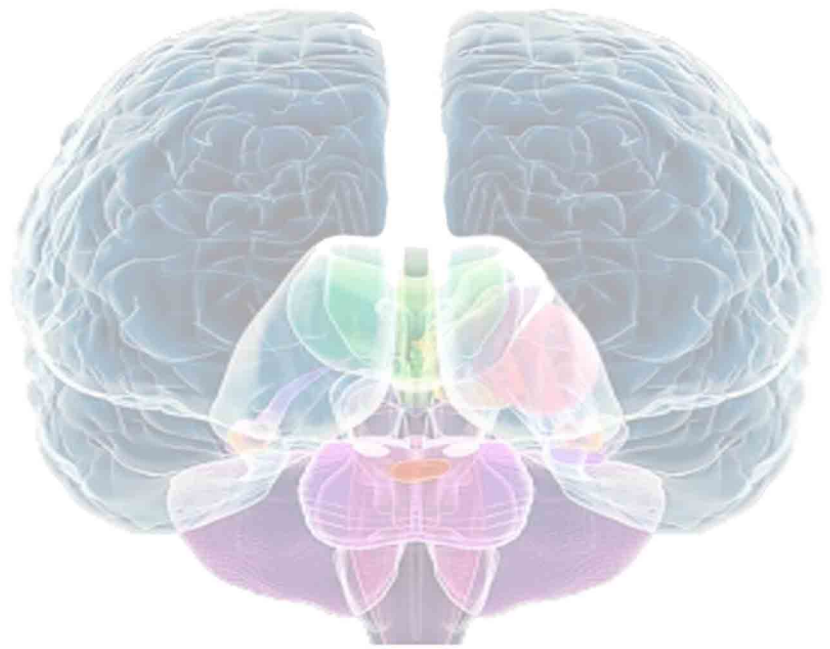
Ph.D. Thesis

DOPAMINE RECEPTOR SUBTYPES, IP3, cAMP AND cGMP FUNCTIONAL REGULATION IN PARKINSONISM INDUCED BY UNILATERAL INFUSION OF ROTENONE IN RATS: RECOVERY WITH BONE MARROW CELLS, SEROTONIN AND GABA SUPPLEMENTATION



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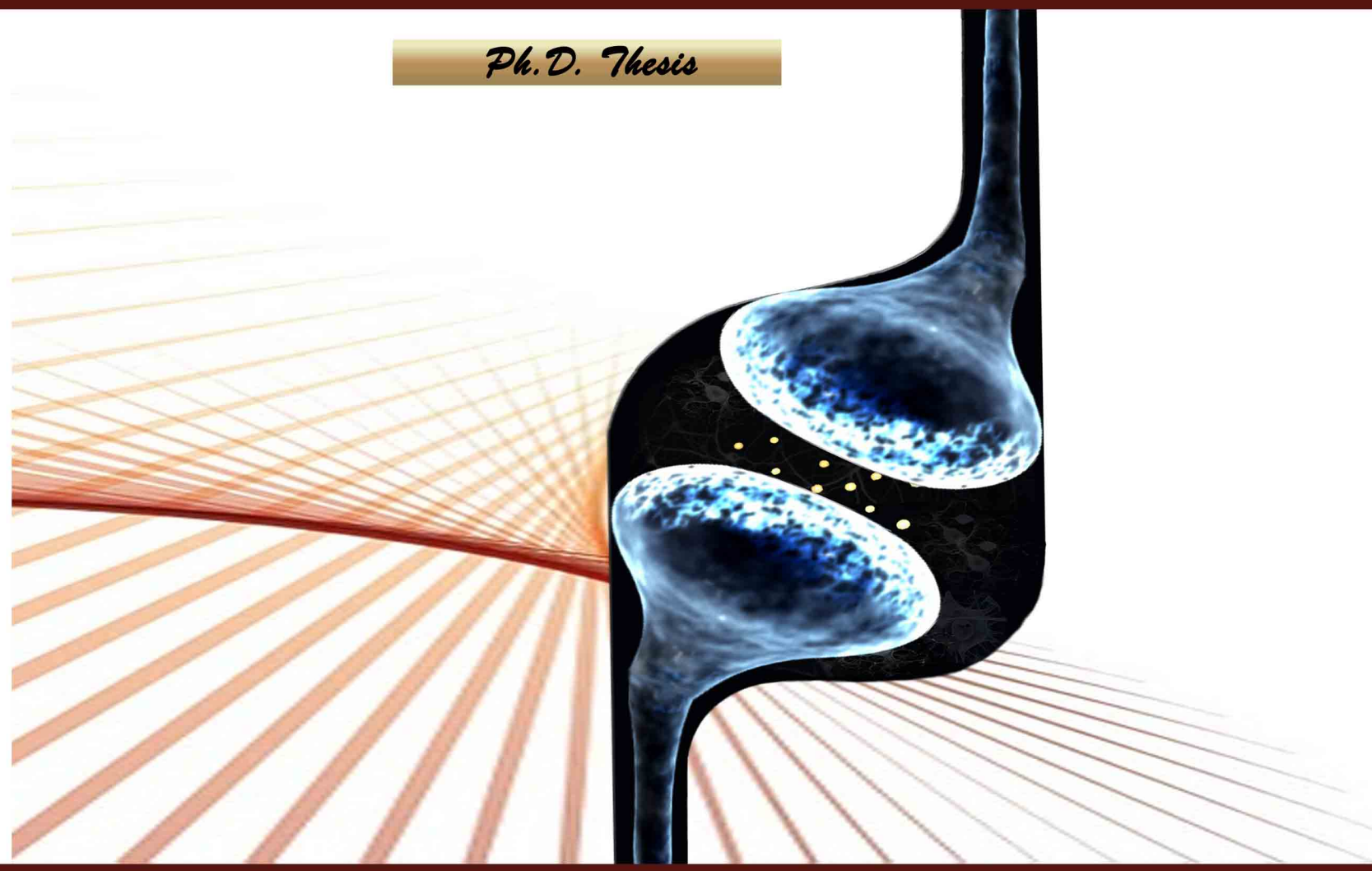

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COCHIN, 682022, KERALA, INDIA

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BY

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CERTIFICATE

This is to certify that the thesis entitled **"Dopamine Receptor Subtypes, IP3, cAMP and cGMP Functional Regulation in Parkinsonism Induced by Unilateral Infusion of Rotenone in Rats: Recovery with Bone Marrow Cells, Serotonin and GABA Supplementation"** is a bonafide record of the research work carried out by **Mr. Jes Paul,** under my guidance and supervision in the Department of Biotechnology, Cochin University of Science and Technology and that no part thereof has been presented for the award of any other degree.

Cochin – 682 022

(C. S. Paulose)

February 28, 2011

DECLARATION

I hereby declare that the thesis entitled **"Dopamine Receptor Subtypes, IP3, cAMP and cGMP Functional Regulation in Parkinsonism Induced by Unilateral Infusion of Rotenone in Rats: Recovery with Bone Marrow Cells, Serotonin and GABA Supplementation"** is the authentic record of research work carried out by me for my doctoral degree under the supervision and guidance of **Dr. C. S. Paulose,** Director, Centre for Neuroscience, Professor, Department of Biotechnology, Cochin University of Science and Technology, Cochin and that no part thereof has previously formed the basis for the award of any degree or diploma, associateship or other similar title or recognition.

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Jes Paul

Dedicated To My Beloved Parents . . .

ABBREVIATIONS

5-HIAA	5-hydroxy indole - 3 acetic acid
5-HT	5-Hydroxy tryptamine
AC	Adenylate cyclase
aCSF	Artificial cerebrospinal fluid
AD	Alzheimer's disease
ACh	Acetylcholine
AChR	Acetylcholine receptor
AIF	Apoptosis inducing factor
ALS	Amyotrophic lateral sclerosis
ANS	Autonomic nervous system
ATP	Adenosine triphosphate
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
BBB	Blood brain barrier
BDNF	Brain derived neurotrophic factor
B_{max}	Maximal binding
BMC	Bone marrow cells
BS	Brainstem
CB	Cerebellum
CC	Cerebral cortex
CAT	Catalase
cAMP	Cylic adenosine monophosphate
cAPK	cAMP-dependent protein kinase
cDNA	Complementary deoxy ribonucleic acid
cGMP	Cyclic guanosine monophosphate
CNS	Central Nervous System
CREB	cAMP regulatory element binding protein

CSF	Cerebrospinal fluid
CT	Crossing threshold
DA	Dopamine
DARRP-32	Dopamine-receptor-associated protein
DAG	Diacylglycerol
DEPC	Di ethyl pyro carbonate
DHPG	3,5-dihydroxyphenylglycine
DNA	Deoxy ribonucleic acid
DOPAC	Dihydroxy phenyl acetic acid
EAA	Excitatory amino acids
EDTA	Ethylene diamine tetra acetic acid
EPI	Epinephrine
ER	Endoplasmic reticulum
Gi	G protein inhibitory
Gs	G protein stimulatory
GABA	Gamma amino butyric acid
GAP	GTPase-activating protein
GDNF	Glial cell line-derived neurotrophic factor
GPCR	G-protein-coupled receptors
GPi	Globus pallidus internus
GPm	Medial globus pallidus
GTP	Guanosine triphosphate
HEPES	[n′ (2-hydroxy ethyl)] piperazine-n′-[2-ethanesulfonic acid]
i.p	Intraperitoneally
IP3	Inositol 1,4,5-triphosphate
IP3R	IP3 receptors
K_d	Dissociation constant
LBD	Lewy body diseases

L-DOPA	L-3,4-dihydroxyphenylalanine
LIDs	Levodopa-induced dyskinesia
LTD	Long term depression
LTP	Long term potentiation
MAO	Monoamine oxidase
MHPG	3-methoxy-4-hydroxyphenylglycol
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydroxypyridine
MPP+	(1-methyl-4-phenylpyridinium)
mRNA	Messenger Ribonucleic acid
NADH	Nicotinamide adenine dinucleotide, reduced form
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
NE	Norepinephrine
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NOS	Nitric-oxide synthase
NPY	Neuropeptide Y
NSB	Non specific binding
O.D.	Optical density
p	Level of significance
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline Triton X- 100
PCP	Phencyclidine
PCPA	Parachlorophenylalanine
PCR	Polymerase chain reaction
PD	Parkinson's Disease
PDE2	phosphodiesterase 2
PFC	Prefrontal cortex
Pi	Inorganic phosphate

PIP2	Phosphatidyl inositol 4,5-bisphosphate
PKC	Protein kinase C
PKG	Protein kinase G
PLC	Phospholipase C
PPN	Pedunculopontine nucleus
ROS	Reactive oxygen species
RNA	Ribonucleic acid
RT-PCR	Reverse-transcription-polymerase chain reaction
SCH 23390	[methyl]-(R)-(+)-8-chloro-2,3,4,5-tetra-hydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol
S.E.M	Standard error of mean
Smac	second mitochondrion-derived activator of caspase
SMC	Smooth muscle cells
SN	Substantia nigra
SNpc	Substantia nigra pars compacta
SNpr	Substantia nigra pars reticulate
SOD	Superoxide dismutase
STN	Subthalamic nucleus
TM	Transmembrane
TH	Tyrosine hydroxylase
TNF-α	Tumor necrosis factor-α
VTA	Ventral tegmental area
YM-09151-2	cis-N-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide

CONTENTS

RESULTS

Introduction

As described by the English physician James Parkinson in "An Essay on the Shaking Palsy," Parkinsonism is clinically characterized by the triad of tremor, rigidity and bradykinesia. Parkinsonism is defined as any combination of six specific, independent motoric features: tremor-at-rest, bradykinesia, rigidity, loss of postural reflexes, flexed posture and the freezing phenomenon (Thomas & Beal 2007; Varanese S *et al*., 2011). Dopamine (DA) and non-DA neurotransmitter systems within the basal ganglia are intimately connected by complex and not totally well understood relationships (Linazasoro *et al*., 2008). Parkinson's disease (PD) is currently regarded as the most common degenerative disorder of the aging brain after the Alzheimer's dementia. Most epidemiological studies estimate that over five million individuals in the world are carrying the diagnosis of PD and that roughly one lakh new cases arise each year (Fahn & Przedborski, 2010). PD is a neurodegenerative disease and these early motoric symptoms appear to be related to striatal dopamine deficiency due to loss of dopaminergic neurons in the substantia nigra *pars compacta*, which sends axons to the striatum.

Parkinson's disease is an age-related disorder, more common in senior citizens than in younger ones. The proper cause of this disease still remains a mystery, despite the role of oxidative stress, free radical formation, genetic susceptibility, programmed cell death and some unknown factor, which is endogenous or exogenous. The disease progresses slowly and ultimately produce complete akinesia. The neuropathology of the disease is based on the depigmentation and cell loss in the dopaminergic nigrostriatal tract of the brain with the corresponding decrease in the striatal dopamine concentration and the presence of eosinophilic inclusions called Lewy bodies (Marley, 2010).

Parkinson's disease is a progressive neurodegenerative disease marked by motor and non-motor abnormalities. The hallmark pathological features of PD are selective nigrostriatal dopaminergic degeneration and formation of filamentous,

cytoplasmic inclusions called Lewy bodies, containing α-synuclein and ubiquitin. Brains of PD patients show evidence of extensive oxidative damage and microglial activation. Additionally, PD patients are characterized by systemic mitochondrial dysfunction, marked by inhibition of complex I of the mitochondrial electron transport chain (Sherer *et al*, 2003). The pathogenesis of idiopathic PD is believed to involve an interaction between genetic and environmental factors. Specifically, PD has been associated with pesticide exposure and rural living. Most insights into PD pathogenesis come from investigations performed in experimental models of PD, especially those produced by neurotoxins.

Increasing evidence has suggested an important role for environmental toxins such as pesticides in the pathogenesis of PD. Chronic exposure to rotenone, a common herbicide, reproduces features of Parkinsonism in rats. Rotenone-induced dopaminergic neurodegeneration has been associated with both its inhibition of neuronal mitochondrial complex I and the enhancement of activated microglia (Hui-Ming *et al*., 2010).

Parkinson's disease appears essentially as a sporadic condition, without any other family members being affected. PD etiology remains mysterious, whereas its pathogenesis begins to be understood as a multifactorial cascade of deleterious factors. Although PD develop at any age, it is most common in older adults, with a peak age at onset around 60 years. The prevalence and incidence increases with age, with a lifetime risk of about 2%.

The early symptoms and signs are rest tremor, bradykinesia and rigidity. Bradykinesia is slowness and reduced amplitude of movement. Features of limb bradykinesia are a smaller and slower of handwriting, difficulty shaving and brushing teeth. Walking becomes slow, with decreased arm swing and with a tendency to shuffle feet. Difficulties arising from a deep chair, getting out of automobiles and turning in bed are symptoms of truncal bradykinesia (Saravanan 2005). Rigidity of muscles is detected by the examiner when he/she moves the

patient's limbs, neck or shoulders and experiences increased resistance. There is often a ratchet-like feel to the muscles, so-called cogwheel rigidity. These early motoric symptoms appear to be related to striatal dopamine deficiency due to loss of dopaminergic neurons in the substantia nigra pars compacta, which sends axons to the striatum. The early features of PD usually respond to medication that activates striatal dopamine receptors, such as levodopa and dopamine agonists, whereas three later motoric symptoms of flexed posture, loss of postural reflexes and freezing of gait do not. This lack of response suggests that these late features of PD are the result of nondopaminergic effects (Nicholson 2002). The neuropathology of PD is far from being restricted to the nigrostriatal pathway and histological abnormalities are also found in many other dopaminergic and nondopaminergic cell groups. Moreover, increasing bradykinesia that is not responsive to levodopa also appears as the disease worsens.

While the motor symptoms of PD dominate the clinical picture – and even define the parkinsonian syndrome – most patients with PD have other features that have been classified as nonmotor. These include bradyphrenia, slowness in mental function, decreased motivation and apathy, dementia, fatigue, depression, anxiety, sleep disturbances, fragmented sleep and REM sleep behaviour disorder, constipation and other autonomic disturbances of sexual and gastrointestinal complaints. Sensory symptoms include pain, numbness, tingling and burning in the affected limbs occurs in about 40% of patients. Dementia is associated with age and has been reported to occur in over 70% of patients with PD eventually.

Patients with PD live 20 or more years, depending on the age at onset. The mortality rate is about 1.5 times that of normal individuals of the same age. Death in PD is usually due to some concurrent unrelated illness or due to the effects of decreased mobility, aspiration, or increased falling with subsequent physical injury.

To model the systemic defect in complex I reported in PD, researchers have used rotenone exposure. Rotenone is a commonly used pesticide and potent,

specific inhibitor of mitochondrial complex I. Rotenone because of its lipophilic nature, crosses biological membranes easily and independent of transporters. As a result, systemic rotenone exposure inhibits complex I uniformly throughout brain (Betarbet *et al*., 2000).

Most studies using the rotenone model of PD use chronic treatment regimens. Rotenone gains access to the brain whether given intravenously, subcutaneously, or intraperitoneally (Alam & Schmidt 2002; Betarbet *et al*., 2000; Sherer *et al*., 2003).

Rotenone infused animals demonstrated reduced locomotor activity, hunched posture and in some cases rigidity and freezing behavior (Betarbet *et al*., 2000; Sherer *et al*., 2003). Specifically, rotenone-treated animals show decreased rearing, line crossing and head dips in open field tests and increased catalepsy. Behavioral deficits in rotenone treated animals correlated with striatal dopamine loss (Alam & Schmidt 2002). An initial study examining the effects of rotenone on the nigrostriatal dopaminergic system demonstrated that direct stereotaxic injection of rotenone into the medial forebrain bundle damaged the nigrostriatal dopaminergic system, marked by reduced dopamine levels in the striatum (Heikkila *et al*., 1985,). While chronic exposure to rotenone at high doses (12 mg/kg/day) failed to cause selective dopaminergic neurodegeneration (Ferrante *et al*., 1997,), chronic systemic low dose (2–3 mg/kg/day) rotenone exposure caused highly selective nigrostriatal dopaminergic degeneration.

Despite causing uniform mitochondrial inhibition throughout the brain, rotenone treatment reproduces many features of PD including motor abnormalities, selective nigrostriatal dopaminergic degeneration and formation of α-synuclein, ubiquitin-positive aggregates in nigral neurons.

Dopamine, a major neurotransmitter in central nervous system is involved in the control of motor and cognitive programmes. Dopaminergic neurons appear early during development, 6-8 weeks in humans. DA is synthesised from tyrosine, stored in vesicles in axon terminals and released when the neuron is depolarised.

DA interacts with specific membrane receptors to produce its effects. These effects are terminated by reuptake of dopamine into the presynaptic neuron by a dopamine transporter or by metabolic inactivation by monoamine oxidase B (MAO-B) or catechol-*O*-methyltransferase (COMT). DA plays an important role both centrally and peripherally. The recent identification of five dopamine receptor subtypes provides a basis for understanding dopamine's central and peripheral actions. DA receptors are classified into two major groups: DA D_1 like and DA D_2 like. DA D_1 like receptors consists of DA D_1 and DA D_5 receptors. DA D_2 like receptors consists of DA D_2, DA D_3 and DA D_4 receptors. Stimulation of the DA D_1 receptor gives rise to increased production of cAMP. DA D_2 receptors inhibit cAMP production, but activate the inositol phosphate-second messenger system (Seeman, 1980). Disturbances of the development of the dopmaninergic system lead to dyskinesia, dystonia, tics and abnormal eye movements. An imbalance between dopaminergic neurotransmission and DA receptors is known to be associated with the symptomatology of numerous neuropsychiatric disorders, like schizophrenia, psychosis, mania and depression as well as neuropathological disorders, like Parkinson's disease and Huntington's disease (Carlsson, 1988, 1993; Bermanzohn & Siris, 1992; Brown & Gershon, 1993; Jakel & Maragos, 2000; Kostrzewa & Segura-Aguilar, 2003,). The dopaminergic cells in particular are highly sensitive to excitotoxicity and oxidative stress when the energy metabolism is impaired (Callahan *et al.*, 1998,). Of all the neurotransmitter systems, DA is of particular interest in relation to the development of cognitive abilities subserved by the prefrontal cortex. The most postsynaptic markers of the DA system are its receptors.

