydryl content, protein-bound sulfhydryl and non-protein bound sulfhydryl content in Group III rats **(Fig 4.7.1-4.7.3)**. This is in line with earlier reported studies (Singal *et al.*, 1983; Ondrejickova *et al.*, 1991). The reactive oxygen species produced by isoproterenol might have induced oxidation of sulfhydryl groups, leading to a decline in thiol concentration in the myocardial tissue. Similar observation reported earlier also (Nirmala & Puvanakrishnan, 1996[b]). Isoproterenol can be converted into o-quinones and undergoes cyclization into aminochromes enzymatically or through autoxidation. Aminochromes are highly reactive molecules that can cause oxidation of protein sulfhydryl groups (Remiao *et al.*, 2002).

In the present investigation, pretreatment with taurine preserved total sulfhydryl as well as protein bound and non-protein bound thiol content in heart tissue of Group IV rats compared to Group III rats. It probably did so by shielding the thiol groups of the myocardial membrane from reactive oxygen species by its antioxidant property (Atmaca, 2004). This observation concurs with an earlier reported study. (Tadros *et al.*, 2005) which showed that taurine exerted neuroprotective role in an experimental animal model of Huntington's disease through its antioxidant effect and γ-amino butyric acid agonistic action. Venkatesan & Chandrakasan (1994) reported that taurine and niacin in combination was efficient in blunting increases in lung content of lipid hydroperoxides that paralleled the decreased levels of both free sulfhydryl groups and total sulfhydryl groups in cyclophosphamide-induced pulmonary damage. There is also evidence that administration

of taurine along with ethanol restored glutathione and tissue thiols in rats (Pushpakiran *et al.*, 2004). Wu *et al.* (1999) reported that taurine reversed carbontetrachloride-induced lactate dehydrogenase release, low cellular thiols and polyamine levels in isolated rat hepatocytes.

4.7.2 Membrane-bound ATPases

There was a significant ($p< 0.001$) reduction noticed in the activities of the membrane-bound ATPases (Na^+, K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase) in the heart tissue of Group III isoproterenol-induced myocardial infarcted rats compared with Group I normal rats **(Fig 4.7.4- 4.7.6)**. This is in line with previous findings (Nirmala & Puvanakrishnan, 1996[b]; Farvin *et al.*, 2005). ATPases are integral membrane proteins which require thiol groups and phospholipids to maintain their structure and function. Isoproterenol administration is known to produce reactive oxygen species, which modifies membrane phospholipids and proteins, leading to lipid peroxidation and oxidation of thiol groups. This ultimately results in the inactivation of the lipid-dependent membrane bound ATPases. Previous reports by Garner *et al.*, (1983) and Adhirai & Selvam (1997) indicated that toxic insult of heart tissue promoted depletion of GSH. This depletion might have affected membrane-bound ATPases. According to Rauchcova *et al.* (1995) and Hazarika & Sarkar (2001), peroxidation of membrane phospholipids not only altered the lipid milieu and structural as well as functional integrity of cell membrane, but also affected the activities of various membrane-bound enzymes including Mg^{2+}-ATPase, Ca^{2+}-ATPase and Na^+,K^+ -ATPase.

In the present study, the Group IV rats pretreated with taurine showed a significant ($p< 0.001$) increase in the level of membrane-bound ATPases compared to Group III isoproterenol-injected rats. It probably did so by its membrane stabilizing action (Heller-

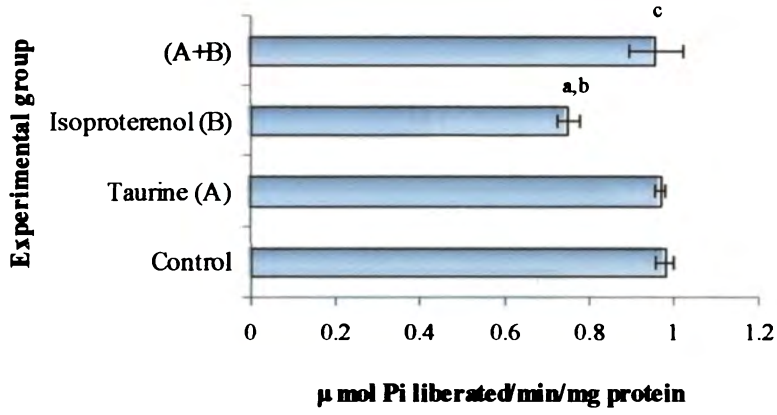


Fig 4.7.4 Activity of Mg^{2+} -ATPase in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

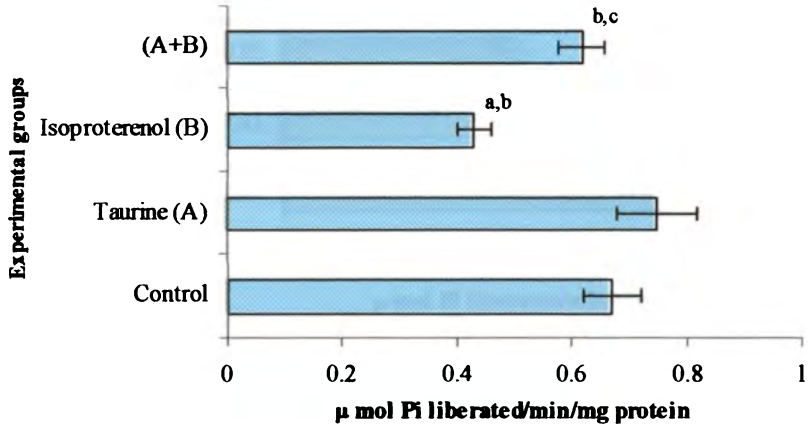


Fig 4.7.5 Activity of Ca^{2+} -ATPase in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

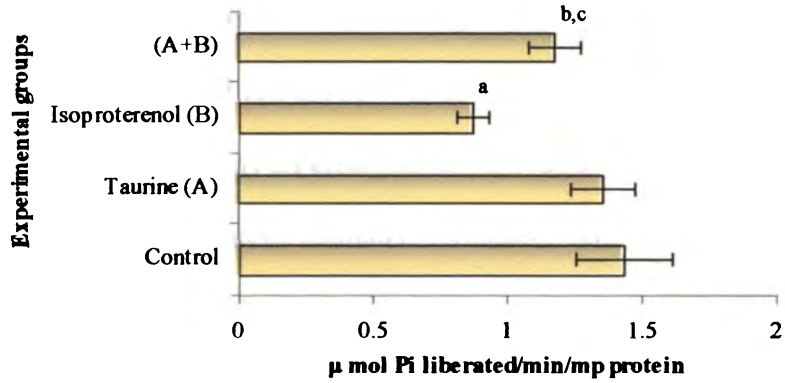


Fig 4.7.6 Activity of Na^+, K^+ -ATPase in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

Stilb *et al.*, 2001). Supplementation of thiol group generating substances and free radical scavengers have been reported to restore the cellular thiol content and membrane functions (Selvam & Kurien, 1992; Selvam *et al.*, 1993). Since ATPases require sulfhydryl groups for their structural and functional integrity, the maintenance of thiol content by taurine might have contributed to its protective effect. Reports by Qi *et al.* (1995) showed that supplementation of taurine restored the ozone exposure-induced reduction in the activity of membrane-bound Na^+, K^+-ATPase by its antioxidant property. Taurine can be considered both as an antioxidant and as a membrane stabilizer. Pushpakiran *et al.* (2005) reported that taurine prevented ethanol-induced alterations in lipids and ATPases in rat tissues. Taurine treatment significantly prevented the inhibition in the activities of Na^+, K^+-ATPase and Ca^{2+}-ATPase in high glucose-treated red blood cells (Nandhini *et al.*, 2003). According to Hastings *et al.* (1985) taurine acted as an endogenous activator or modulator of Na^+, K^+-ATPase and a membrane-bound protein mediated its action. Mankovskaya *et al.* (2000) reported that taurine prevented hypoxia-induced lactate accumulation and lipid peroxidation in brain, liver, and heart tissues and prevented the decrease of Na^+, K^+ ATPase activity in the liver. According to Di Leo *et al.* (2003) taurine supplementation better preserved ATPase activity in comparison with vitamin E and selenium in experimental animals. Taurine acted as an antioxidant of ONOO- to decrease lipid peroxidation and restored liver plasma membrane Na^+-K^+-ATPase activity (Kocak-Toker *et al.*, 2005).

4.7.3 Mineral status

A significant ($p< 0.001$) rise was observed in the level of calcium in the plasma and heart tissue of isoproterenol-administered Group III rats compared with Group I control animals **(Fig 4.7.7- 4.7.12)**. This is in accordance with an earlier reported study

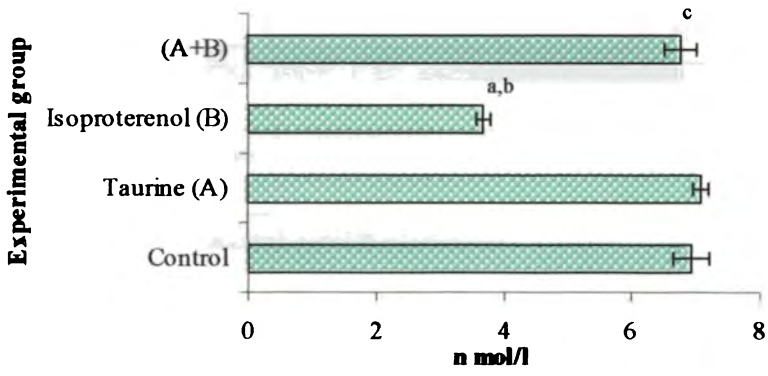


Fig 4.7.7 Level of potassium in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

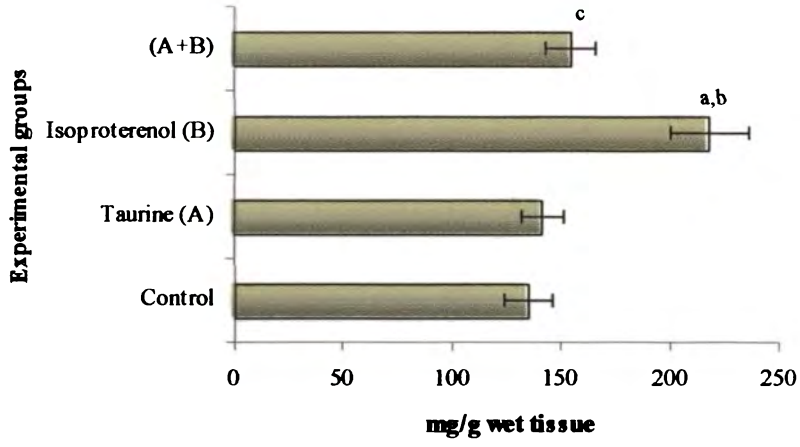


Fig 4.7.8 Level of potassium in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

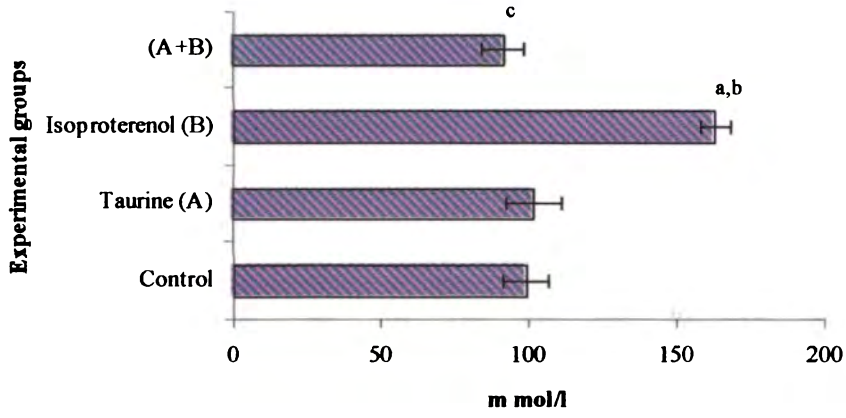


Fig 4.7.9 Level of sodium in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

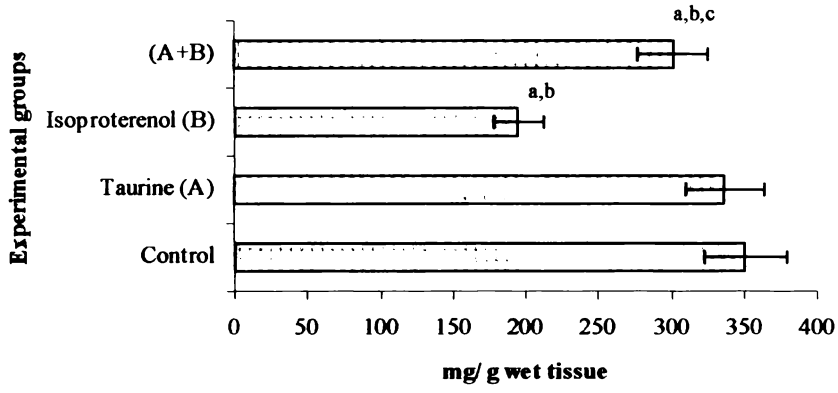


Fig 4.7.10 Level of sodium in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

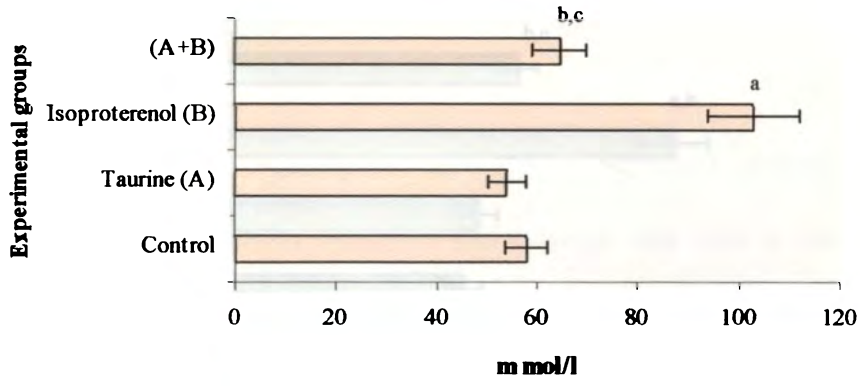


Fig 4.7.11 Level of calcium in plasma of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

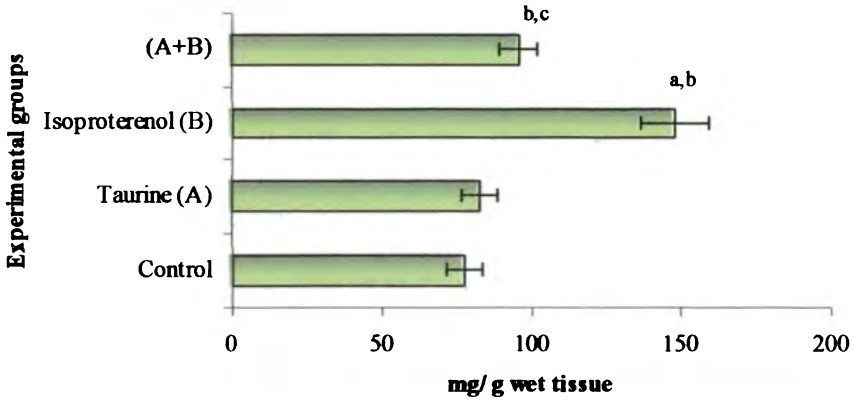


Fig 4.7.12 Level of calcium in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

(Namikawa *et al.*, 1991). Isoproterenol administration is reported to induce lipolysis and production of reactive oxygen species that destabilized the myocardial membrane. This resulted in the inactivation of Na^+, K^+-ATPase, resulting in the depletion of plasma potassium and rise in sodium concentration. Inhibition of Ca^{2+}ATPase under conditions of isoproterenol-induced myocardial infarction resulted in calcium accumulation (Sathish *et al.*, 2003[a]). This leads to a disturbance in the equilibrium between the intracellular and extracellular calcium concentrations (Kristof *et al.*, 1999). Active calcium transport and resultant low calcium concentrations are requirement for active Na^+ /K^+ transport. Since calcium and sodium ions are competitive at a number of membrane sites, a high concentration of calcium ions in the cells of ischemic heart would compete with sodium specific sites at the inner surface of the membrane, leading to a decrease in myocardial sodium content (Vincenzi, 1971).

Pretreatment with taurine significantly ($p< 0.001$) prevented isoproterenol-induced alterations in the levels of sodium, potassium and calcium ions both in plasma and heart tissue of Group IV rats. The transport of Na^+ and K^+ between intra and extracellular pools and the maintenance of the transmembrane gradients are important to cell function and integrity. The Na^+/taurine co-transport mechanism might have played an important role in the protection against Na^+/K^+ imbalance (Bkaily *et al.*, 1996) and intracellular calcium overload. Taurine treatment has probably improved Ca^{2+} homeostasis by facilitating the efflux of Ca^{2+} via the Na^+/Ca^{2+} exchanger (Schaffer *et al.*, 1995; Suleiman *et al.*, 1992). In addition to this, the ability of taurine to maintain the integrity of membrane-bound ATPases might have contributed significantly to its role in maintaining the ionic equilibrium in Group IV rats. This is in agreement with studies by Nandhini & Anuradha (2003) which indicated that taurine inhibited lipid peroxidation and prevented the suppression of membrane-bound ATPases in high glucose treated red blood cells. Taurine is also reported

to normalize the content of potassium and calcium ions both *in vivo* and *in vitro* (Shustova *et al.*, 1986). Chovan *et al.* (1979) reported that taurine antagonized the inhibition of calcium binding to the sarcolemma caused by both verapamil and lanthanum. El Idrissi (2006) reported that role of taurine in modulating mitochondrial calcium homeostasis could be of particular importance under pathological conditions characterized by excessive calcium overloads.

4.8 Effect of taurine on mitochondrial function

Mitochondria are intracellular centers for aerobic metabolism (Hatefi, 1985). In morphological terms, mitochondria are relatively large particles characterized by the presence of two membranes, a smooth outer membrane, which is permeable to most metabolites and an inner membrane, which has unique transport properties. Many of the proteins and enzymes of the respiratory metabolism are present in the inner membrane of the mitochondria. The enzymes of the tricarboxylic acid cycle and oxidative phosphorylation are present in the mitochondrial matrix region. Mitochondria are the main consumers of molecular oxygen in the cardiac cell and provide the energy required for ATP synthesis in the oxidative phosphorylation (Echtay *et al.*, 2002). Therefore the function of mitochondria in ischemic heart disease is significant. Ebenezar *et al.* (2003[b]) have reported that myocardial infarction correlated with increased oxidative stress and loss of mitochondrial function.

Mitochondrial respiration refers to all those processes concerned with the uptake of oxygen and associated production of ATP, including the activity of the citric acid cycle and respiratory chain. The citric acid cycle is the central metabolic pathway for all aerobic processes and provides metabolic intermediates for biosynthetic purposes. The enzymes and intermediates of the citric acid cycle are found inside the mitochondrial matrix, where

the reductive power of NADH and $FADH_2$ can directly be fed (in the form of electrons) into the electron transport chain of the oxidative phosphorylation process in the inner membrane of this organelle. This electron flow is coupled to proton flow which is temporarily stored as an electrochemical gradient which in turn is harvested by the ATP synthase to produce chemical energy. The major source of energy for the cardiac muscle contraction comes from the oxidative metabolism of mitochondria in the myocardial cell (Brown, 1992).

4.8.1 TCA cycle enzymes and respiratory marker enzymes

In the present study, there was a significant ($p< 0.001$) reduction in the level of myocardial ATP content with a concomitant decline in the activities of TCA cycle enzymes (isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, α-ketoglutarate dehydrogenase) and respiratory marker enzyme (NADH dehydrogenase) **(Fig 4.8.1- 4.8.6)** in heart mitochondria of Group III isoproterenol-administered rats as compared to Group I control rats. This concurs with earlier reported studies (Sathish *et al.*, 2002 Chagoya de Sanchez *et al.*, 1997; Kumar & Anandan, 2007). The free radicals generated by isoproterenol might have damaged the mitochondrial membrane and hence inactivated these enzymes (Ithayarasi & Shyamala, 1998). In contrast with all other enzymes of the TCA cycle, which are soluble proteins found in the mitochondrial matrix, succinate dehydrogenase is an integral membrane protein. It is tightly attached to the inner membrane and is directly linked to the electron transport, transferring electrons to the respiratory chain (Singh *et al.*, 1990). Succinate dehydrogenase is a site for metabolic control in TCA cycle (Papa *et al.*, 1969) and contains many cysteine rich sulfur clusters and can be inhibited by agents that modify sulfhydryl groups. Isoproterenol administration is known to alter protein-bound sulfhydryl groups and hence might have resulted in the inactivation of the

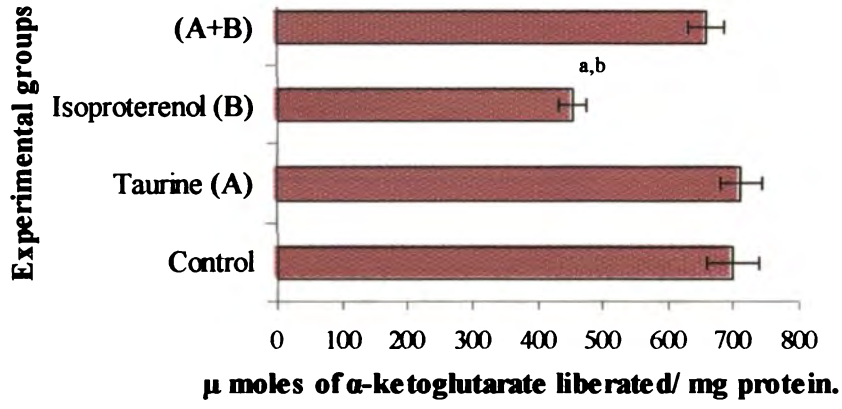


Fig 4.8.1 Activity of isocitrate dehydrogenase in heart mitochondria of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

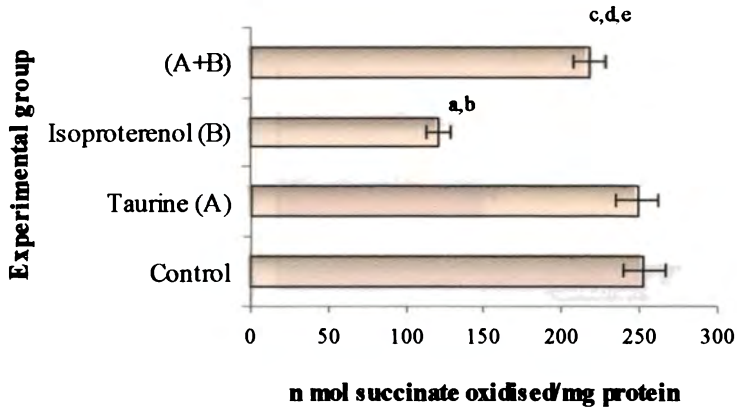


Fig 4.8.2 Activity of succinate dehydrogenase in heart mitochondria of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group I control animals

[e] $p<0.05$ significantly different compared with Group II taurine-administered animals

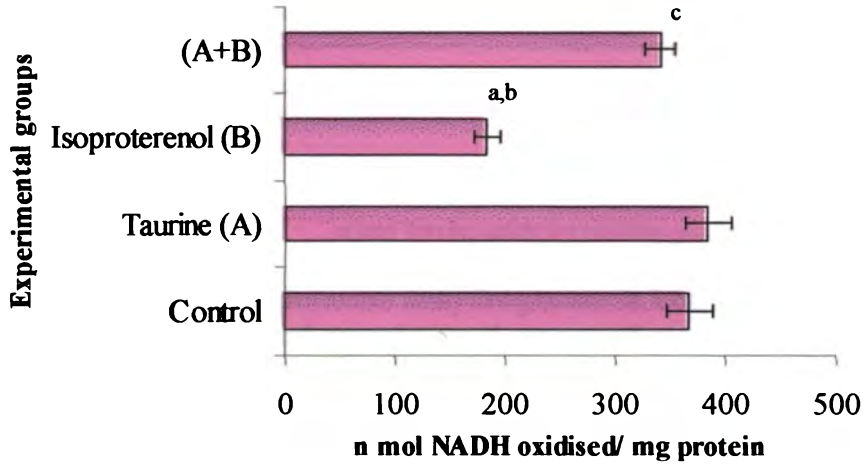


Fig 4.8.3 Activity of malate dehydrogenase in heart mitochondria of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

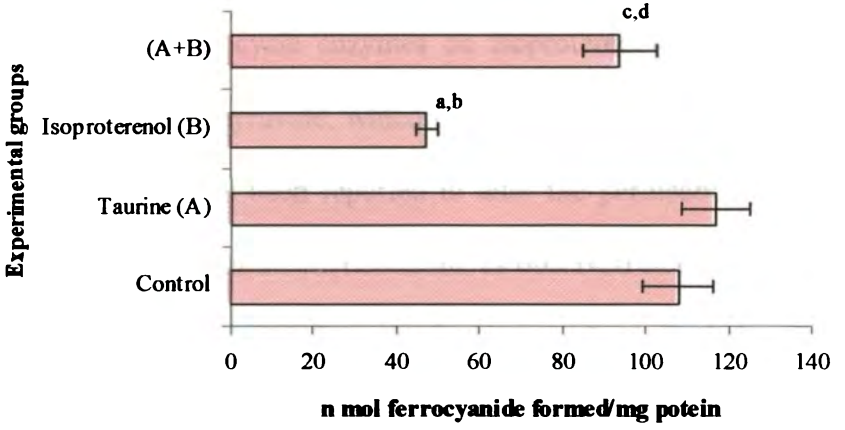


Fig 4.8.4 Activity of α-ketoglutarate dehydrogenase in heart mitochondria of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group II taurine-administered animals

enzyme. The respiratory marker enzyme NADH dehydrogenase is located in the mitochondrial membrane (Nicolay *et al.*, 1985) and isoproterenol administration lowers its activity. Decrease in the activity of the respiratory enzymes in rats with experimentally induced myocardial infarction has been previously reported (Calva *et al.*, 1966; Sathish *et al.*, 2002). This could be due to the free radical induced destabilization of mitochondrial membrane or due to the enhanced phospholipid degradation (Padma & Devi, 2001). The free radicals produced as a result of this lipid peroxidation might have attacked the RNA polymerase corresponding to these mitochondrial enzymes. Reduction noticed in the activities of tricarboxylic acid cycle enzymes on isoproterenol administration proved a defect in aerobic oxidation of pyruvate, which might have resulted in lowered production of ATP. Lipid peroxidation has been reported to alter the permeability of mitochondrial membrane and to deplete ATP in the myocardium, as observed in the present study. Under conditions of ischemia when supply of oxygen is compromised, the balance between ATP production and consumption is disturbed, resulting in the decrease in ATP level (Sathish *et al.*, 2003[a]).