Non-dopaminergic neurotransmission is also affected in PD. The dysfunction of non-dopaminergic systems explains the principal non-dopaminergic symptoms, such as ‘axial’ signs and cognitive impairment.

The non-dopaminergic neurotransmitters affected in PD are noradrenaline (norepinephrine), serotonin (5-hydroxytryptamine; 5-HT), glutamate, gamma-

aminobutyric acid (GABA), acetylcholine and neuropeptides (Bonnet, 2000). Dysfunction of these systems lead to some of the motor symptoms of the disease and provide targets for pharmacological interventions to treat these symptoms. For example, antagonists of certain glutamate receptors have been found to improve Parkinsonian symptoms when given with levodopa, although adverse effects limit their use.

The dysfunction of non-dopaminergic neurotransmitter systems in PD is also important because it leads to non-motor symptoms that are not responsive to dopaminergic therapy and can be a major cause of disability during disease evolution (Zhang Y, 2010). Dysautonomia is not infrequent in individuals with PD and is characterised by constipation, urinary disorders and orthostatic hypotension, the latter resulting from deficits in adrenergic and noradrenergic neurotransmission. Postural instability is caused by abnormalities in both dopaminergic and non-dopaminergic pathways. Depression is partially a result of dopaminergic denervation, but also of a decrease of serotonergic transmission. Cognitive impairment with frontal lobe-like symptomatology is a result of the dopaminergic deficit but also, at least in part, a cholinergic and noradrenergic deficit.

Long term use of levodopa is associated with complications such as dyskinesias. Although these are treated initially with other dopaminergic treatments, including changes in the levodopa administration schedule and dopamine receptor agonists, there have been attempts to treat dyskinesias with non-dopaminergic drugs (Phylinda 2010). Agents such as glutamate antagonists and opioid antagonists have been found to be useful.

Stem cell study is one of the most fascinating areas of research. Stem cells have the ability to differentiate under appropriate conditions to the required mature cell types (Kaplitt 2001). Bone marrow has stem cells, mesenchymal and hematopoetic, which can be used for therapeutic applications (Bjorklund, 1999). This promising area of science led scientists to investigate the possibility of cell-

based therapies to treat diseases like Parkinson's, Alzheimer's, spinal cord injury, epilepsy, stroke, heart disease and diabetes. In the present study, we administrated bone marrow derived stem cells in combination with Serotonin and GABA for Parkinson's disease management and the brain dopaminergic functional regulation by Serotonin, GABA and bone marrow cells at the molecular level.

5-HT receptor subtypes and their putative role in the control of movement suggest possible novel intervention strategies for modulating dopaminergic and non-dopaminergic systems in PD patients based on the distribution, localization and function in the basal ganglia (Barnes 1999). 5-HT receptor subtypes and serotonergic modulation of dyskinesia syndrome and psychosis in PD has made significant progress with the availability of these selective serotonergic agents. Serotonin has been recognized to cause proliferation of a variety of cells in culture and the activation of tyrosine kinase as done by many of the novel mitogens (Di Matteo *et al*., 2010)

GABA, the main inhibitory neurotransmitter in the mature CNS is implicated in playing a complex role during neurogenesis. Through embryonic development, GABA was demonstrated as acting as a chemo-attractant and being involved in the regulation of progenitor cell proliferation (Behar *et al*., 2000; Haydar *et al*., 2000). GABA acts as a trophic factor, being involved in neurogenesis, neuron development and migration. GABA and its receptors play a key role in neuroblast proliferation, migration and differentiation in nervous system development (Owens 2002).

Cell transplantation to replace lost neurons is a promising approach for the treatment of progressive neurodegenerative diseases. Induced pluripotent stem cells derived from somatic cells of PD patients used for mechanistic studies of PD pathogenesis and drug screening (Soldner et al, 2009). Hematopoietic system is used as a source of progenitor cells for the central nervous system (CNS) and it also has the property to differentiate into both microglia and macroglia when injected directly to the brain of adult rats (Martin & Eva 1997). The success of the

cell transplantation will depend on the ability of the cells to replace those neurons lost as a result of the disease process in the DA-deficient striatum and reverse, at least in part, the major symptoms of the disease. Serotonin (5HT) and Gamma aminobutyric acid (GABA) as therapeutic agents for cell proliferation and differentiation is a novel approach.

The signaling from the neurotransmitters is carried to the cell nucleus by second messengers like IP3, cAMP and cGMP. Their expression and changes play a major role in the signaling cascade (Hajnoczky *et al*., 2010). Different transcriptions factors, α-synuclein, ubiquitin and Bax are modulated during Parkinson's disease to overcome the neurodegeneration. Cyclic AMP responsive element binding protein (CREB) plays an important role in a variety of cellular processes, including proliferation, differentiation and adaptive responses. Increased CREB phosphorylation during Parkinson's disease recovery is reported to be associated with neuronal survival. (Walton & Dragunow 2000, Finkbeiner 2000; Shimamura *et al*., 2000).

In the present work, the effects of serotonin, GABA and bone marrow cells supplementation intranigrally to substantia nigra as treatment individually and in combination on rotenone induced Hemiparkinson's disease in rats were analyzed. Dopaminergic binding parameters investigated its role in the regulation of dopamine receptor subtypes in the brain regions of the experimental rats. Gene expression analysis of receptor specific probes for Dopamine D1, D2, pro-apoptotic protein bax, transcription factor CREB, regulatory protein ubiquitin and neural protein of α-synuclein in the brain regions of control and experimental groups of rats were studied. Immunohistochemistry of brain slices were done to confirm the binding studies and gene expression analysis using specific antibodies. Behavioural responses in Rotarod, Social interaction, Elevated plus maze, Grid walking and Narrow beam tests were carried out to assess the motor learning deficit in rotenone induced PD rats.

OBJECTIVES OF THE PRESENT STUDY

1) To induce unilateral Parkinsonism in adult male Wistar Rats using rotenone and to study the effect of 5-HT, GABA and BMC treatment individually and in combinations.

2) To quantify dopamine in various brain regions of control and experimental groups of PD rats.

3) To study the differentiation of bone marrow cells using cell tracker dye PKH2GL and Nestin to the premature neurons in the substantia nigra of the control and experimental groups of PD rats.

4) To study the dopamine receptor subtypes binding parameters and their functional regulation in Parkinson's disease using Bone marrow cells and neurotransmitters combinations.

5) To study the gene expression of dopamine D_1 and D_2 receptor subtypes using Real time PCR in the brain regions of control and experimental groups of PD rats.

6) To study the regulation of second messengers-cyclic AMP, cyclic GMP , inositol triphosphate in the brain regions of control and experimental groups of PD rats.

7) To study the transcription factor CREB, pro-apoptotic protein Bax, regulatory protein ubiquitin carboxy-terminal hydrolase and neural protein of α-synuclein in the brain regions of control and experimental groups of PD rats.

8) To study the localisation and expression status of dopamine D_1 and D_2 in the brain regions of control and experimental groups of PD rats using specific antibodies in confocal microscope.

9) To study the behaviour changes in control and experimental PD rats using rotarod test, elevated plus maze, social interaction test, grid walk test and narrow beam test to assess the motor learning deficit

Literature Review

Parkinson's disease (PD), one of the most prevalent neurodegenerative disorders among the elderly population, is charecterised by dopamine neurons degeneration in the substantia nigra pars compacta which makes an impact on ascending adrenergic and serotonergic networks, frontocortical cholinergic projections and a diversity of neuronal circuits located not only in the brain (from the cortex to the medulla), but even in the spinal cord and sympathetic nervous system (Smith, 2008; Djaldetti, 2009). It often impairs the sufferer's motor skills, speech and other functions. It is characterized by muscle rigidity, tetrad of tremor at rest with a frequency of about 4 Hz, postural instability, a slowing of physical movement (bradykinesia) and, in extreme cases, a loss of physical movement (akinesia). Parkinson's disease also affects movement (motor symptoms), mood, behaviour, thinking and sensation (non-motor symptoms). This disease is characterized by Lewy body formation and neuronal loss in brain-stem nuclei, particularly the substantia nigra, leads to movement disorder. The motor abnormalities are caused by alterations in basal ganglia network activity of the subthalamic nucleus (STN) and excessive activity of the major output nuclei (Choi *et al*., 2011). The symptoms are the results of decreased stimulation of the motor cortex by the basal ganglia, normally caused by the insufficient formation and action of dopamine, which is produced in the dopaminergic neurons of the substantia nigra pars compacta (SNpc) of brain.

Epidemiology

Parkinson's disease is the second most common neurodegenerative disorder, trailing Alzheimer disease. Charcot, in the late nineteenth century first suggested that the shaking palsy be given the name "Parkinson's disease" (Goetz *et al*., 2000). In the general population, the prevalence of PD is approximately 100 in 100,000. However,

PD is an age-related illness and in individuals aged sixty-five or older, its prevalence go to 1–2%. The average age of symptom onset is sixty to sixty-five, but approximately 10% of PD patients develop motor symptoms before age forty. Investigators have never identified a uniform etiology for PD and it has become increasingly clear in recent years that there is probably no single cause and that PD is, in fact, not a disease in the strict sense, but rather a syndrome with multiple etiologies: some environmental, some genetic and perhaps the majority a combination of the two.

Various environmental factors are hypothesized to be operative in the development of PD. Rural living with its agricultural chemical exposure, certain industrial environments and even occupations such as the teaching and medical professions are reported to confer an increased risk for development of PD (Marras & Tanner, 2004).

Pathophysiology of Parkinson's Disease

Investigators have long considered the progressive destruction of nigrostriatal dopaminergic neurons with consequent striatal dopamine deficiency as the pathological hallmark of PD. The presence of Lewy bodies in surviving dopaminergic neurons in the substantia nigra is also considered central to the pathologic confirmation of PD. In recent years it has become evident, however, that neither of these dogmas is absolutely true. While nigrostriatal dopaminergic cellular loss is certainly a central feature of the disease process, the damage is not confined to this pathway and neuronal loss in other locations within the central nervous system has clearly been identified. Moreover, damage in PD is not even confined to the central nervous system. Neuronal loss and even dopamine deficiency are documented within the enteric nervous system of the gastrointestinal tract (Singaram *et al*., 1995). Peripheral cardiac sympathetic denervation is also identified in the setting of PD (Goldstein, 2003).

Even the Lewy body is no longer sacrosanct in that the parkin mutation that results in an autosomal recessive form of young-onset PD is not accompanied by the presence of Lewy bodies (Mouradian, 2002).

Clinical Features Of Parkinson's Disease

Premonitory Features

Shoulder discomfort was the first symptom in 8% of PD patients preceded the emergence of more typical PD features by as much as two years (Nutt *et al*., 1992). A sense of undue fatigue and diminished energy also herald the onset of clinical PD (Hoehn & Yahr, 1967). Patients present with dizziness, constipation, urinary dysfunction, seborrheic dermatitis, or sweating abnormalities. Impaired olfaction, with consequent impairment of taste, also antedate motor features of PD (Furtado & Wszolek, 2004).

Cardinal Features

Tremor is the feature of PD that is most readily recognized by the lay public and the one that most often prompts its diagnosis. It is the initially identified clinical feature in 50–70% of PD patients, but never develops at all in up to 15% (Jankovic 2003; Pal *et al.*, 2002). As tremor first emerges, it only intermittent and even fleeting and it is brought out or accentuated by stress, fatigue, or anxiety. In advanced PD, tremor diminishes in prominence and sometimes virtually disappear (Toth *et al*., 2004). In animal models lesioning of the nigrostriatal tract alone does not produce Parkinsonian's tremor (Pechadre *et al*., 1976). This explains why tremor often responds incompletely to dopaminergic medication in the treatment of PD.

Rigidity is characterized by increased muscle tone that produces abnormal resistance to passive movement. The resistance is velocity-independent in that

thespeed of passive movement does not significantly affect the degree of resistance to the movement (Dewey, 2000).

Bradykinesia is the most disabling component of PD and the cardinal feature that most closely correlates with nigrostriatal dopaminergic cell loss and dopamine deficiency. It is the feature most responsive to dopaminergic therapy. Some degree of bradykinesia eventually develops in virtually all PD patients (Selby, 1990).

Postural instability appears to be the consequence of loss or diminution of the normal anticipatory responses or reflexes that appear following postural perturbations or the expectation of such (Hallett, 2003).

Secondary Features

Micrographia **-** changes in handwriting frequently develop in individuals with PD and are occasionally the presenting feature. Impairment of speech or voice eventually develops in over 75% of persons with PD (Ramig, *et al*., 2002).

Non – Motor Features

Depression is the most frequently encountered psychological problem in PD patients. Reported prevalence figures vary considerably, but some degree of depression appears to be present in approximately 50% of PD patients, although moderate to severe depression is present in approximately only 5% (Burke *et al*., 2004; Tandberg *et al*., 1996). Depression antedates the appearance of motor dysfunction in some patients. Anxiety, including panic attacks, also becomes problematic for PD patients, sometimes in conjunction with depression but also independently (Fernandez & Simuni, 2004). Anxiety also appears as a wearingoff phenomenon in individuals on dopaminergic therapy. Obsessive-compulsive features have also been noted in individuals with PD (Bruneau, 2004).

Gastrointestinal dysfunction is the most widely recognized element of autonomic dysfunction in PD (Pfeiffer, 2003; Pfeiffer & Quigley, 2004). Excess saliva is often present and sometimes very troubling to PD patients. Gastrointestinal dysfunction in PD appears to be due to central nervous system dysfunction combined with the documented loss of dopaminergic neurons and formation of Lewy bodies in the enteric nervous system within the gastrointestinal tract itself (Singaram, *et al*., 1995; Wakabayashi, *et al*., 1990). Urinary dysfunction also develops frequently in PD. Symptoms of bladder irritability, with urinary frequency and urgency; develop in 57 to 83% of persons with PD (Singer, 2004). Carlucci and Hauser (2004) suggested that obstructive sleep apnea which occurs more frequently in PD than in the normal population.

Rotenone

Among the toxic animal models of PD, rotenone represents one of the most recently used approaches (Betarbet *et al*., 2000). Rotenone is the most potent member of the rotenoids, a family of natural cytotoxic compounds extracted from various parts of *Leguminosa* plants (Mulcahy, 2011). Rotenone is widely used around the world as insecticide and pesticide (Hisata, 2002).

Rotenone is highly lipophilic and thus readily gains access to all organs including the brain. After a single intravenous injection, rotenone reaches maximal concentration in the CNS within 15 min and decays to about half of this level in less than 2 h (Talpade *et al*., 2000). Its brain distribution is heterogeneous (Talpade *et al*., 2000), paralleling regional differences in oxidative metabolism. Rotenone also freely crosses all cellular membranes and accumulates in subcellular organelles such as mitochondria. In mitochondria, rotenone impairs oxidative phosphorylation by inhibiting reduced nicotinamide adenine dinucleotide (NADH)-ubiquinone reductase activity through its binding to the PSST subunit of the multipolypeptide enzyme complex I of the electron transport chain (Schuler & Casida, 2001). Aside from its

action on mitochondrial respiration, rotenone also inhibits the formation of microtubules from tubulin (Brinkley *et al*., 1974; Marshall & Himes, 1978). This effect is quite relevant to the mechanism of dopaminergic neurodegeneration because excess of tubulin monomers is toxic to cells (Burke *et al*., 1989; Weinstein & Solomon, 1990). Interestingly, a protein implicated in some familial forms of PD, parkin, appears to bind to tubulin, thereby enhancing the ubiquitination and degradation of misfolded tubulins, an effect that is lacking with the PD-linked parkin mutants (Ren *et al*., 2003).

Rotenone has been used extensively as a prototypic mitochondrial poison in cell cultures, but less frequently in living animals. Exposure of embryonic ventral midbrain cultures to rotenone causes major neurotoxicity (Marey-Semper *et al*., 1995), especially in the presence of microglial cells (Gao *et al*., 2002). In these two studies, markers of dopaminergic neurons were altered than those of γ-aminobutyric acid (GABA) neurons, suggesting greater susceptibility of dopaminergic neurons to such an insult. In animals, rotenone has been administered by different routes. Oral delivery of rotenone appears to cause little neurotoxicity in animals (Marking, 1988). Systemic administration, on the other hand, often causes toxicity and lethality, the degree of which is related to the dose used. Stereotaxic injection of rotenone into the median forebrain bundle depletes striatal dopamine and serotonin (Heikkila *et al*., 1985). By quantitative analysis, it appears that substantia nigra dopaminergic neuron numbers are reduced by about 30% in rotenone-infused rats compared with vehicle controls (Hoglinger *et al*., 2003). The numbers of mesolimbic dopaminergic neurons, the cell bodies of which reside adjacent to the substantia nigra in the ventral tegmental area (VTA), are unaffected by rotenone administration (Hoglinger *et al*., 2003). In the striatum, the average loss of dopaminergic fibers is estimated to be 55% after rotenone infusion in rats (Hoglinger *et al*., 2003), that, like in PD, is greater than the loss of substantia nigra dopaminergic neurons. Despite the use of the exact same regimen of rotenone, the severity of the striatal dopaminergic damage in rats within a given

experiment appears highly variable, ranging from none to near complete (Betarbet *et al*., 2000; Sherer *et al*., 2003; Hoglinger *et al*., 2003; Lapointe *et al*., 2004; Zhu *et al*., 2004). After the infusion of rotenone, the loss of tyrosine hydroxylase-positive fibers in the striatum is either focal, showing a zone of maximal loss at the center, or diffuse (Betarbet *et al*., 2000; Sherer *et al*., 2003; Hoglinger *et al*., 2003; Lapointe *et al*., 2004; Zhu *et al*., 2004) in rotenone-infused rats, some of the remaining substantia nigra dopaminergic neurons contain proteinaceous inclusions (Betarbet *et al*., 2000; Sherer *et al*., 2003; Hoglinger *et al*., 2003). Like Lewy bodies in PD, these inclusions are immunoreactive for both ubiquitin and α-synuclein (Betarbet *et al*., 2000), and by electron microscopy they appear composed of a dense core with fibrillar peripheral elements (Betarbet *et al*., 2000). Likewise in PD in which neurodegeneration extends beyond the dopaminergic system (Agid *et al*., 1987), rotenone infusion is associated with 35% reduction in serotonin transporter density in the striatum, 26% reduction of noradrenergic neurons in the locus coeruleus, and 29% reduction in cholinergic neurons in the pedunculopontine nucleus (Hoglinger *et al*., 2003).

Although the initial descriptive studies did not report any striatal lesion (Betarbet *et al*., 2000), the number of dopamine-regulated phosphoprotein-32 projecting neurons, cholinergic interneurons and reduced nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase-positive neurons in the striatum were all found significantly reduced by the infusion of rotenone in rats (Hoglinger *et al*., 2003; Lapointe *et al*., 2004).