Pretreatment with taurine maintained the activities of TCA cycle enzymes and the level of myocardial ATP content at near normalcy in Group IV rats compared to Group III rats, reflecting its ability to maintain the function of the heart mitochondria at near normal status. Taurine is well known to counteract reactive oxygen species and to increase antioxidant defense status in experimental animals (Pasantes-Morales *et al.*, 1985). Taurine has also been reported to increase the GSH content and to protect the reduced glutathione from the free radical attack (Tadros *et al.*, 2005; Lee *et al.*, 2004). This may help to maintain the activity of enzymes like dehydrogenases that requires or possesses sulfhydryl groups at the active site. The membrane stabilizing property of taurine is already recognized (Heller-Stilb *et al.*, 2001). Since tricarboxylic acid cycle enzymes are located in

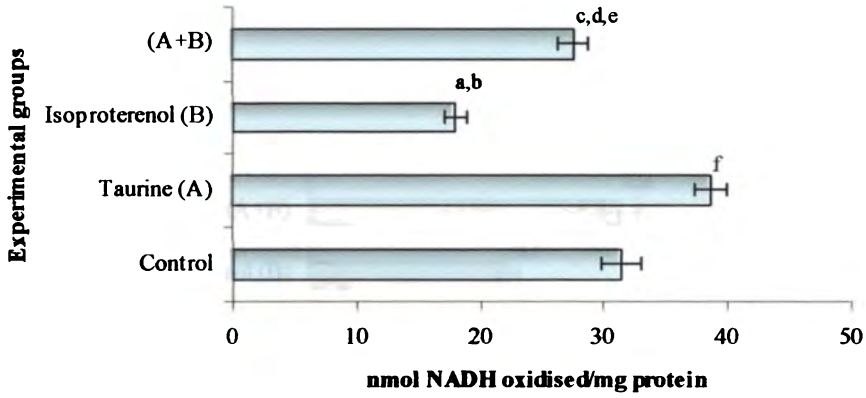


Fig 4.8.5 Activity of NADH dehydrogenase in heart mitochondria of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.01$ significantly different compared with Group II taurine-administered animals

[e] $p<0.05$ significantly different compared with Group I control animals

[f] $p<0.01$ significantly different compared with Group I control animals

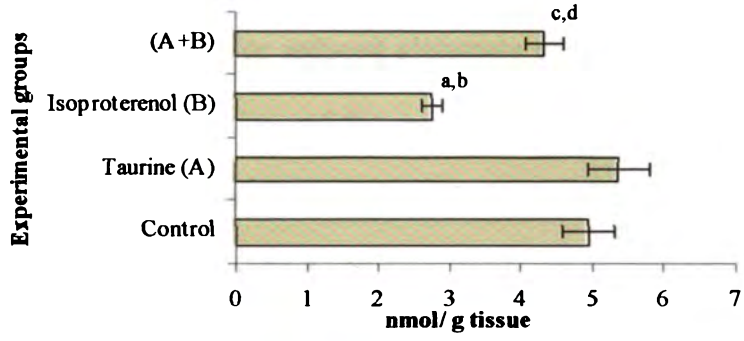


Fig 4.8.6 Level of ATP in heart tissue of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group II taurine-administered animals

the mitochondrial inner membrane and matrix region, the ability of taurine to maintain the mitochondrial membrane intact offers important protective mechanism against isoproterenol-induced alterations in the mitochondrial architecture. Yan & Sohal (1998) have reported that adenine nucleotide translocase is subjected to oxidative modification. The ability of taurine to prevent oxidative stress may beneficially effect the translocase activity and hence ATP synthesis and transportation.

Chepkova *et al.* (2006) have suggested that taurine may rescue the mechanisms of hippocampal synaptic plasticity by improving mitochondrial function under hyperammonemic conditions. Findings by Takatani *et al.* (2004) demonstrated that taurine effectively prevented myocardial ischemia-induced, mitochondria-mediated apoptosis by inhibiting the assembly of the Apaf-1/caspase-9 apoptosome. According to El Idrissi & Trenkner (2003) taurine prevented or reduced glutamate excitotoxicity through both the enhancement of mitochondrial function and the regulation of intracellular (cytoplasmic and mitochondrial) calcium homeostasis. Reports by Palmi *et al.* (1999) showed that the role of taurine in modulating mitochondrial calcium homeostasis might be of particular importance under pathological conditions characterized by cell calcium overload such as ischaemia and oxidative stress. Waterfield *et al.* (1993) reported that taurine reduced the depletion of ATP caused by 1, 4-naphthoquinone. According to studies by El Idrissi (2006), the overall mitochondrial function was increased in the presence of taurine, when assessed by rhodamine accumulation into mitochondria and total cellular ATP levels.

4.8.2 Mitochondrial antioxidant status

A close association between reactive oxygen species and defects in the electron transport function leads to higher generation of reactive oxygen species, which plays an important role in the development and progression of heart failure. Increased mitochondrial

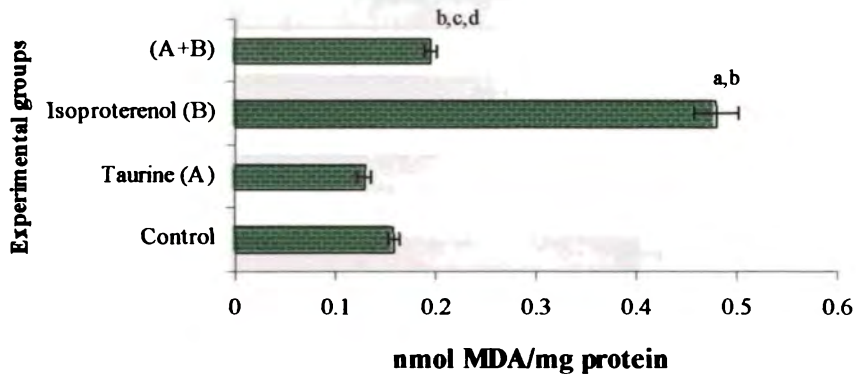


Fig 4.8.7 Level of lipid peroxides in heart mitochondria of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group I control animals

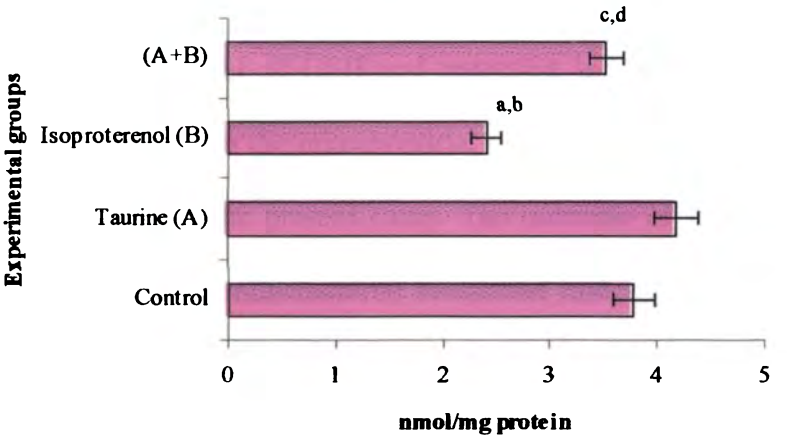


Fig 4.8.8 Level of reduced glutathione (GSH) in heart mitochondria of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.01$ significantly different compared with Group II taurine-administered animals

free radical production is observed under conditions such as ischemia and is associated with impairment of mitochondrial structure and function (Ebenezar *et al.*, 2003[b]).

In the present study, the level of lipid peroxidation was significantly ($p< 0.001$) higher in heart mitochondria of Group III isoproterenol-administrated rats as compared to Group I control **(Fig 4.8.7)**. This was paralleled by a significant ($p< 0.001$) reduction in the level of reduced glutathione and the activities of glutathione-dependent antioxidant enzymes and antiperoxidative enzymes in the heart mitochondria of myocardial infarction induced Group III rats **(Fig 4.8.8-4.8.12)**. This is in line with an earlier reported study (Padma & Devi, 2002), which indicated that free radicals were produced in substantial amounts in heart mitochondria during myocardial infarction. Overproduction of reactive oxygen species lead to decreased level of GSH and reduction in the activities of GSH related enzymes and SOD and CAT in the mitochondria, (Sathish *et al.*, 2003[b]; Fernandez-Checa *et al.*, 1997) as observed in the present study. The outcome of lipid peroxidation is the development of alterations in mitochondrial membrane integrity and permeability leading to irreversible myocardial injury (Burton *et al.*, 1984; Spector & Yorek, 1985). Zhang *et al.* (1990) and Ide *et al.* (2001) reported that hydroxyl radicals, which originated from the superoxide anion and lipid peroxide formation in the mitochondria, were both increased during myocardial infarction. Lower amounts of thiols are indicative of oxidative stress and damage (Soszynski *et al.*, 1997). The decline observed in the levels of mitochondrial GSH content and in the activities of antioxidant enzymes was an indicative of the increased oxidative stress mitochondria induced by isoproterenol. Depletion in the levels of mitochondrial GSH seems to be a major mechanism in inducing imbalance of mitochondrial function (Anderson *et al.*, 1990). GST is a critical detoxification enzyme that functions primarily in conjugating functionalized P450 metabolites with endogenous ligands like GSH favoring its elimination (Mohandas *et al.*, 1984).

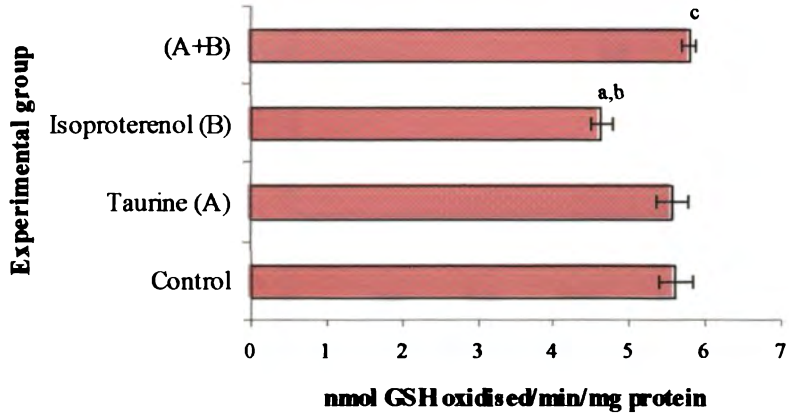


Fig 4.8.9 Activity of glutathione peroxidase (GPx) in heart mitochondria of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.01$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

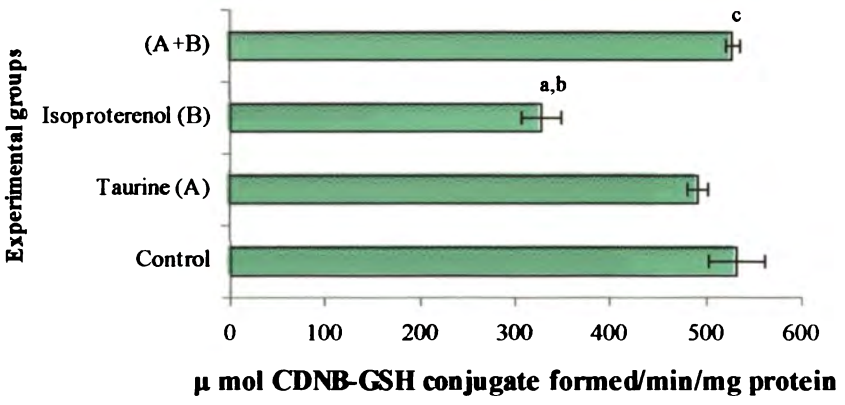


Fig 4.8.10 Activity of glutathione-S-transferase (GST) in heart mitochondria of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

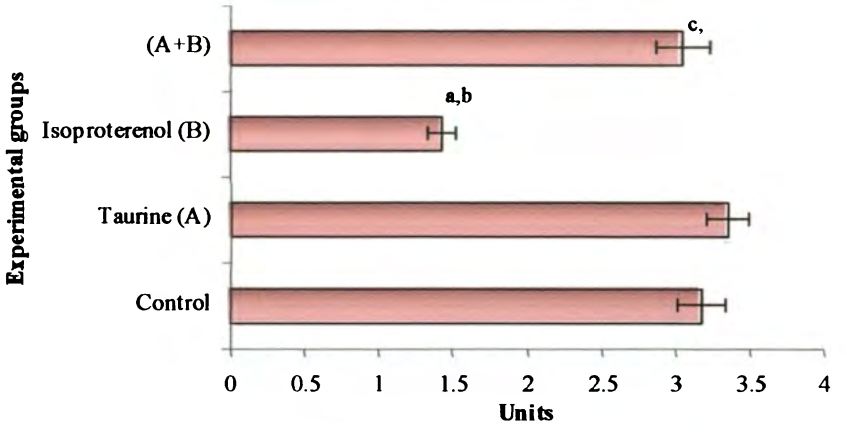


Fig 4.8.11 Activity of superoxide dismutase (SOD) in heart mitochondria of control and experimental groups of rats

Unit: One unit of the SOD activity is the amount of protein required to give 50% inhibition of adrenaline autoxidation.

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

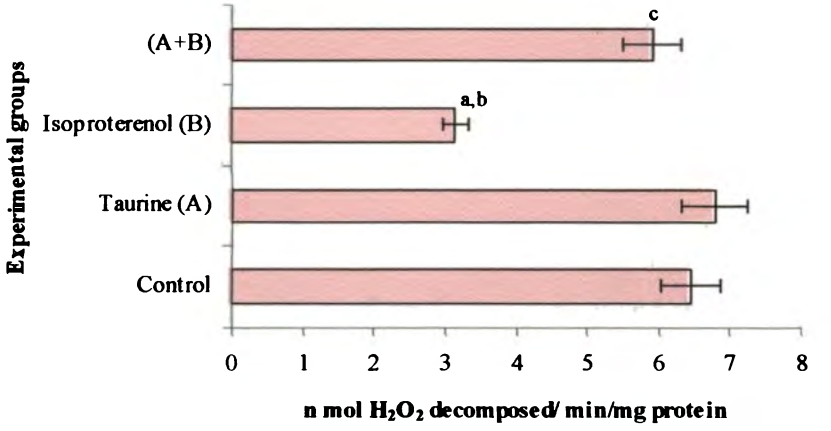


Fig 4.8.12 Activity of catalase (CAT) in heart mitochondria of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.001$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

In the present study, pretreatment with taurine significantly ($p< 0.001$) counteracted the isoproterenol-induced lipid peroxidation and maintained the level of GSH and the activities of GSH-dependent antioxidant enzymes in heart mitochondria at near normalcy compared to that of Group III rats. Taurine is reported to prevent lipid peroxidation and to offer protection against cytotoxicity of different compounds such as hydrazine, carbon tetra chloride and 1, 4-naphthaquinone in isolated rat hepatocytes (Waterfield *et al.*, 1993). You & Chang (1998[b]) reported that taurine inhibited lipid peroxidation, altered the activity of the defense enzymes and protected the liver against membrane disintegration in rat hepatocarcinogenesis. Colell *et al.*, (2001) reported that tauroursodeoxycholic acid protectsed hepatocytes in long-term ethanol-fed rats through modulation of mitochondrial membrane fluidity and subsequent normalization of mitochondrial GSH levels. Reports by Hansen *et al.* (2006) demonstrated the role of taurine as mitochondrial matrix buffer for stabilizing the mitochondrial oxidation. Taurine is reported to increase the GSH content in rat liver and to improve the levels of SOD and CAT in perchloroethylene-induced cytotoxicity in mice (Ebrahim & Sakthisekaran, 1997; Hwang *et al.*, 2000). Present results also confirmed same pattern and showed significant rise in the level of GSH and antioxidant enzymes in heart mitochondria of Group IV rats. The ability of taurine to combat reactive oxygen species and to stabilize glutathione metabolizing system under oxidative stress induced by 6-hydroxydopamine neurotoxicity has already been reported (Hayes *et al.*, 2001). Previous reports showed that supplementation of taurine effectively attenuated the hyperhomocysteinemia-induced reactive oxygen species production and inhibition of Mn-superoxide dismutase and catalase activities in the myocardial mitochondria (Chang *et al.*, 2004[b]).

4.9 Effect of taurine on lysosomal function

Lysosomes are intracytoplasmic organelles defined by an acidic milieu of pH 4.5 and surrounded by a single membrane. Lysosomes contain numerous enzymes allowing the

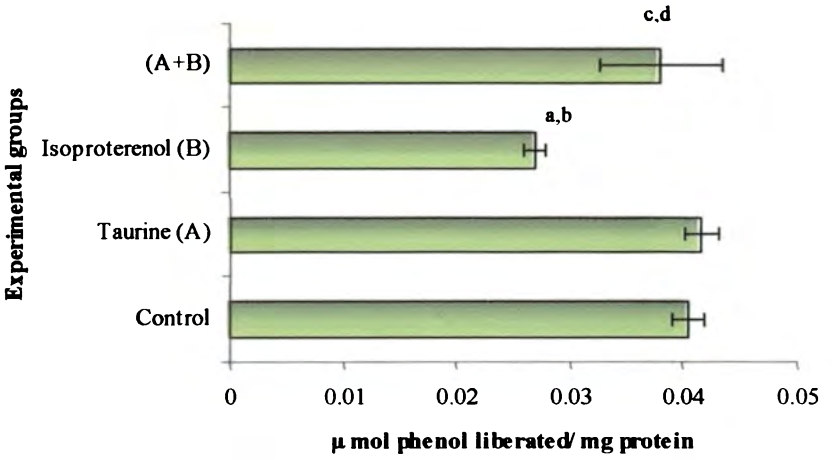


Fig 4.9.1 Activity of acid phosphatase in heart lysosomal fraction of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.01$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

[d] $p<0.05$ significantly different compared with Group I control animals

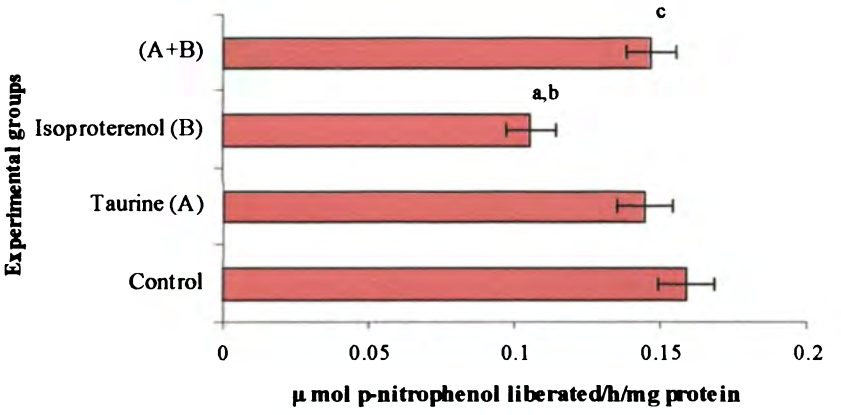


Fig 4.9.2 Activity of β-glucosidase in heart lysosomal fraction of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.01$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

degradation of proteins, nucleic acids, polysaccharides, lipids and their conjugates. Lysosomal hydrolases are also involved in the digestion of foreign material engulfed by phagocytosis or pinocytosis and pathologically in necrosis and other degenerative changes (De duve, 1959).

In the present study, administration of isoproterenol resulted in a significant ($p< 0.001$) reduction in the activities of lysosomal enzymes (acid phosphatase, β-D glucosidase and β-D-galactosidase) in the lysosomal fraction of the heart tissue of Group III rats **(Fig 4.9.1- 4.9.3)** compared to Group I control. This is in agreement with the earlier reports (Ravichandran *et al.*, 1990; Noronha-Dutra *et al.*, 1985; Ebenezar *et al.*, 2003[a]). Isoproterenol administration is known for its enhanced reactive oxygen production and induction of lysosomal enzyme activity changes both *in vivo* and *in vitro*. Alterations in the activities of lysosomal enzymes are believed to be responsible for aberration in the rate of degradation of many tissue components. Lysosomes are organelles particularly vulnerable to oxidative stress since they exhibit the most important pool of reactive iron in the cell (Ollinger & Brunk, 1995). Oxidative stress can induce very fast lysosomal disruption creating intralysosomal iron-mediated redox reactions (Persson *et al.*, 2003). A necrotic cell death can be triggered by a too strong lysosomal membrane permeabilization (Li *et al.*, 2000) and oxygen free radicals are primarily responsible for the release of lysosomal hydrolases. It has also been suggested that abnormal release and activation of lysosomal enzymes during ischaemia and other potentially lethal events may contribute to the tissue damage. Therefore, oxygen free radicals in addition to their direct myocardial damaging effect may also be responsible for the cardiac damage through the release of lysosomal enzymes (Kalra *et al.*, 1989).