Behaviourally, rotenone-infused rats exhibit reduced mobility, flexed posture, and in some cases rigidity and even catalepsy (Alam & Schmidt, 2002). Twenty four hours after the infusion of rotenone, rats show more than 70% reduction in spontaneous motor activity.

Role of Neurotransmitters in PD

Dopamine

Dopamine is the predominant catecholamine neurotransmitter in the mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake and endocrine regulation. This catecholamine also plays multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function and gastrointestinal motility (Missale *et al*., 1998).

DA containing neurons arise mainly from DA cell bodies in the substantia nigra and ventral tegmental area in mid-brain region (Royh *et al*., 1991; Carlsson, 1993; Lookingland *et al*., 1995; Tarazi *et al*., 2001; Tepper *et al*, 1997). Dopaminergic system is organized into four major subsystems (i) the *nigrostriatal* system involving neurons projecting from the substantia nigra *pars compacta* to the caudate-putamen of the basal ganglia. This is the major DA system in the brain as it accounts for about 70% of the total DA in the brain and its degeneration makes a major contribution to the pathophysiology of Parkinson's disease; (ii) *the mesolimbic system* that originates in the midbrain tegmentum and projects to the nucleus accumbens septi and lateral septal nuclei of the basal forebrain as well as the amygdala, hippocampus and the entorhinal cortex. They are all considered components of the limbic system and hence of particular interest for the pathophysiology of idiopathic psychiatric disorders; (iii) the *mesocortical* system, which also arises from neuronal cell bodies in the tegmentum which project their axons to the cerebral cortex, particularly the medial prefrontal regions; (iv) the *tuberinfundibular* pathway, which is a neuroendocrinological pathway arising from the arcuate and other nuclei of the hypothalamus and ending in the median eminence of the inferior hypothalamus. DA released in this system exerts regulatory effects in the anterior pituitary and inhibits the release of prolactin. DA is involved in the

control of both motor and emotional behaviour. Despite the large number of crucial functions it performs, DA is found in a relatively small number of brain cells. In fact, while there are a total of 10 billion cells in the cerebral cortex alone, there are only one million dopaminergic cells in the entire brain (Missale *et al*., 1998).

Biosynthesis of dopamine

DA is synthesized from the amino acid L-tyrosine. L-tyrosine is hydroxylated by the enzyme tyrosine hydroxylase (TH) to give L-3, 4-dihydroxyphenylalanine (L-DOPA) which is the rate limiting step. L-DOPA is subsequently decarboxylated to DA by the enzyme aromatic L-amino acid decarboxylase. Therefore, it is not possible to enhance the levels of DA by providing L-tyrosine. The activity of tyrosine hydroxylase is regulated by several endogenous mechanisms. For example, the enzyme is activated by increased neuronal impulse flow, but is inactivated either by DA itself as an end product inhibitor or by activation of presynaptic DA receptors. On the other hand, the enzyme aromatic L-amino acid decarboxylase converts L-DOPA to DA instantaneously. Therefore, providing L-DOPA creates a possibility to enhance the formation of DA.

Dopamine reuptake and metabolism

DA exerts its functions mediated through various receptors and these actions are terminated to prevent continuous stimulation of the receptors. This inactivation is brought about by reuptake mechanisms and metabolism of DA. Reuptake of DA is accomplished by a high affinity carrier present in the membrane, the dopamine transporter (DAT). The DA transporter recycles extracellular DA by actively pumping it back into the nerve terminal. The DA content which is about 70 to 80 % in the striatal synaptic cleft is inactivated by this process. Drugs, such as cocaine are able to block the action of the DA transporter, thereby sustaining the presence of DA in the synaptic cleft and its action on DA receptors. Part of the DA is inactivated by

conversion to inactive compounds by metabolic enzymes, which are present both intra and extraneuronally. Monoamine oxidase (MAO), aldehyde dehydrogenase (ALDH) and catechol-*O*-methyltransferase (COMT) are responsible for the metabolism of DA. DA after reuptake is intraneuronally deaminated by MAO to give dihydroxyphenyl acetaldehyde which subsequently is converted to 3, 4-dihydroxyphenylacetic acid (DOPAC) by ALDH. DOPAC is then methylated by COMT to give homovanillic acid (HVA). Extraneuronally, DA is metabolized by an alternative route in which it is first *O*-methylated to 3-methoxytyramine (3-MT) through the action of COMT and subsequently oxidized by MAO and ALDH to HVA.

Dopamine receptors

DA mediates its actions *via* membrane receptor proteins. DA receptors are found on postsynaptic neurons in brain regions that are DA enriched. In addition, they reside presynaptically on DA neuronal cell bodies and dendrites in the midbrain as well as on their terminals in the forebrain. DA receptors belong to a family of large peptides that are coupled to G-proteins which are modified by attached carbohydrate, lipid-ester or phosphate groups. The topologies of the five DA receptors are predicted to be the same as all the other G-protein-coupled receptors. They are characterized by having seven hydrophobic transmembrane-spanning regions. The third intra cytoplasmic loop is functionally critical and interacts with G-proteins and other effector molecules to mediate the physiological and neurochemical effects (Royh *et. al*., 1991; Carlsson, 1993; Tarazi *et al*., 1997; Tepper *et al*., 1997). In their putative transmembrane domains, the DA D_1 and DA D_5 receptors are 79% identical to each other, while they are only 40–45% identical to the DA D_2, DA D_3, and DA D_4 receptors. Conversely, the DA D_2, DA D_3, and DA D_4 receptors are between 75% and 51% identical to each other. They contain seven putative membrane spanning helices which would form a narrow dihedral hydrophobic cleft surrounded by three extracellular and three intracellular loops. The receptor polypeptides are probably

further anchored to the membranes through palmitoylation of a conserved Cys residue found in their carboxy tails, 347 in DA D_1, the C-terminus in DA D_2 like receptors. The DA receptors are glycosylated in their N-terminal domains. DA D_1 like subtypes has potential glycosylation sites in their first extra cytoplasmic loop.

DA receptors are divided into two families on the presence or absence of ability of DA to stimulate adenylyl cyclase and produce the second-messenger molecule cyclic AMP (cAMP) (Kebabian & Calne, 1979; Schwartz *et al*., 1992; Civelli *et al.*, 1993; O'Dowd, 1993; Jackson & Westlind, 1994; Ogawa, 1995; Strange, 1996). This classification is based on similarities in structure, pharmacology, function and distribution. DA D_1 like receptors are characterized initially as mediating the stimulation of cAMP production. DA D_2 like receptors inhibits the production of cAMP. This pharmacological characterization is based on the ability of some DA agents to block adenylyl cyclase activity to inhibit the release of prolactin *in vivo* and *in vitro* in a cAMP independent fashion (Seeman, 1980). Applications of recent technical advances in molecular genetics have greatly facilitated the isolation and characterization of novel DA receptors, DA D_3, DA D_4 and DA D_5, with different anatomical localization from traditional DA D_1 or DA D_2 receptors. Based upon their pharmacological profiles, including their effects on different signal transduction cascades, these receptors are currently divided into two families: the DA D_1 like family which includes DA D_1 and DA D_5 receptors. The DA D_2 like family includes DA D_2, DA D_3 and DA D_4 receptors (Schwartz *et al*., 1992; Grandy *et al*., 1993; Sibley *et al*., 1993). The genomic organizations of the DA receptors demonstrate that they are derived from the divergence of two gene families that mainly differ in the absence or the presence of introns in their coding sequences. DA D_1 like receptors genes do not contain introns in their coding regions, a characteristic shared with most G-protein coupled receptors. The genes encoding the DA D_2 like receptors are interrupted by introns (Gingrich & Marc, 1993). Furthermore, most of the introns in the DA D_2 like receptor genes are located in similar positions.

Dopamine D_1 like family

The DA D_1 receptor is the most abundant DA receptor in the CNS. The DA D_1 like receptors are characterized by a short third loop as in many receptors coupled to Gs protein (Civelli *et al*., 1993; Gingrich & Canon, 1993; O'Dowd, 1993). The DA D_1 like receptors has short third intracellular loops and long carboxy terminal tails. The DA D_1 like receptors is classified into DA D_1 and DA D_5. In the DA D_1 and DA D_5 receptor third intracellular loop and the carboxy terminus are similar in size but divergent in their sequence. In contrast, the small cytoplasmic loops 1 and 2 are highly conserved so that any difference in the biology of these receptors is related to the third cytoplasmic loop and the carboxy terminal tail (Civelli *et al*., 1993; Gingrich & Marc, 1993; O'Dowd, 1993). The external loop between transmembrane domain (TM) TM4 and TM5 is considerably different in the two receptor subtypes, being shorter (27 amino acids) in the DA D_1 receptor than in the DA D_5 receptor (41 amino acids). The amino acid sequence of this loop is divergent in the DA D_5 receptor (Marc *et al*., 1998).

Dopamine D_1 receptor

DA D_1 receptors are found at high levels in the typical DA regions of brain such as the neostriatum, substantia nigra, nucleus accumbens and olfactory tubercles. DA D_1 receptor seems to mediate important actions of DA to control movement, cognitive function and cardiovascular function. The DA D_1 receptor gene, which lacks introns, encodes a protein that extends for 446 amino acids (Dohlman *et al*., 1991). In humans DA D_1 receptor gene has been localized to chromosome 5 (Sunahara *et al*., 1990). The DA D_1 receptors show characteristic ability to stimulate adenylyl cyclase and generate inositol 1, 4, 5-trisphosphate (IP3) and diacylglycerol (DAG) *via* the activation of phospholipase C (Monsma *et al*., 1990; Sibley *et al*., 1990). DA D_1 receptors are highly expressed in basal ganglia followed by cerebral cortex, hypothalamus and thalamus. DA D_1 receptors messenger ribonucleic acid

(mRNA) is colocalized in striatal neurons of the basal ganglia with mRNA for DA receptor phosphor protein (DARPP-32; 32 kDa) which is a DA and cAMP-regulated phosphoprotein. DA Receptor Phosphor Protein contributes to the actions of DA D_1 receptor (Hemmings & Greengard, 1986; Greengard *et al*., 1987). The DA D_1 receptors in the brain are linked to episodic memory, emotion and cognition.

Dopamine D_5 receptors

DA D_5 receptors are localized in the substantia nigra pars compacta, hypothalamus, striatum, cerebral cortex, nucleus accumbens and olfactory tubercle (Khan *et al*., 2000, Beaulieu & Gainetdinov, 2011). The DA D_5 receptor gene is intronless and encodes a protein that extends for 47 amino acids (George *et al*., 1991). This protein has an overall 50% homology with DA D_1 receptor and 80% if only the seven transmembrane segments are considered. The gene encoding the human DA D_5 protein is located at the short arm of chromosome 4, the same region where the Huntington's disease gene has been located. Two DA D_5 receptor pseudogenes having 154 amino acids have been identified with 90% homology (Gusella, 1989). These pseudogenes, however, contain stop codons in their coding regions that prevent them from expressing functional receptors. The functions of these pseudogenes, which appear so far to be specific to humans, are not yet known (Grandy *et al*., 1991).

DA D_5 receptor mRNA expression is unique and limited to the hippocampus and parafascicular nucleus of the thalamus (Civelli *et al*., 1992). It is involved in the thalamic processing of painful stimuli (Giesler *et al*., 1979). DA D_5 receptors appear to interact with G-proteins and stimulate adenylyl cyclase, with relatively high affinity for DA and DA D_1 selective agonists (George *et al*., 1991). Studies by Holmes *et al*., (2001) concluded that DA D_5 contributes to the pharmacological activation of dopaminergic pathways relevant to exploratory locomotion, startle, and prepulse inhibition.

Dopamine D_2 like family

DA D_2 like receptors belong to the G-protein coupled receptors and has 400 amino acid residues. DA D_2 like receptors is characterized by a long extracellular amino terminus which has several glycosylation sites and a shorter carboxy terminal tail with putative phosphorylation sites. The function of sugar moieties is not clear (Marc *et al*., 1998; Sibley, 1999). It is generally believed that the membrane enclosed part of the amino acid chain of G-protein coupled receptors is folded into seven α-helices. The transmembrane helices consist primarily of hydrophobic amino acid residues. The unique feature of DA D_2 like receptors family is that they posses a bigger third cytoplasmic (intracellular) loop in common, which is thought to be the site where the G-protein couples (Marc *et al*., 1998). Between the different DA receptors, the third loop also displays the greatest variability in amino acid sequence. This has consequences for their respective second messenger systems. The DA D_2 like receptors are coupled to G_i protein and inhibit the formation of cAMP. The DA D_2 receptors tertiary structure is stabilized by two cysteine disulphide bridges.

Dopamine D_2 receptors

The DA D_2 receptor gene encodes a protein that extends for 415 amino acids. Similar to other G-protein coupled receptors, the DA D_2 receptor has seven transmembrane segments, but in contrast to DA D_1 like receptors, the third cytoplasmic domain is long and the carboxy terminus is short. Unlike the DA D_1 like receptor genes, the DA D_2 receptor gene contains seven introns that are spliced out during mRNA transcription (Fischer *et al*., 1989). The gene encoding this receptor was found to reside on q22-q23 of human chromosome 11 (Makam *et al*., 1989). The DA D_2 receptor was the first receptor to be cloned (Chrisre *et al*., 1988). The DA D_2 receptors are involved in several signal transduction cascades, including inhibition of cAMP production (Vallar & Meldolesi, 1989), inhibition of phosphoinositide turnover (Epelbaum *et al*., 1986), activation of potassium channels and potentiation of

arachidonic acid release (Axelrod *et al*., 1991). The DA D_2 receptors are highly expressed in basal ganglia, nucleus accumbens septi and ventral tegmental area (Schwartz *et al*., 1992).

The DA D_2 receptor exist as two alternatively spliced isoforms differing in the insertion of a stretch of 29 amino acids in the third intracellular loop and are designated as DA D_{2S} and DA D_{2L} (Seeburg *et al*.,1989; Marc *et al*., 1998). Because this loop seems to play a central role in receptor coupling, the existence of a splicing mechanism at this level could imply functional diversity. However, in spite of the efforts of several groups, no obvious differences have emerged so far between the two DA D_2 receptor isoforms. The two isoforms are derived from the same gene by alternative RNA splicing which occurs during the maturation of the DA D_2 receptor pre-mRNA (Schwartz *et al*., 1989). DA D_2 receptor isoforms, DA D_{2S} and DA D_{2L} vary within each species by the presence or absence of a 29-amino acid sequence in the third cytoplasmic domain of the DA D_2 receptor peptide chain. Both variants share the same distribution pattern. The shorter form is less abundantly transcribed and they appear to differ in their mode of regulation (Marc *et al*., 1998). Pharmacologically, both isoforms exhibit nearly similar profiles in terms of their affinities to different DA D_2 selective agents and inhibit adenylyl cyclase activity. However, these isoforms display an opposite regulatory effect (Sibley *et al*., 1993). These isoforms have the same pharmacological profile, even though a marginal difference in the affinity of some substituted response to DA treatment is reported. DA induces the up regulation of DA D_{2L} isoform of DA D_2 receptors (Castro & Strange, 1993). When expressed in host cell lines, both isoforms inhibited adenylyl cyclase (Marc *et al*., 1998; Sibley, 1999). However, the DA D_{2S} receptor isoform displayed higher affinity than the DA D_{2L} in this effect (Seeburg *et al*., 1993). The isoforms of DA D_2 mediate a phosphatidylinositol-linked mobilization of intracellular calcium in mouse Ltk [-] fibroblasts. Protein kinase C (PKC), however, differentially

modulates DA D_{2S} and D_{2L} activated transmembrane signalling in this system with a selective inhibitory effect on the DA D_{2S} mediated response.

Dopamine D_3 receptors

DA D_3 receptor gene contains five introns and encodes a 446 amino acid protein (Schwartz *et al*., 1992). The gene encoding this receptor resides on chromosome 3 (Giros *et al*., 1990). The DA D_3 receptors bear close structural and pharmacological similarities to the DA D_2 receptors. DA D_3 mRNA occurs in longer and shorter spliced forms generated from the same gene (Schwartz *et al*., 1992). Distribution of DA D_3 receptor mRNA are distributed and expressed mainly in subcortical limbic regions including islands of Calleja, nucleus accumbens septi and olfactory tubercle, with low levels of expression in the basal ganglia. DA D_3 receptor mRNA has also been found in neurons of the cerebellum, which regulate eye movements (Levesque *et al*., 1992). The status of the DA D_3 molecular entity as a functional receptor remains uncertain since it neither couples to G-proteins nor consistently transduces an effector mechanism. However, the structural similarity with DA D_2 receptor raises the possibility that DA D_3 receptor also inhibit adenylyl cyclase activity in its normal cellular setting. Studies have reported that DA D_3 receptors mediate positive regulatory influences of DA on production of the peptide neurotensin (Schwartz *et al*., 1992; Sokoloff *et al*., 1990).

Dopamine D_4 receptors

DA D_4 receptor gene contains four introns and encodes a 387 amino acid protein (Van Tol *et al*., 1991). The overall homology of the DA D_4 receptor to the DA D_2 and DA D_3 receptors is about 41% and 39% respectively, but this homology increases to 56% for both receptors when only the transmembrane spanning segments are considered. The gene encoding the human DA D_4 protein is located at the tip of the short arm of chromosome 11 (Civelli & Bunzow, 1993; Missale *et al*., 1998). DA

D_4 receptor gene has been localized in brain regions like hippocampus and frontal cortex using specific histoprobes. The stimulation of DA D_4 receptor inhibits adenylyl cyclase activity and release arachidonic acid in brain neurons (Huff *et al*., 1994; Misalle *et al*., 1998). In humans, DA D_4 receptor occurs in several genomic polymorphic variants that contain two to eleven repeats of a 48 base pair segment that is expressed in the third cytoplasmic domain (Van Tol *et al*., 1992; Misalle *et al*., 1998). These are called the DA D_4 alleles which are represented as DA $D_{4.2}$, DA $D_{4.4}$ and DA $D_{4.7.}$ These contribute to the pathophysiology of certain neuropsychiatric disorders (Jackson & Westlind-Danielsson, 1994).

Most dorsal hypothalamic spinal projection neurons are dopaminergic and appear to be involved in autonomic function (Cechetto *et al*., 1988). DA terminal axon density is highest in the intermediolateral cell columns of the spinal cord, where preganglionic sympathetic nervous system neurons originate and microelectrophoretic DA application there inhibits sympathetic preganglionic neurons (Lindvall *et al*., 1983). Retrograde labeling has identified caudal lateral hypothalamic area neurons as likely sympathetic nervous system "central command neurons" (Jansen *et al*., 1995). In humans, DA D_2 agonists cause inhibition of sympathetic output that is abolished by DA D_2 antagonists but only at higher degrees of sympathetic stimulation (Mannelli *et al*., 1997). These data are consistent with previously discussed anatomical evidence for dopaminergic inhibition of sympathetic nervous system function and indicate that state dependent factors mediate DA D_2 antagonist effects.