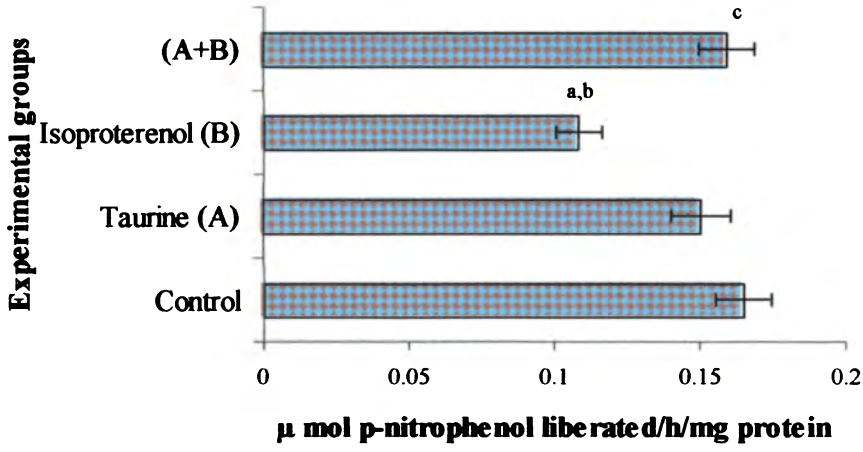


Fig 4.9.3 Activity of β-galactosidase in heart lysosomal fraction of control and experimental groups of rats

(A): Taurine, 100mg kg^{-1} body wt day^{-1}, i.p. for 15 days

(B): Isoproterenol, 11mg $100g^{-1}$ body wt day^{-1}, i.p. for 2 days

Results are mean ± SD for 6 animals; Oneway ANOVA; Tukey's multiple comparison test.

[a] $p<0.001$ significantly different compared with Group I control animals

[b] $p<0.01$ significantly different compared with Group II taurine-administered animals

[c] $p<0.001$ significantly different compared with Group III isoproterenol-induced myocardial infarcted rats

Pretreatment with taurine preserved lysosomal integrity as evidenced by near normal levels of lysosomal hydrolases in heart tissue of Group IV rats compared to Group III isoproterenol-injected rats, indicating the cytoprotective effect of taurine. Antioxidant nature of taurine might have protected the lysosomal membrane from isoproterenol-induced reactive oxygen species and maintained the lysosomal membrane integrity. The ability of taurine to complex with metal ions like iron may further reduce iron-mediated redox reactions induced by lysosomal hydrolases (Pasantes-Morales *et al.*, 1985). Since the release of lysosomal enzymes plays an important role in the progression of myocardial injury, maintaining the stability of lysosomal membrane and hence prevention of release of its contents is a vital concept involved in the mechanism of cardioprotection. Marinelli *et al.* (1997) reported that taurolithocholate inhibited the biliary discharge of lysosomes in the rat without altering the functional integrity of these organelles. According to Vadgama *et al.* (1991), rat liver lysosomes possessed a high Km system for taurine transport which is sensitive to changes in K^+ gradient and valinomycin induced diffusional membrane potential. Pasantes-Morales *et al.* (1985) have examined the membrane stabilizing effect of taurine and indicated that taurine increases cell viability in cultured lymphoblastoid cells.

The results of the present study indicated that the pre-treatment with taurine significantly prevented isoproterenol-induced myocardial infarction in rats. The overall cytoprotective effect of taurine is probably due to its membrane stabilizing action by maintenance of calcium homeostasis and osmoregulation or to an inhibition of lipid accumulation by its hypolipidemic property, or to a counteraction of free radicals by its antioxidant nature, or to its ability to maintain near to normal status, the activities of the free radical scavenging enzymes and the level of reduced glutathione, which protect myocardial membrane against peroxidative damage by decreasing lipid peroxidation.

Summary and Conclusion

5. SUMMARY AND CONCLUSION

Despite wide distribution in animal kingdom and high concentration in tissues, taurine remains a mysterious substance, very little being known about its physiology and biochemistry. In the present investigation, an attempt has been made to examine the cardioprotective effects of taurine against isoproterenol-induced myocardial infarction in rats, an animal model for myocardial infarction of human beings, by virtue of its cytoprotective, hypolipidemic, antioxidant and membrane stabilizing properties. The salient features of the study are summed up below,

1. Isoproterenol-induced myocardial infarction was manifested by a significant increase in the levels of diagnostic markers such as, marker enzymes (AST, ALT, LDH, CPK, ACP and ALP), troponin T and homocysteine in plasma. The histopathological observation of heart tissue also confirmed the necrotic damage induced by isoproterenol. Pretreatment with taurine for 15 days (100mg kg^{-1} body wt day^{-1}, i. p.) significantly prevented the isoproterenol-induced elevation in the levels of these diagnostic markers in plasma and maintained the normal architecture of heart tissue evidenced by histopathological studies, indicating the cytoprotective activity of taurine.

2. Administration of isoproterenol significantly decreased the level of proteins and glycoproteins in plasma and in the heart tissue of experimental animals. Oxidation of protein is a common phenomenon mediated by highly reactive agents in myocardial infarction condition and oxidized proteins are in turn capable of inducing oxidative stress, a potential mediator of the pathogenesis. This protein oxidation might also be a possible reason for the decline noted in the protein and glycoprotein levels in the heart tissue of isoproterenol-injected rats. The disaggregation of polyribosomes might be

associated with the inhibition of protein synthesis and the decreased protein synthesis in turn might have lead to reduced glycoprotein synthesis. Pretreatment with taurine resulted in near normal levels of protein and glycoprotein components. It probably did so by preventing the isoproterenol-induced necrotic damage to the myocardial cell membrane by inhibiting the disaggregation of polyribosomes or by attenuating the isoproterenol-induced oxidation of myocardial proteins.

3. Significant reduction was observed in the level of taurine, aspartate and glutamate with a concomitant increase in arginine content in the heart tissue of isoproterenol administered rats compared to that of normal controls. Supplementation of taurine prevented these isoproterenol-induced alterations in free amino acids. It preserved the myocardial taurine content for other biological functions by its uptake probably mediated through taurine transporters. It also blocked the availability of NO required for the formation of peroxynitrite radicals probably by inhibiting the nitric oxide synthase enzyme. It prevented isoproterenol-induced alterations noted in the levels of aspartate and glutamate probably by its involvement in cell volume regulation and Na dependent transport.

4. The major disorder encountered in isoproterenol-induced myocardial infarction is fat accumulation in the heart. The levels of total cholesterol and LDL-cholesterol, apolipoprotein B, lipoprotein (a) were significantly elevated in isoproterenol-administered rats, indicating the isoproterenol-induced hypercholesterolemic condition. This was paralleled by a decline in the level of HDL-cholesterol and apolipoprotein A1. Also the levels of triglycerides and free fatty acids were significantly increased in experimentally induced myocardial infarction. But significant reduction in the level of phospholipids was observed, which might be due

to increased necrotic damage to the myocardial membrane. Taurine pretreatment reversed the isoproterenol-induced alterations in the levels of cholesterol, triglycerides, free fatty acids and phospholipids in the experimental group and maintained their levels at near normalcy. It probably did so by its involvement in bile acid conjugation, and indicated the antilipidemic nature of taurine.

5. The intraperitoneal administration of isoproterenol significantly altered the fatty acid composition in the myocardial infarcted rats. There was a significant change in the distribution of saturated, monounsaturated and polyunsaturated fatty acid in isoproterenol-injected rats compared to control rats. Prior supplementation with taurine significantly prevented the isoproterenol-induced alterations in the fatty acid composition probably by protecting the myocardium from isoproterenol-induced free radicals indicating its antilipid peroxidative property.

6. Isoproterenol induced significant rise in the level of lipid peroxides in plasma and heart tissue, which was paralleled by a significant decrease in the level of reduced glutathione content and a decrease in the activities of glutathione dependent antioxidant enzymes (GPx and GST) and antiperoxidative enzymes (CAT and SOD) in the heart tissue as compared with the activities of the control rats. Prior supplementation with taurine significantly prevented the isoproterenol-induced alterations in the tissue defense system and maintained the antioxidant status at near normal level, establishing the antioxidant nature of taurine. The overall antioxidant effect of taurine might be due to the increase in reduced glutathione synthesis and hence increased activities of the free radical scavenging enzymes or to a counteraction of the free radicals by its antioxidant nature.

7. Significant reduction noticed in the total sulfhydryl, protein bound sulfhydryl and non protein bound sulfhydryl content and decrease in the activities of membrane-bound ATPases (Na^+, K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase) in isoproterenol-induced myocardial infarction, indicated a severe derangement of subcellular metabolism and structural alterations of cardiac cell membranes. Administration of isoproterenol significantly altered the mineral metabolism. This might be due to increased lipid peroxidative damage of cell membranes. In the present study, taurine administration significantly prevented the isoproterenol-induced alterations in the activities of membrane-bound ATPases and maintained the levels of minerals and sulfhydryl content at near normal levels by its membrane stabilizing property.

8. Significant reduction was observed in the activities of TCA-cycle enzymes, respiratory marker enzymes and ATP content in the heart mitochondria. Mitochondrial antioxidant system was also found to be operating in diminished manner in isoproterenol-induced condition reflecting an increased lipid peroxidation, reduction in mitochondrial-reduced glutathione, glutathione dependent antiperoxidative enzymes and antioxidant enzymes. Taurine pretreatment prevented these adverse changes induced by isoproterenol, which was probably due to its stabilization of mitochondrial membrane in isoproterenol-induced myocardial infarction, or by its antioxidant capacity against isoproterenol induced lipid peroxidation, which is mainly responsible for the inhibition of both oxidation and phosphorelation. The ability of taurine to counteract free radicals induced by isoproterenol, offers significant protection against mitochondrial oxidative damage. Hence, it is possible that stabilization of the mitochondrial membranes by taurine may prolong the viability of ischemic cardiac muscle from isoproterenol-induced peroxidative damage.

9. Isoproterenol administration to rats resulted in decreased stability of the membranes, which was reflected by the lowered activity of heart lysosomal hydrolases in the lysosomal fraction of heart tissue indicating the severity of myocardial infarction. Pretreatment with taurine significantly inhibited the isoproterenol-induced release of these hydrolytic enzymes from the lysosomes. This might be due to the stabilizing effect of taurine on the lysosomal membranes. Since the release of lysosomal enzymes is crucial in the pathogenesis of ischemic myocardial injury and related inflammation process, it is likely that the reduction of such enzyme release would prove beneficial which confirms the beneficial effect of taurine.

In conclusion, the results of the present study indicate that prior administration of taurine is effective in minimizing all the deleterious effects induced by isoproterenol, thereby justifying its use as a potent cytoprotective agent. The overall cardioprotective effect of taurine is probably related to its antioxidant property evidenced by its ability to reduce lipid peroxidation and to maintain the activities of free radical enzymes and non-enzymatic antioxidants, its membrane stabilizing action and to its hypolipidemic property.

References

6. REFERENCES

Acikel M, Buyukokuroglu ME, Aksoy H, Erdogan F, Erol MK. Protective effects of melatonin against myocardial injury induced by isoproterenol in rats. J Pineal Res. 2003; 35: 75-79.

Acikel M, Buyukokuroglu ME, Erdogan F, Aksoy H, Bozkurt E, Senocak H. Protective effects of dantrolene against myocardial injury induced by isoproterenol in rats: biochemical and histological findings. Int J Cardiol. 2005; 98: 389-394.

Adhirai M, Selvam R. Effect of cyclosporin A on tissue lipid peroxidation and membrane bound phosphatases in hyperoxaluric rat and the protection by vitamin E pretreatment. Jpn J Med Sci Biol. 1997; 50: 9-17.

Aghi M, Husain Z, Pillai KK, Qadry JS, Balani DK, Imran M. Cardioprotective effect of cromakalim (potassium channel opener) in isoproterenol induced myocardial infarction in rats.Indian J Exp Biol. 1992; 30: 611-614.

Ahmed KK, Rana AC, Dixit VK. Effect of Calotropis procera latex on isoproterenol induced myocardial infarction in albino rats. Phytomedicine. 2004; 11: 327-330.

Al Makdessi S, Andrieu JL, Herilier H, Faucon G. Effect of isoproterenol on the metabolism of myocardial fatty acids. J Mol Cell Cardiol. 1987; 19: 141-149.

Al Makdessi S, Sweidan H, Jacob R. n-3 versus n-6 fatty acid incorporation into the phospholipids of rat heart sarcolemma. A comparative study of four different oil diets. J Mol Cell Cardiol. 1994; 26: 23-29.

Albrecht J. Roles of neuroactive amino acids in ammonia neurotoxicity. J Neurosci Res. 1998; 51: 133-138.

Allen MJ, Lowry RW. Successful Reversal of Retinitis Pigmentosa. J Orthomol Med. 1998; 13: 41-44.

Alliende C, Esponda P. Nucleolar changes induced by isoproterenol in mouse acinar parotid cells. J Submicrosc Cytol Pathol. 1988; 20: 67-72.

Alvarez JG, Storey BT. Taurine, hypotaurine, epinephrine and albumin inhibit lipid peroxidation in rabbit spermatozoa and protect against loss of motility. Biol Reprod. 1983; 29: 548-555.

Alvaro-Gonzalez LC, Freijo-Guerrero MM, Sadaba-Garay F. Inflammatory mechanisms, arteriosclerosis and ischemic stroke: clinical data and perspectives. Rev Neurol. 2002; 35: 452-462.

Amann S, Reinke C, Valet G, Moser U, Leuenberger H. Flow-cytometric investigation of cellular metabolism during oxidative stress and the effect of tocopherol. Int J Vitam Nutr Res. 1999; 69: 356-361.

Anandan R, Asha KK, Ammu K, Mathew S, Nair PGV: Effects of peroxidised PUFA on tissue defense system in experimentally induced myocardial infarction in rats. In: Seafood Safety (Surendran PK, Mathew PT, Thampuran N, Nambiar N, Joseph J, Boopendranath MR, Lakshmanan PT, Nair PGV, eds.), Society of Fisheries Technologists, Cochin, India. 2003; 330-335.

Andersson BS, Rundgren M, Nelson SD, Harder S. N-acetyl-p-benzoquinone imine-induced changes in the energy metabolism in hepatocytes. Chem Biol Interact. 1990; 75: 201-211.

Angles-Cano E, de la Pena Diaz A, Loyau S. Inhibition of fibrinolysis by lipoprotein(a). Ann N Y Acad Sci. 2001; 936: 261-275.

AOAC. Atomic absorption methods of fish. Official Methods of Analysis of the Association of Official Analytical Chemists. 13th edition , Washington, DC. 1980; 399.

AOAC. Fatty acid analysis. Official methods of Analysis of the Association of Official Analytical chemists. 12th Edn. Washington, D.C. 1975; 497-500.

Arstall MA, Sawyer DB, Fukazawa R, Kelly RA. Cytokine-mediated apoptosis in cardiac myocytes: the role of inducible nitric oxide synthase induction and peroxynitrite generation. Circ Res. 1999; 85: 829-840.

Aruoma OI, Halliwell B, Hoey BM, Butler J. The antioxidant action of taurine, hypotaurine and their metabolic precursors. Biochem J. 1988; 256: 251-255.

Atmaca G. Antioxidant effects of sulfur-containing amino acids. Yonsei Med J. 2004; 45: 776-788.

Backstrom T, Goiny M, Lockowandt U, Liska J, Franco-Cereceda A. Cardiac outflow of amino acids and purines during myocardial ischemia and reperfusion. J Appl Physiol. 2003; 94: 1122-1128.

Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, Joshi SS, Pruess HG. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology. 2000; 148: 187-197.

Balkan J, Parldar FH, Dogru-Abbasoglu S, Aykac-Toker G, Uysal M. The effect of taurine or betaine pretreatment on hepatotoxicity and prooxidant status induced by lipopolysaccharide treatment in the liver of rats. Eur J Gastroenterol Hepatol. 2005; 17: 917-21.

Banks MA, Porter DW, Pailes WH, Schwegler-Berry D, Martin WG, Castranova V. Taurine content of isolated rat alveolar type I cells. Comp Biochem Physiol B. 1991; 100: 795-799.

Bantseev V, Bhardwaj R, Rathbun W, Nagasawa H, Trevithick JR. Antioxidants and cataract: (cataract induction in space environment and application to terrestrial aging cataract). Biochem Mol Biol Int. 1997; 42: 1189-1197.

Barada KA, Atallah JB, Nassar CF. Age influence on intestinal taurine transport in mice. Comp Biochem Physiol A Physiol. 1997; 118: 159-163.

Barnett PA, Spence JD, Manuck SB, Jennings JR. Psychological stress and the progression of carotid artery disease. J Hypertens. 1997; 15: 49-55.

Barter P, Kastelein J, Nunn A, Hobbs R. High density lipoproteins (HDLs) and atherosclerosis; the unanswered questions. Atherosclerosis. 2003; 168: 195-211.

Bartlett GR. Phosphorus assay in column chromatography. J Biol Chem. 1959; 234: 466-468.

Barua M, Liu Y, Quinn MR. Taurine chloramine inhibits inducible nitric oxide synthase and TNF-alpha gene expression in activated alveolar macrophages: decreased NF-kappaB activation and IkappaB kinase activity. J Immunol. 2001; 167: 2275-2281.

Bauersachs J, Fraccarollo D. Aldosterone antagonism in addition to angiotensin-converting enzyme inhibitors in heart failure. Minerva Cardioangiol. 2003; 51: 155-164.

Bell JL, Baron DN. A colorimetric method for determination of Isocitate dehydrogenase. 1960; Clin Chim Acta. 5: 740.

Belli DC, Levy E, Darling P, Leroy C, Lepage G, Giguere R, Roy CC. Taurine improves the absorption of a fat meal in patients with cystic fibrosis. Pediatrics. 1987; 80: 517-523.

Benjamin IJ, Jalil JE, Tan LB, Cho K, Weber KT, Clark WA. Isoproterenol-induced myocardial fibrosis in relation to myocyte necrosis. Circ Res. 1989; 65: 657-670.

Berlett BS, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. J Biol Chem. 1997; 272: 20313-20316.

Bernacca GM, Wheatley DJ. Surface modification of polyurethane heart valves: effects on fatigue life and calcification. Int J Artif Organs. 1998; 21: 814-819.

Berwanger CS, Jeremy JY, Stansby G. Homocysteine and vascular disease. Br J Surg. 1995; 82: 726-731.

Birdsall TC. Therapeutic applications of taurine. Altern Med Rev. 1998; 3: 128-136.

Bkaily G, Haddad G, Jaalouk D, Gros-Louis N, Benchekroun MT, Naik R, Pothier P, D'Orleans-Juste P, Bui M, Wang S, Sperelakis N. Modulation of Ca2+ and Na+ transport by taurine in heart and vascular smooth muscle. Adv Exp Med Biol. 1996; 403: 263-273.

Blasig IE, Blasig R, Lowe H. Myocardial lipid peroxidation during isoproterenol-induced blood flow reduction in rat myocardium. Biomed Biochim Acta. 43: S171-S174.

Bobkova D, Poledne R. Lipid metabolism in atherogenesis. Cesk Fysiol. 2003; 52: 34-41.

Bonting SL. Sodium-Potassium Activated Adenosinetriphosphate and Cation Transport. In: Bittar, E.E. (Ed.); Wiley-Interscience, New York. 1970; 257-363.

Braverman ER. The Healing Nutrition Within (3rd ed.). Basic Health Publications, 8200 Blvd. E, North Bergen,New Jersey 07047 USA. 2003.

Brembilla-Perrot B, Terrier de la Chaise A, le Van D, Beurrier D. Effect of isoproterenol on serum potassium and magnesium. Eur Heart J. 1993; 14: 677-681.

Brezinski DA, Tofler GH, Muller JE, Pohjola-Sintonen S, Willich SN, Schafer AI, Czeisler CA, Williams GH. Morning increase in platelet aggregability. Association with assumption of the upright posture. Circulation. 1988; 78: 35-40.

Brown GC. Control of respiration and ATP synthesis in mammalian mitochondria and cells. Biochem J. 1992; 284: 1-13.

Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science. 1986; 232: 34-47.

Buerke M, Rupprecht HJ. Antithrombotic therapy in acute coronary syndromes. EXS. 2000; 89: 193-209.

Buring JE, O'Connor GT, Goldhaber SZ, Rosner B, Herbert PN, Blum CB, Breslow JL, Hennekens CH. Decreased HDL2 and HDL3 cholesterol, Apo A-I and Apo A-II, and increased risk of myocardial infarction. Circulation. 1992; 85: 22-29.

Burstein M, Scholnick HR. Precipitaion of chylomicrons and very low density lipoproteins from human serum with sodium lauryl sulphate. Life Sci. 1972; 11: 177-184.

Burton KP, McCord JM, Ghai G. Myocardial alterations due to free-radical generation. Am J Physiol. 1984; 246: H776-H783.

Buyukuysal RL. Ischemia and reoxygenation induced amino acid release and tissue damage in the slices of rat corpus striatum. Amino Acids. 2004; 27: 57-67.

Calva E, Mujica A, Nunez R, Aoki K, Bisteni A, Sodi-Pallares D. Mitochondrial biochemical changes and glucose-KCl-insulin solution in cardiac infarct. Am J Physiol. 1966; 211: 71-76.

Cao G, Giovanoni M, Prior RL. Antioxidant capacity of different tissues of young and old rats. Proc Soc Exp Biol Med. 1996; 211: 359-365.

Caplice NM, Panetta C, Peterson TE, Kleppe LS, Mueske CS, Kostner GM, Broze GJ Jr, Simari RD. Lipoprotein (a) binds and inactivates tissue factor pathway inhibitor: a novel link between lipoproteins and thrombosis. Blood. 2001; 98: 2980-2987.

Capozza G, Muolo L, Jirillo E, Guerrieri F. Isoproterenol causes changes in the mitochondrial energy metabolism in the rat heart. Cardiologia. 1992; 37: 663-665.

Casey RG, Gang C, Joyce M, Bouchier-Hayes DJ. Taurine Attenuates Acute Hyperglycaemia-Induced Endothelial Cell Apoptosis, Leucocyte-Endothelial Cell Interactions and Cardiac Dysfunction. J Vasc Res. 2006; 44: 31-39.

Castelli WP. Cholesterol and lipids in the risk of coronary artery disease- the Framingham Heart study. Can J Cardiol. 1988; 4: A: 5A-10A.

Cebelin MS, Hirsch CS. Human stress cardiomyopathy. Myocardial lesions in victims of homicidal assaults without internal injuries. Hum Pathol. 1980; 11: 123-132.

Cetiner M, Sener G, Sehirli AO, Eksioglu-Demiralp E, Ercan F, Sirvanci S, Gedik N, Akpulat S, Tecimer T, Yegen BC. Taurine protects against methotrexate-induced toxicity and inhibits leukocyte death. Toxicol Appl Pharmacol. 2005; 209: 39-50.