Activation of these presynaptic receptors inhibits the release from their respective nerve terminals of other neurotransmitters such as NE, ACh and GABA (Hársing & Zigmond, 1997) from the striatum. Dopaminergic innervation of the medial and dorsolateral prefrontal cortex appears to be particularly vulnerable to stress and relatively low intensity levels of stress are capable of promoting significant responses. The prefrontal dopaminergic neurons have a number of higher functions including attention, 'working' memory and the acquisition of coping patterns in

response to stress (Castellano *et al*., 1999). Amphetamines and cocaine agonise these receptors and have a similar effect as stress, resulting in symptoms such as anxiety, panic, hypervigilence, exaggerated startle reflexes and paranoia (Horger *et al*., 1999). Malondialdehyde (MDA) and opiate receptors are plentiful in this area and stress-induced innervation of the fronto-cortical neurons is prevented if these receptors are selectively blocked. This increase of DA from the dendrites of DA neurons is due to an alteration in GABA regulation of the DA neurons. As with noradrenergic systems, single or repeated exposures to stress potentiates the capacity of a subsequent stressor to increase DA function in the forebrain without altering basal DA turnover, suggesting that the receptors have been hyper-sensitized (Basso *et al*., 1999).

Sensory and cognitive dissociations resulting from dopaminergic hyperfunction produce a state of fear and anxiety *via* direct anatomic connections from cortical brain structures to the limbic system principally through mesolimbic pathways (Iturriaga *et al*., 1996). This disinhibition of mesolimbic dopaminergic neurons cause the bizarre behavioural and cognitive symptoms experienced by patients in schizophrenia and by extension, with delirium (Harrison, 1999). Delirium resulting from dopaminergic hyperfunction is characterized by global disorders of cognition and wakefulness by impairment of psychomotor behavior (Miller *et al*., 1991). Major cognitive functions such as perception, deductive reasoning, memory, attention and orientation are all globally disordered. Excessive motor activity frequently accompanies severe cases of delirium and, when this occurs, the resulting constellation of symptoms is called 'agitated delirium' (Crippen, 1994).

Serotonin and serotonin receptors

Serotonin synthesis and metabolism

Serotonin was initially discovered as a vasoconstrictor substance in blood and later in blood vessel walls, platelets and in enterochromafine cells of the gastrointestinal system, the lungs and the heart (Rapport *et al*., 1948, Markoutsaki *et al*., 2011). Outside the CNS, 5-HT acts on autonomic smooth muscle cells, e.g. in blood vessels and the digestive tract (Zifa & Fillion, 1992). More than 50 years ago the chemical structure of 5-HT was identified and it was synthesised (Twarog & Page, 1953, Lee *et al*., 2010). Later, the function of 5-HT as a neurotransmitter in the CNS was proposed (Bogdanski *et al*., 1956) and 5-HT has been studied intensively since its identification in the pituitary gland (Hyyppa & Wurtman, 1973). In the CNS, serotonin is a two step pathway from the essential amino-acid tryptophan. Serotonin is synthesised in the perikarya of the neuron where tryptophan is hydroxylated to the 5-HT precursor 5-hydroxytryptophan (5-HTP) which is then decarboxylated to 5-HT (Hamon *et al*., 1982). To avoid immediate enzymatic oxidation to 5-hydroxy-indol acetic acid (5-HIAA) by monoamine oxidase (MAO), 5-HT is contained in neuronal vesicles until it is released into the synaptic cleft (Fisar *et al*., 2011). Serotonin then activates either postsynaptic or presynaptic receptors or is reuptaken *via* the 5-HT transporters molecule into the neuron (Hamon *et al*., 1982). The principle route of metabolism of 5-HT involves monoamine oxidase forming 5-Hydroxyindole acetic acid by a two step process. In addition to metabolism by MAO, a Na^+ dependent carrier mediated uptake process exists and is involved in terminating the action of 5-HT. The 5-HT transporters are localized in the outer membrane of serotonergic axon terminals and in the outer membrane of platelets. This uptake system is the only way that platelets acquire 5-HT since they do not have the enzymes required for synthesis of 5-HT. The degradation processes are very fast due to a large surplus of monoamine

oxidase. Therefore, concentrations of 5-HT in cerebral extra cellular space and in peripheral plasma are low and do not reflect serotonergic activity.

Anatomy of Serotonin System

The serotonin systems are widespread throughout the brain, with most of the cell bodies of serotonergic neurons located in the raphe nuclei of the midline brainstem (Palacios *et al*., 1990). The largest collections of 5-HT neurons are in the dorsal and median raphe nuclei of the caudal midbrain (Jacobs *&* Azmitia 1992). The neurons of these nuclei project widely over the thalamus, hypothalamus, basal ganglia, basal forebrain and the entire neocortex. Interestingly, these 5-HT neurons also provide a dense subependymal plexus throughout the lateral and third ventricles (Roth, 2011). Activation of this innervations result in 5-HT release into the cerebrospinal fluid (CSF) and measurement of 5-HT content in CSF in disease states will largely reflect this pool (Chan-Palay, 1976; Richerson & Buchanan, 2011). This is another interesting aspect of the 5-HT neuron innervation of forebrain. Descarries *et al*., (1975) has shown that the terminals of 5-HT neurons in forebrain, unlike terminals from other systems, only infrequently form synaptic complexes. Thus, when 5-HT neurons innervating forebrain are activated, 5-HT will be released into the extracellular fluid and its action will depend on the location of nearby 5-HT receptors. The organization of the ascending 5-HT neuron projections, the nature of their interaction with postsynaptic elements and the widespread distribution of 5-HT terminals in cortical and limbic areas indicate that these projections are most likely to be involved in the regulation of behavioural state and the modulation of more specific behaviours. The second 5-HT neuron system is comprised of 5-HT neurons in the pontine and medullary raphe with projections principally to brainstem, cerebellum and spinal cord. This system appears primarily to be involved in modulation of sensory input and motor control (Meltzer *et al*., 1998). During brain development, 5-HT provides essential neurotrophic signal. 5-HT is known to play an important role in

several physiological functions (Jackson & Paulose, 1999). Evidence from animal and human studies suggests that 5-HT is linked to many functions, such as mood, aggression, feeding and sleep. Dysregulation of 5-HT function is believed to be involved in depression, impulsivity and suicide (Meltzer, 1998). Additionally, modulation of cholinergic neuronal activity by 5-HT plays a role in higher cognitive processes such as memory and learning (Altman *et al*., 1990; Richter-Levin *&* Segal, 1990). Accordingly, alterations in serotonergic function accounts for behavioural disturbances commonly observed during PD. There is conflicting evidence from animal studies, post mortem work and limited clinical trails as to the direction, magnitudes and significance of these findings.

While Parkinson's disease is undoubtedly a disorder with a primary pathology of dopamine neuronal loss, that loss of dopamine and subsequent dopamine replacement therapy leads to imbalances in many non-dopaminergic transmitter systems, including 5-hydroxytryptamine (5-HT). Recent advances in understanding the role of 5-HT in parkinsonism and the generation of side-effects of dopamine replacement therapy (e.g. wearing-off and levodopa-induced dyskinesia) have identified 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2C} receptors as potential therapeutic targets in Parkinson's disease (Nicholson & Brotchie, 2002).

Dysfunction in the 5-HT system and reduced serotonin concentrations have been reported in patients with PD. Serotonin concentrations in neural tissue are controlled by a presynaptic serotonin transporter protein that is encoded by a single gene. However, investigations by Sally *et al*., (2000), suggest that defects in serotonin concentrations in patients with PD are unlikely to be due to polymorphisms in the serotonin transporter gene.

Dopamine uptake inhibitors provide a means of sustaining endogenous and exogenous striatal dopamine levels in Parkinson's disease, but most are not selective and also inhibit the noradrenaline and 5-hydroxytryptamine (5-HT) transporters. Lane

et al., (2005) suggest that inhibition of the 5-HT and noradrenaline transporters modulate dopamine uptake inhibitor-mediated motor activity.

GABA Receptors

Gamma- aminobutyric acid, also known as GABA was discovered over 40 years ago as a key inhibitory neurotransmitter in the brain (Bazemore *et al*., 1957; Krnjevic & Phillis, 1963). Since then, evidence has accumulated that this amino acid function as a neurotransmitter not only in the CNS but also in the peripheral nervous system, including the mesenteric plexus (Amenta, 1986), major pelvic ganglia (Akasu *et al*., 1999), sympathetic ganglia, encompassing the rat superior cervical ganglion (Wolff *et al*., 1986; Kasa *et al*., 1988) and abdominal prevertebral ganglia (Parkman & Stapelfeldt, 1993). In the mammalian central nervous system, GABA is the most important inhibitory neurotransmitter occurring in 30-40% of all synapses. Three types of GABA receptors have been identified: $GABA_A$ and $GABA_C$ receptors are ligand-gated Cl^- channels, while $GABA_B$ receptors are G-protein coupled (Chebib & Johnston, 1999; Perfilova & Tiurenkov, 2011). $GABA_A$ receptors are ligand gated Cl^- channels that consist of a heteromeric mixture of protein subunits forming a pentameric structure and $GABA_B$ receptors couple to Ca^{2+} and K^+ channels *via* G-proteins and second messengers (Johnston, 1996). In the CNS, application of GABA reduces excitability by a combination of $GABA_A$ and $GABA_B$ receptor activation, leading to membrane re-polarization, reduced Ca^{2+} influx and suppression of neurotransmitter release (Tiurenkov & Perfilova, 2010). The genetic diversity of multiple $GABA_A$ receptor subunits permits the assembly of a vast number of receptor heteromeric isoforms. Apparently, the subunit composition determines the pharmacological profile of the resulting receptor subtypes (Barnard *et al*., 1998). Mechanisms that modulate the stability and function of postsynaptic $GABA_A$ receptor subtypes and that are implicated in functional plasticity of inhibitory transmission in the brain are of special interest (Luscher & Keller, 2004).

GABA is the most widely distributed inhibitory neurotransmitter (Sivilotti, 1991) and because during nervous system development, GABA can act as a trophic factor, being involved in neurogenesis, neuron development and migration (Owens, 2002). Receptors for GABA are divided into three main classes: $GABA_A$ and $GABA_C$ receptors, which are members of the ligand-gated ion, channel superfamily and $GABA_B$ receptor, which is a member of the Gprotein- linked receptor superfamily (Bormann, 2000; Lujan, 2005; McKernan, 1996). The $GABA_B$ receptor exists as a heterodimer with the subunits designated $GABA_{B1}$ and $GABA_{B2}$ (Jones, 1998). Both $GABA_A$ and $GABA_B$ receptors seem to be required for GAB_A functioning as neuron developmental signal (Owens, 2002).

It should be considered that the GABAergic system appears early in development, behaving as a developmental signal for neurons during nervous system development (Owens, 2002) or promoting neuronal differentiation of adult progenitor cells (Tozuka, 2005). Interestingly, the GABAB receptors on neurons seem to play important roles in axon growth, neuron migration and modulation of neuron activity during development (Owens, 2002; Tozuka, 2005; Represa, 2005). Furthermore, GABAB-evoked neuroblast migration has been shown to be dependent on activation of G proteins negatively coupled to adenylate cyclase (Behar, 1995).

Neurotransmitter Receptors and their Role in Parkinson's disease

The brain neurotransmitter receptor activity and hormonal pathways control many physiological functions in the body. The pharmacological challenge strategy involves administering a test agent under controlled conditions to elucidate some aspect of biological or behavioural function in the organism being studied. It is based on the assumption that true functional abnormalities will not be evident in the basal state because of the action of compensatory mechanisms. Under such circumstances, pharmacological perturbation of a specific target system will reveal information about

the functional integrity of both that system and systems that modulate it (Lawrence *et al*., 2000). Basing a treatment on symptoms alone (traditional medicine) will not provide the information needed to address the underlying brain imbalance. New sophisticated equipments and tests are now available to evaluate neurotransmitter imbalances. This provides a neurotransmitter baseline assessment and is useful in determining the root causes for many diseases and illnesses. Laboratory analysis can now provide precise information on brain neurotransmitter deficiencies or overloads as well as detect hormonal and nutrient cofactor imbalances which influence neurotransmitter production. Testing helps to determine exactly which neurotransmitters are out of balance and helps to determine which therapies are needed for an individualized treatment plan. It also helps in monitoring the effectiveness of an individual's treatment.

Bone Marrow Cells

The existence of stem cells for nonhematopoietic cells in bone marrow was proposed over 100 years ago, but the isolation and differentiation of marrow stromal cells into osteoblasts, chondroblasts, adipocytes and myoblasts was only recently demonstrated. Nonhematopoietic precursors from bone marrow stroma have been referred to as colony-forming-unit fibroblasts, mesenchymal stem cells or bone marrow stromal cells (BMSC). Although BMSC can naturally be expected to be a source of surrounding tissue of bone, cartilage and fat, several recent reports demonstrate that these cells, under specific experimental conditions, can differentiate into muscle, glia, and hepatocytes (Azizi, 1999; Ferrari, *et al* 1998; Petersen *et al*., 1999). Bone marrow cells also have the capacity to migrate extensively. Transplantation of genetically labeled bone marrow cells into immunodeficient mice has been reported to result in migration of marrow cells into a region of chemically induced muscle degeneration (Ferrari, *et al*., 1998). These marrow-derived cells underwent myogenic differentiation and participated in the regeneration of the

damaged muscle fibers (Sugiyama *et al*., 2011). Systemic infusion of genetically labeled bone marrow cells into irradiated female rat resulted in an influx of labeled cells into the brain over days to weeks (Eglitis & Mezey, 1997). Marrow-derived cells were found throughout regions of the brain, from cortex to brain stem. Some bone marrow-derived cells were positive for the microglial antigenic marker F4/80. Other marrow-derived cells expressed the astroglial marker glial fibrillary acidic protein (GFAP) (Eglitis & Mezey, 1997). These results indicated that some microglia and astroglia arose from a precursor that is a normal constituent of adult bone marrow. Infusion of human BMSC into rat striatum resulted in engrafting, migration, and survival of cells (Azizi S A, 1999). After engraftment, these cells lost markers typical of marrow stromal cells in culture, such as immunoreactivity to antibodies against collagen and fibronectin. BMSC developed many of the characteristics of astrocytes, and their engraftment and migration markedly contrasted with fibroblasts that continued to produce collagen and undergo gliosis after implantation (Recio *et al*., 2011). Grafting of BMSC into the lateral ventricle of neonatal mice resulted in their migration throughout the forebrain and cerebellum without disruption of host brain architecture (Kopen *et al*., 1996). Some BMSC in striatum and hippocampus were reported to express GFAP. Moreover, occasional neurofilament-positive BMSC were found in the brain stem suggesting that some BMSC differentiated into a neuronal phenotype (Kopen *et al*., 1996). All of these reports provide impetus to investigate the potential of bone marrow cells to develop into nonhematopoietic cells and, in particular, to generate neural lineages.

One of the therapeutic successes in the treatment of this disease has been the use of L-dopa. However, dopamine replacement fails to slow the rate of loss of neurons and the beneficial effects wear off with time. Surgical treatment is not a better option because the unstable efficiency and shortage of donated embryonic mesencephalic tissue limit the application of embryonic tissue transplantation as a

therapeutic option. Therefore, recent advances in stem cell research have inspired high hopes for cell based therapy as the answer to this disease (Wang *et al*., 2007; Baier, 2004). Three types of stem cells (neural stem cells, mesenchymal stem cells and embryonic stem cells) are currently being tested in stem cell therapy for Parkinson's disease. Parish *et al*., (2008) found that, using *in vitro* differentiation of embryonic stem cells resulted in an increase in dopaminergic differentiation and, upon transplantation into Rotenone lesioned Parkinson's rats, facilitated behaviour and anatomical recovery in the animals tested. Using tyrosine hydroxylase and GTP cyclohydrolase 1 transduced human neuronal stem cells in brain transplantation of Parkinsonian rats, Kim *et al*., (2010) reported functional improvement in animal models of Parkinson's disease. Similarly, mesenchymal stem cells derived from bone marrow and umbilical cord was reported to be capable of producing functional benefits in animals with Parkinson's disease (Wang 2007).

Behavioural Changes Associated with Parkinson's disease

The temporal lobes and the Hesh gyrus receive auditory information, modulate memory and language skills and relay information to the cortex where cognitive judgments are made and motor responses are integrated (Davidson & Irwin, 1999). The thalamus and basal ganglia act as relay stations between lower centres and the cortex (Kropotov & Etlinger, 1999). The locus coeruleus is a small structure on the upper brain stem under the fourth ventricle and is involved in the regulation of wakefulness, attention and orientation (Smythies, 1997).

Some parts of the brain that are especially involved in higher cognitive functions (including consciousness) must be very dependent on a rich supply of energy – presumably because they are extremely active. In the first place, as their neurons continually generate many synaptic and action potentials, resulting in large inward and outward fluxes of ions, cellular and ionic homeostasis can be preserved only by the ATP-consuming Na^+ -K^+ pump, which maintains the trans-membrane Na^+

and K^+ gradients and thus indirectly supports such vital transport processes as uptake of sugars and amino acids (Leentjens, 2011). Even more than for the pump, 60% of ATP consumption is utilized for protein synthesis (Hochachka, 1996), presumably required to maintain the cell's structure, as well as the rapid turnover of enzymes, receptors and other proteins involved in neurotransmitter release, action and transport. These processes are crucial for synaptic transmission and plasticity and the closely related cognitive processes of memory, learning and selective attention.

Brain Wave Activity as a Result of Parkinson's disease

The frontal lobe is in charge of voluntary motion. The left lobe controls the motor movements involved in language (speech and writing). The right lobe is usually involved in non-verbal activities. Damage to one frontal lobe usually results in a person's inability to move the opposite side of his body. Moreover, damage to the frontal lobes can also cause the inability to initiate or respond to speech even though language can still be understood.

Second Messengers

Inositol 1,4,5-trisphosphate

Inositol 1,4,5-trisphosphate (IP3) receptors are the IP3 gated intracellular Ca^{2+} channels that are mainly present in the endoplasmic reticulum (ER) membrane. Many biological stimuli, such as neurotransmitters and hormones, activate the hydrolysis of phosphatidyl inositol 4,5-bisphosphate, generating the second messenger IP3. The IP3 mediates Ca^{2+} release from intracellular Ca^{2+} stores by binding to IP3 receptors (IP3R). The IP3 induced Ca^{2+} signaling plays a crucial role in the control of diverse physiological processes such as contraction, secretion, gene expression and synaptic plasticity (Berridge, 1993).

In mammalian cells, there are three IP3R subtypes, type 1 (IP3R1), type 2 (IP3R2), and type 3 (IP3R3), which are expressed to varying degrees in individual cell types (Wojcikiewicz, 1995; Taylor *et al*., 1999) and form homotetrameric or heterotetrameric channels (Monkawa *et al*., 1995). In previous studies, we constructed a plasmid vector containing full-length rat IP3R3 linked to green fluorescent protein (GFP-IP3R3) and visualized the distribution of GFP-IP3R3 in living cells (Morita *et al*., 2002; Morita *et al*., 2004). The confocal images obtained in these studies provided strong evidence that IP3Rs are distributed preferentially on the ER network. Furthermore, Morita *et al*., (2004) demonstrated that the expressed GFP-IP3R3 acts as a functional IP3-induced Ca^{2+} channel. Frequently, IP3Rs are not uniformly distributed over the membrane but rather form discrete clusters (Bootman *et al*., 1997). The clustered distribution of IP3Rs has been predicted to be important in controlling elementary Ca^{2+} release events, such as Ca^{2+} puffs and blips, which act as triggers to induce the spatiotemporal patterns of global Ca^{2+} signals, such as waves and oscillations (Thomas *et al*., 1998; Swillens *et al*., 1999; Shuai & Jung, 2003). Recently, Tateishi *et al*., (2005) reported that GFP-IP3R1 expressed in COS-7 cells aggregates into clusters on the ER network after agonist stimulation. They concluded that IP3R clustering is induced by its IP3-induced conformational change to the open state, not by Ca^{2+} release itself, because IP3R1 mutants that do not undergo an IP3 induced conformational change failed to form clusters (Higley & Sabatini, 2010). However, their results are inconsistent with studies by other groups (Wilson *et al*., 1998; Chalmers *et al*., 2006), which suggested that IP3R clustering is dependent on the continuous elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]i$). Thus, the precise mechanism underlying IP3R clustering remains controversial. Studies by Tojyo *et al*., (2008) have shown that IP3 binding to IP3R, not the increase in $[Ca^{2+}]i$, is absolutely critical for IP3R clustering. We also found that depletion of intracellular Ca^{2+} stores facilitates the generation of agonist-induced IP3R clustering.

The elevated IP3 level causes extra cellular release of Ca^{2+}, which in turn enhanced metabolic stress on mitochondria that leads to excessive oxidative phosphorylation and increased production of reactive oxygen species. If the matrix Ca^{2+} level rises too high, then deleterious changes in mitochondrial structure occur. In particular, mitochondria can swell and rupture or undergo permeability transition, thereby releasing several pro-apoptotic factors into the cytoplasm, such as cytochrome C, second mitochondrial activator of caspases (SMAC/Diablo) or apoptosis-inducing factor (AIF).

Cyclic Adenosine Monophosphate (cAMP)

The second messenger concept of signalling was born with the discovery of cyclic AMP (cAMP) and its ability to influence metabolism, cell shape and gene transcription (Sutherland, 1972) *via* reversible protein phosphorylations. cAMP is produced from ATP adenylyl cyclase (AC) in response to a variety of extracellular signals such as hormones, growth factors and neurotransmitters. Elevated levels of cAMP in the cell lead to activation of different cAMP targets. It was long thought that the only target of cAMP was the cAMP-dependent protein kinase (cAPK), which has become a model of protein kinase structure and regulation (Doskeland *et al*., 1993; Francis & Corbin, 1999; Canaves & Taylor, 2002). In recent years it has become clear that not all effects of cAMP are mediated by a general activation of cAPK (Dremier *et al*., 1997). Several cAMP binding proteins have been described: cAPK (Walsh *et al*., 1968), the cAMP receptor of *Dictyostelium discoideum*, which participates in the regulation of development (Klein *et al*., 1998), cyclic nucleotide gated channels involved in transduction of olfactory and visual signals (Kaupp *et al*., 1989; Goulding *et al*., 1992) and the cAMP-activated guanine exchange factors Epac 1,2, which specifically activate the monomeric G protein Rap (Rooij *et al*., 1998; Kawasaki *et al*., 1998).

The DA D_1 like receptors were assumed to couple to the adenylate cyclase stimulatory G protein G_s. Because G_s is ubiquitously expressed, the ability of DA D_1 like receptors to stimulate adenylate cyclase in virtually any cell line (Huff, 1997), together with physical and functional coupling of both DA D_1 and DA D_5 receptors to G_s (Sidhu 1998; Jin *et al*., 2001), strongly support the notion that G_s mediates the DA D_1 like receptor signaling in some tissues.

DA D_1 receptor stimulation simultaneously activates PKA by stimulating the production of cAMP and disinhibits PKA by phosphorylation dependent activation of protein phosphatase-2A and Thr75 dephosphorylation of DARPP-32. PKA increases the phosphorylation of numerous voltage- and ligand-gated ion channels by various combinations of direct PKA catalyzed phosphorylation of channel subunits and DARPP-32-mediated inhibition of PP1. DA D_1 receptor stimulation induces the expression of a number of transcription factors (Liu & Graybiel,1996; Zhang *et al*., 2002) which are dependent on initial activation of the transcription factor CREB (Liu & Graybiel,1996; Konradi *et al*., 1994).

DA D_2 like receptor signalling is mediated primarily by activation of the heterotrimeric G proteins $G\alpha_{i/o}$, a class of G proteins inactivated by pertussis toxin catalyzed ADP-ribosylation (Kurose *et al*., 1983; Bokoch *et al*., 1983). There is considerable disagreement in the literature concerning which G proteins interact with D_{2S} and D_{2L} (Robinson & Caron, 1997, Neve *et al*., 2003). It seems likely that both receptor isoforms are inherently able to activate multiple $G\alpha_{i/o}$ subtypes, including $G\alpha_{i2}$, $G\alpha_{i3}$, and $G\alpha_o$ (Lledo *et al*., 1992; Liu *et al*., 1994), but that interactions with particular G proteins are restricted in a cell-type dependent manner due to compartmentalization or the availability of appropriate effectors and scaffolding proteins. DA D_{2S} and DA D_{2L} can also activate the pertussis toxin insensitive G protein $G\alpha_z$ (Wong *et al*., 1992; Obadiah, 1999). The first signaling pathway identified for DA D_2 like receptors was inhibition of cAMP accumulation (De Camilli *et al*., 1979; Stoof & Kebabian, 1981).

DA D_2 like receptor inhibition of adenylate cyclase is mediated by $G\alpha_{i/o}$, because adenylate cyclase 5 is directly inhibited by $G\alpha_i$ and is insensitive to $G_{\beta\gamma}$ (Taussig *et al*., 1994). $G\alpha_i$ binds primarily to the C1 cytosolic domain of $G\alpha_i$ inhibited forms of adenylate cyclase and reduces C1/C2 domain interaction (Dessauer *et al*., 2002). DA D_2 receptor signalling occurs *via* inhibition of adenylate cyclase act in opposition to agents that stimulate adenylate cyclase, decreasing the phosphorylation of PKA substrates.

Cyclic Guanosine Monophospahte (cGMP)

cGMP generation has been associated with neurotransmission (Hofmann *et al*., 2000), vascular smooth muscle relaxation (Fiscus *et al*., 1985) and and inhibition of aldosterone release from adrenal glomerulosa cell suspension (Matsuoka *et al*., 1985; Nandhu *et al*., 2010). The most extensively studied cGMP signal transduction pathway is that triggered by nitric oxide (NO) (Bredt & Snyder, 1990). cGMP effects are primarily mediated by the activation of cGMP-dependent protein kinases (PKGs). Two distinct mammalian PKGs, PKG-I and PKG-II, have been identified, as well as two splice variants of PKG-I (PKG-Iα and -Iβ). In the brain, PKG-I is highly expressed in cerebellar Purkinje cells and, to a lesser extent, in striatal medium spiny neurons (De Camilli *et al*., 1984). PKG-II is a membrane-associated protein that is expressed throughout the brain (de Vente *et al*., 2001). The effects produced by the cGMP signaling pathway modulate drug-induced neural plasticity leading to behavioural alterations (Jouvert *et al*., 2004).

Activation of the dopamine receptor increases cAMP in the CA1 region of the hippocampus; this increase is mediated through Ca^{2+} calmodulin-dependent adenylyl cyclase (Chetkovich & Sweatt, 1993). The influx of Ca^{2+} also stimulates Ca^{2+} calmodulin-dependent nitric-oxide (NO) synthase (NOS) type to produce NO, which stimulates guanylyl cyclase to produce cGMP (Garthwaite, 1991).

Cyclic nucleotide pathways can cross talk to modulate each other's synthesis, degradation, and actions. Increased cGMP can increase the activity of cGMP stimulated PDE2 to enhance hydrolysis of cAMP or it can inhibit the PDE3 family and decrease the hydrolysis of cAMP (Pelligrino & Wang, 1998). cAMP and cGMP are involved in DA receptor-mediated signaling in cerebral cortical and hippocampal neuronal cultures. The influx of Ca^{2+}*via* the NMDA receptor stimulates calcium/calmodulin dependent adenylyl cyclase, leading to production of cAMP. This increase in cAMP seems to be tightly regulated by PDE4. The Ca^{2+} influx also stimulates the production of NO and subsequent activation of guanylyl cyclase, leading to cGMP production (Suvarna & O'Donnell, 2002).

Cyclic AMP responsive element binding protein (CREB)

The cyclic AMP responsive element binding protein is a nuclear protein that modulates the transcription of genes with cAMP responsive elements in their promotors. Increase in the concentration of either Ca^{2+} or cAMP trigger the phosphorylation and activation of CREB. This transcription factor is a component of intracellular signaling events that regulate a wide range of biological functions, from spermatogenesis to circadian rhythms and memory. Evidence from mice and rats showed that CREB-dependent transcription is required for the cellular events underlying long-term memory (Byrne *et al*., 1993).

CREB is a transcription factor that plays an important role in neuronal survival, in part by controlling the transcription of neuroprotective genes (Finkbeiner *et al*., 2000). The promoter regions of the genes for brain-derived neurotrophic factor (BDNF) and the pro-survival protein Bcl-2 contain cAMP response elements (CREs) (Mayr *et al*.,2001). Rotenone administration causes

a decrease in transactivation of the CRE promotor, resulting in reduced expression of downstream CREB-regulated genes (Chalovich *et al*., 2006).

Enhanced activation of the DA receptors leads to the production of second messengers. But its acute and prolonged action triggers the cell death pathways by activating pro apoptotic genes like bax, bad and destabilizing jun-fos complex. The activation of apoptotic pathways down regulates the CREB expression thereby blocking the cAMP signaling cascade in PD rats.

Bax Protein

The Bax gene was the first identified pro-apoptotic member of the Bcl-2 protein family (Oltvai *et al*., 1993). Bcl-2 family members share one or more of the four characteristic domains of homology entitled the Bcl-2 homology (BH) domains (named BH1, BH2, BH3 and BH4) and can form hetero- or homodimers. Bcl-2 proteins act as anti- or pro-apoptotic regulators that are involved in a wide variety of cellular activities.(Stormo, 2001)

Apoptosis has been implicated in the pathophysiology of PD. Components of signaling pathways that initiate cell death are highly concentrated in vulnerable substantia nigra neurons and may therefore contribute to the relentless demise of dopamine cells (Kim *et al*., 2011). Neurons positive for Bax-IR exhibited a discrete cytoplasmic and dendritic labeling that was conspicuously interspersed with previously unrecognized axonal spheroid-like inclusions (Kipreos, 2000). Direct comparisons revealed a difference in the aggregation of Bax-rich inclusions, with the parkinsonism brain containing more SN inclusions. Bax, one of the major pro-apoptotic family members, exerts its effects by compromising the membrane integrity leading to leakage of apoptogenic factors such as cytochrome c into the cytosol, resulting in caspase-3 activation and demise of the cell (Shacka, 2005).

The ubiquitin and alpha synuclein

The ubiquitin-proteasome (UPS) system is an important regulator of cell growth and apoptosis. The discovery of PD genes has led to the hypothesis that misfolding of proteins and dysfunction of the ubiquitin-proteasome pathway are pivotal to PD pathogenesis. Proteolytic degradation of unwanted proteins by the UPS is critical for normal maintenance of various cellular functions. Parkinson's disease one of the most prevalent neurodegenerative disorders, is characterized by prominent and irreversible nigral dopaminergic neuronal loss and intracellular protein aggregations.

The group of a-synucleinopathies comprises the neurodegenerative disorders, Parkinson's disease, Lewy body dementia, Lewy body variant of Alzheimer's disease, multiple system atrophy and neurodegeneration with brain iron accumulation type I (Spillantini, 1997; Wakabayashi, 1997; Arawaka, 1998; Gai, 1998; Spillantini, 1998; Wakabayashi, 1998). They are all brain amyloidoses unified by pathological intracellular inclusions of aggregates having the a-synuclein protein as a key component (Spillantini, 1997; Wakabayashi, 1997). The inclusions are designated Lewy bodies when found in the neuronal cell body, Lewy neurites in axons and glial cytoplasmic inclusions when found in oligodendrocytes. The latter is the pathognomonic cellular lesion in multiple system atrophy (Forno, 1996; Papp, 1989; Wang *et al*., 2011).

The central aspect of PD involves dysmetabolism of specific proteins resulting in aggregation, aborted protein degradation and/or formation of Lewy bodies. PD involves the progressive loss of dopamine-containing neurons from the substantia nigra. This loss is frequently associated with the presence of neuronal cytoplasmic inclusions, Lewy bodies and/or Pael-R associated ER stress (Masliah, 1998; Koo *et al*., 1999; Ramassamy *et al*., 1999 Ferrigno & Silver, 2000). Most cases of PD are (primary Parkinsonism) late-onset, sporadic and idiopathic (of unknown cause) (Hashimoto *et al*., 2001). However early- and late-onset genetically-linked

forms of PD (familial PD) have been identified and associated with several gene mutations including the α-synuclein (SNCA), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), ubiquitin-protein ligase (Parkin), DJ-1 (PARK-1), leucine-rich repeat kinase 2 (dardarin) (LRRK2), and PTEN induced putative kinase 1 (PINK1) genes.

Lewy bodies are proteinaceous cytoplasmic inclusions that promote neural cell death. Factors that promote the formation of Lewy bodies within nigra dopaminergic neurons promote PD. Lewy bodies result from dysfunctions affecting the ubiquination and proteosome-mediated degradation of specific proteins. Alpha synculein, synphilin-1 and parkin are component of Lewy bodies (Sonja, 2010). Mutation in α-synuclein can lead to mis-folding, aggregation and resistance to protein degradation. Parkin is a multi-faceted ubiquitin-protein ligase whose substrates include CDCrel-1, synphilin-1 (an α-synuclein-interacting protein), o-glycosylated forms of α-synuclein (αSp22) and Parkin associated endothelin-receptor like receptor (Pael-R) (Kasuga, 2010). Parkin promotes the survival of dopaminergic neurons by facilitating the degradation of α-synuclein, synphilin-1 and other proteins (Paleologou, 2005). Under some conditions, Parkin enhance the formation of Lewy-body like inclusions by protesomal-independent ubiquination of Lewy body proteins; parkin ubiquitinates synphilin-1 *via* nonclassical K63-linked ubiquitin chains. Pael-R induces endoplasmic reticulum (ER) stress and consequent apoptotic dopaminergic neuron death. Parkin together with HRD1 protects dopaminergic cells from ER stress induced death by facilitating Pael-R proteosome-mediated degradation (Cookson, 2009).

In the present study, we investigated the changes in the DA D_1 and DA D_2 receptors, gene expression of dopamine receptor subtypes, transcription factors – CREB, pro-apoptotic protein Bax, α- synuclein, ubiquitin and second messengers – IP3, cAMP, cGMP content in brain regions of rotenone induced Parkinsonism induced rats and 5-HT, GABA and Bone marrow cells

supplemented groups. Also, the behavioural studies highlights the deficits in motor learning and cognition in Parkinson's rats exposed to rotenone. 5-HT, GABA and Bone marrow Cells in combinations functionally reversed the alterations of DA receptors in rotenone induced hemi-parkinson's rat. This has clinical significance in the therapeutic management of Parkinson's disease.

Materials and Methods

Chemicals used and their sources

Biochemicals

Rotenone, DA, 5-hydroxy tryptamine, ascorbic acid, pargyline, calcium chloride, sulpiride, SCH 23390, amphetamine and apomorphine were purchased from Sigma Chemical Co., St Louis, MI, USA. All other reagents were of analytical grade purchased locally. Tissue freezing medium Jung was purchased from Leica Microsystems Nussloch GmbH, Germany. HPLC solvents were of HPLC grade obtained from SRL, India. Sigma, Chemical Co., St. Louis, MI, USA.

Radiochemicals

[^{3}H]Dopamine (Sp. activity- 45.1Ci/mmol), [^{3}H]SCH 23390 (Sp. activity 83Ci/mmol) and [^{3}H]YM-09151-2 (*cis–N-(1-benzyl-2-methylpyrrolidine-3-yl)-5-chloro-2-methoxy-4-methylaminobenzamide* Sp. activity - 85.0Ci/mmol) were purchased from NEN Life Sciences Products, Inc. Boston, USA. The [^{3}H]IP3, [^{3}H]cGMP and [^{3}H]cAMP Biotrak Assay Systems were purchased from G.E Healthcare Limited, UK.

Molecular Biology Chemicals

Tri-reagent kit was purchased from Sigma Chemical Co., St. Louis, MI, USA. ABI PRISM High Capacity cDNA Archive kit, Primers and Taqman probes for Real-Time PCR - Dopamine D_1 (Rn_02043440) Dopamine D_2 (Rn_00561126), Bax (Rn_01480160_g1), CREB (Rn 00578826_ml), α-Synuclein (Rn_00569821_m1), ubiquitin carboxy-terminal hydroxylase (Rn

00568258), endogenous control (β-actin) were purchased from Applied Biosystems, FosterCity, CA, USA.

Animals

Experiments were carried out adult male Wistar rats of 250-300g body weight purchased from Kerala Agricultural University, Mannuthy. They were housed in separate cages under 12 hrs light and 12 hrs dark periods and were maintained on standard food pellets and water *ad libitum*. Adequate measures were also taken to minimize pain and discomfort of the animals. All animal care and procedures were taken in accordance with the Institutional, National Institute of Health guidelines and CPCSEA guidelines.

Experimental design

The experimental rats were divided into the following groups i) Control ii) Rotenone infused (Rot) iii) Rotenone infused supplemented with Serotonin (Rot + 5-HT) and iv) Rotenone infused supplemented with GABA (Rot + GABA) v) Rotenone infused supplemented with Bone marrow cells (isolated from rats on femur) (Rot + BMC) vi) Rotenone infused supplemented with 5-HT and BMC (Rot +5-HT+BMC) vii) Rotenone infused supplemented with GABA and BMC (Rot + GABA+BMC) viii) Rotenone infused supplemented with 5-HT, GABA and BMC (Rot +5-HT+GABA+BMC). Each group consisted of 6-8 animals.

Unilateral intranigral infusion of Rotenone

Rats were anesthetized with Chloryl Hydrate (450 mg/kg body weight. i.p.). The animal was placed in the flat skull position on a cotton bed on a stereotaxic frame (Benchmark^{TM}, USA) with incisor bar fixed at 3.5 mm below the interaural line. Rotenone dissolved in DMSO: PEG (1:1) was infused 1µl into the right SNpc at a flow rate of 0.2µl/min. After stopping the infusion of the toxin, the probe was kept in the same position for a further 5 min for complete diffusion of

the drug and then slowly retracted. The stereotaxic coordinates for SNpc were: lateral (L) =+0.20 cm, antero-posterior (AP, from the bregma point) =–0.53 cm and dorsi-ventral (DV) = +0.75 cm. The stereotaxic co-ordinates were calculated for the dopaminergic neuronal cell body region, SNpc following the "Rat Brain Atlas" (Paxinos G, 1998). All the groups except Control group were infused with Rotenone and in control animals, 1 µl of the vehicle (DMSO: PEG (1:1)) was infused into the right SNpc. Proper postoperative care was provided till the animals recovered completely.

Rotational behaviour

Amphetamine-induced rotational behaviour was assessed as described earlier (Ungerstedt, 1971). Rats were tested with amphetamine on the 14^{th} day after intranigral injection of Rotenone and with apomorphin on the 16^{th} day. Animals that had completed a 360° circle towards the intact (contralateral) and the lesioned (ipsilateral) sides were counted for 60 min continuously and recorded separately. Animals that showed no significant contralateral rotations were excluded from the study.

Treatment

On the 18^{th} day and Stereotaxic single dose of 1µl of 5-HT (10µg/µl), GABA (10µg/µl) and 10µl of Bone marrow cell (BMC) (10^6 Cells/10µl) suspension individually and in combination was infused into the right SNpc at a flow rate of 0.2 µl/min into the respective groups. On the 30^{th} day and the apomorphine-induced rotations were recorded for every 10 min duration for a period of 70 min.