Chagoya de Sanchez V, Hernandez-Munoz R, Lopez-Barrera F, Yanez L, Vidrio S, Suarez J, Cota-Garza MD, Aranda-Fraustro A, Cruz D. Sequential changes of energy metabolism and mitochondrial function in myocardial infarction induced by isoproterenol in rats: a long-term and integrative study. Can J Physiol Pharmacol. 1997; 75: 1300-1311.

Chang L, Xu J, Yu F, Zhao J, Tang X, Tang C. Taurine protected myocardial mitochondria injury induced by hyperhomocysteinemia in rats. Amino Acids. 2004[b]; 27: 37-48.

Chang L, Xu JX, Zhao J, Pang YZ, Tang CS, Qi YF. Taurine antagonized oxidative stress injury induced by homocysteine in rat vascular smooth muscle cells. Acta Pharmacol Sin. 2004[a]; 25: 341-346.

Chattopadhyay A, Biswas S, Bandyopadhyay D, Sarkar C, Datta AG. Effect of isoproterenol on lipid peroxidation and antioxidant enzymes of myocardial tissue of mice and protection by quinidine. Mol Cell Biochem. 2003; 245: 43-49.

Chen W, Matuda K, Nishimura N, Yokogoshi H. The effect of taurine on cholesterol degradation in mice fed a high-cholesterol diet. Life Sci. 2004; 74: 1889-1898.

Chepkova AN, Sergeeva OA, Haas HL. Taurine rescues hippocampal long-term potentiation from ammonia-induced impairment. Neurobiol Dis. 2006; 23: 512-521.

Cherif H, Reusens B, Ahn MT, Hoet JJ, Remacle C. Effects of taurine on the insulin secretion of rat fetal islets from dams fed a low-protein diet. J Endocrinol. 1998; 159: 341-348.

Chesney RW, Helms RA, Christensen M, Budreau AM, Han X, Sturman J A. The role of taurine in infant nutrition. Adv Exp Med Biol 1998; 442: 463-476.

Chiba Y, Ando K, Fujita T. The protective effects of taurine against renal damage by salt loading in Dahl salt-sensitive rats. J Hypertens. 2002; 20: 2269-2274.

Chien, KR, Abrams J, Serroni A, Martin JT, Farber JL. Accelerated phospholipid degradation and associated membrane dysfunction in irreversible, ischemic liver cell injury. J Biol Chem. 1978; 253: 4809-4817.

Chovan JP, Kulakowski EC, Benson BW, Schaffer SW. Taurine enhancement of calcium binding to rat heart sarcolemma. Biochim Biophys Acta. 1979; 551: 129-135.

Christensen B, Refsum H, Vintermyr O, Ueland PM. Homocysteine export from cells cultured in the presence of physiological or superfluous levels of methionine: methionine loading of non-transformed, transformed, proliferating, and quiescent cells in culture. J Cell Physiol. 1991; 146: 52-62.

Colell A, Coll O, Garcia-Ruiz C, Paris R, Tiribelli C, Kaplowitz N, Fernandez-Checa JC. Tauroursodeoxycholic acid protects hepatocytes from ethanol-fed rats against tumor necrosis factor-induced cell death by replenishing mitochondrial glutathione. Hepatology. 2001; 34: 964-71.

Collarini EJ, Oxender DL. Mechanisms of transport of amino acids across membranes. Annu Rev Nutr. 1987; 7: 75-90.

Collinson PO, Stubbs PJ, Kessler AC. Multicentre evaluation of the diagnostic value of cardiac troponin T, CK-MB mass, and myoglobin for assessing patients with suspected acute coronary syndromes in routine clinical practice. Heart. 2003; 89: 280-286.

Colombo C, Battezzati PM, Podda M, Bettinardi N, Giunta A.Ursodeoxycholic acid for liver disease associated with cystic fibrosis: a double-blind multicenter trial. Hepatology. 1996; 23: 1484-1490.

Conchie J, Gelman AL, Levvy GA. Inhibition of glycosidases by aldonolactones of corresponding configuration. The C-4 and C-6 specificity of beta glucosidase and beta galactosidase. Biochem J. 1967; 103: 609-615.

Condron CM, Toomey DM, Casey RG, Creagh T, Bouchier-Hayes DJ. Taurine protects against PMN dysfunction and death in urine. Urol Res. 2004; 32: 338-345.

Cortinovis C, Klimek F, Nogueira E. Adenylate cyclase activity in microdissected rat liver tissue: periportal to pericentral activity gradient. Hepatology. 1993; 18: 160-164.

Csernansky JG, Bardgett ME, Sheline YI, Morris JC, Olney JW. CSF excitatory amino acids and severity of illness in Alzheimer's disease. Neurology. 1996; 46: 1715-1720.

Curti C, Uyemura SA, Grecchi MJ, Leone FA. Kinetic properties of mitochondrial ATPase during isoproterenol-induced cardiomyopathy. Int J Biochem. 1990; 22: 611-615.

Dawson R Jr, Biasetti M, Messina S, Dominy J. The cytoprotective role of taurine in exercise-induced muscle injury. Amino Acids. 2002; 22: 309-324.

Dawson R Jr, Pelleymounter MA, Cullen NJ, Gollub M, Liu S. An age-related decline in striatal taurine is correlated with a loss of dopaminergic markers. Brain Res Bull. 1999; 48: 319-24

De Duve C. The Cytochemistry of Enzymes and Antigens: The function of intracellular hydrolases. Experimental Cell Research. 1959; 7: 169-182.

Degim Z, Celebi N, Sayan H, Babul A, Erdogan D, Take G. An investigation on skin wound healing in mice with a taurine-chitosan gel formulation. AminoAcids. 2002; 22: 187-198.

Demarcay H. Ueber die Natur der Galle. Annalen der Pharmacie. 1838; 27: 271-291.

Dhalla KS, Rupp H, Beamish RE, Dhalla NS. Mechanisms of alterations in cardiac membrane calcium transport due to excess catecholamines. Cardiovasc Drugs Ther. 1996; 1: 231-238.

Dhillon SK, Davies WE, Hopkins PC, Rose SJ. Effects of dietary taurine on auditory function in full-term infants. Adv Exp Med Biol. 1998; 442: 507-514.

Di Leo MA, Ghirlanda G, Gentiloni Silveri N, Giardina B, Franconi F, Santini SA. Potential therapeutic effect of antioxidants in experimental diabetic retina: a comparison between chronic taurine and vitamin E plus selenium supplementations. Free Radic Res. 2003; 37: 323-330.

Ding W, Li D, Zhang J. Influences of taurine on thrombolysis. Zhonghua Nei Ke Za Zhi. 1996; 35: 378-381.

Djandjighian L, Planchenault J, Finance O, Pastor G, Gautier P, Nisato D. Hemodynamic and antiadrenergic effects of dronedarone and amiodarone in animals with a healed myocardial infarction. J Cardiovasc Pharmacol. 2000; 36: 376-383.

Dogru-Abbasoglu S, Kanbagli O, Balkan J, Cevikbas U, Aykac-Toker G, Uysal M. The protective effect of taurine against thioacetamide hepatotoxicity of rats. Hum Exp Toxicol. 2001; 20: 23-27.

Dohovics R, Janaky R, Varga V, Hermann A, Saransaari P, Oja SS. Regulation of glutamatergic neurotransmission in the striatum by presynaptic adenylyl cyclase-dependent processes. Neurochem Int. 2003; 42: 1-7.

Dorian P, Dunnmon P, Elstun L, Newman D. The effect of isoproterenol on the class III effect of Azimilide in Humans. J Cardiovasc Pharmacol Ther. 2002; 7: 211-217.

Dudnakova TV, Lakomkin VL, Tsyplenkova VG, Shekhonin BV, Shirinskii VP, Kapel'ko VI. Isoproterenol induced changes of protein expression and myocardial ultra structure. Kardiologiia. 2002; 42: 57-63.

Dumelin EE, Tappel AL. Hydrocarbon gases produced during in vitro peroxidation of polyunsaturated fatty acids and decomposition of preformed hydroperoxides. Lipids. 1977; 12: 894-900.

Duncan JJ, Farr JE, Upton SJ, Hagan RD, Oglesby ME, Blair SN. The effects of aerobic exercise on plasma catecholamines and blood pressure in patients with mild essential hypertension. JAMA. 1985; 254: 2609-2613.

Ebenezar KK, Sathish V, Devaki T. Effect of arginine and lysine on mitochondrial function during isoproterenol induced myocardial infarction in rats. Nutrition Research. 2003[b]; 23: 1417-1425.

Ebenezar KK, Sathish V, Devaki T. Effect of L-arginine and L-lysine on lysosomal hydrolases and membrane bound phosphatases in experimentally induced myocardial infarction in rats. Mol Cell Biochem. 2003[a]; 247: 163-169.

Ebrahim AS, Sakthisekaran D. Effect of vitamin E and taurine treatment on lipid peroxidation and antioxidant defense in perchloroethylene-induced cytotoxicity in mice. Nutr Biochem. 1997; 8: 270-274.

Eby G, Halcomb WW. Elimination of cardiac arrhythmias using oral taurine with L-arginine with case histories: Hypothesis for nitric oxide stabilization of the sinus node. Med Hypotheses. 2006; 67: 1200-1204.

Echtay KS, Murphy MP, Smith RA, Talbot DA, Brand MD. Superoxide activates mitochondrial uncoupling protein 2 from the matrix side. Studies using targeted antioxidants. J Biol Chem. 2002; 277: 47129-47135.

El Idrissi A, Trenkner E. Taurine regulates mitochondrial calcium homeostasis. Adv Exp Med Biol. 2003; 526: 527-536.

El Idrissi A, Trenkner E. Taurine as a modulator of excitatory and inhibitory neurotransmission. Neurochem Res. 2004; 29: 189-197.

El Idrissi A. Taurine increases mitochondrial buffering of calcium: role in neuroprotection. Amino Acids. 2006; Sep 8.

Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82: 70-77.

Elzinga BM, Baller JF, Mensenkamp AR, Yao Z, Agellon LB, Kuipers F, Verkade HJ. Inhibition of apolipoprotein B secretion by taurocholate is controlled by the N-terminal end of the protein in rat hepatoma McArdle-RH7777 cells. Biochim Biophys Acta. 2003; 1635: 93-103.

Endo Y, Hanada K, Miyake M, Ogawara K, Higaki K, Kimura T. Mechanisms of cytoprotective effect of amino acids on local toxicity caused by sodium laurate, a drug absorption enhancer, in intestinal epithelium. J Pharm Sci. 2002; 91: 730-743.

Eppler B, Dawson R Jr. Cysteine sulfinate decarboxylase and cysteine dioxygenase activities do not correlate with strain-specific changes in hepatic and cerebellar taurine content in aged rats. Mech Ageing Dev. 1999; 110: 57-72.

Fang J, Alderman MH. Serum uric acid and cardiovascular mortality the NHANES I epidemiologic follow-up study, 1971-1992. National Health and Nutrition Examination Survey. JAMA. 283: 2404-2410.

Farber JL, Young EE. Accelerated phospholipid degradation in anoxic rat hepatocytes. Arch Biochem Biophys. 1981; 211: 312-320.

Farriol M, Venereo Y, Rossello J, Gomez P, Palao R, Orta X, Segovia-Silvestre T. Effects of taurine on polymorphonuclear phagocytosis activity in burned patients. Amino Acids. 2002; 23: 441-445.

Farvin KHS, Anandan R, Kumar SHS, Shiny KS, Mathew S, Sankar TV, Nair PGV. Cardioprotective effect of squalene on lipid profile in isoprenaline-induced myocardial infarction in rats. J Med Food. 2006; 9: 531-6.

Farvin KHS, Anandan R, Sankar TV, Nair PGV. Cardioprotective effect of squalene against isoproterenol-induced myocardial infarction in rats. J Clin Biochem Nutr. 2005; 37: 55-60.

Farvin KHS, Anandan R, Kumar SHS, Shiny KS, Sankar TV, Thankappan TK. Effect of squalene on tissue defence system in isoproterenol-induced myocardial infarction in rats. Pharmacol. Res. 2004; 50: 231–236.

Fein FS, Sonnenblick EH. Diabetic cardiomyopathy. Cardiovasc Drugs Ther. 1994; 8: 65-73.

Fernandez-Checa JC, Kaplowitz N, Garcia-Ruiz C, Colell A, Miranda M, Mari M, Ardite E, Morales A. GSH transport in mitochondria: defense against TNF-induced oxidative stress and alcohol-induced defect. Am J Physiol. 1997; 273: G7-G17.

Feuer L, Fekete M, Kadar T, Telegdy G. Effect of intraventricular administration of glutaurine on norepinephrine, dopamine and serotonin turnover in different brain regions in rats. Acta Physiol Hung. 1983; 61: 163-167.

Field FJ, Born E, Chen H, Murthy S, Mathur SN. Regulation of apolipoprotein B secretion by biliary lipids in CaCo-2 cells. J Lipid Res. 1994; 35: 749-762.

Finnegan NM, Redmond HP, Bouchier-Hayes DJ. Taurine attenuates recombinant interleukin-2-activated, lymphocyte-mediated endothelial cell injury. Cancer. 1998; 82: 186-199.

Fiske CH, Subbarow Y. The colorimetric determination of phosphorus. J. Biol Chem. 1925; 66: 375-400.

Fletcher EL, Kalloniatis M. Neurochemical development o the degenerating rat retina. J Comp Neurol. 1997; 388: 1-22.

Folch J, Lees M, Stanely GHS. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 1957; 226: 497-509.

Ford ES. Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults. Epidemiology. 2002; 13: 561-568.

Franconi F, Bennardini F, Mattana A, Miceli M, Ciuti M, Mian M, Gironi A, Anichini R, Seghieri G. Plasma and platelet taurine are reduced in subjects with insulin-dependent diabetes mellitus: effects of taurine supplementation. Am J Clin Nutr. 1995; 61: 1115-1119.

Frank S, Durovic S, Kostner K, Kostner GM. Inhibitors for the in vitro assembly of Lp(a). Arterioscler Thromb Vasc Biol. 1995; 15: 1774-1780.

Fredrickson DS. The role of lipids in acute myocardial infarction. Circulation. 1969; 39: IV99- IV111.

Freedman AM, Kramer JH, Mak IT, Cassidy MM, Weglicki WB. Propranolol preserves ultrastructure in adult cardiocytes exposed to anoxia/reoxygenation: a morphometric analysis. Free Radic Biol Med. 1991; 11: 197-206.

Freedman DS, Gruchow HW, Anderson AJ, Rimm AA, Barboriak JJ. Relation of triglyceride levels to coronary artery disease: the Milwaukee Cardiovascular Data Registry. Am J Epidemiol. 1988; 127: 1118-1130.

Fukuda K. Molecular characterization of regenerated cardiomyocytes derived from adult mesenchymal stem cells. Congenit Anom (Kyoto). 2002; 42: 1-9.

Garg A, Grundy SM. Management of dyslipidemia in NIDDM. Diabetes Care. 1990; 13: 153-169.

Garner WH, Garner MH, Spector A. H2O2-induced uncoupling of bovine lens Na^+K^+ ATPase. Proc Natl Acad Sci U S A. 1983; 80: 2044-2048.

Gaull GE, Pasantes-Morales H, Wright CE. Taurine in human nutrition: overview. Prog Clin Biol Res. 1985; 179: 3-21.

Gaull GE. Taurine in pediatric nutrition: review and update. Pediatrics. 1989; 83: 433-442.

Geng B, Chang L, Du JB, Tang CS. A new strategy to treat hyperhomocysteinemia. Beijing Da Xue Xue Bao. 2005; 37: 215-219.

Gersh BJ. The changing prognosis of myocardial infarction in the reperfusion era: implications for evaluation and management of ventricular arrhythmias. Rev Esp Cardiol. 2003; 56: 535-542.

Giri SN, Blaisdell R, Rucker RB, Wang Q, Hyde DM. Amelioration of bleomycin-induced lung fibrosis in hamsters by dietary supplementation with taurine and niacin: biochemical mechanisms. Environ Health Perspect. 1994; 10: 137-147.

Giri SN, Wang Q. Taurine and niacin offer a novel therapeutic modality in prevention of chemically-induced pulmonary fibrosis in hamsters. Adv Exp Med Biol. 1992; 315: 329-340.

Goldstein L, Davis EM. Taurine, betaine, and inositol share a volume-sensitive transporter in skate erythrocyte cell membrane. Am J Physiol. 1994; 267: R426-R431.

Gordon RE, Park E, Laskin D, Schuller-Levis GB. Taurine protects rat bronchioles from acute ozone exposure: a freeze fracture and electron microscopic study. Exp Lung Res. 1998; 24: 659-674.

Gottlieb SS, McCarter RJ. Comparative effects of three beta blockers (atenolol, metoprolol, and propranolol) on survival after acute myocardial infarction. Am J Cardiol. 2001; 87: 823-826.

Gotto AM Jr, Assmann G, Carmena R, Davignon J, Fruchart JC, Kastelein JJP, Paoletti R, Tonkin A. The ILIB lipid handbook for clinical practice: blood lipids and coronary heart disease, International Lipid Information Bureau. 2nd edn. NewYork. 2000.

Griffin BA, Freeman DJ, Tait GW, Thomson J, Caslake MJ, Packard CJ, Shepherd J. Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small, dense LDL to coronary heart disease risk. Atherosclerosis. 1994; 106: 241-253.

Grundy SM. Cholesterol and coronary heart disease. A new era. JAMA. 1986; 256: 2849-2858.

Gudbjarnason S, Braasch W, Cowan C, Bing RJ. Metabolism of infarcted heart muscle during tissue repair. Am J Cardiol. 1968; 22: 360-369.

Gudbjarnason S. Dynamics of n-3 and n-6 fatty acids in phospholipids of heart muscle. J Intern Med Suppl. 1989; 731: 117-128.

Gupta R, Gupta VP. Lessons for prevention of coronary heart disease epidemiological study in western India. Curr Sci. 1998; 74: 1074–1077.

Gupta SK, Mohanty I, Talwar KK, Dinda A, Joshi S, Bansal P, Saxena A, Arya DS. Cardioprotection from ischemia and reperfusion injury by Withania somnifera: a hemodynamic, biochemical and histopathological assessment. Mol Cell Biochem. 2004; 260: 39-47.

Gurer H, Ozgunes H, Saygin E, Ercal N. Antioxidant effect of taurine against lead-induced oxidative stress. Arch Environ Contam Toxicol. 2001; 41: 397-402.

Gurujeyalakshmi G, Hollinger MA, Giri SN. Regulation of transforming growth factor-beta1 mRNA expression by taurine and niacin in the bleomycin hamster model of lung fibrosis. Am J Respir Cell Mol Biol. 1998; 18: 334-342.

Gurujeyalakshmi G, Wang Y, Giri SN. Suppression of bleomycin-induced nitric oxide production in mice by taurine and niacin. Nitric Oxide. 2000; 4: 399-411.

Gustafsson AB, Brunton LL. beta-adrenergic stimulation of rat cardiac fibroblasts enhances induction of nitric-oxide synthase by interleukin-1beta via message stabilization. Mol Pharmacol. 2000; 58: 1470-1478.

Guz G, Oz E, Lortlar N, Ulusu NN, Nurlu N, Demirogullari B, Omeroglu S, Sert S, Karasu C. The effect of taurine on renal ischemia/reperfusion injury. Amino Acids. 2006; Sep 29.

Habig WH, Pabst MJ, Jakoby WB. Glutathione-s-transferases.The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974; 249: 7130-7139.

Hagar HH, El Etter E, Arafa M.Taurine attenuates hypertension and renal dysfunction induced by cyclosporine-A in rats. Clin Exp Pharmacol Physiol. 2006; 33: 189-196.

Hagar HH. Folic acid and vitamin B (12) supplementation attenuates isoprenaline-induced myocardial infarction in experimental hyperhomocysteinemic rats. Pharmacol Res. 2002; 46: 213-219.

Hagar HH.The protective effect of taurine against cyclosporine A-induced oxidative stress and hepatotoxicity in rats. Toxicol Lett. 2004; 151: 335-343.

Hamaguchi T, Azuma J, Schaffer S. Interaction of taurine with methionine: inhibition of myocardial phospholipid methyltransferase. J Cardiovasc Pharmacol. 1991; 18: 224-230.

Hansen SH, Andersen ML, Birkedal H, Cornett C, Wibrand F. The important role of taurine in oxidative metabolism. Adv Exp Med Biol. 2006; 583: 129-135.

Hashimoto H, Ogawa K. Effects of sulfinpyrazone, aspirin and propranolol on the isoproterenol-induced myocardial necrosis. Jpn Heart J. 1981; 22: 643-652.

Hastings DF, Welty JD, Rohani F. Taurine stimulation of isolated hamster brain Na+, K+-ATPase: activation kinetics and chemical specificity. J Neurochem. 1985; 44: 1764-1770.

Hatefi Y.The mitochondrial electron transport and oxidative phosphorylation. Annu Rev Biochem. 1985; 54: 1015-1069.

Hayes J, Tipton KF, Bianchi L, Corte LD. Complexities in the neurotoxic actions of 6-hydroxydopamine in relation to the cytoprotective properties of taurine. Brain Res Bull. 2001; 55: 239–245.

Hayes KC, Sturman JA. Taurine in metabolism. Ann Rev Nutr. 1981; 1: 401-425.

Hazarika A, Sarkar SN. Effect of isoproturon pretreatment on the biochemical toxicodynamics of anilofos in male rats. Toxicology. 2001; 165: 87-95.

Heller-Stilb B, van Roeyen C, Rascher K, Hartwig HG, Huth A, Seeliger MW, Warskulat U, Haussinger D. Disruption of the taurine transporter gene (taut) leads to retinal degeneration in mice. FASEB J. 2002; 16: 231–233.

Herold BC, Kirkpatrick R, Marcellino D, Travelstead A, Pilipenko V, Krasa H, Bremer J, Dong LJ, Cooper MD. Bile salts: natural detergents for the prevention of sexually transmitted diseases. Antimicrob Agents Chemother. 1999. 43: 745-751.

Hilgier W, Zielinska M, Borkowska HD, Gadamski R, Walski M, Oja SS, Saransaari P, Albrecht J. Changes in the extracellular profiles of neuroactive amino acids in the rat striatum at the asymptomatic stage of hepatic failure. J Neurosci Res. 1999; 56: 76-84.

Hjerten S, Pan H. Purification and characterization of two forms of a low-affinity Ca2+-ATPase from erythrocyte membranes. Biochim Biophys Acta. 1983; 728: 281-288.

Hopkins PN, Hunt SC, Schreiner PJ, Eckfeldt JH, Borecki IB, Ellison CR, Williams RR, Siegmund KD. Lipoprotein(a) interactions with lipid and non-lipid risk factors in patients with early onset coronary artery disease: results from the NHLBI Family Heart Study. Atherosclerosis. 1998; 141: 333-345.

Horn WT, Menahan LA. A sensitive method for the determination of free fatty acids in plasma. J Lipid Res. 1981; 22: 377–381.

Hu A, Jiao X, Gao E, Koch WJ, Sharifi-Azad S, Grunwald Z, Ma XL, Sun JZ. Chronic beta-adrenergic receptor stimulation induces cardiac apoptosis and aggravates myocardial ischemia/reperfusion injury by provoking inducible nitric-oxide synthase-mediated nitrative stress. J Pharmacol Exp Ther. 2006; 318: 469-475.

Huxtable RJ. Physiological actions of taurine. Physiol Rev. 1992; 72: 101-163.

Hwang DF, Hour JL, Cheng HM. Effect of taurine on toxicity of oxidized fish oil in rats. Food Chem Toxicol. 2000; 38: 585-591.

Hwang DF, Wang LC, Cheng HM. Effect of taurine on toxicity of copper in rats. Food Chem Toxicol. 1998; 36: 239-244.

Hwang DF, Wang LC. Effect of taurine on toxicity of cadmium in rats. Toxicology. 2001; 167: 173-180.

Ide T, Tsutsui H, Hayashidani S, Kang D, Suematsu N, Nakamura K, Utsumi H, Hamasaki N, Takeshita A. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing heart after myocardial infarction. Circ Res. 2001; 88: 529- 535.

Ishida Y, Fujita T, Asai K. New detection and separation method for amino acids by high-performance liquid chromatography. J Chromatogr. 1981; 204: 143-148.

Itaya K. A more sensitive and stable colorimetric determination of free fatty acids in blood. J Lipid Res. 1977; 18: 663-665.

Ithayarasi AP, Padmavathy VN, Shyamala Devi C. Effect of alpha-tocopherol on isoproterenol induced myocardial infarction in rats--electrocardiographic biochemical and histological evidences. Indian J Physiol Pharmacol. 1996; 40: 297-302.

Ithayarasi AP, Shyamala Devi C. Effect of alpha-tocopherol on mitochondrial electron transport in experimental myocardial infarction in rats. Indian J Biochem Biophys. 1998; 35: 115-119.

Jacobi CA, Sabat R, Ordemann J, Wenger F, Volk HD, Muller JM. Peritoneal instillation of taurolidine and heparin for preventing intraperitoneal tumor growth and trocar metastases in laparoscopic operations in the rat model. Langenbecks Arch Chir. 1997; 382: S31-S36.

Jaffe AS, Ravkilde J, Roberts R, Naslund U, Apple FS, Galvani M, Katus H. It's time for a change to a troponin standard. Circulation. 2000; 102: 1216-1220.

Jalal S, Alai MS, Khan KA, Jan VM, Rather HA, Iqbal K, Tramboo NA, Lone NA, Dar MA, Hayat A, Abbas SM. Silent myocardial ischemia and cardiac autonomic neuropathy in diabetics. J Assoc Physicians India. 1999; 47: 767-769.

Jalil JE, Janicki JS, Pick R, Abrahams C, Weber KT. Fibrosis-induced reduction of endomyocardium in the rat after isoproterenol treatment. Circ Res. 1989; 65: 258-264.

Janeke G, Siefken W, Carstensen S, Springmann G, Bleck O, Steinhart H, Hoger P, Wittern KP, Wenck H, Stab F, Sauermann G, Schreiner V, Doering T. Role of taurine accumulation in keratinocyte hydration. J Invest Dermatol. 2003; 121: 354-361.

Janousek, S. The search for the best reperfusion therapy in the treatment of acute myocardial infarct. Vnitr Lek. 2003; 49: 88-90.

Jenkins RR, Del Signore CM, Sauer P, Skelly C. The effect of beta blocking drugs on lipid peroxidation in rat heart in vitro. Lipids. 1992; 27: 539-542.

Joan F, Peter Z, Philip, D. Cholesterol in Clinical Chemistry on Diagnosis and Treatments, ed. By Amold, E and Lyold, L. PG publishing, New Delhi. 1984; 230-256.

Jones RL, Miller JC, Hagler HK, Chien KR, Willerson JT, Buja LM. Association between inhibition of arachidonic acid release and prevention of calcium loading during ATP depletion in cultured rat cardiac myocytes. Am J Pathol. 1989; 135: 541-556.

Jurewitsch B, Lee T, Park J, Jeejeebhoy K. Taurolidine 2% as an antimicrobial lock solution for prevention of recurrent catheter-related bloodstream infections. JPEN J Parenter Enteral Nutr. 1998; 22: 242-244.

Kagan A, Dawber TR, Kannel WB, Revotskie N. The Framingham study: a prospective study of coronary heart disease. Fed Proc. 1962; 21: 52-57.

Kalra J, Chaudhary AK, Prasad K. Role of oxygen free radicals and pH on the release of cardiac lysosomal enzymes. J Mol Cell Cardiol. 1989; 21: 1125-1136.

Kamata K, Sugiura M, Kojima S, Kasuya Y. Restoration of endothelium-dependent relaxation in both hypercholesterolemia and diabetes by chronic taurine. Eur J Pharmacol. 1996; 303: 47-53.

Kannel WB, Wilson PW, D'Agostino RB, Cobb J. Sudden coronary death in women. Am Heart J. 1998; 136: 205-212.

Kardaras F, Kardara DF. Hyperhomocysteinemia: An independent risk factor for atherosclerotic vascular disease. Hell J Cardiol. 1995; 36: 275-279.

Karmazyn M. Myocardial Ischemia: Mechanisms, Reperfusion, Protection. ed. Birkhäuser Verlag. Basel, Switzerland. 1996; 115-133.

Karthikeyan K, Bai BR, Gauthaman K, Sathish KS, Devaraj SN. Cardioprotective effect of the alcoholic extract of Terminalia arjuna bark in an in vivo model of myocardial ischemic reperfusion injury. Life Sci. 2003; 73: 2727-2739.

Katona PG, McLean M, Dighton DH, Guz A. Sympathetic and parasympathetic cardiac control in athletes and nonathletes at rest. J Appl Physiol. 1982; 52: 1652-1657.

Kaul S, Kapoor NK. Reversal of changes of lipid peroxide, xanthine oxidase and superoxide dismutase by cardio-protective drugs in isoproterenol induced myocardial necrosis in rats. Indian J Exp Biol. 1989; 27: 625-627.

Keith ME, Ball A, Jeejeebhoy KN, Kurian R, Butany J, Dawood F, Wen WH, Madapallimattam A, Sole MJ. Conditioned nutritional deficiencies in the cardiomyopathic hamster heart. Can J Cardiol. 2001; 17: 449-458.

Kennon S, Price CP, Mills PG, MacCallum PK, Cooper J, Hooper J, Clarke H, Timmis AD. Cumulative risk assessment in unstable angina: clinical, electrocardiographic, autonomic, and biochemical markers. Heart. 2003; 89: 36-41.

Kerai MD, Waterfield CJ, Kenyon SH, Asker DS, Timbrell JA. Reversal of ethanol-induced hepatic steatosis and lipid peroxidation by taurine: a study in rats. Alcohol Alcohol. 1999; 34: 529-541.

Kerai MD, Waterfield CJ, Kenyon SH, Asker DS, Timbrell JA. Taurine: protective properties against ethanol-induced hepatic steatosis and lipid peroxidation during chronic ethanol consumption in rats. Amino Acids. 1998; 15: 53-76.

Keys SA, Zimmerman WF. Antioxidant activity of retinol, glutathione, and taurine in bovine photoreceptor cell membranes. Exp Eye Res. 1999; 68: 693-702.

Khattar RS. Effects of ACE-inhibitors and beta-blockers on left ventricular remodeling in chronic heart failure. Minerva Cardioangiol. 2003; 51: 143-154.

King J. Lactate dehydrogenase n practical clinical enzymology. (Van. D., Ed.) Nostrand Co, London. 1965; 83-93.

Kishida T, Ishikawa H, Tsukaoka M, Ohga H, Ogawa H, Ebihara K. Increase of bile acids synthesis and excretion caused by taurine administration prevents the ovariectomy-

induced increase in cholesterol concentrations in the serum low-density lipoprotein fraction of Wistar rats. J Nutr Biochem. 2003; 14: 7-16.

Kocak-Toker N, Giris M, Tulubas F, Uysal M, Aykac-Toker G. Peroxynitrite induced decrease in Na+, K+-ATPase activity is restored by taurine. World J Gastroenterol. 2005; 11: 3554-3557.

Kodama M, Tsukada H, Ooya M, Onomura M, Saito T, Fukuda K, Nakamura H, Taniguchi T, Tominaga M, Hosokawa M, Fujita J, Seino Y. Gastric mucosal damage caused by monochloramine in the rat and protective effect of taurine: endoscopic observation through gastric fistula. Endoscopy. 2000; 32: 294-299.

Kondo T, Ogawa Y, Sugiyama S, Ito T, Satake T, Ozawa T. Mechanism of isoproterenol induced myocardial damage. Cardiovasc Res. 1987; 21: 248-254.

Konovalova GG, Lankin VZ, Beskrovnova NN. The role of free radical inhibitors of lipid peroxidation in protecting the myocardium from ischemic damage. Arkh Patol. 1989; 51: 19-24.

Konukoglu D, Iynem H, Ziylan E. Antioxidant status in experimental peritonitis: effects of alpha tocopherol and taurolin. Pharmacol Res. 1999; 39: 247-251.

Kozumbo WJ, Agarwal S, Koren HS. Break-age and binding of DNA by reaction products of hypochlorous acid with aniline, l-naphthy-lamine or l-naphthol. Toxicol Appl Pharmacol 1992; 115: 107-115.

Kramer JH, Chovan JP, Schaffer SW. Effect of taurine on calcium paradox and ischemic heart failure. Am J Physiol. 1981; 240: H238-H246.

Krishnaswami S. Observations on serial changes in coronary artery disease in Indians. Curr Sci. 1998; 74: 1064-1068.

Kristof E, Szigeti G, Papp Z, Bodi A, Ball NA, Walsh RA, Edes I. The effects of levosimendan on the left ventricular function and protein phosphorylation in post-ischemic guinea pig hearts. Basic Res Cardiol. 1999; 94: 223-230.

Kukreja RC, Hess ML. The oxygen free radical system: from equations through membrane-protein interactions to cardiovascular injury and protection. Cardiovasc Res. 1992; 26: 641-655.

Kumar SHS, Anandan R, Devaki T, Kumar MS. Cardioprotective effects of Picrorhiza kurroa against isoproterenol-induced myocardial stress in rats. Fitoterapia. 2001; 72: 402-405.

Kumar SHS, Anandan R. Biochemical studies on the Cardioprotective Effect of Glutamine on Tissue Antioxidant Defense system in Isoprenaline-Induced Myocadial Infarction in Rats. J Clin Biochem Nutr. 2007; 40: 49-55.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227: 680-685.

Lake N. Alterations of ventricular contractility and myofibril loss in taurine-deficient hearts. Adv Exp Med Biol. 1994; 359: 335-342.

Lampson WG, Kramer JH, Schaffer SW. Potentiation of the actions of insulin by taurine. Can J Physiol Pharmacol. 1983; 61: 457-463.

Lee JY, Jung DW, Park HA, Kim SJ, Chung JH, Moon CK, Kim YC. Effect of taurine on biliary excretion and metabolism of acetaminophen in male hamsters. Biol Pharm Bull. 2004; 27: 1792-1796.

Lewczuk B, Przybylska-Gornowicz B. Effects of sympathicolytic and sympathicomimetic drugs on pineal ultrastructure in the domestic pig. J Pineal Res. 1997; 23: 198-208.

Li JB, Jefferson LS. Effect of isoproterenol on amino acid levels and protein turnover in skeletal muscle. Am J Physiol. 1977; 232: E243-E249.

Li W, Yuan X, Nordgren G, Dalen H, Dubowchik GM, Firestone RA, Brunk UT. Induction of cell death by the lysosomotropic detergent MSDH. FEBS Lett. 2000; 470: 35-39.

Lieber CS. Mechanisms of ethanol-drug-nutrition interactions. J Toxicol Clin Toxicol. 1994; 32: 631-681.

Lim E, Park S, Kim H. Effect of taurine supplementation on the lipid peroxide formation and the activities of glutathione-related enzymes in the liver and islet of type I and II diabetic model mice. Adv Exp Med Biol. 1998; 442: 99-103.

Lin Y, Havinga R, Schippers IJ, Verkade HJ, Vonk RJ, Kuipers F. Characterization of the inhibitory effects of bile acids on very-low-density lipoprotein secretion by rat hepatocytes in primary culture. Biochem J. 1996; 316: 531-538.

Lombardini JB. Taurine depletion in the intact animal stimulates in vitro phosphorylation of an approximately 44-kDa protein present in the mitochondrial fraction of the rat heart. J Mol Cell Cardiol. 1996; 28: 1957-1961.

Lopez-Jimenez, F. Heavy meals may trigger heart attacks. American Heart Association Scientific Sessions. New Orleans. Science News. 2000; 158: 366.

Loria P, Bozzoli M, Concari M, Guicciardi ME, Carubbi F, Bertolotti M, Piani D, Nistri A, Angelico M, Romani M, Carulli N. Effect of taurohyodeoxycholic acid on bilary lipid secretion in humans. Hepatology. 1997; 25: 1306-1314.

Loscalzo J, Weinfeld M, Fless GM, Scanu AM. Lipoprotein(a), fibrin binding, and plasminogen activation. Arteriosclerosis. 1990; 10: 240-245.

Lowry OH, Rosebrough NJ, Farr AL. Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193: 265-275.

Luthra K, Misra A, Srivastava L M. Lipoprotein (a): biology and role in atherosclerotic vascular disease. Curr Sci. 1999; 76: 1553-1560.

Macickova T, Navarova J, Urbancikova M, Horakova K. Comparison of isoproterenol-induced changes in lysosomal enzyme activity in vivo and in vitro. Gen Physiol Biophys. 1999; 18: 86-91.

Manfredini R, Boari B, Gallerani M, Portaluppi F; Myocardial Infarction Study on Timing and Outcome (MISTO) Investigators. Typical chest pain and onset of acute myocardial infarction in a prospective cohort of emergency calls. Ann Emerg Med. 2003; 41: 759-760.

Manikandan P, Sumitra M, Kumar DA, Gayathri C, Arutselvan N, Manohar BM, Puvanakrishnan R. Antioxidant potential of a novel tetrapeptide derivative in isoproterenol-induced myocardial necrosis in rats. Pharmacology. 2002; 65: 103-109.

Manjula TS, Geetha A, Devi CS. Effect of aspirin on isoproterenol induced myocardial infarction--a pilot study. Indian J Biochem Biophys. 1992; 29: 378-379.

Mankovskaya IN, Serebrovskaya TV, Swanson RJ, Vavilova GL, Kharlamova ON. Mechanisms of taurine antihypoxic and antioxidant action. High Alt Med Biol. 2000; 1: 105-110.

Marcil M, Brooks-Wilson A, Clee SM, Roomp K, Zhang LH, Yu L, Collins JA, van Dam M, Molhuizen HO, Loubster O, Ouellette BF, Sensen CW, Fichter K, Mott S, Denis M, Boucher B, Pimstone S, Genest J Jr, Kastelein JJ, Hayden MR. Mutations in the ABC1 gene in familial HDL deficiency with defective cholesterol efflux. Lancet. 1999; 354: 1341-1346.

Marinelli RA, Pellegrino JM, Larocca MC. Taurolithocholate can inhibit the biliary discharge of lysosomes in the rat. Biochim Biophys Acta. 1997; 1334: 155-160.

Marshall S, Bacote V, Traxinger RR. Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. J Biol Chem. 1991; 266: 4706-4712.

Mayer EL, Jacobsen DW, Robinson K. Homocysteine and coronary atherosclerosis. J Am Coll Cardiol. 1996; 27: 517-527.

McCarty MF. Exploiting complementary therapeutic strategies for the treatment of type II diabetes and prevention of its complications. Med Hypotheses. 1997; 49: 143-152.

McCarty MF. Magnesium taurate for the prevention and treatment of pre-eclampsia/eclampsia. Med Hypotheses. 1996; 47: 269-272.

McCarty MF. Vascular nitric oxide may lessen Alzheimer's risk. Med Hypotheses. 1998; 51: 465-476.

Meerson FZ, Belkina LM, Isaev VA, Saltykova VA, Arkhipenko IuV, Rozhitskaia II, Pshennikova MG, Golubeva LIu, Shimkovich MV, Sian' TsF, Fu Xiangqun and Vertikin A. L. The effect of tissue fish oil with a high content of polyunsaturated fatty acids (eiconol) on lipid peroxidation, arrhythmias and mortality in experimental acute myocardial ischemia, reperfusion and infarct. Kardiologiia. 1993; 33: 43-48.

Mehler AH, Korenberg A, Crisolia S, Ocho, S. The enzymatic mechanism of oxidation reductions between malate or isocitrate or pyruvate. J Biol Chem. 1948; 174: 961-977.

Meister A, Anderson ME. Glutathione. Annu Rev Biochem. 1983; 52: 711-760.

Meister A. Glutathione metabolism and its selective modification. J Biol Chem. 1988; 263: 17205-17208.

Meister A. Transport and metabolism of glutathione and gamma-glutamyl amino acids. Biochem Soc Trans. 1983; 11: 793-794.

Metcalfe LD, Schmitz AA. Petka JR. Rapid preparation of fatty acid esters from lipids for chromatographic analysis. Anal Chem. 1966; 38: 514-515.

Meyer M, Trost SU, Bluhm WF, Knot HJ, Swanson E, Dillmann WH. Impaired sarcoplasmic reticulum function leads to contractile dysfunction and cardiac hypertrophy. Am J Physiol Heart Circ Physiol. 2001; 280: H2046-H2052.

Minakami S, Ringler RL, Singer TP. Studies on the respiratory chain-linked dihydrodiphosphopyridine nucleotide dehydrogenase. I. Assay of the enzyme in particulate and in soluble preparations. J Biol Chem. 1962; 237: 569-576.

Minor T, Yamaguchi T, Klauke H, Wingenfeld P, Michalk D, Isselhard W. Taurine reduces experimental liver injury after cold ischemic preservation and a period of rewarming prior to reperfusion. Adv Exp Med Biol. 1996; 403: 157-161.

Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247: 3170-3175.

Mitra SK, Venkataranganna MV, Sundaram R, Gopumadhavan S. Antioxidant activity of AO-8, a herbal formulation in vitro and in vivo experimental models. Phytother Res. 1999; 13: 300-303.

Mizushima S, Nara Y, Sawamura M, Yamori Y. Effects of oral taurine supplementation on lipids and sympathetic nerve tone. Adv Exp Med Biol. 1996; 403: 615-622.

Mochizuki H, Oda H, Yokogoshi H. Increasing effect of dietary taurine on the serum HDL-cholesterol concentration in rats. Biosci Biotechnol Biochem. 1998; 62: 578-579.

Mohan P, Bloom S. Lipolysis is an important determinant of isoproterenol-induced myocardial necrosis. Cardiovasc Pathol. 1999; 8: 255-261.

Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller DJ. Low activities of glutathione-related enzymes as factors in the genesis of urinary bladder cancer. Cancer Res. 1984; 44: 5086-5091.

Mohur A, Cook IJY. Simple methods for measuring serum levels of glutamic-oxalo acetic and glutamic-pyruvic transaminase in routine laboratories. J Clin Pathol. 1957; 10: 394-399.

Molchanova SM, Oja SS, Saransaari P. Inhibitory effect of taurine on veratridine-evoked d-[(3)H]aspartate release from murine corticostriatal slices: Involvement of chloride channels and mitochondria. Brain Res. 2007; 1130: 95-102.

Molchanova SM, Oja SS, Saransaari P. Taurine attenuates D-[3H] aspartate release evoked by depolarization in ischemic corticostriatal slices. Brain Res. 2006; 1099: 64-72.

Mori TA, Beilin LJ. Long-chain omega 3 fatty acids, blood lipids and cardiovascular risk reduction. Current Opinion in Lipidology. 2001; 12: 11-17.

Morris SM Jr. Regulation of enzymes of urea and arginine synthesis. Ann Rev Nutr. 1992; 12: 81-101.

Motulsky HJ, Insel PA. Adrenergic receptors in man: direct identification, physiologic regulation and clinical alterations. N Engl J Med. 1982; 307: 18-29.

Mukherjee AK, Ghosal SK, Maity CR. Lysosomal membrane stabilization by alpha-tocopherol against the damaging action of Vipera russelli venom phospholipase A2. Cell Mol Life Sci. 1997; 53: 152-155.

Murakami S, Kondo-Ohta Y, Tomisawa K. Improvement in cholesterol metabolism in mice given chronic treatment of taurine and fed a high-fat diet. Life Sci. 1999; 64: 83-91.

Nag TC, Jotwani G, Wadhwa S. Immunohistochemical localization of taurine in the retina of developing and adult human and adult monkey. Neurochem Int. 1998; 33: 195-200.

Nageswari K, Banerjee R, Menon VP. Effect of saturated, ω-3 and ω-6 polyunsaturated fatty acids on myocardial infarction. Journal of Nutritional Biochemistry. 1999; 10: 338-344.

Nagl M, Larcher C, Gottardi W. Activity of N-chlorotaurine against herpes simplex- and adenoviruses. Antiviral Res. 1998; 38: 25-30.

Nair SS, Leitch JW, Falconer J, Garg ML. Prevention of cardiac arrhythmia by dietary (n-3) polyunsaturated fatty acids and their mechanism of action. J Nutr. 1997; 127: 383-393.

Nakamori K, Koyama I, Nakamura T, Yoshida T, Umeda M, Inoue K. Effectiveness of taurine in protecting biomembrane against oxidant. Chem Pharm Bull (Tokyo). 1990; 38: 3116-3119.

Nakashima T, Takino T, Kuriyama K. Pretreatment with taurine facilitates hepatic lipid peroxide formation associated with carbon tetrachloride intoxication. Jpn J Pharmacol. 1983; 33: 515-523.

Nalbone G, Grynberg A, Chevalier A, Leonardi J, Termine E, Lafont H. Phospholipase A activity of cultured rat ventricular myocyte is affected by the nature of cellular polyunsaturated fatty acids. Lipids. 1990; 25: 301-306.

Namikawa K, Okazaki Y, Nishida S, Kadota E, Tomura T, Hashimoto S. Studies on early stage changes of peroxide lipid in isoproterenol-induced myocardial injury. Yakugaku Zasshi. 1992; 112: 557-562.

Namikawa K, Okazaki Y, Nishida S, Kimoto S, Akai F, Tomura T, Hashimoto S. Changes in myocardial metallothionein on isoproterenol-induced myocardial injury. Yakugaku Zasshi. 1993; 113: 591-595.

Namikawa K, Okazaki Y, Nishida S, Tomura T, Hashimoto S. Studies on early changes in myocardial electrolytes and histological reaction in isoproterenol-induced myocardial injury. Yakugaku Zasshi. 1991; 111: 247-252.

Nandhini AT, Anuradha CV. Inhibition of lipid peroxidation, protein glycation and elevation of membrane ion pump activity by taurine in RBC exposed to high glucose. Clin Chim Acta. 2003; 336: 129-135.

Nandhini AT, Balakrishnan SD, Anuradha CV. Taurine improves lipid profile in rats fed a high fructose-diet. Nutrition Research. 2002; 22: 343–354.

Nandhini AT, Thirunavukkarasu V, Anuradha CV. Stimulation of glucose utilization and inhibition of protein glycation and AGE products by taurine. Acta Physiol Scand. 2004; 181: 297-303.

Neri Serneri GG, Gensini GF, Bonechi F. Blood clotting active drugs in long-term post-myocardial infarction treatment. J Cardiovasc Pharmacol. 1989; 14: S72-S74.

Nicolay K, van der Neut R, Fok JJ, de Kruijff B. Effects of adriamycin on lipid polymorphism in cardiolipin-containing model and mitochondrial membranes. Biochim Biophys Acta. 1985; 819: 55-65.

Niebes P. Determination of enzymes and degradation of the products of glycosylamino-glycan metabolism in healthy and various subjects. Clin Chim Acta. 1972; 42: 399-408.