Tissue preparation

Control and experimental groups of rats were sacrificed by decapitation. The brain regions and body parts were dissected out quickly over ice according to

the procedure of Glowinski and Iversen (1966) and the tissues were stored at $^{-}$80°C for various experiments.

All animal care and procedures were in accordance with Institutional and National Institute of Health guidelines.

Quantification of brain monoamines and their metabolites in the control and experimental groups of rats

The monoamines were assayed according to the modified procedure of Paulose *et al*., (1988). The substantia nigra *pars compacta* (SNpc), corpus striatum (CS), cerebral cortex (CC), cerebellum (CB) and brain stem (BS) of experimental groups of rats was homogenized in 0.4N perchloric acid. The homogenate was then centrifuged at 5000 x g for 10 minutes at 4°C in a Sigma 3K30 refrigerated centrifuge and the clear supernatant was filtered through 0.22µm HPLC grade filters and used for HPLC analysis.

Dopamine (DA) content was determined in high performance liquid chromatography (HPLC) with electrochemical detector (ECD) (Waters, USA) fitted with CLC-ODS reverse phase column of 5 µm particle size. The mobile phase consisted of 50mM sodium phosphate dibasic, 0.03M citric acid, 0.1mM EDTA, 0.6mM sodium octyl sulfonate, 15% methanol. The pH was adjusted to 3.25 with orthophosphoric acid, filtered through 0.22µm filter (Millipore) and degassed. A Waters (model 515, Milford, USA) pump was used to deliver the solvent at a rate of 1 ml/minute. The neurotransmitters and their metabolites were identified by amperometric detection using an electrochemical detector (Waters, model 2465) with a reduction potential of +0.80 V. Twenty microlitre aliquots of the acidified supernatant were injected into the system for detection. The peaks were identified by relative retention times compared with external standards and quantitatively estimated using an integrator (Empower software) interfaced with the detector. Data from different brain regions of the experimental and control rats were statistically analysed and tabulated.

DOPAMINE RECEPTOR BINDING STUDIES USING [^{3}H] RADIOLIGANDS IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS

Dopamine receptor binding studies using [^{3}H] Dopamine

DA receptor assay was done using [^{3}H]DA according to Madras *et al*., (1988) and Hamblin and Creese, (1982). Brain tissues were homogenised in a polytron homogeniser with 20 volumes of cold 50mM Tris-HCl buffer, along with 1mM EDTA, 0.01% ascorbic acid, 4mM $MgCl_2$, 1.5mM $CaCl_2$, pH. 7.4 and centrifuged at 38,000 x g for 30min at 4°C. The pellet was washed twice by rehomogenization and centrifuged twice at 38,000 x g for 30min at 4°C. This was resuspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.25nM-1.5nM of [^{3}H]DA in 50mM Tris-HCl buffer, along with 1mM EDTA, 0.01% ascorbic acid, 1mM $MgCl_2$, 2mM $CaCl_2$, 120mM NaCl, 5mM KCl, pH.7.4 in a total incubation volume of 250µl containing 200-300µg of protein. Specific binding was determined using 100µM unlabelled dopamine.

Tubes were incubated at 25°C for 60min. and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 5.0ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Dopamine D_1 receptor binding studies using [^{3}H]SCH 23390

Dopamine D_1 receptor binding assay using [^{3}H]SCH 23390 in the brain regions were done according to the modified procedure of Mizoguchi *et al*., (2000). The tissues were weighed and homogenized in 10 volumes of ice cold 50mM Tris-HCl buffer, along with 1mM EDTA, 4mM $MgCl_2$, 1.5mM $CaCl_2$,

5mM KCl, pH. 7.4. The homogenate was centrifuged at 40,000 x g for 30min. The pellet was washed and centrifuged with 50 volumes of the buffer at 40,000 x g for 30min. This was suspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.5 - 5.0nM of [^{3}H]SCH 23390 in 50mM Tris-HCl buffer, along with 1mM EDTA, 4mM $MgCl_2$, 1.5 mM $CaCl_2$, 5mM KCl with 12µM pargyline and 0.1% ascorbic acid in a total incubation volume of 250µl containing 150-200µg protein. Specific binding was determined using 50µM unlabelled SCH 23390.

Tubes were incubated at 25°C for 60 min. and filtered rapidly through GF/B filters. The filters were washed quickly by three successive washing with 5.0ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Dopamine D_2 receptor binding studies using [^{3}H]YM-09151-2

Dopamine D_2 receptor binding assay was done according to the modified procedure of Unis *et al*., (1998) and Madras *et al*., (1988). The dissected brain tissues were weighed and homogenized in 10 volumes of ice cold 50mM Tris-HCl buffer, along with 1mM EDTA, 5mM $MgCl_2$, 1.5mM $CaCl_2$, 120mM NaCl, 5mM KCl, pH 7.4. The homogenate was centrifuged at 40,000 x g for 30min. The pellet was washed and centrifuged with 50 volumes of the buffer at 40,000 x g for 30min. This was suspended in appropriate volume of the buffer containing the above mentioned composition.

Binding assays were done using different concentrations i.e., 0.1 - 2.0nM of [^{3}H]YM-09151-2 in 50mM Tris-HCl buffer, along with 1mM EDTA, 5mM $MgCl_2$, 1.5mM $CaCl_2$, 120mM NaCl, 5mM KCl with 10µM pargyline and 0.1% ascorbic acid in a total incubation volume of 300µl containing 200-300µg of protein. Specific binding was determined using 5.0µM unlabelled sulpiride. Tubes were incubated at 25°C for 60 min. and filtered rapidly through GF/B filters

(Whatman). The filters were washed quickly by three successive washing with 5.0ml of ice cold 50mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter.

Protein Determination

Protein was measured by the method of Lowry *et al*., (1951) using bovine serum albumin as standard. The intensity of the purple blue colour formed was proportional to the amount of protein which was read in Spectrophotometer at 660nm.

ANALYSIS OF THE RECEPTOR BINDING DATA

Linear regression analysis for Scatchard plots

The data was analysed according to Scatchard (1949). The specific binding was determined by subtracting non-specific binding from the total. The binding parameters, maximal binding (B_{max}) and equilibrium dissociation constant (K_d), were derived by linear regression analysis by plotting the specific binding of the radioligand on X-axis and bound/free on Y-axis. The maximal binding is a measure of the total number of receptors present in the tissue and the equilibrium dissociation constant is the measure of the affinity of the receptors for the radioligand. The K_d is inversely related to receptor affinity.

GENE EXPRESSION STUDIES OF DOPAMINE D_1 AND D_2 RECEPTOR IN DIFFERENT BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS

Preparation of RNA

RNA was isolated from the different brain regions of control and experimental rats using the TRI reagent from Sigma Aldrich.

Isolation of RNA

Tissue (25-50mg) homogenates were made in 0.5ml TRI Reagent and was centrifuged at 12,000 x g for 10 minutes at 4°C. The clear supernatant was transferred to a fresh tube and it was allowed to stand at room temperature for 5min. 100µl of chloroform was added to it, mixed vigorously for 15sec and allowed to stand at room temperature for 15min. The tubes were then centrifuged at 12,000 x g for 15min at 4°C. Three distinct phases appear after centrifugation. The bottom red organic phase contained protein, interphase contained DNA and a colourless upper aqueous phase contained RNA. The upper aqueous phase was transferred to a fresh tube and 250µl of isopropanol was added and the tubes were allowed to stand at room temperature for 10min. The tubes were centrifuged at 12,000 x g for 10min at 4°C. RNA precipitate forms a pellet on the sides and bottom of the tube. The supernatants were removed and the RNA pellet was washed with 500µl of 75% ethanol, vortexed and centrifuged at 12,000 x g for 5 min at 4°C. The pellets were semi dried and dissolved in minimum volume of DEPC-treated water. 2µl of RNA was made up to 1ml and absorbance was measured at 260nm and 280nm. For pure RNA preparation the ratio of absorbance at 260/280 was ≥ 1.7. The concentration of RNA was calculated as one $absorbance_{260} = 42\mu g$.

cDNA Synthesis

Total cDNA synthesis was performed using ABI PRISM cDNA Archive kit in 0.2ml microfuge tubes. The reaction mixture of 20µl contained 0.2µg total RNA, 10X RT buffer, 25X dNTP mixture, 10X Random primers, MultiScribe RT (50U/µl) and RNase free water. The cDNA synthesis reactions were carried out at 25°C for 10min and 37°C for 2 hours using an Eppendorf Personal Cycler. The

primers and probes were purchased from Applied Biosystems, Foster City, CA, USA designed using Primer Express Software Version (3.0).

Real-Time PCR Assay

Real Time PCR assays were performed in 96-well plates in ABI 7300 Real Time PCR instrument (Applied Biosystems). To detect gene expression, a TaqMan quantitative 2-step reverse transcriptase polymerase chain reaction (RT-PCR) was used to quantify mRNA levels. First-strand cDNA was synthesized with use of a TaqMan RT reagent kit, as recommended by the manufacturer. PCR analyses were conducted with gene-specific primers and fluorescently labeled TaqMan probe (designed by Applied Biosystems). Endogenous control, β-actin, was labelled with a reporter dye VIC. All reagents were purchased from Applied Biosystems. The probes for specific gene of interest were labeled with FAM at the 5' end and a quencher (Minor Groove Binding Protein - MGB) at the 3' end. The real-time data were analyzed with Sequence Detection Systems software version 1.7. All reactions were performed in duplicate.

The TaqMan reaction mixture of 20µl contained 25ng of total RNA-derived cDNAs, 200nM each of the forward primer, reverse primer and TaqMan probe for Dopamine DA D_1 (Rn_02043440_s1) and DA D_2 (Rn_00561126_m1) gene, endogenous control β-actin and 12.5µl of TaqMan 2X Universal PCR Master Mix (Applied Biosystems). The volume was made up with RNAse free water. Each run contained both negative (no template) and positive controls.

The thermocycling profile conditions were as follows:

50ºC -- 2 minutes	---- Activation	
95ºC -- 10 minutes	---- Initial Denaturation	
95ºC -- 15 seconds	---- Denaturation	40 cycles
50ºC -- 30 seconds	--- Annealing	
60ºC -- 1 minutes	--- Final Extension	

Fluorescence signals measured during amplification were considered positive if the fluorescence intensity was 20-fold greater than the standard deviation of the baseline fluorescence. The ΔΔCT method of relative quantification was used to determine the fold change in expression. This was done by first normalizing the resulting threshold cycle (CT) values of the target mRNAs to the CT values of the internal control β-actin in the same samples ($\Delta CT = CT_{Target} - CT_{\beta\text{-actin}}$). It was further normalized with the control ($\Delta\Delta CT = \Delta CT - CT_{Control}$). The fold change in expression was then obtained ($2^{-\Delta\Delta CT}$).

IP3 CONTENT IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS *IN VIVO*

Brain tissues were homogenised in a polytron homogeniser in 50mM Tris-HCl buffer, pH 7.4, containing 1mM EDTA to obtain a 15% homogenate. The homogenate was then centrifuged at 40,000 x g for 15 minutes and the supernatant was transferred to fresh tubes for IP3 assay using [^{3}H]IP3 Biotrak Assay System kit.

Principle of the assay

The assay was based on competition between [^{3}H]IP3 and unlabelled IP3 in the standard or samples for binding to a binding protein prepared from bovine adrenal cortex. The bound IP3 was then separated from the free IP3 by centrifugation. The free IP3 in the supernatant was then discarded by simple decantation, leaving the bound fraction adhering to the tube. Measurement of the radioactivity in the tube enables the amount of unlabelled IP3 in the sample to be determined.

Assay Protocol

Standards, ranging from 0.19 to 25pmoles/tube, [^{3}H]IP3 and binding protein were added together and the volume was made up to 100µl with assay buffer. Samples of appropriate concentration from experiments were used for the assay. The tubes were then vortexed and incubated on ice for 15min and they were centrifuged at 2000 x g for 10min at 4°C. The supernatant was aspirated out and the pellet was resuspended in water and incubated at room temperature for 10min. The tubes were then vortexed and decanted immediately into scintillation vials. The radioactivity in the suspension was determined using liquid scintillation counter.

A standard curve was plotted with %B/Bo on the Y-axis and IP3 concentration (pmoles/tube) on the X-axis of a semi-log graph paper. %B/B$_o$ was calculated as:

$$\frac{\text{(Standard or sample cpm – NSB cpm)}}{\text{(B}_0\text{ cpm – NSB cpm)}} \times 100$$

NSB- non specific binding and B_0 - zero binding. IP3 concentration in the samples was determined by interpolation from the plotted standard curve.

cGMP CONTENT IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS *IN VIVO*

Brain tissues were homogenised in a polytron homogeniser with cold 50mM Tris-HCl buffer, pH 7.4, containing 1mM EDTA to obtain a 15% homogenate. The homogenate was then centrifuged at 40,000 x g for 15min and the supernatant was transferred to fresh tubes for cGMP assay using [^{3}H]cGMP Biotrak Assay System kit.

Principle of the assay

The assay is based on the competition between unlabelled cGMP and a fixed quantity of the [^{3}H]cGMP for binding to an antiserum, which has a high specificity and affinity for cGMP. The amount of [^{3}H]cGMP bound to the antiserum is inversely related to the amount of cGMP present in the assay sample. Measurement of the antibody bound radioactivity enables the amount of unlabelled cGMP in the sample to be calculated. Separation of the antibody bound cGMP from the unbound nucleotide was done by ammonium sulphate precipitation, followed by centrifugation. The precipitate which contains the antibody bound complex was dissolved in water and its activity was determined by liquid scintillation counting. The concentration of unlabelled cGMP in the sample was determined from a linear standard curve.

Assay Protocol

Standards, ranging from 0.5 to 4.0pmoles/tube, and [^{3}H]cGMP were added together and the volume was made up to 100µl with assay buffer. Samples of appropriate concentration from experiments were used for the assay. The antiserum was added to all the assay tubes and then vortexed. The tubes were incubated for 90min at 2 - 8°C. Ammonium sulphate was added to all tubes, mixed and allowed to stand for 5min in ice bath. The tubes were centrifuged at 12000 x g for 2min at room temperature. The supernatant was aspirated out and the pellet was dissolved in water and decanted immediately into scintillation vials. The radioactivity in the suspension was determined using liquid scintillation counter.

A standard curve was plotted with C_o/C_x on the Y-axis and cGMP concentration (pmoles/tube) on the X-axis of a linear graph paper. C_o- the cpm bound in the absence of unlabelled cGMP; C_x- the cpm bound in the presence of standard/unknown cGMP. cGMP concentration in the samples was determined by interpolation from the plotted standard curve.

cAMP CONTENT IN THE BRAIN REGIONS OF CONTROL AND EXPERIMENTAL RATS *IN VIVO*

Brain tissues-CS, CC, CB, BS and HIPPO were homogenised in a polytron homogeniser with cold 50mM Tris-HCl buffer, pH 7.4, containing 1mM EDTA to obtain a 15% homogenate. The homogenate was then centrifuged at 40,000 x g for 15min and the supernatant was transferred to fresh tubes for cAMP assay using [^{3}H]cAMP Biotrak Assay System kit.

Principle of the assay

cAMP assay kit was used. The assay is based on the competition between unlabelled cAMP and a fixed quantity of [^{3}H]cAMP for binding to a protein which has a high specificity and affinity for cAMP. The amount of labeled protein - cAMP complex formed is inversely related to the amount of unlabelled cAMP present in the assay sample. Measurement of the protein-bound radioactivity enables the amount of unlabelled cAMP in the sample to be calculated.

Free [^{3}H] cAMP + Binding protein = Bound [^{3}H] cAMP-binding protein

cAMP + cAMP-binding protein

Separation of the protein bound cAMP from unbound nucleotide is achieved by adsorption of the free nucleotide on to a coated charcoal followed by centrifugation. An aliquot of the supernatant is then removed for liquid scintillation counting. The concentration of unlabelled cAMP in the sample is then determined from a linear standard curve.

Assay Protocol

The tubes were placed on a water bath at 0°C. The assay mixture consisted of different concentrations of standard, [^{3}H]cAMP and binding protein in case of standards; buffer, [^{3}H]cAMP and binding protein for zero blank and unknown samples, [^{3}H]cAMP and binding protein for determination of unknown samples. The mixture was incubated at 2°C for 2h. Cold charcoal reagent was added to the tubes and the tubes were immediately centrifuged at 12,000 x g for 2min at 2°C. Aliquots of the supernatant was immediately transferred to scintillation vials and mixed with cocktail-T and counted in a liquid scintillation counter (Wallac, 1409).

C_o/C_x is plotted on the Y-axis against picomoles of inactive cAMP on the X- axis of a linear graph paper, where C_o is the counts per minute bound in the absence of unlabelled cAMP and C_x is the counts per minute bound in the presence of standard or unknown unlabelled cAMP. From the C_o/C_x value for the sample, the number of picomoles of unknown cAMP was calculated.

Dopamine D_1 and Dopamine D_2 Receptor Expression using Confocal Microscope

Animals were deeply anesthetized with ether. The rat was transcardially perfused with PBS (pH- 7.4) followed by 4% paraformaldehyde in PBS (Chen *et al*., 2007). After perfusion the brains were dissected and immersion fixed in 4% paraformaldehyde for 1 hr and then equilibrated with 30% sucrose solution in 0.1M PBS. 40 µm sections were cut using Cryostat (Leica, CM1510 S). The sections were treated with PBST (PBS in 0.05% Triton X-100) for 20 min. Brain slices were incubated overnight at 4 °C with either rat primary antibody for Dopamine D_1(No: NRG 01691597 Millipore, diluted in PBST at 1: 500 dilution) and Dopamine D_2. (No: LV 1583420 Millipore, diluted in PBST at 1: 500 dilution). After overnight incubation brain slices were incubated with the secondary antibody of FITC (No: AB7130F, Chemicon, diluted in PBST at 1:1000

dilution). The sections were observed and photographed using confocal imaging system (Leica SP 5).

Bone marrow cell differentiation pattern studies using PKH2GL cell linker dye

Bone marrow cells were tagged with PKH2GL cell linker dye according to the kit protocol Sigma Chemical Co., St. Louis, USA. Tagged BMC (10^6 Cells/10µl) suspension was infused individually and in combinations stereotaxically into the right SNpc at a flow rate of 0.2 µl/min to the respective groups. 10 µm brain sections were cut using Cryostat (Leica, CM1510 S). Brain slices were incubated overnight at 4°C with primary antibody for Nestin. After overnight incubation brain slices were incubated with the secondary antibody with CY5. The sections were observed and photographed using confocal imaging system (Leica SP 5).

Tyrosine hydrxylase enzyme expression using Confocal Microscope

The rat was transcardially perfused with PBS, pH- 7.4, followed by 4% paraformaldehyde in PBS (Chen *et al*., 2007). 10 µm brain sections were cut using Cryostat (Leica, CM1510 S). Brain slices were incubated overnight at 4°C with rat primary antibody for tyrosine hydroxylase. After overnight incubation brain slices were incubated with the secondary antibody of FITC. The sections were observed and photographed using confocal imaging system (Leica SP 5).