Nirmala C, Puvanakrishnan R. Effect of curcumin on certain lysosomal hydrolases in isoproterenol-induced myocardial infarction in rats. Biochem Pharmacol. 1996[a]; 51: 47-51.

Nirmala C, Puvanakrishnan R. Isoproterenol induced myocardial infarction in rats: Functional and Biochemical alterations. Med Sci Res. 1994; 22: 575-577.

Nirmala C, Puvanakrishnan R. Protective role of curcumin against isoproterenol induced myocardial infarction in rats. Mol Cell Biochem. 1996[b]; 159: 85-93.

Norberg S, Powell TL, Jansson T. Intrauterine growth restriction is associated with a reduced activity of placental taurine transporters. Pediatr Res. 1998; 44: 233-238.

Noronha-Dutra AA, Steen EM, Woolf N. The correlation between catecholamine and lipid peroxidation induced damage in heart cells. Basic Res Cardiol. 1985; 80: 133-136.

Obinata K, Maruyama T, Hayashi M, Watanabe T, Nittono H. Effect of taurine on the fatty liver of children with simple obesity. Adv Exp Med Biol. 1996; 403: 607-613.

O'Brien PJ, Dameron GW, Beck ML, Kang YJ, Erickson BK, Di Battista TH, Miller KE, Jackson KN, Mittelstadt S. Cardiac troponin T is a sensitive, specific biomarker of cardiac injury in laboratory animals. Lab Anim Sci. 1997; 47: 486-495.

Obrosova IG, Fathallah L, Stevens MJ. Taurine counteracts oxidative stress and nerve growth factor deficit in early experimental diabetic neuropathy. Exp Neurol. 2001; 172: 211-219.

Obrosova IG, Stevens MJ. Effect of dietary taurine supplementation on GSH and NAD(P)-redox status, lipid peroxidation, and energy metabolism in diabetic precataractous lens. Invest Ophthalmol Vis Sci. 1999; 40: 680-688.

Ocaranza MP, Diaz-Araya G, Chiong M, Munoz D, Riveros JP, Ebensperger R, Sabat S, Irarrazaval P, Jalil JE, Lavandero S. Isoproterenol and angiotesin-I-converting enzyme in lung, left ventricle and plasma during myocardial hypertrophy and fibrosis. J Cardiovasc Pharmacol. 2002; 40: 246-254.

Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95: 351-358.

Ohnishi T, Suzuki T, Suzuki Y, Ozawa K. A comparative study of plasma membrane Mg2+ -ATPase activities in normal, regenerating and malignant cells. Biochim Biophys Acta. 1982; 684: 67-74.

Okinaka S, Kumagai H, Ebashi S. Sugaita H, Momoi H, Toyokura Y, Fujie Y. Serum creatine phosphokinase. Activity in progressive muscular dystrophy and neuromuscular diseases. Arch Neurol. 1961; 4: 520-525.

Ollinger K, Brunk UT. Cellular injury induced by oxidative stress is mediated through lysosomal damage. Free Radic Biol Med. 1995; 19: 565-574.

Olson JE. Osmolyte contents of cultured astrocytes grown in hypoosmotic medium. Biochim Biophys Acta. 1999; 1453: 175-179.

Ondrejickova O, Dzurba A, Sedlak J, Tokarova J, Macickova T, Benes L. Processes linked to the formation of reactive oxygen species are not necessarily involved in the development of isoproterenol-induced hypertrophy of the heart. The effect of stobadine. Biomed Biochim Acta. 1991; 50: 1251-1254.

Ono T, Imai K, Kohno H, Uchida M, Takemoto Y, Dhar DK, Nagasue N. Tauroursodeoxycholic acid protects cholestasis in rat reperfused livers: its roles in hepatic calcium mobilization. Dig Dis Sci. 1998; 43: 2201-2210.

Oudit GY, Trivieri MG, Khaper N, Husain T, Wilson GJ, Liu P, Sole MJ, Backx PH. Taurine supplementation reduces oxidative stress and improves cardiovascular function in an iron-overload murine model. Circulation. 2004; 109: 1877-1885.

Packard CJ, O'Reilly DS, Caslake MJ, McMahon AD, Ford I, Cooney J, Macphee CH, Suckling KE, Krishna M, Wilkinson FE, Rumley A, Lowe GD. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. West of Scotland Coronary Prevention Study Group. N Engl J Med. 2000; 343: 1148-1155.

Padma V, Devi CS, Ramkumar KM. Effect of fish oil pretreatment on isoproterenol-induced changes in myocardial membrane phospholipids. Nutrition. 2006[a]; 2: 1171-1176

Padma V, Devi CS. Effect of fish oil on mitochondrial respiration in isoproterenol induced myocardial infarction in rats. Ind J Exp Biol. 2001; 40: 268-272.

Padma VV, Devi CS, Ramkumar KM. Modulatory effect of fish oil on the myocardial antioxidant defense system in isoproterenol-induced myocardial infarction. J Basic Clin Physiol Pharmacol. 2006[b]; 17: 1-15.

Padma VV, Devi CS. Effect of fish oil on mitochondrial respiration in isoproterenol induced myocardial infarction in rats. Indian J Exp Biol. 2002; 40: 268-272.

Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutothione peroxidase. J Lab Clin Med. 1967; 70: 158-169.

Palmi M, Youmbi GT, Fusi F, Sgaragli GP, Dixon HB, Frosini M, Tipton KF. Potentiation of mitochondrial Ca2+ sequestration by taurine. Biochem Pharmacol. 1999; 58: 1123-1131.

Panasenko OM, Evgina SA, Driomina ES, Sharov VS, Sergienko VI, Vladimirov YA. Hypochlorite induces lipid peroxidation in blood lipoproteins and phospholipid liposomes. Free Radic Biol Med. 1995; 19: 133-140.

Papa S, Lofrumento NE, Paradies G, Quagliariello E. Mechanism of inhibition by uncouples of succinate oxidation in isolated mitochondria. Biochim Biophys Acta. 1969; 180: 35-44.

Parekh AC, Jung DH. Cholestrol determination with ferric acetate- uranil acetate and sulphuric acid – ferrus sulphate reagents. Anal Chem. 1970; 42: 1423-1427.

Park T, Lee K. Dietary taurine supplementation reduces plasma and liver cholesterol and triglyceride levels in rats fed a high-cholesterol or a cholesterol-free diet. Adv Exp Med Biol. 1998; 442: 319-325.

Pasantes-Morales H, Quesada O, Moran J. Taurine: an osmolyte in mammalian tissues. Adv Exp Med Biol. 1998; 442: 209-217.

Pasantes-Morales H, Wright CE, Gaull GE. Taurine protection of lymphoblastoid cells from iron-ascorbate induced damage. Biochem Pharmacol. 1985; 34: 2205-2207.

Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. Circulation. 2000; 102: 2165-2168.

Patriarca S, Furfaro AL, Domenicotti C, Odetti P, Cottalasso D, Marinari UM, Pronzato MA,Traverso N. Supplementation with N-acetylcysteine and taurine failed to restore glutathione content in liver of streptozotocin-induced diabetics rats but protected from oxidative stress. Biochim Biophys Acta. 2005; 1741: 48-54.

Pecci L, Montefoschi G, Fontana M, Dupre S, Costa M, Cavallini D. Hypotaurine and superoxide dismutase: protection of the enzyme against inactivation by hydrogen peroxide and peroxidation to taurine. Adv Exp Med Biol. 2000; 483: 163-168.

Penninx BW, Beekman AT, Honig A, Deeg DJ, Schoevers RA, van Eijk JT, van Tilburg W. Depression and cardiac mortality: results from a community-based longitudinal study. Arch Gen Psychiatry. 2001; 58: 221-227.

Pepe S, McLennan PL. Cardiac membrane fatty acid composition modulates myocardial oxygen consumption and postischemic recovery of contractile function. Circulation. 2002; 105: 2303-2308.

Persad S, Elimban V, Siddiqui F, Dhalla NS. Alterations in cardiac membrane beta-adrenoceptors and adenylyl cyclase due to hypochlorous acid. J Mol Cell Cardiol. 1999; 31: 101-111.

Persson HL, Yu Z, Tirosh O, Eaton JW, Brunk UT. Prevention of oxidant-induced cell death by lysosomotropic iron chelators. Free Radic Biol Med. 2003; 34: 1295-1305.

Peskin AV, Winterbourn CC. Kinetics of the reactions of hypochlorous acid and amino acid chloramines with thiols, methionine, and ascorbate. Free Radic Biol Med. 2001; 30: 572-579.

Pierno S, De Luca A, Camerino C, Huxtable RJ, Camerino DC. Chronic administration of taurine to aged rats improves the electrical and contractile properties of skeletal muscle fibers. J Pharmacol Exp Ther. 1998; 286: 1183-1190.

Pike RL, Brown ML. Nutrition-An integrated Approach. 3ed. Macmillan Publishing Company. 1984; 736-794.

Pion PD, Kittleson MD, Rogers QR, Morris JG. Myocardial failure in cats associated with low plasma taurine: a reversible cardiomyopathy. Science. 1987; 237: 764–768.

Pisarenko O, Studneva I, Khlopkov V, Solomatina E, Ruuge E. An assessment of anaerobic metabolism during ischemia and reperfusion in isolated guinea pig heart. Biochim Biophys Acta. 1988; 934: 55-63.

Pisarenko OI, Oleinikov OD, Shul'zhenko VS, Studneva IM, Kapel'ko VI. Relations of glutamate and aspartate contents of the heart and its energy state after ischaemia. Kardiologiia. 1990; 30: 65-69.

Pisarenko OI, Rosenfeldt FL, Langley L, Conyers RA, Richards SM. Differing protection with aspartate and glutamate cardioplegia in the isolated rat heart. Ann Thorac Surg. 1995; 59: 1541-1548.

Plummer DT. An introduction to Practical Biochemistry. Third Edition.Mc Graw-Hill Book Company (U. K) Limited. 1998; 268-269.

Pushpakiran G, Mahalakshmi K, Anuradha CV. Taurine restores ethanol-induced depletion of antioxidants and attenuates oxidative stress in rat tissues. AminoAcids. 2004; 27: 91-96.

Pushpakiran G, Mahalakshmi K, Viswanathan P, Anuradha CV. Taurine prevents ethanol-induced alterations in lipids and ATPases in rat tissues. Pharmacological Reports. 2005; 57: 578-587.

Qi B, Yamagami T, Naruse Y, Kagamimori S. Effects of taurine on depletion of erythrocyte membrane Na+K+ ATPase activity de to ozone exposure or cholesterol enrichment. J Nutr Sci Vitaminol. 1995; 41: 627-634.

Quertemont E, Goffaux V, Vlaminck AM, Wolf C, DeWitte P. Oral taurine supplementation modulates ethanol-conditioned stimulus preference. Alcohol. 1998; 16: 201-206.

Rao SV, Donahue HL, Pi-Sunyer FX, Fuster V. Results of Expert Meetings: Obesity and Cardiovascular Disease. Obesity as a risk factor in coronary artery disease. Am Heart J. 2001; 142: 1102-1107.

Raschke P, Massoudy P, Becker BF. Taurine protects the heart from neutrophil induced reperfusion injury. Free Radic Biol Med. 1995; 19: 461-471.

Rathore N, John S, Kale M. and Bhatnagar D. Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. Pharmacol Res. 1998; 38: 297-303.

Rauchcova H, Ledvinkova J, Kalous M, Drahota Z. The effect of lipid peroxidation on the activity of various membrane-bound ATPases in rat lier kidney. Intl J Biochem Cell Biol. 1995; 27: 251-255.

Ravichandran LV, Puvanakrishnan R, Joseph KT. Alterations in the heart lysosomal stability in isoproterenol induced myocardial infarction in rats. Biochem Int. 1990; 22: 387-396.

Ravichandran LV, Puvanakrishnan R. and Joseph KT. Influence of isoproterenol-induced myocardial infarction on certain glycohydrolases and cathepsins in rats. Biochem Med Metab Biol. 1991; 45: 6-15.

Ravichandran LV, Puvanakrishnan R. Collagen level in isoproterenol induced myocardial infarction in rats. Indian J Exp Biol. 1993; 31: 825-830.

Reaven GM. Diet and Syndrome X. Curr Atheroscler Rep. 2000; 2: 503-507.

Redmond HP, Stapleton PP, Neary P, Bouchier-Hayes D. Immunonutrition: the role of taurine. Nutrition. 1998; 14: 599–604.

Redtenbacher J. uber die Zusammensetzung. des Taurins. Ann Chem Pharmakol. 1846; 57: 170-174.

Reed LJ, Mukherjee RB. Methods Enzymol. 1969; 13: 53.

Refik Mas M, Comert B, Oncu K, Vural SA, Akay C, Tasci I, Ozkomur E, Serdar M, Mas N, Alcigir G, Yener N. The effect of taurine treatment on oxidative stress in experimental liver fibrosis. Hepatol Research. 2004; 28: 207-215.

Remiao F, Carmo H, Carvalho FD, Bastos ML. Inhibition of glutathione reductase by isoproterenol oxidation products. J Enzyme Inhib. 2000; 15: 47-61.

Remiao F, Carvalho M, Carmo H, Carvalho F, Bastos ML. $Cu2^{+}$-induced isoproterenol oxidation into isoprenochrome in adult rat calcium-tolerant cardiomyocytes. Chem Res Toxicol. 2002; 6: 861-869.

Remla A, Menon PV, Kurup PA. Effect of coconut oil and safflower oil on lipids in isoproterenol induced myocardial infarction in rats. Indian J Med Res. 1991; 94: 151-155.

Rendon DA, Lopez LF. Activation of mitochondrial oxidative phosphorylation during (+/-)-isoproterenol-induced cell injury of myocardium. Arch Cardiol Mex. 2001; 71: 13-19.

Rendon DA, Lopez LF. Mitochondrial oligomycin-sensitive ATPase during isoproterenol induced cell injury of myocardium. Arch Inst Cardiol. 2000; 70: 130-135.

Renteria RC, Johnson J. and Copenhagen DR. Need rods? Get glycine receptors and taurine. Review. Neuron. 2004; 41: 839-841.

Rice EW. In: Roedrick P, McDonold RP, editors. Standard methods in clinical chemistry. NewYork. Academic Press. 1970; 215-216.

Ridker PM. Evaluating novel cardiovascular risk factors: can we better predict heart attacks? Ann Intern Med. 1999; 130: 933-937.

Ridker PM. Novel risk factors and markers for coronary disease. Adv Intern Med. 2000; 45: 391-418.

Robinson KA, Weintein ML, Lindenmayer GE, Buse MG. Effects of diabetes and hyperglycemia on the hexosamine synthesis pathway in rat muscle and liver. Diabetes. 1995; 44: 1438-1446.

Robison GA, Butcher RW, Sutherland EW. Cyclic AMP. Annu Rev Biochem. 1968; 37: 149-174

Rodgers GM, Kane WH. Activation of endogenous factor V by a homocysteine-induced vascular endothelial cell activator. J Clin Invest. 1986; 77: 1909-1916.

Rodriguez-Martinez E, Rugerio-Vargas C, Rodriguez AI, Borgonio-Perez G, Rivas-Arancibia S. Antioxidant effects of taurine, vitamin C and vitamin E on oxidative damage in hippocampus caused by the administration of 3-nitropropionic acid in rats. Int J Neurosci. 2004; 114: 1133-1145.

Roig-Perez S, Guardiola F, Moreto M, Ferrer R. Lipid peroxidation induced by DHA enrichment modifies paracellular permeability in Caco-2 cells: protective role of taurine. J Lipid Res. 2004; 45: 1418-1428.

Rona G, Boutet M. and Hittner I. Reperfusion injury. A possible link between catecholamine-induced and ischemic myocardial alterations. Adv Myocardiol. 1983; 4: 427-439.

Rote WE, Connor JD. Autonomic mechanism in heart rate variability after isoproterenol induced myocardial damage in rats. J Auton Nerv Syst. 1992; 38: 37-44.

Roth J, Goebeler M, Ludwig S, Wagner L, Kilian K, Sorg C, Harms E, Schulze-Osthoff K, Koch H. Homocysteine inhibits tumor necrosis factor-induced activation of endothelium via modulation of nuclear factor-kappa b activity. Biochim Biophys Acta. 2001; 1540: 154-165.

Roth RA, Harkema JR, Pestka JP, Ganey PE. Is exposure to bacterial endotoxin a determinant of susceptibility to intoxication from xenobiotic agents?. Toxicol Appl Pharmacol. 1997; 147: 300-311.

Ryder JM. Determination of adenosine tri phosphate and its break down products in fish muscle by high performance liquid chromatography. J Agric Food Chem. 1985; 33: 678-680.

Ryder RE, Hayes TM, Mulligan IP, Kingswood JC, Williams S. and Owens DR. How soon after myocardial infarction should plasma lipid values be assessed? Br Med J (Clin Res Ed). 1984; 289: 1651-1653.

Saad SY, Al-Rikabi AC. Protection effects of Taurine supplementation against cisplatin-induced nephrotoxicity in rats. Chemotherapy. 2002; 48: 42-48.

Sandkamp M, Funke H, Kohler E, Assmann G. Lipoprotein (a) is an independent risk factor for myocardial infaction at a young age. Clin Chem. 1990; 36: 20-23.

Sangeetha T, Quine DS. Preventive effect of S-allyl cysteine sulfoxide (alliin) on cardiac marker enzymes and lipids in isoproterenol-induced myocardial injury. J Pharm Pharmacol. 2006; 58: 617-623.

Saravanan G, Prakash J. Effect of garlic (Allium sativum) on lipid peroxidation in experimental myocardial infarction in rats. J Ethnopharmacol. 2004; 94: 155–158.

Sardesai VM. Fundamentals of nutrition. In: Introduction to Clinical Nutrition edited by Dekker M, New York. 1998; 1–13.

Sasikumar CS, Devi CS. Effect of abana an ayurvedic formulation, on lipid peroxidation in experimental myocardial infarction in rats. Indian J Exp Biol. 2000; 38: 827-830.

Sathish V, Ebenezar KK, Devaki T. Biochemical changes on the cardioprotective effect of nicorandil and amlodipine during experimental myocardial infarction in rats. Pharmacol Res. 2003[a]; 48: 565-570.

Sathish V, Ebenezar KK, Devaki T. Synergistic effect of Nicorandil and Amlodipine on tissue defense system during experimental myocardial infarction in rats. Mol Cell Biochem. 2003[b]; 243: 133-138.

Sathish V, Ebenezar KK, Devaki T. Synergistic effect of nicorandil and amlodipine on lysosomal hydrolases during experimental myocardial infarction in rats. Biomed Pharmacother. 2003[c]; 57: 309-313.

Sathish V, Vimal V, Ebenezar KK, Devaki T. Synergistic effect of nicornadil and amlodipine on mitochondrial function during isoproterenol-induced myocardial infarction in rats. J Pharm Pharmacol. 2002; 54: 133-137.

Satoh H. Cardiac actions of taurine as a modulator of the ion channels. Adv Exp Med Biol. 1998; 442: 121-128.

Schaffer S, Azuma J, Takahashi K, Mozaffari M. Why is taurine cytoprotective?. Adv Exp Med Biol. 2003; 526: 307-321.

Schaffer SW, Azuma J, Madura LD. Mechanisms underlying taurine-mediated alterations in membrane fuction. Amino Acids. 1995; 8: 231-246.

Schaur RJ, Jerlich A, Stelmaszynska T. Hypochlorous acid as reactive oxygen species. Curr Top Biophys. 1998; 22: 176-185.

Scioscia KA, Snyderman CH, Wagner R. Altered serum amino acid profile in head and neck cancer. Nutr Cancer. 1998; 30: 144-147.

Sedlak J, Lindsay RH. Ellman Assay. Anal Biochem. 1968; 25: 192-205.

Selvam R, Kurien TB. Restoration of antioxidants in liver by methionine feeding in experimental rat urolithiasis. Indian J Biochem Biophys. 1992; 29: 364-370.

Selvam R, Ravichandran V. Restoration of tissue antioxidants and prevention of renal stone deposition in vitamin B6 deficient rats fed with vitamin E or methionine. Indian J Exp Biol. 1993; 31: 882-887.

Senaratne MP, Griffiths J. and Nagendran J. Elevation of plasma homocysteine levels associated with acute myocardial infarction. Clin Invest Med. 2000; 23: 220-226.

Sener G, Sehirli O, Ipci Y, Cetinel S, Cikle E, Gedik N, Alican I. Protective effects of taurine against nicotine-induced oxidative damage of rat urinary bladder and kidney. Pharmacology. 2005; 74: 37-44.

Shai I, Stampfer MJ, Mab J, Manson JE, Hankinson SE, Cannuscio C, Selhub J, Curhanc G, Rimma EB. Homocysteine as a risk factor for coronary heart diseases and its association with inflammatory biomarkers, lipids and dietary factors. Atherosclerosis. 2004; 177: 375-381.

Sharma M, Kishore K, Gupta SK, Joshi S, Arya DS. Cardioprotective potential of ocimum sanctum in isoproterenol induced myocardial infarction in rats. Mol Cell Biochem. 2001; 225: 75-83.

Shiny KS, Kumar SHS, Farvin KHS, Anandan R, Devadasan K. Protective effect of taurine on myocardial antioxidant status in isoprenaline-induced myocardial infarction in rats. J Pharm Pharmacol. 2005; 57: 1313-1318.

Shustova TI, Mashkova N, Iu Cherkashina EM, Dokshina GA. Effect of taurine on potassium, calcium and sodium levels in the blood and tissues of rats. Vopr Med Khim. 1986; 32: 113-116.

Silverman MD, Tumuluri RJ, Davis M, Lopez G, Rosenbaum JT, Lelkes PI. Homocysteine upregulates vascular cell adhesion molecule-1 expression in cultured human aortic endothelial cells and enhances monocyte adhesion. Arterioscler Thromb Vasc Biol. 2002; 22: 587-592.

Singal PK, Beamish RE. and Dhalla NS. Potential oxidative pathways of catecholamines in the formation of lipid peroxides and genesis of heart disease. Adv Exp Med Biol. 1983; 161: 391-401.

Singal PK, Kapur N, Dhillon KS, Beamish RE, Dhalla NS. Role of free radicals in catecholamine induced cardiomyopathy. Can J Physiol Pharmacol. 1982; 60: 1390-1397.

Singh UK, Agarwal KN, Shankar R. Effect of under nutrition on succinate dehydrogenase and acetylcholine esterase in developing rat brain. Ind J Exp Biol. 1990; 28: 868-870.

Slater EC, Bonner WD. The effect of fluoride on succinic oxidase system. Biochem J. 1952; 52: 185-196.

Smith LJ, Lacaille F, Lepage G, Ronco N, Lamarre A, Roy CC. Taurine decreases fecal fatty acid and sterol excretion in cystic fibrosis. A randomized double-blind trial. Am J Dis Child. 1991; 145: 1401-1404.

Smith ML, Hudson DL, Graitzer HM, Raven PB. Exercise training bradycardia: the role of autonomic balance. Med Sci Sports Exerc. 1989; 21: 40-44.

Son M, Kim HK, Kim WB, Yang J, Kim BK. Protective effect of taurine on indomethacin-induced gastric mucosal injury. Adv Exp Med Biol. 1996; 403: 147-155.

Son MW, Ko JI, Doh HM, Kim WB, Park TS, Shim MJ, Kim BK. Protective effect of taurine on TNBS-induced inflammatory bowel disease in rats. Arch Pharm Res. 1998; 21: 531-536.

Song D, O' Regan MH, Phillis JW. Amino acid release during volume regulation by cardiac cells: Cellular mechanisms. Eur J of pharmacol. 1998[b]; 341: 273-280.

Song D, O'Regan MH, Phillis JW. Release of the excitotoxic amino acids, glutamate and aspartate, from the isolated ischemic/anoxic rat heart. Neurosci Lett. 1996; 220: 1-4.

Song L, Wang D, Cui X, Hu W. The protective action of taurine and L-arginine in radiation pulmonary fibrosis. J Environ Pathol Toxicol Oncol. 1998[a]; 17: 151-157.

Soszynski M, Bartoz G. Decrease in accessible thiols as an index of oxidative damage to membrane proteins. Free Radic Biol Med. 1997; 23: 463-469.

Spanagel R, Zieglgansbereger W. Anti-craving compounds for ethanol: new pharmacological tools to study addictive processes. Trends Pharmacol Sci 1997; 18: 54-59.

Spector AA, Yorek MA. Membrane lipid composition and cellular function. J Lipid Res. 1985; 26: 1015-1035.

Sreepriya M, Devaki T, Nayeem M. Effects of L-Arginine pre treatment on isoproterenol-induced changes in lipid metabolism during experimental myocardial injury in rats. J Clin Biochem Nutr. 1998; 25: 169-175.

Stampfer MJ, Mailnow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, Tishler PV, Hennekens CH. A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians. JAMA. 1992; 268: 877-881.

Stapleton PP, Molloy AM, Rogers S, Bloomfield FJ. Neutrophil taurine in psoriasis. Ir J Med Sci. 1996; 165: 173-176.

Stephan ZF, Lindsey S, Hayes KC. Taurine enhances low density lipoprotein binding. Internalization and degradation by cultured Hep G2 cells. J Biol Chem. 1987; 262: 6069-6073.

Stipanuk MH, Coloso RM, Garcia RAG, Banks MF. Cysteine concentration regulates cysteine metabolism to glutathione, sulfate and taurine in rat hepatocytes. J Nutr. 1992; 122: 420-427.

Stoney CM, Engebretson TO. Plasma homocysteine concentrations are positively associated with hostility and anger. Life Sci. 2000; 66: 2267-2275.

Subhash D, Narinder KK, Nityanand S. Effect of isoprenaline on lipid profile and cardiac enzymes in rats. Indian J Exp Biol. 1978; 16: 376-378.

Suchalatha S, Shyamala Devi CS. Protective effect of Terminalia chebula against experimental myocardial injury induced by isoproterenol. Indian J Exp Biol. 2004; 42: 174-178.

Suleiman MS, Dihmis WC, Caputo M, Angelini GD, Bryan AJ. Changes in myocardial concentration of glutamate and aspartate during coronary artery surgery. Am J Physiol. 1997; 272: H1063-H1069.

Suleiman MS, Rodrigo GC, Chapman RA. Interdependence of intracellular taurine and sodium in guinea pig heart. Cardiovasc Res. 1992; 26: 897-905.

Superko HR. New aspects in lipidology and atherosclerosis. Can J Cardiol. 1995; 11: 4C-8C.

Swahn E. The care of patients with ischaemic heart disease from a gender perspective. Eur Heart J. 1998; 19: 1758-1765.

Szymanski LM, Pate RR. Fibrinolytic responses to moderate intensity exercise. Comparison of physically active and inactive men. Arterioscler Thromb.1994; 14: 1746-1750.

Tabassum H, Rehman H, Banerjee BD, Raisuddin S, Parvez S. Attenuation of tamoxifen-induced hepatotoxicity by taurine in mice. Clin Chim Acta. 2006; 370: 129-136.

Tadros MG, Khalifa AE, Abdel-Naim AB, Arafa HM. Neuroprotective effect of taurine in 3-nitropropionic acid-induced experimental animal model of Huntington's disease phenotype. Pharmacol Biochem Behav. 2005; 82: 574-582.

Takahama M, Barka T. Electron microscopic alterations of submaxillary gland produced by isoproterenol. J Ultrastruct Res. 1967; 17: 452-474.

Takahara S, Hamilton BH, Nell JV, Kobra TY, Ogawa Y. and Nishimura ET. Hypocatalasemia: A new genetic carrier state. J Clin Invest. 1960; 29: 610-619.

Takahashi K, Azuma M, Yamada T, Ohyabu Y, Takahashi K, Schaffer SW, Azuma J. Taurine transporter in primary cultured neonatal rat heart cells: a comparison between cardiac myocytes and nonmyocytes. Biochem Pharmacol. 2003; 65: 1181-1187.

Takatani T, Takahashi K, Uozumi Y, Shikata E, Yamamoto Y, Ito T, Matsuda T, Schaffer SW, Fujio Y, Azuma J. Taurine inhibits apoptosis by preventing formation of the Apaf-1/caspase-9 apoptosome. Am J Physiol Cell Physiol. 2004; 287: C949-C953.

Tang L, Mamotte CD, Van Bockxmeer FM, Taylor RR. The effect of homocysteine on DNA synthesis in cultured human vascular smooth muscle. Atherosclerosis. 1998; 136: 169-173.

Tawakol A, Forgione MA, Stuehlinger M, Alpert NM, Cooke JP, Loscalzo J, Fischman AJ, Creager MA, Gewirtz H, Tawakol A, Forgione MA, Stuehlinger M, Alpert NM, Cooke JP, Loscalzo J, Fischman AJ, Creager MA, Gewirtz H. Homocysteine impairs coronary microvascular dilator function in humans. J Am Coll Cardiol. 2002; 40: 1051-1058.

Teerlink JR, Pfeffer JM, Pfeffer MA. Progressive ventricular remodeling in response to diffuse isoproterenol induced myocardial necrosis in rats. Circ Res. 1994; 75: 105-113.

Thompson D, Edelsberg J, Kinsey KL, Oster G. Estimated economic costs of obesity to U.S. business. Am J Health Promot. 1998; 13: 120-127.

Tiedemann F, Gmelin L. Einige neue Bestandteile der Galle des Ochsen. Ann Physik Chem. 1827; 9: 326-337.

Timbrell JA, Seabra V, Waterfield CJ. The in vivo and in vitro protective properties of taurine. Gen Pharmacol. 1995; 26: 453-462.

Tipnis UR, He GY, Li S, Campbell G. and Boor PJ. Attenuation of isoproterenol-mediated myocardial injury in rat by an inhibitor of polyamine synthesis. Cardiovasc Pathol. 2000; 9: 273-280.

Tomaszewski A, Kleinrok A, Zackiewicz A, Gorny D, Billewicz-Stankiewicz J. Effect of various amino acids on acetylcholine metabolism in brain tissue. Ann Univ Mariae Curie Sklodowska(Med). 1982; 37: 61-70.

Trachtman H, Futterweit S, Maesaka J, Ma C, Valderrama E, Fuchs A, Tarectecan AA, Rao PS, Sturman JA, Boles TH. Taurine ameliorates chronic streptozocin-induced diabetic nephropathy in rats. Am J Physiol. 1995; 269: F429-F438.

Trachtman H, Del Pizzo R, Futterweit S, Levine D, Rao PS, Valderrama E, Sturman JA. Taurine attenuates renal disease in chronic puromycin aminonucleoside nephropathy. Am J Physiol. 1992; 262: F117-F123.

Trachtman H, Del Pizzo R, Sturman JA. Taurine and osmoregulation III. Taurine deficiency protects against cerebral edema during acute hyponatremia. Pediatr Res. 1990; 27: 85-88.

Tsai JC, Perrella MA, Yoshizumi M, Hsieh CM, Haber E, Schlegel R. and Lee ME. Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. Proc Natl Acad Sci. 1994; 91: 6369-6373.

Tyson JE, Lasky R, Flood D, Mize C, Picone T, Paule CL. Randomized trial of taurine supplementation for infants less than or equal to 1,300 gram birth weight: effect on auditory brainstem-evoked responses. Pediatrics. 1989; 83: 406-415.

Uchida A, Yamada T, Hayakawa T, Hoshino M. Taurochenodeoxycholic acid ameliorates and ursodeoxycholic acid exacerbates small intestinal inflammation. Am J Physiol. 1997; 272: G1249-G1257.

Vadgama JV, Chang K, Kopple JD, Idriss JM, Jonas AJ. Characteristics of taurine transport in rat liver lysosomes. J Cell Physiol. 1991; 147: 447-454.

van Aken BE, Jansen J, van Deventer SJ, Reitsma PH. Elevated levels of homocysteine increase IL-6 production in monocytic Mono Mac 6 cells. Blood Coagul Fibrinolysis. 2000; 11: 159-164.

van Bilsen M, van der Vusse GJ, Willemsen PH, Coumans WA, Roemen TH, Reneman RS. Lipid alterations in isolated, working rat heart during ischemia and reperfusion: its relation to myocardial damage. Cir Res. 1989; 64: 304-314.

Van Gelder NM. Neuronal discharge hypersynchrony and the intracranial water balance in relation to glutamic acid and taurine redistribution: migraine and epilepsy. Prog Clin Biol Res. 1990; 351: 1-20.

Vecer J, Kvapil M, Sprongl L, Kubatova H, Hoch J, Jech Z, Charvat J. Tissue amino acids in patients with colorectal carcinoma. Vnitr Lek. 1998; 44: 192-194.

Vendemiale G, Grattagliano I, Signorile A, Altomare E. Ethanol-induced changes of intracellular thiol compartmentation and protein redox status in the rat liver: effect of tauroursodeoxycholate. J Hepatol. 1998; 28: 46-53.

Venkatesan N, Chandrakasan G. In vivo administration of taurine and niacin modulate cyclophosphamide-induced lung injury. Eur J Pharmacol. 1994; 292: 75-80.

Vente JP, von Meyenfeldt MF, van Eijk HM, van Berlo CL, Gouma DJ, van der Linden CJ, Soeters PB. Plasma-amino acid profiles in sepsis and stress. Ann Surg. 1989; 209: 57-62.

Vimal V, Devaki T. Linear furanocoumarin protects rat myocardium against lipidperoxidation and membrane damage during experimental myocardial injury. Biomed Pharmacother. 2004; 58: 393-400.

Vincenzi FF. A calcium pump in red cell membranes, In Cellular mechanisms for calcium transfer and homeostasis, (Nicholas, G. and Wasserman, R. H. Eds.), Academic press. New York. 1971; 135-148.

Vinten-Johansen J. Physiological effects of peroxynitrite. Circ Res. 2000; 87: 170-172.

Wan FS, Li GH. Protective effects of taurine on myocardial injury in severely burned rats. Zhonghua Shao Shang Za Zhi. 2005; 21: 173-176.

Wang JH, Redmond HP, Watson RW, Condron C, Bouchier-Hayes D. The beneficial effect of taurine on the prevention of human endothelial cell death. Shock . 1996; 6: 331-338.

Wang Q, Hollinger MA, Giri SN. Attenuation of amiodarone-induced lung fibrosis and phospholipidosis in hamsters by taurine and/or niacin treatment. J Pharmacol Exp Ther. 1992; 262: 127-132.

Wang WY. Intestinal endotoxin translocation in endotoxemic rats. Sheng Li Ke Xue Jin Zhan. 1995; 26: 41-44.

Warren L. The thiobarbituric acid assay of sialic acids. J Biol Chem. 1959; 234: 1971-1975.

Warskulat U, Flogel U, Jacoby C, Hartwig HG, Thewissen M, Merx MW, Molojavyi A, Heller-Stilb B, Schrader J, Haussinger D. Taurine transporter knockout depletes muscle taurine levels and results in severe skeletal muscle impairment but leaves cardiac function uncompromised. FASEB J. 2004; 18: 577–579.

Waterfield CJ, Asker DS, Patel S, Timbrell JA. Is there a correlation between taurine levels and xenobiotic-induced perturbations in protein synthesis?. a study with tetracycline in rats. Amino Acids. 1998; 15: 161-177.

Waterfield CJ, Mesquita M, Parnham P, Timbrell JA. Taurine protects against the cytotoxicity of hydrazine, 1, 4-naphthaquinone and carbon tetra chloride in isolated rat hepatocytes. Biochem Pharmac. 1993; 46. 589- 595.

Wenger NK. The current state of hormonal prevention of coronary heart disease in menopausal women. Rev Esp Cardiol. 2003; 56: 1-8.

Wettstein M, Haussinger D. Taurine attenuates cold ischemia-reoxygenation injury in rat liver. Transplantation. 2000; 69: 2290-2296.

Wexler BC. Myocardial infarction in young vs old male rats. Pathophysiological changes. Am Heart J. 1978; 96: 70-80.

Wexler BC. Protective effects of propranolol on isoproterenol- induced myocardial infarction in arteriosclerotic and non- arteriosclerotic rats. Atherosclerosis. 1973; 18: 11-42.

Whitney EN, Rolfes SR. Understanding Normal and Clinical Nutrition. 5th ed. Belmont, CA. Wadsworth Publishing Co. 1998; 256-277.

Wilhelmsen L, Svardsudd K, Korsan-Bengtsen K, Larsson B, Welin L, Tibblin G. Fibrinogen as a risk factor for stroke and myocardial infarction. N Engl J Med. 1984; 311: 501-505.

Williams JE, Paton CC, Siegler IC, Eigenbrodt ML, Nieto FJ, Tyroler HA. Anger proneness predicts coronary heart disease risk: prospective analysis from the atherosclerosis risk in communities (ARIC) study. Circulation. 2000; 101: 2034-2039.

Willich SN, Goldberg RJ, Maclure M, Perriello L, Muller JE. Increased onset of sudden cardiac death in the first three hours after awakening. Am J Cardiol. 1992; 70: 65-68.

Winterbourn CC, Van den Berg JJM, Roitman E, Kuypers FA. Chlorohydrin formation from unsaturated fatty acids reacted with hypochlorous acid. Arch Biochem Biophys. 1992; 296: 547-555.

Woo KS, Chook P, Lolin YI. Hyperhomocysteinemia is a risk factor for arterial endothelial dysfunction in humans. Circultion. 1997; 96: 2542-2544.

Wood D, De Backer, Faergeman O, Graham I, Mancia G, Pyorala K. Prevention of coronary heart disease in clinical practice: recommendations of the Second Joint Task Force of European and other societies on Coronary Prevention. Eur Heart J. 1998; 19: 1434-1503.

Wright CE, Lin TT, Lin YY, Sturman JA, Gaull GE. Taurine scavenges oxidized chlorine in biological systems. Prog Clin Biol Res. 1985; 179: 137-147.

Wright CE, Tallan HH, Lin YY, Gaull GE. Taurine: biological update. Annu Rev Biochem. 1986; 55: 427-453.

Wu C, Kennedy, DO, Yano Y, Otani S, Matsui-Yuasa I. Thiols and polyamines in the cytoprotective effect of taurine on carbon tetrachloride-induced hepatotoxicity. J Biochem Mol Toxicol. 1999; 13: 71-76.

Xia Y, Dawson VL, Dawson TM, Snyder SH, Zweier JL. Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. Proc Natl Acad Sci. U S A. 1996; 93: 6770-6774.

Yagi K. A simple fluorometric assay for lipoperoxide in blood plasma. Biochem Med. 1976; 15: 212-216.

Yamaguchi Y, Itami S, Nishida, K, Ando Y, Okamoto S, Hosokawa K, Yoshikawa K. Taurine-conjugated ursodeoxycholic acid has a reversible inhibitory effect on human keratinocyte growth. J Dermatol Sci. 1998; 18: 35-42.

Yamakura F, Taka H, Fujimura T, Murayama K. Inactivation of human manganese-superoxide dismutase by peroxynitrite is caused by exclusive nitration of tyrosine 34 to 3-nitrotyrosine. J Biol Chem. 1998; 273: 14085-14089.

Yamamoto T, Katori R. Isoproterenol induces ST-segment elevation without wall motion aggravation and myocardial ischemia in experimental myocardial infarction. J Cardiol. 1995; 26: 167-175.

Yamazaki T, Komuro I, Zou Y, Kudoh S, Mizuno T, Hiroi Y, Shiojima I, Takano H, Kinugawa K, Kohmoto O, Takahashi T, Yazaki Y. Protein kinase A and protein kinase C synergistically activate the Raf-1 kinase/mitogen-activated protein kinase cascade in neonatal rat cardiomyocytes. J Mol Cell Cardiol. 1997; 29: 2491-501.

Yamazaki T, Yazaki Y. Molecular basis of cardiac hypertrophy. Z Kardiol. 2000; 89: 1-6.

Yan LJ, Sohal RS. Mitochondrial adenine nucleotide translocase is modified oxidatively during aging. Proc Natl Acad Sci. USA. 1998; 95: 12896– 12901.

Yany Y, Xiao R, Qiu F, Li X. Effect of taurine on blood and liver lipids in rats. Wei Sheng Yan Jiu. 2002; 31: 63-65.

Yates JC, Beamish RE, Dhalla NS. Ventricular dysfunction and necrosis produced by adrenochrome metabolite of epinephrine: Relation to pathogenesis of catecholamine cardiomyopathy. Am Heart J. 1981; 102: 210-221.

Ye J, Yang L, Sethi R, Copps J, Ramjiawan B, Summers R, Deslauriers RA new technique of coronary artery ligation: experimental myocardial infarction in rats in vivo with reduced mortality. Mol Cell Biochem. 1997; 176: 227-233.

Yeoh D, Zhao XT, Sanders SL, Elashoff JD, Bonorris G, Lin HC. Bile salt inhibits acid-promoting feeding tube occlusion. Nutr Clin Pract. 1996; 11: 105-107.

Yogeeta SK, Raghavendran HR, Gnanapragasam A, Subhashini R, Devaki T. Ferulic acid with ascorbic acid synergistically extenuates the mitochondrial dysfunction during beta-adrenergic catecholamine induced cardiotoxicity in rats. Chem Biol Interact. 2006; 163: 160-9.

You JS, Chang KJ. Effects of taurine supplementation on lipid peroxidation, blood glucose and blood lipid metabolism in streptozotocin-induced diabetic rats. Adv Exp Med Biol. 1998[a]; 442: 163-168.

You JS, Chang KJ. Taurine protects the liver against lipid peroxidation and membrane disintegration during rat hepatocarcinogenesis. Adv Exp Med Biol. 1998[b]; 442: 105-112.

Zeybek A, Ercan F, Cetinel S, Cikler E, Saglam B, Sener G. Taurine Ameliorates Water Avoidance Stress-Induced Degenerations of Gastrointestinal Tract and Liver. Dig Dis Sci. 2006; 51: 1853-1861.

Zhang X, Du W, Shen F, Wang J. Research on effects of taurine on the transplanted tumor of mice. Wei Sheng Yan Jiu. 1997; 26: 321-324.

Zhang XQ, Moore RL, Tillotson DL, Cheung JY. Calcium currents in post infarction rat cardiac myocytes. Am J Physiol. 1995; 269: C1464-C1473.

Zhang Y, Marcillat O, Giulivi C, Ernster L, Davies KJA. The oxidative inactivation of mitochondrial electron transport chain components and ATPase. J Biol Chem. 1990; 265: 16330- 16336.

ZhaoXi-he. Dietary protein, amino acids and their relation to health. Asia Pacific J Clin Nutr. 1994; 3: 131-134.

Zou Y, Komuro I, Yamazaki T, Kudoh S, Uozumi H, Kadowaki T, Yazaki Y. Both Gs and Gi proteins are critically involved in isoproterenol induced cardiomyocyte hypertrophy. J Biol Chem. 1999; 274: 9760-9770.

LIST OF PUBLICATIONS

Shiny KS, Kumar SHS, Farvin KHS, Anandan R, Devadasan K. Protective effect of taurine on myocardial antioxidant status in isoprenaline-induced myocardial infarction in rats. J Pharm Pharmacol. 2005; 57: 1313-7.

Farvin KHS, Anandan R, Kumar SHS, **Shiny KS**, Sankar TV, Thankappan TK. Effect of squalene on tissue defence system in isoproterenol-induced myocardial infarction in rats. Pharmacol. Res. 2004; 50: 231–236.

Farvin KHS, Anandan R, Kumar SHS, **Shiny KS**, Mathew S, Sankar TV, Nair PGV. Cardioprotective effect of squalene on lipid profile in isoprenaline-induced myocardial infarction in rats. J Med Food. 2006; 9: 531-6.

JPP Journal of Pharmacy and Pharmacology

JPP 2005, 57: 1313–1317
© 2005 The Authors
Received February 25, 2005
Accepted May 26, 2005
DOI 10.1211/jpp.57.10.0010
ISSN 0022-3573

Protective effect of taurine on myocardial antioxidant status in isoprenaline-induced myocardial infarction in rats

K. S. Shiny, S. Hari Senthil Kumar, K. H. Sabeena Farvin, R. Anandan and K. Devadasan

Abstract

We have examined the protective effect of taurine on the myocardial antioxidant defense system in isoprenaline (isoproterenol)-induced myocardial infarction in rats, an animal model of myocardial infarction in man. Levels of diagnostic marker enzymes in plasma, lipid peroxides and reduced glutathione, and the activity of glutathione-dependent antioxidant enzymes and antiperoxidative enzymes in the heart tissue were determined. Intraperitoneal administration of taurine significantly prevented the isoprenaline-induced increases in the levels of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and creatine phosphokinase in the plasma of rats. Taurine exerted an antioxidant effect against isoprenaline-induced myocardial infarction by preventing the accumulation of lipid peroxides and by maintaining the level of reduced glutathione and the activity of glutathione peroxidase, glutathione-S-transferase, catalase and superoxide dismutase at near normality. The results indicated that the cardioprotective potential of taurine was probably due to the increase of the activity of the free radical enzymes, or to a counteraction of free radicals by its antioxidant nature, or to a strengthening of myocardial membrane by its membrane stabilizing property.

Introduction

Despite improved clinical care, heightened public awareness, and widespread use of health innovations, myocardial infarction remains a leading cause of death. It is estimated that by the year A.D. 2020, up to three quarters of deaths in developing countries will result from non-communicable diseases and that myocardial infarction will top the list of killers (Gupta & Gupta 1998). With changing life style in developing countries like India, particularly in urban areas, myocardial infarction is making an increasingly important contribution to mortality statistics of such countries (Farvin et al 2004). In India, the number of patients being hospitalized for heart attack has increased over the past 35 years, with male patients showing a striking increase (Krishnaswami 1998).

Taurine (2-aminoethanesulfonic acid), a non-protein sulfur containing amino acid, is the most abundant free amino acid and has been shown to play several essential roles in the human body (Lombardini 1996). It is widely distributed in very high concentrations in brain, heart, kidney, lens, and reproductive organs (Huxtable 1992). It is involved in various important biological and physiological functions, which include cell membrane stabilization (Heller-Stilb et al 2002), antioxidation (Atmaca 2004), detoxification (Birdsall 1998), osmoregulation (Timbrell et al 1995), neuromodulation, and brain (Renteria et al 2004) and retinal development (Wright et al 1986). Taurine accounts for more than 50% of the total amino acid pool in the mammalian heart (Lombardini 1996). Earlier studies (Keith et al 2001; Warskulat et al 2004) demonstrated that pathology develops in the myocardium if an animal is depleted of taurine stores either through a taurine-deficient diet or use of taurine transport antagonists. Pion et al (1987) were the first to explain the role of dietary taurine deficiency associated with a dilated cardiomyopathy observed in experimental animals. Other studies by Keith et al (2001) and Lake et al (1994) have explored the relationship between

Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Matsyapuri (PO) Cochin-682029, India

K. S. Shiny, S. Hari Senthil Kumar, K. H. Sabeena Farvin, R. Anandan, K. Devadasan

Correspondence: R. Anandan, Scientist Sr. Scale, Biochemistry and Nutrition Division, Central Institute of Fisheries Technology, Cochin-682029, India. E-mail: kranandan@email.com

Funding and acknowledgements: We acknowledge greatly the financial aid from the Indian Council of Agricultural Research for K. S. Shiny. We thank the Director, Central Institute of Fisheries Technology, Cochin, for granting permission to publish this paper. We gratefully acknowledge the technical assistance rendered by B. Ganesan.

taurine deficiency and cardiac contractility, loss of cardiac myofibrils, and arrhythmogenesis. Though there is considerable evidence concerning the pharmacological significance of taurine in maintaining the integrity of an organism, the protective effect of taurine on myocardial antioxidant status in experimentally-induced myocardial infarction in rats has not been explored in detail.