Rotarod Test

Rotarod has been used to evaluate motor coordination by testing the ability of rats to remain on revolving rod (Dunham, 1957). The apparatus has a horizontal rough metal rod of 3cm diameter attached to a motor with variable speed. This 70cm long rod was divided into four sections by wooden partitions. The rod was placed at a height of 50cm to discourage the animals to jump from

the rotating rod. The rate of rotation was adjusted to allow the normal rats to stay on it for five minutes. Each rat was given five trials before the actual reading was taken. The readings were taken at 10, 15 and 25rpm after 15 days of treatment in all groups of rats.

Grid Walk Test

Grid Walk Test has been used to evaluate motor coordination. (Graggen *et al.*, 1998). Motor coordination was examined by assessing the ability to navigate across a 1m long runway with irregularly assigned gaps (0.5–5 cm) between round metal bars. Crossing this runway requires that animals accurately place their limbs on the bars. In baseline training and postoperative testing, every animal had to cross the grid for at least three times. The number of foot falls/3 minutes (errors) was counted in each crossing and a mean error rate was calculated.

Narrow Beam Test

Narrow Beam Test has been used to evaluate motor control (Haydn & Jasmine, 1975). A rectangular beam 1.2cm wide, 1.05m long and elevated 30cm from the ground was used for the study. After training, normal rats were able to traverse the horizontal beams with less than three foot falls. When occasionally their feet slipped off the beam, they were retrieved and repositioned precisely. The time, the rats could remain balanced on the beam, was noted.

Elevated plus maze

The elevated plus-maze is a widely used animal model of anxiety that is based on two conflicting tendencies; the rodent's drive to explore a novel environment and it's aversion to heights and open spaces. Four arms were arranged in the shape of a cross. Two arms had side walls and an end wall ("closed arms") - the two other arms had no walls ("open arms"). The open arms were surrounded by small ledges to prevent the animal from falling from the maze. The

maze was fastened to a light-weight support frame. Thus "anxious" animals spent most of the time in the closed arms while less anxious animals explored open areas longer.

Animals were placed individually into the centre of elevated plus-maze consisting of two open arms (38L x 5W cm) and two closed arms (38L x 5W x 15H cm), with a central intersection (5cm x 5cm) elevated 50 cm above the floor. Behaviour was tested in a dimly lit room with a 40W bulb hung 60 cm above the central part of the maze. The investigator sitting approximately 2 metre apart from the apparatus observes and detects the movements of the rats for a total of 5 minutes. The experimental procedure was similar to that described by Pellow *et al.*, (1985). During the 5 minutes test period the following parameters were measured to analyze the behavior changes of the experimental rats using elevated plus-maze: open arm entry, closed arm entry, percentage arm entry, total arm entry, time spent in open arm, time spent in closed arm, percentage of time spent in open arm, head dipping, stretched attend posture and grooming (Holmes & Rodgers, 1998). An entry was defined as entering with all four feet into one arm.

Social interaction test

Social interaction test was done during post- treatment period once daily for 3 days (Millan *et al*., 2005). Weight-matched rats receiving the same treatment were placed in opposite corners for 10 min into a brightly-lit chamber (30 × 30 × 60 cm; width, length and height, respectively) with floor covered with wood shavings. The total time spent in active social behavior - allogrooming, sniffing the partner, crawling under and over, following was recorded, for each rat separately.

STATISTICS

Statistical evaluations were done by ANOVA using InStat (Ver.2.04a) computer programme. Linear regression Scatchard plots were made using SIGMA

PLOT (Ver 2.03). Sigma Plot software (version 2.0, Jandel GmbH, Erkrath, Germany). Empower software were used for HPLC analysis. Relative Quantification Software was used for analyzing Real-Time PCR results.

RESULTS

Rotational Behaviour

Behavioural effects of the Parkinsonism induced rat model by Rotenone were confirmed by using apomorphine and amphetamine. Stereotaxic single dose infusion of BMC, 5-HT and GABA were given individually and in combinations after the confirmation of the disease in 18 days. After 12 days of treatment significant reversal in the apomorphine induced rotational behaviour were observed; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant change (Table-1, Figure-1).

Substantia nigra *pars compacta*

Dopamine content (nmoles/g wet wt.) in the Subtantia nigra *pars compacta* of Control and Experimental Groups of Rats

Dopamine content in the Subtantia nigra *pars compacta* showed a significant decrease ($p<0.001$) in Rotenone induced PD rats compared to Control. A significant reversal of the dopamine content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-2).

Real Time PCR amplification of Dopamine D_1 receptor mRNA in the Substantia nigra *pars compacta* of Control and Experimental Groups of Rats.

The gene expression studies using specific probe by real-time PCR analysis showed that DA D_1 receptor mRNA was significantly ($p<0.001$) down regulated in Rotenone induced PD rats compared to Control. A significant reversal

of the DA D_1 gene expression was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control (Table-3, Figure-2).

Real Time PCR amplification of Dopamine D_2 receptor mRNA in the Substantia nigra *pars compacta* of Control and Experimental Groups of Rats.

The gene expression studies using specific probe by real-time PCR analysis showed that DA D_2 receptor mRNA was significantly ($p<0.001$) up regulated in Rotenone induced PD rats compared to Control. A significant reversal of the DA D_2 gene expression was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control (Table-4, Figure-3).

Real Time PCR amplification of Nestin mRNA in the Substantia nigra *pars compacta* of Control and Experimental Groups of Rats.

The gene expression studies using specific probe by real-time PCR analysis showed that Nestin mRNA was significantly ($p<0.001$) up regulated in rats treated with Rotenone, serotonin 5-HT and GABA ($p<0.05$). The expression was further increased when the rats were treated with BMC ($p<0.01$) and the most prominent gene expression was seen in 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$) (Table-5, Figure-4).

***In vivo* expression studies of PKH2GL tagged bone marrow cells and Nestin in the Substantia nigra of experimental rats**

In vivo differentiation of BMC tagged with PKH2GL and nestin to premature neurons in SNpc was done. Cellular morphology was changed once the BMC was injected along with 5-HT and GABA in to SNpc. Our results showed

that the PKH2GL tagged BMC differentiated to neuronal cells when 5-HT and GABA was given to SNpc. Nestin stained newly formed premature neurons. PKH2GL tagged BMC transformation to newly formed premature neurons was confirmed by superimposing the images. There was increased transformation pixel intensity in 5-HT, GABA and BMC combinations. Maximum transformation mean pixel intensity was observed in the PD rats treated with 5-HT, GABA and BMC (Table-6, Figure-5, 6).

Tyrosine hydroxylase antibody staining in Substantia nigra *pars compacta* of Control and Experimental Groups of Rats.

Gene expression studies were confirmed with the specific antibody staining using confocal microscope. The tyrosine hydroxylase antibody expression showed significant ($p<0.001$) down regulation in the pixel intensity of Rotenone induced PD rats compared to control. A significant reversal of the tyrosine hydroxylase antibody staining was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control (Table-7, Figure-7).

Corpus Striatum

Dopamine content (nmoles/g wet wt.) in the Corpus Striatum of Control and Experimental Groups of Rats

Dopamine content in the Corpus Striatum showed a significant decrease ($p<0.001$) in Rotenone induced PD rats compared to Control group. A significant reversal of the dopamine content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-8).

Scatchard Analysis of [^{3}H]Dopamine binding against Dopamine in the Corpus
Striatum of Control and Experimental Groups of Rats

Scatchard analysis of [^{3}H] dopamine against dopamine in the corpus striatum of Rotenone infused PD rats showed a significant ($p<0.001$) increase in B_{max} and K_d compared to control. A significant reversal of the dopamine receptor number and affinity was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-9, 10, Figure-8, 9).

Scatchard analysis using [^{3}H]SCH 23390 against SCH 23390 in the Corpus Striatum of Control and Experimental Groups of Rats

Binding studies of [^{3}H]SCH 23390 against SCH 23390 for DA D_1 receptors showed a significant ($p<0.001$) down regulation in Rotenone infused PD rats compared to Control. A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$).

BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-11, 12, Figure-10, 11).

Scatchard analysis using [^{3}H]YM-09151-2 against sulpiride in the Corpus Striatum of Control and Experimental Groups of Rats

Binding studies of [^{3}H]YM-09151-2 against sulpiride for DA D_2 receptors showed a significant ($p<0.001$) up regulation in Rotenone induced PD rats compared to control. A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-13, 14, Figure-12, 13).

Real Time PCR amplification of Dopamine D_1 receptor mRNA in the Corpus Striatum of Control and Experimental Groups of Rats

The gene expression studies using specific probe by real-time PCR analysis showed that DA D_1 receptor mRNA was significantly ($p<0.001$) down regulated in Rotenone induced PD rats compared to Control. A significant reversal of the DA D_1 gene expression was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control. K_d showed no significant change in experimental rats (Table-15, Figure-14).

Real Time PCR amplification of Dopamine D_2 receptor mRNA in the Corpus Striatum of Control and Experimental Groups of Rats

The gene expression studies using specific probe by real-time PCR analysis showed that DA D_2 receptor mRNA was significantly ($p<0.001$) up regulated in Rotenone induced PD rats compared to Control. A significant reversal

of the DA D_2 gene expression was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control (Table-16, Figure-15).

Dopamine D_1 and Dopamine D_2 receptor specific antibody staining in the Corpus Striatum of Control and experimental groups of rats

Scatchard analysis and the gene expression studies were confirmed with the specific antibody staining using confocal microscope. The DA D_1 receptor antibody expression showed significant ($p<0.001$) down regulation in the mean pixel intensity in Rotenone infused PD rats compared to control and DA D_2 receptor antibody expression showed significant ($p<0.001$) up regulation in the pixel intensity of Rotenone infused PD rats compared to control. A significant reversal of the DA D_1 and DA D_2 antibody staining was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control (Table-17, 18, Figure-16, 17).

IP3 content in the Corpus Striatum of Control and experimental groups of rats

Our study showed that the IP3 content in the corpus striatum of Rotenone infused PD rats was significantly ($p<0.001$) up regulated compared to the control. A significant reversal of the IP3 content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-19, Figure-18).

cAMP content in the Corpus Striatum of Control and experimental groups of rats

cAMP content showed a significant ($p<0.001$) up regulation in corpus striatum of Rotenone infused PD rats compared to control. A significant reversal of the cAMP content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-20, Figure-19).

cGMP content in the Corpus Striatum of Control and experimental groups of rats

Our study showed that the cGMP content in the corpus striatum of Rotenone infused PD rats was significantly ($p<0.001$) down regulated compared to the control. A significant reversal of the cGMP content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$).BMC alone treated group did not show any significant reversal (Table-21, Figure-20).

Real Time PCR amplification of CREB mRNA in the Corpus Striatum of Control and Experimental Groups of Rats

Gene expression studies of CREB (cAMP response element-binding protein) showed a significant ($p<0.001$) down regulation in Rotenone infused PD rats compared to control. A significant reversal of the CREB was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$).BMC alone treated group did not show any significant reversal (Table-22, Figure-21).

Real Time PCR amplification of Bax mRNA in the Corpus Striatum of Control and Experimental Groups of Rats

Gene expression studies of Bax showed a significant ($p<0.001$) up regulation in the corpus striatum of the Rotenone infused PD rats. A significant reversal of the Bax was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-23, Figure-22).

Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the Corpus Striatum of Control and Experimental Groups of Rats

Gene expression studies of ubiquitin carboxy-terminal hydrolase showed a significant ($p<0.001$) up regulation in Rotenone infused PD rats compared to control. A significant reversal of the ubiquitin carboxy-terminal hydrolase was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-24, Figure-23).

Real Time PCR amplification of ά-Synuclien mRNA in the Corpus Striatum of Control and Experimental Groups of Rats

Gene expression studies of ά-Synuclien showed a significant ($p<0.001$) up regulation in Rotenone infused PD rats compared to control. A significant reversal of the ά-Synuclien was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-25, Figure-24).

Cerebral Cortex

Dopamine Content (nmoles/g wet wt.) in the Cerebral Cortex of Control and Experimental Groups of Rats

Dopamine content in the Cerebral Cortex showed a significant decrease ($p<0.001$) in Rotenone induced PD rats compared to Control group. A significant reversal of the dopamine content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-26).

Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Cerebral

Cortex of Control and Experimental Groups of Rats

Scatchard analysis of [^{3}H] dopamine against dopamine in the Cerebral Cortex of Rotenone infused PD rats showed a significant ($p<0.001$) increase in B_{max} compared to control rats. A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-27, 28, Figure-25, 26).

Scatchard analysis using [^{3}H]SCH 23390 against SCH 23390 in the Cerebral Cortex of Control and Experimental Groups of Rats

Binding studies of [^{3}H]SCH 23390 against SCH 23390 for DA D_1 receptors showed a significant ($p<0.001$) up regulation in Rotenone induced PD rats compared to Control. A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$).

BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-29, 30, Figure-27, 28).

Scatchard analysis using [^{3}H]YM-09151-2 against sulpiride in the Cerebral Cortex of Control and Experimental Groups of Rats

Binding studies of [^{3}H]YM-09151-2 against sulpiride for DA D_2 receptors showed a significant ($p<0.001$) up regulation in Rotenone induced PD rats compared to Control. A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-31, 32, Figure-29, 30).

Real Time PCR amplification of Dopamine D_1 receptor mRNA in the Cerebral Cortex of Control and Experimental Groups of Rats

The gene expression studies using specific probe by real-time PCR analysis showed that DA D_1 receptor mRNA was significantly ($p<0.001$) up regulated in Rotenone induced PD rats compared to Control. A significant reversal of the DA D_1 gene expression was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control (Table-33, Figure-31).

Real Time PCR amplification of Dopamine D_2 receptor mRNA in the Cerebral Cortex of Control and Experimental Groups of Rats

The gene expression studies using specific probe by real-time PCR analysis showed that DA D_2 receptor mRNA was significantly ($p<0.001$) up regulated in Rotenone induced PD rats compared to Control. A significant reversal of the DA D_2 gene expression was observed in the treatment groups; 5-HT

($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control (Table-34, Figure-32).

Dopamine D_1 and Dopamine D_2 receptor specific antibody staining in the Cerebral Cortex of Control and experimental rats

Scatchard analysis and the gene expression studies were confirmed with the specific antibody staining using confocal microscope. The Dopamine D_1 and D_2 receptor antibody expression showed significant ($p<0.001$) up regulation in the pixel intensity of Rotenone infused PD rats compared to control ($p<0.001$). A significant reversal of the DA D1 and DA D2 antibody staining was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control (Table-35, 36, Figure-33, 34).

IP3 content in the Cerebral Cortex of Control and experimental groups of rats

Our study showed that the IP3 content in the Cerebral Cortex of Rotenone infused PD rats was significantly ($p<0.001$) up regulated compared to the control. A significant reversal of the IP3 content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-37, Figure-35).

cAMP content in the Cerebral Cortex of Control and experimental groups of rats

cAMP content showed a significant ($p<0.001$) up regulation in Cerebral Cortex of Rotenone infused PD rats compared to control. A significant reversal of

the cAMP content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-38, Figure-36).

cGMP content in the Cerebral Cortex of Control and experimental groups of rats

Our study showed that the cGMP content in the Cerebral Cortex of Rotenone infused PD rats was significantly ($p<0.001$) down regulated compared to the control. A significant reversal of the cGMP content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-39, Figure-37).

Real Time PCR amplification of CREB mRNA in the Cerebral Cortex of Control and Experimental Groups of Rats

Gene expression studies of CREB (cAMP response element-binding protein) showed a significant ($p<0.001$) down regulation in Rotenone infused PD rats compared to control. A significant reversal of the CREB was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-40, Figure-38).

Real Time PCR amplification of Bax mRNA in the Cerebral Cortex of Control and Experimental Groups of Rats

Gene expression studies of Bax showed a significant ($p<0.001$) up regulation in the corpus striatum of the Rotenone infused PD rats. A significant reversal of the Bax was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC

($p<0.001$). BMC alone treated group did not show any significant reversal (Table-41, Figure-39).

Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the Cerebral Cortex of Control and Experimental Groups of Rats

Gene expression studies of ubiquitin carboxy-terminal hydrolase showed a significant ($p<0.001$) up regulation in Rotenone infused PD rats compared to control. A significant reversal of the ubiquitin carboxy-terminal hydrolase was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-42, Figure-40).

Real Time PCR amplification of ά-Synuclien mRNA in the Cerebral Cortex of Control and Experimental Groups of Rats

Gene expression studies of ά-Synuclien showed a significant ($p<0.001$) up regulation in Rotenone infused PD rats compared to control. A significant reversal of the ά-Synuclien was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-43, Figure-41).

Cerebellum

Dopamine Content (nmoles/g wet wt.) in the Cerebellum of Control and Experimental Groups of Rats

Dopamine content in the Cerebellum showed a significant decrease ($p<0.001$) in Rotenone induced PD rats compared to Control group. A significant reversal of the dopamine content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-44).

Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Cerebellum of Control and Experimental Groups of Rats

Scatchard analysis of [^{3}H] dopamine against dopamine in the Cerebellum of Rotenone infused rats showed a significant ($p<0.001$) up regulation in B_{max} compared to control rats. A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-45, 46, Figure-42, 43).

Scatchard analysis using [^{3}H]SCH 23390 against SCH 23390 in the Cerebellum of Control and Experimental Groups of Rats

Binding studies of [^{3}H]SCH 23390 against SCH 23390 for DA D_1 receptors showed a significant ($p<0.001$) up regulation in Rotenone induced PD rats compared to Control. A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$).

BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-47, 48, Figure-44, 45).

Scatchard analysis using [^{3}H]YM-09151-2 against sulpiride in the Cerebellum of Control and Experimental Groups of Rats

Binding studies of [^{3}H]YM-09151-2 against sulpiride for DA D_2 receptors showed a significant ($p<0.001$) up regulation in Rotenone induced PD rats compared to Control A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-49, 50, Figure-46, 47)

Real Time PCR amplification of Dopamine D_1 receptor mRNA in the Cerebellum of Control and Experimental Groups of Rats

The gene expression studies using specific probe by real-time PCR analysis showed that DA D_1 receptor mRNA was significantly ($p<0.001$) up regulated in Rotenone induced PD rats compared to Control. A significant reversal of the DA D_1 gene expression was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control (Table-51, Figure-48).

Real Time PCR amplification of Dopamine D_2 receptor mRNA in the Cerebellum of Control and Experimental Groups of Rats

The gene expression studies using specific probe by real-time PCR analysis showed that DA D_1 receptor mRNA was significantly ($p<0.001$) up regulated in Rotenone induced PD rats compared to Control. A significant reversal of the DA D_1 gene expression was observed in the treatment groups; 5-HT

(p<0.05), GABA (p<0.05), 5-HT+BMC (p<0.01), GABA+ BMC (p<0.01) and 5-HT+GABA+BMC (p<0.001). BMC alone treated group did not show any significant reversal to control (Table-52, Figure-49).

Dopamine D_1 and Dopamine D_2 receptor antibody staining in Control and experimental groups of rats

Scatchard analysis and the gene expression studies were confirmed with the specific antibody staining using confocal microscope. The Dopamine D_1 and D_2 receptor antibody expression showed significant (p<0.001) up regulation in the pixel intensity of Rotenone infused PD rats compared to control. A significant reversal of the DA D_1 and DA D_2 antibody staining was observed in the treatment groups; 5-HT (p<0.05), GABA (p<0.05), 5-HT+BMC (p<0.01), GABA+ BMC (p<0.01) and 5-HT+GABA+BMC (p<0.001). BMC alone treated group did not show any significant reversal to control (Table-53, 54, Figure-50, 51).