Intraperitoneal administration of isoprenaline (isoproterenol; L-β-(3,4-dihydroxyphenyl)-α-isopropylaminoethanol hydrochloride), a β-adrenergic agonist, produces acute irreversible myocardial injury in rats that morphologically resembles myocardial infarction in man (Ravichandran et al 1990; Geng et al 2004). It induces myocardial necrosis by a multiple-step mechanism (Chagoya de Sanchez et al 1997). Peroxidation of endogenous lipids has been shown to be a major factor in the cardiotoxic action of isoprenaline (Kumar et al 2001; Chattopadhyay et al 2003). Isoprenaline-induced myocardial infarction is generally attributed to the formation of the highly reactive hydroxyl radical (OH^*), stimulator of lipid peroxidation and source for the destruction and damage to cell membranes (Farvin et al 2004). Alterations in tissue defense systems including chemical scavengers or antioxidant molecules and the antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase, and glutathione-S-transferase have been reported in isoprenaline-induced myocardial infarction (Sharma et al 2001; Saravanan & Prakash 2004).

In this study, an attempt has been made to assess the preventive effects of taurine against isoprenaline-induced myocardial infarction in rats by virtue of its hypolipidaemic (Takenaga et al 2000), antioxidant (Rodriguez-Martinez et al 2004) and membrane stabilizing properties (Birdsall 1998).

Materials and Methods

Chemicals

Taurine, adrenaline (epinephrine), tetramethoxypropane and isoprenaline were obtained from Sigma Chemical Company (St Louis, MO). All the other chemicals used were of analytical grade.

Animals

Wistar strain male albino rats (100–120 g) were housed individually in polyurethane cages under standard environmental conditions and allowed free access to food and water. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee.

Induction of myocardial infarction

Myocardial infarction was induced in experimental rats by injecting isoprenaline (11 mg (dissolved in physiological saline)/100 g/day), intraperitoneally for two days (Anandan et al 2003).

Experimental protocol

The rats were divided into four groups of six rats and housed individually in polyurethane cages. Group 1 served as the control. Group 2 animals were intraperitoneally (i.p.) injected with taurine (100 mg kg^{-1}/day, dissolved in physiological saline) for 15 days. Group 3 rats were injected intraperitoneally with isoprenaline (11 mg (dissolved in physiological saline)/100 g/day), for two days for the induction of myocardial infarction. Group 4 animals were injected with taurine at the above dosage for 15 days and then injected intraperitoneally with isoprenaline (11 mg/100 g/day) for two days.

At the end of the experimental period, i.e. 24 h after the last injection of isoprenaline, the rats were killed. Blood was collected using heparin as the anticoagulant. The plasma was separated and then used for the determination of alanine aminotransferase [EC 2.6.1.2] (ALT) (Mohur & Cook 1957), aspartate aminotransferase [EC 2.6.1.1] (AST) (Mohur & Cook 1957), lactate dehydrogenase [EC 1.1.1.27] (LDH) (King 1965) and creatine phosphokinase [EC 2.7.3.2] (CPK) (Okinaka et al 1961). The heart tissue was excised immediately and washed with chilled isotonic saline. The heart tissue homogenates prepared in ice-cold 0.1 M Tris-HCl buffer, pH 7.2, were used for the determination of lipid peroxides (LPO) (Ohkawa et al 1979), reduced glutathione (GSH) (Ellman 1959), glutathione peroxidase (GPx) [EC 1.11.1.9] (Pagila & Valentine 1967), glutathione-S-transferase (GST) [EC 2.5.1.18] (Habig et al 1974), catalase [EC 1.11.1.6] (Takahara et al 1960) and superoxide dismutase (SOD) [EC 1.15.1.1] (Misra & Fridovich 1972). The protein content was estimated by the method of Lowry et al (1951).

Statistics

Results are expressed as mean ± s.d. Multiple comparisons of the significant analysis of variance were performed by Tukey's multiple comparison test. A P-value < 0.05 was considered as statistically significant. All data were analysed with the aid of a statistical package program, SPSS 10.0 for Windows.

Results

Table 1 shows the levels of diagnostic marker enzymes (AST, ALT, LDH and CPK) in plasma of normal and of experimental rats. Injection of isoprenaline caused significant elevation in the levels of these marker enzymes in the plasma of group 3 rats as compared with group 1 normal controls. Prior treatment with taurine significantly ($P < 0.001$) prevented the isoprenaline-induced elevation in the levels of diagnostic marker enzymes in plasma of group 4 animals as compared with group 3 rats.

A significant rise in the level of lipid peroxidation was observed in the heart tissue of group 3 isoprenaline-administered rats as compared with controls (Table 2). This was paralleled by a significant decline in the level of reduced glutathione and the activity of glutathione-

Table 1 Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) in plasma of normal and experimental rats

Diagnostic marker	Group 1 (control)	Group 2 (administered taurine)	Group 3 (administered isoprenaline)	Group 4 (administered taurine and isoprenaline)
ALT	95.8 ± 7.1	90.3 ± 6.5	$408 \pm 35.3^{a,b}$	103 ± 7.5^{c}
AST	132 ± 9.4	127 ± 8.1	$376 \pm 28.7^{a,b}$	145 ± 8.7^{c}
LDH	169 ± 14.8	165 ± 12.4	$412 \pm 36.1^{a,b}$	188 ± 14.2^{c}
CPK	118 ± 7.2	115 ± 7.6	$343 \pm 18.5^{a,b}$	$142 \pm 8.9^{c,d,e}$

Results are mean ± s.d. of six animals. Values expressed: ALT, AST, and LDH, μmol pyruvate liberated $h^{-1} L^{-1}$; CPK, μmol creatine liberated $h^{-1} L^{-1}$. $^{a}P < 0.001$ significantly different compared with group 1 control; $^{b}P < 0.001$ significantly different compared with group 2 taurine-administered animals; $^{c}P < 0.001$ significantly different compared with group 3 isoprenaline-induced myocardial infarcted rats; $^{d}P < 0.05$ significantly different compared with group 1; $^{e}P < 0.05$ significantly different compared with group 2.

Table 2 Levels of lipid peroxides (LPO) and reduced glutathione (GSH), and the activity of glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase and superoxide dismutase (SOD) in the heart tissue of normal and experimental rats

Diagnostic marker	Group 1 (control)	Group 2 (administered taurine)	Group 3 (administered isoprenaline)	Group 4 (administered taurine and isoprenaline)
LPO	0.95 ± 0.04	0.92 ± 0.04	$2.79 \pm 0.15^{a,b}$	1.05 ± 0.18^{c}
GSH	5.17 ± 0.27	5.99 ± 0.35^{f}	$2.12 \pm 0.15^{a,b}$	$5.34 \pm 0.25^{c,e}$
GPx	3.15 ± 0.29	3.27 ± 0.24	$1.45 \pm 0.16^{a,b}$	2.98 ± 0.26^{c}
GST	1193 ± 84	1218 ± 91	$827 \pm 71^{a,b}$	$1105 \pm 88^{c,e}$
Catalase	10.4 ± 0.76	9.98 ± 0.84	$3.76 \pm 0.18^{a,b}$	$9.02 \pm 0.55^{c,d,e}$
SOD	3.73 ± 0.24	3.85 ± 0.32	$1.01 \pm 0.05^{a,b}$	$3.51 \pm 0.21^{c,e}$

Results are mean ± s.d. of six animals. Values expressed: LPO, nmol malondialdehyde (mg protein)$^{-1}$; GSH, μmol (g wet tissue)$^{-1}$; GPx, nmol GSH oxidized min^{-1} (mg protein)$^{-1}$; GST, μmol 1-chloro-2,4-dinitrobenzene conjugate formed min^{-1} (mg protein)$^{-1}$; catalase, nmol H_2O_2 decomposed min^{-1} (mg protein)$^{-1}$; SOD, one unit of the SOD activity is the amount of protein required to give 50% inhibition of adrenaline autoxidation. $^{a}P < 0.001$ significantly different compared with group 1 control; $^{b}P < 0.001$ significantly different compared with group 2 taurine-administered animals; $^{c}P < 0.001$ significantly different compared with group 3 isoprenaline-induced myocardial infarcted rats; $^{d}P < 0.05$ significantly different compared with group 1; $^{e}P < 0.05$ significantly different compared with group 2; $^{f}P < 0.001$ significantly different compared with group 1.

dependent antioxidant enzymes (GPx and GST) and anti-peroxidative enzymes (SOD and catalase) (Table 2). Administration of taurine significantly prevented all the isoprenaline-induced alterations in the tissue antioxidant system and maintained the rats at near normal status. The normal rats receiving taurine alone (group 2) did not show any significant change when compared with the normal rats, showing that taurine itself did not have any adverse effects.

Discussion

The significant rise noticed in the levels of AST, ALT, LDH and CPK in plasma of group 3 isoprenaline-administered rats as compared with group 1 normal controls was indicative of the severity of isoprenaline-induced myocardial infarction. This was in line with an earlier study (Suchalatha & Shyamala Devi 2004), which indicated that increased susceptibility of myocardial cell membrane to the isoprenaline-mediated peroxidative damage might lead to an increased release of these diagnostic marker enzymes into the systemic circulation. In this study, administration of taurine resulted in a significant reduction in the levels of these marker enzymes towards near normality as compared with group 3 isoprenaline-administered rats, indicating the cytoprotective effect of taurine (Schaffer et al 2003). It probably did so by its membrane stabilizing action (Redmond et al 1998). Timbrell et al (1995) had reported that taurine exerted membrane stabilization against carbon tetrachloride, hydrazine and 1,4-naphthoquinone-induced necrotic damages by modulating intracellular calcium levels and osmoregulation.

Biological membranes are sensitive to lipid peroxidation induced by reactive oxygen species. The oxidation of polyunsaturated fatty acids in biological membranes may cause impairment of membrane function, decrease in membrane fluidity, inactivation of membrane receptors and enzymes, increase of non-specific permeability to ions and disruption of membrane structure. In this investigation, the level of lipid peroxides in the heart tissue of group 3 isoprenaline-administered rats was significantly ($P < 0.001$) higher compared with group 1. This was in agreement with Nirmala & Puvanakrishnan (1996), who indicated that a lack of antioxidant defense might lead to an increase in lipid peroxidation and subsequent deleterious effects on the myocardial membrane in the isoprenaline-induced myocardial infarction condition. The rats pretreated with taurine showed a significant ($P < 0.001$) decrease in the level of lipid peroxidation in the heart tissue. This was probably achieved by means of its antioxidant nature (Rodriguez-Martinez 2004) against lipid peroxidation induced by isoprenaline. The unpaired electron present in the hydroxyl radicals generated by isoprenaline might have been trapped and subsequently dismuted by taurine. Obrosova et al (2001) reported that supplementation of taurine counteracted oxidative stress through the ascorbate system of antioxidant defenses in experimental diabetic nephropathy.

The glutathione antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species (Meister & Anderson 1983). The cellular tripeptide GSH exerts protective antioxidant influence through a complex enzyme system including GPx and GST. In this study, a significant reduction in

the activity of glutathione dependent antioxidant enzymes and antiperoxidative enzymes were observed in the heart tissue of group 3 isoprenaline-administered rats as compared with group 1 control animals. Also, the level of GSH was significantly reduced in isoprenaline-induced myocardial infarction. Decline in the activity of GPx in the heart tissue of infarction-induced rats made the myocardial cells more sensitive to oxidative damage, leading to a change in the cell composition and function.

The significant decrease noted in the activity of GST, another scavenging enzyme involved in the removal of toxic metabolites by glutathione conjugation reactions, in the heart tissue of group 3 myocardial infarction-induced rats might have been due to the reduced availability of GSH. This was in accordance with Sathish et al (2002), who indicated that GSH- and GSH-dependent enzyme systems might be directly related to the pathogenic mechanism of isoprenaline-induced myocardial infarction. Significant reduction observed in the activity of antiperoxidative enzymes SOD and catalase in the heart tissue of group 3 rats might have led to the formation of O^{2-} and H_2O_2, which in turn formed hydroxyl radical ($OH^{\bullet}$) and brought about a number of reactions harmful to the myocardial cell membranes. Similar observations were reported by Farvin et al (2004) and Gupta et al (2004).

This investigation has shown that prior treatment with taurine significantly prevented the isoprenaline-induced reduction in the level of GSH and the activity of catalase, SOD, GPx and GST in the heart tissue of group 4 rats as compared with group 3 rats. It probably did so either by increasing the level of GSH or by counteraction of isoprenaline-generated free radicals by its antioxidant nature. Hwang et al (2000) indicated that the level of thiobarbituric acid reactive substances was reduced and the level of GSH was elevated in the liver when the rats were fed with a taurine supplement.

Conclusion

Pretreatment with taurine prevented isoprenaline-induced myocardial infarction in rats. The overall cardioprotective effect of taurine was probably due to its membrane stabilizing action, or to a counteraction of free radicals by its antioxidant nature, or to its ability to maintain near to the normal status the activity of the free radical scavenging enzymes and the level of reduced glutathione, which protected the myocardial membrane against peroxidative damage by decreasing lipid peroxidation and strengthening the myocardial membrane.

References

Anandan, R., Asha, K. K., Ammu, K., Suseela, M., Viswanathan Nair, P. G. (2003) Effects of peroxidised PUFA on tissue defense system in experimentally induced myocardial infarction in rats. In: Surendran, P. K., Mathew, P. T., Thampuran, N., Nambiar, N., Joseph, J., Boopendranath, Laksmanan, P. T., Viswanathan Nair, P. G. (eds) *Seafood safety*. Society of Fisheries Technologists (India), Cochin, pp 330–335

Atmaca, G. (2004) Antioxidant effects of sulfur-containing amino acids. *Yonsei Med. J.* **45**: 776–788

Birdsall, T. C. (1998) Therapeutic applications of taurine. *Altern. Med. Rev.* **3**: 128–136

Chagoya de Sanchez, V., Hernandez-Munoz, R., Lopez-Barrera, F., Yanez, L., Vidrio, S., Suarez, J., Cota-Garza, M. D., Aranda-Fraustro, A., Cruz, D. (1997) Sequential changes of energy metabolism and mitochondrial function in myocardial infarction induced by isoproterenol in rats: a long-term and integrative study. *Can. J. Physiol. Pharmacol.* **75**: 1300–1311

Chattopadhyay, A., Biswas, S., Bandyopadhyay, D., Sarkar, C., Datta, A. G. (2003) Effect of isoproterenol on lipid peroxidation and antioxidant enzymes of myocardial tissue of mice and protection by quinidine. *Mol. Cell. Biochem.* **245**: 43–49

Ellman, G. L. (1959) Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **82**: 70–71

Farvin, K. H. S., Anandan, R., Kumar, S. H. S., Shiny, K. S., Sankar, T. V., Thankappan, T. K. (2004) Effect of squalene on tissue defence system in isoproterenol-induced myocardial infarction in rats. *Pharmacol. Res.* **50**: 231–236

Geng, B., Chang, L., Pan, C., Qi, Y., Zhao, J., Pang, Y., Du, J., Tang, C. (2004) Endogenous hydrogen sulfide regulation of myocardial injury induced by isoproterenol. *Biochem. Biophys. Res. Commun.* **318**: 756–763

Gupta, R., Gupta, V. P. (1998) Lessons for prevention of coronary heart disease epidemiological study in western India. *Curr. Sci.* **74**: 1074–1077

Gupta, S. K., Mohanty, I., Talwar, K. K., Dinda, A., Joshi, S., Bansal, P., Saxena, A., Arya, D. S. (2004) Cardioprotection from ischemia and reperfusion injury by Withania somnifera: a hemodynamic, biochemical and histopathological assessment. *Mol. Cell. Biochem.* **260**: 39–47

Habig, W. H., Pabst, M. J., Jackoby, W. B. C. (1974) Glutathione-S-transferases: the first enzymatic step in enzymatic stepping mercapturic acid formation. *J. Biol. Chem.* **249**: 7130–7139

Heller-Stilb, B., Van Roeyen, C., Rascher, K., Hartwig, H. G., Huth, A., Seeliger, M. W., Warskulat, U., Haussinger, D. (2002) Disruption of the taurine transporter gene (*taut*) leads to retinal degeneration in mice. *FASEB J.* **16**: 231–233

Huxtable, R. J. (1992) Physiological actions of taurine. *Physiol Rev.* **72**: 101–163

Hwang, D. F., Hour, J. L., Cheng, H. M. (2000) Effect of taurine on toxicity of oxidized fish oil in rats. *Food Chem. Toxicol.* **38**: 585–591

Keith, M. E., Ball, A., Jeejeebhoy, K. N., Kurian, R., Butany, J., Dawood, F., Wen, W. H., Madapallimattam, A., Sole, M. J. (2001) Conditioned nutritional deficiencies in the cardiomyopathic hamster heart. *Can. J. Cardiol.* **17**: 449–458

King, J. (1965) Lactate dehydrogenase. In: Van, D. (ed.) *Practical clinical enzymology*. Nostrand Co., London, pp 83–93

Krishnaswami, S. (1998) Observations on serial changes in coronary artery disease in Indians. *Curr. Sci.* **74**: 1064–1068

Kumar, S. H. S., Anandan, R., Devaki, T., Kumar, M. S. (2001) Cardioprotective effects of *Picrorhiza kurroa* against isoproterenol-induced myocardial stress in rats. *Fitoterapia* **72**: 402–405

Lake, K. (1994) Alterations of ventricular contractility and myofibril loss in taurine-deficient hearts. *Adv. Exp. Med. Biol.* **359**: 335–342

Lombardini, J. B. (1996) Taurine depletion in the intact animal stimulates *in vitro* phosphorylation of a ~44-kDa protein pre-

sent in the mitochondrial fraction of the rat heart. *J. Mol. Cell. Cardiol.* **28**: 1957–1961

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275

Meister, A., Anderson, M. E. (1983) Glutathione. *Annu. Rev Biochem.* **52**: 711–760

Misra, H. P., Fridovich, T. (1972) The role of superoxide ion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* **247**: 3170–3175

Mohur, A., Cook, I. J. Y. (1957) Simple methods for measuring serum levels of glutamic-oxalo acetic and glutamic-pyruvic transaminase in routine laboratories. *J. Clin. Pathol.* **10**: 394–399

Nirmala, C., Puvanakrishnan, R. (1996) Protective role of curcumin against isoproterenol induced myocardial infarction in rats. *Mol. Cell. Biochem.* **159**: 85–93

Obrosova, I. G., Fathallah, L., Stevens, M. J. (2001) Taurine counteracts oxidative stress and nerve growth factor deficit in early experimental diabetic neuropathy. *Exp. Neurol.* **172**: 211–219

Ohkawa, H., Onishi, N., Yagi, K. (1979) Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal. Biochem.* **95**: 351–358

Okinaka, S., Kumagai, H., Ebashi, E., Sugaita, M., Momoi, Y., Toyokura, Y. (1961) Serum creatine phosphokinase activity in progressive muscular dystrophy and neuromuscular disease. *Arch. Neurol.* **4**: 520–526

Pagila, D. E., Valentine, W. N. (1967) Studies on the glutathione characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **70**: 158–169

Pion, P. D., Kittleson, M. D., Rogers, Q. R., Morris, J. G. (1987) Myocardial failure in cats associated with low plasma taurine: a reversible cardiomyopathy. *Science* **237**: 764–768

Ravichandran, L. V., Puvanakrishnan, R., Joseph, K. T. (1990) Alterations in the heart lysosomal stability in isoproterenol induced myocardial infarction in rats. *Biochem. Int.* **22**: 387–396

Redmond, H. P., Stapleton, P. P., Neary, P., Bouchier-Hayes, D. (1998) Immunonutrition: the role of taurine. *Nutrition* **14**: 599–604

Renteria, R. C., Johnson, J., Copenhagen, D. R. (2004) Need rods? Get glycine receptors and taurine. *Neuron* **41**: 839–841

Rodriguez-Martinez, E., Rugerio-Vargas, C., Rodriguez, A.I., Borgonio-Perez, G., Rivas-Arancibia, S. (2004) Antioxidant effects of taurine, vitamin C, and vitamin E on oxidative damage in hippocampus caused by the administration of 3-nitropropionic acid in rats. *Int. J. Neurosci.* **114**: 1133–1145

Saravanan, G., Prakash, J. (2004) Effect of garlic *(Allium sativum)* on lipid peroxidation in experimental myocardial infarction in rats. *J. Ethnopharmacol.* **94**: 155–158

Sathish, V., Vimal, V., Ebenezar, K. K., Devaki, T. (2002) Synergistic effect of nicorandil and amlodipine on mitochondrial function during isoproterenol-induced myocardial infarction in rats. *J. Pharm. Pharmacol.* **54**: 133–137

Schaffer, S., Azuma, J., Takahashi, K., Mozaffari, M. (2003) Why is taurine cytoprotective? *Adv. Exp. Med. Biol.* **526**: 307–321

Sharma, M., Kishore, K., Gupta, S. K., Joshi, S., Arya, D. S. (2001) Cardioprotective potential of *Ocimum sanctum* in isoproterenol induced myocardial infarction in rats. *Mol. Cell. Biochem.* **225**: 75–83

Suchalatha, S., Shyamala Devi, C. S. (2004) Protective effect of *Terminalia chebula* against experimental myocardial injury induced by isoproterenol. *Indian J. Exp. Biol.* **42**: 174–178

Takahara, S., Hamilton, B. H., Nell, J. V., Kobra, T. Y, Ogawa, Y., Nishimura, E. T. (1960) Hypocatalasemia: a new genetic carried state. *J. Clin. Invest.* **29**: 610–619

Takenaga, T., Imada, K., Otomo, S. (2000) Hypolipidemic effect of taurine in golden Syrian hamsters. *Adv. Exp. Med. Biol.* **483**: 187–192

Timbrell, J. A., Seabra, V., Waterfield, C. J. (1995) The in vivo and in vitro protective properties of taurine. *Gen. Pharmacol.* **26**: 453–462

Warskulat, U., Flogel, U., Jacoby, C., Hartwig, H. G., Thewissen, M., Merx, M. W., Molojavyi, A., Heller-Stilb, B., Schrader, J., Haussinger, D. (2004) Taurine transporter knockout depletes muscle taurine levels and results in severe skeletal muscle impairment but leaves cardiac function uncompromised. *FASEB J.* **18**: 577–579

Wright, C. E., Tallan, H. H., Lin, Y. Y., Gaull, G. E. (1986) Taurine: biological update. *Annu. Rev. Biochem.* **55**: 427–453

T 180