IP3 content in the Cerebellum of Control and experimental groups of rats

Our study showed that the IP3 content in the Cerebellum of Rotenone infused PD rats was significantly (p<0.001) up regulated compared to the control. A significant reversal of the IP3 content was observed in the treatment groups; 5-HT (p<0.05), GABA (p<0.05), 5-HT+BMC (p<0.01), GABA+ BMC (p<0.01) and 5-HT+GABA+BMC (p<0.001). BMC alone treated group did not show any significant reversal (Table-55, Figure-52).

cAMP content in the Cerebellum of Control and experimental groups of rats

cAMP content showed a significant (p<0.001) up regulation in Cerebellum of Rotenone infused PD rats compared to control. A significant reversal of the cAMP content was observed in the treatment groups; 5-HT (p<0.05), GABA (p<0.05), 5-HT+BMC (p<0.01), GABA+ BMC (p<0.01) and 5-

HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-56, Figure-53).

cGMP content in the Cerebellum of Control and experimental groups of rats

Our study showed that the cGMP content in the Cerebellum of Rotenone infused PD rats was significantly ($p<0.001$) down regulated compared to the control. A significant reversal of the cGMP content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-57, Figure-54).

Real Time PCR amplification of CREB mRNA in the Cerebellum of Control and Experimental Groups of Rats

Gene expression studies of CREB (cAMP response element-binding protein) showed a significant ($p<0.001$) down regulation in Rotenone infused PD rats compared to control. A significant reversal of the CREB content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-58, Figure-55).

Real Time PCR amplification of Bax mRNA in the Cerebellum of Control and Experimental Groups of Rats

Gene expression studies of Bax showed a significant ($p<0.001$) up regulation in the corpus striatum of the Rotenone infused PD rats. A significant reversal of the Bax expression was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-59, Figure-56).

Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the Cerebellum of Control and Experimental Groups of Rats

Gene expression studies of ubiquitin carboxy-terminal hydrolase showed a significant ($p<0.001$) up regulation in Rotenone infused PD rats compared to control. A significant reversal of the ubiquitin carboxy-terminal hydrolase content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-60, Figure-57).

Real Time PCR amplification of ά-Synuclien mRNA in the Cerebellum of Control and Experimental Groups of Rats

Gene expression studies of ά-Synuclien showed a significant ($p<0.001$) up regulation in Rotenone infused PD rats compared to control. A significant reversal of the ά-Synuclien content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-61, Figure-58).

Brain Stem

Dopamine Content (nmoles/g wet wt.) in the Brain Stem of Control and Experimental Groups of Rats

Dopamine content in the Brain Stem showed a significant decrease ($p<0.001$) in Rotenone induced PD rats compared to Control group. A significant reversal of the dopamine content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-62)

Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Brain Stem of Control and Experimental Groups of Rats

Scatchard analysis of [^{3}H] dopamine against dopamine in the Brain Stem of Rotenone infused rats showed a significant ($p<0.001$) increase in B_{max} compared to control rats. A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-63, 64, Figure-59, 60).

Scatchard analysis using [^{3}H]SCH 23390 against SCH 23390 in the Brain Stem of Control and Experimental Groups of Rats

Binding studies of [^{3}H]SCH 23390 against SCH 23390 for DA D_1 receptors showed a significant ($p<0.001$) up regulation in Rotenone induced PD rats compared to Control. A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$).

BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-65, 66, Figure-61, 62).

Scatchard analysis using [^{3}H]YM-09151-2 against sulpiride in the Brain Stem of Control and Experimental Groups of Rats

Binding studies of [^{3}H]YM-09151-2 against sulpiride for DA D_2 receptors a significant ($p<0.001$) up regulation in Rotenone induced PD rats compared to Control. A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-67, 68, Figure-63, 64).

Real Time PCR amplification of Dopamine D_1 receptor mRNA in the Brain Stem of Control and Experimental Groups of Rats

The gene expression studies using specific probe by real-time PCR analysis showed that DA D_1 receptor mRNA was significantly ($p<0.001$) up regulated in Rotenone induced PD rats compared to Control. A significant reversal of the DA D_1 gene expression was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control (Table-69, Figure-65).

Real Time PCR amplification of Dopamine D_2 receptor mRNA in the Brain Stem of Control and Experimental Groups of Rats

The gene expression studies using specific probe by real-time PCR analysis showed that DA D_1 receptor mRNA was significantly ($p<0.001$) up regulated in Rotenone induced PD rats compared to Control. A significant reversal of the DA D_1 gene expression was observed in the treatment groups; 5-HT

($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control (Table-70, Figure-66).

Dopamine D_1 and Dopamine D_2 receptor antibody staining in the Brain Stem of Control and experimental rats

Scatchard analysis and the gene expression studies were confirmed with the specific antibody staining using confocal microscope. The Dopamine D_1 and D_2 receptor antibody expression showed significant ($p<0.001$) up regulation in the pixel intensity of Rotenone infused PD rats compared to control. A significant reversal of the DA D_1 and DA D_2 antibody staining was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control (Table-71, 72, Figure-67, 68).

IP3 content in the Brain Stem of Control and experimental groups of rats

Our study showed that the IP3 content in the Brain Stem of Rotenone infused PD rats was significantly ($p<0.001$) up regulated compared to the control. A significant reversal of the IP3 content content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-73, Figure-69).

cAMP content in the Brain Stem of Control and experimental groups of rats

cAMP content showed a significant ($p<0.001$) up regulation in Brain Stem of Rotenone infused PD rats compared to control. A significant reversal of the cAMP content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC

($p<0.001$). BMC alone treated group did not show any significant reversal (Table-74, Figure-70).

cGMP content in the Brain Stem of Control and experimental groups of rats

Our study showed that the cGMP content in the Brain Stem of Rotenone infused PD rats was significantly ($p<0.001$) down regulated compared to the control. A significant reversal of the cGMP content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-75, Figure-71).

Real Time PCR amplification of CREB mRNA in the Brain Stem of Control and Experimental Groups of Rats

Gene expression studies of CREB (cAMP response element-binding protein) showed a significant ($p<0.001$) down regulation in Rotenone infused PD rats compared to control. A significant reversal of the CREB expression was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-76, Figure-72).

Real Time PCR amplification of Bax mRNA in the Brain Stem of Control and Experimental Groups of Rats

Gene expression studies of Bax showed a significant ($p<0.001$) up regulation in the corpus striatum of the Rotenone infused PD rats. A significant reversal of the Bax was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-77, Figure-73).

Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the Brain Stem of Control and Experimental Groups of Rats

Gene expression studies of ubiquitin carboxy-terminal hydrolase showed a significant ($p<0.001$) up regulation in Rotenone infused PD rats compared to control. A significant reversal of the ubiquitin carboxy-terminal hydrolase was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-78, Figure-74).

Real Time PCR amplification of ά-Synuclien mRNA in the Brain Stem of Control and Experimental Groups of Rats

Gene expression studies of ά-Synuclien showed a significant ($p<0.001$) up regulation in Rotenone infused PD rats compared to control. A significant reversal of the ά-Synuclien was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-79, Figure-75).

Hippocampus

Dopamine Content (nmoles/g wet wt.) in the Hippocampus of Control and Experimental Groups of Rats

Dopamine content in the Hippocampus showed a significant decrease ($p<0.001$) in Rotenone induced PD rats compared to Control group. A significant reversal of the dopamine content was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-80).

Scatchard Analysis of [^{3}H]Dopamine Binding against Dopamine in the Hippocampus of Control and Experimental Groups of Rats

Scatchard analysis of [^{3}H]dopamine against dopamine in the Hippocampus of Rotenone infused rats showed a significant ($p<0.001$) increase in B_{max} compared to control rats. A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-81, 82, Figure-76, 77).

Scatchard analysis using [^{3}H]SCH 23390 against SCH 23390 in the Hippocampus of Control and Experimental Groups of Rats

Binding studies of [^{3}H]SCH 23390 against SCH 23390 for DA D_1 receptors showed a significant ($p<0.001$) up regulation in Rotenone induced PD rats compared to control. A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$).

BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-83, 84, Figure-78, 79).

Scatchard analysis using [^{3}H]YM-09151-2 against sulpiride in the Hippocampus of Control and Experimental Groups of Rats

Binding studies of [^{3}H]YM-09151-2 against sulpiride for DA D_2 receptors showed a significant ($p<0.001$) up regulation in Rotenone induced PD rats compared to control. A significant reversal of the dopamine receptor number was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal. K_d showed no significant change in experimental rats (Table-85, 86, Figure-80, 81).

Real Time PCR amplification of Dopamine D_1 receptor mRNA in the Hippocampus of Control and Experimental Groups of Rats

The gene expression studies using specific probe by real-time PCR analysis showed that DA D_1 receptor mRNA was significantly ($p<0.001$) up regulated in Rotenone induced PD rats compared to Control. A significant reversal of the DA D_1 gene expression was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal to control (Table-87, Figure-82).

Real Time PCR amplification of Dopamine D_2 receptor mRNA in the Hippocampus of Control and Experimental Groups of Rats

The gene expression studies using specific probe by real-time PCR analysis showed that DA D_2 receptor mRNA was significantly ($p<0.001$) up regulated in Rotenone induced PD rats compared to Control. A significant reversal of the DA D_2 gene expression was observed in the treatment groups; 5-HT

(p<0.05), GABA (p<0.05), 5-HT+BMC (p<0.01), GABA+ BMC (p<0.01) and 5-HT+GABA+BMC (p<0.001). BMC alone treated group did not show any significant reversal to control (Table-88, Figure-83).

Dopamine D_1 and Dopamine D_2 receptor antibody staining in the Hippocampus of Control and experimental groups of rats

Scatchard analysis and the gene expression studies were confirmed with the specific antibody staining using confocal microscope. The Dopamine D_1 and D_2 receptor antibody expression showed significant (p<0.001) up regulation in the pixel intensity of Rotenone infused PD rats compared to control. A significant reversal of the DA D_1 and DA D_2 antibody staining was observed in the treatment groups; 5-HT (p<0.05), GABA (p<0.05), 5-HT+BMC (p<0.01), GABA+ BMC (p<0.01) and 5-HT+GABA+BMC (p<0.001). BMC alone treated group did not show any significant reversal to control (Table-89, 90, Figure-84, 85).

IP3 content in the Corpus Striatum of Control and experimental groups of rats

Our study showed that the IP3 content in the Hippocampus of Rotenone infused PD rats was significantly (p<0.001) up regulated compared to the control. A significant reversal of the IP3 content was observed in the treatment groups; 5-HT (p<0.05), GABA (p<0.05), 5-HT+BMC (p<0.01), GABA+ BMC (p<0.01) and 5-HT+GABA+BMC (p<0.001). BMC alone treated group did not show any significant reversal (Table-91, Figure-86).

cAMP content in the Hippocampus of Control and experimental groups of rats

cAMP content showed a significant (p<0.001) up regulation in Hippocampus of Rotenone infused PD rats compared to control. A significant reversal of the cAMP content was observed in the treatment groups; 5-HT

(p<0.05), GABA (p<0.05), 5-HT+BMC (p<0.01), GABA+ BMC (p<0.01) and 5-HT+GABA+BMC (p<0.001). BMC alone treated group did not show any significant reversal (Table-92, Figure-87).

cGMP content in the Hippocampus of Control and experimental groups of rats

Our study showed that the cGMP content in the Hippocampus of Rotenone infused PD rats was significantly (p<0.001) down regulated compared to the control. A significant reversal of the cGMP content was observed in the treatment groups; 5-HT (p<0.05), GABA (p<0.05), 5-HT+BMC (p<0.01), GABA+ BMC (p<0.01) and 5-HT+GABA+BMC (p<0.001). BMC alone treated group did not show any significant reversal (Table-93, Figure-88).

Real Time PCR amplification of CREB mRNA in the Hippocampus of Control and Experimental Groups of Rats

Gene expression studies of CREB (cAMP response element-binding protein) showed a significant (p<0.001) down regulation in Rotenone infused PD rats compared to control. A significant reversal of the CREB was observed in the treatment groups; 5-HT (p<0.05), GABA (p<0.05), 5-HT+BMC (p<0.01), GABA+ BMC (p<0.01) and 5-HT+GABA+BMC (p<0.001). BMC alone treated group did not show any significant reversal (Table-94, Figure-89).

Real Time PCR amplification of Bax mRNA in the Hippocampus of Control and Experimental Groups of Rats

Gene expression studies of Bax showed a significant (p<0.001) up regulation in the Hippocampus of the Rotenone infused PD rats. A significant reversal of the Bax was observed in the treatment groups; 5-HT (p<0.05), GABA (p<0.05), 5-HT+BMC (p<0.01), GABA+ BMC (p<0.01) and 5-HT+GABA+BMC

($p<0.001$). BMC alone treated group did not show any significant reversal (Table-95, Figure-90).

Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the Hippocampus of Control and Experimental Groups of Rats

Gene expression studies of ubiquitin carboxy-terminal hydrolase showed a significant ($p<0.001$) up regulation in Rotenone infused PD rats compared to control. A significant reversal of the ubiquitin carboxy-terminal hydrolase was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-96, Figure-91).

Real Time PCR amplification of ά-Synuclien mRNA in the Hippocampus of Control and Experimental Groups of Rats

Gene expression studies of ά-Synuclien showed a significant ($p<0.001$) up regulation in Rotenone infused PD rats compared to control. A significant reversal of the ά-Synuclien was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-97, Figure-92).

Behavioural study

Rotarod Performance of control and experimental groups of rats

Rotarod experiment showed a significantly down regulated in the retention time on the rotating rod in Rotenone infused PD rats compared to control. A significant reversal of the retention time on the rotating rod was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-98, Figure-93).

Behavioural response of control and experimental rats on Grid Walk test

There was a significant up regulation ($p<0.001$) in the foot falls in infused PD rats compared to control. A significant reversal of the foot falls was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-99, Figure-94).

Behavioural response of control and experimental rats on Narrow Beam test

There was a significant down regulation ($p<0.001$) in the retention time in Rotenone infused PD rats compared to control. A significant reversal of the retention time was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-100, Figure-95).

Social Interaction Performance of control and experimental groups of rats

There was less time in active interactions in the novel environment. Attempts at allogrooming, sniffing the partner, following were reduced in Rotenone infused PD rats when compared to control rats. A significant reversal of

the active interactions was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-101).

Behavioural response of control and experimental rats on Elevated Plus Maze test

There was less time in exploring the new areas of environment in Rotenone infused PD rats when compared to control rats. A significant reversal of the active interactions was observed in the treatment groups; 5-HT ($p<0.05$), GABA ($p<0.05$), 5-HT+BMC ($p<0.01$), GABA+ BMC ($p<0.01$) and 5-HT+GABA+BMC ($p<0.001$). BMC alone treated group did not show any significant reversal (Table-102, Figure-96).

Figure - 1
Apomorphine induced rotational behaviour in control and experimental groups of rats.

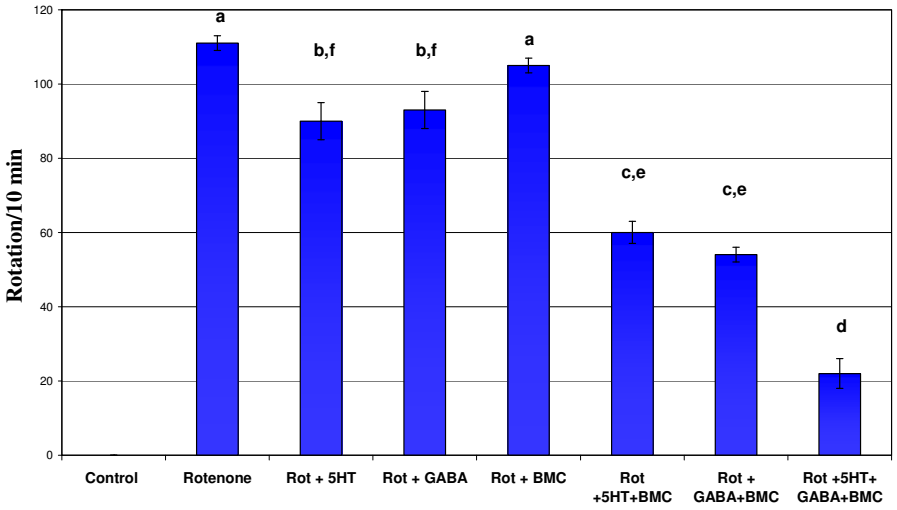


Table - 1
Apomorphine induced rotational behaviour in control and experimental groups of rats.

Experimental groups	Log RQ
Control	0
Rotenone	111 ± 10.31[a]
Rot + 5-HT	90 ± 8.29 [b, f]
Rot + GABA	93 ± 7.26 [b, f]
Rot + BMC	105 ± 9.24 [a]
Rot + 5-HT + BMC	60 ± 5.09 [c, e]
Rot + GABA + BMC	54 ± 6.12 [c, e]
Rot + 5-HT + GABA + BMC	22 ± 3.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Table - 2
Dopamine content in the substantia nigra *pars compacta* of control and experimental groups of rats

Experimental groups	DA(nmoles/g wet wt.)
Control	27.87 ± 1.20
Rotenone	5.90 ± 0.90 [a]
Rot + 5-HT	12.67 ± 1.33 [b, f]
Rot + GABA	13.32 ± 1.33 [b, f]
Rot + BMC	12.33 ± 1.20 [a, f]
Rot + 5-HT + BMC	18.00 ± 1.58 [c, e]
Rot + GABA + BMC	20.00 ± 1.35 [c, e]
Rot + 5-HT + GABA + BMC	23.33 ± 1.88 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 2
Real Time PCR amplification of Dopamine D_1 receptor mRNA in the substantia nigra *pars compacta* of control and experimental groups of rats

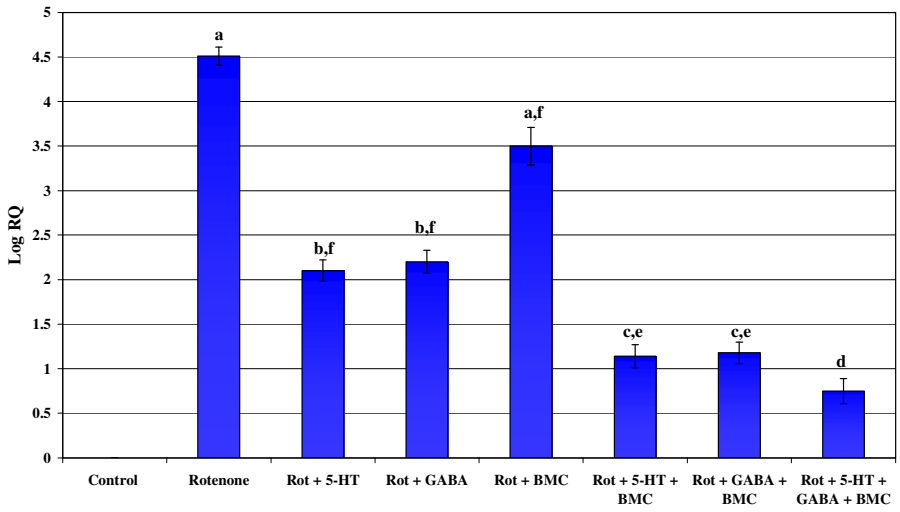


Table - 3
Real Time PCR amplification of Dopamine D_1 receptor mRNA in the substantia nigra *pars compacta* of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	4.51 ± 0.37 [a]
Rot + 5-HT	2.10 ± 0.27 [b, f]
Rot + GABA	2.20 ± 0.18 [b, f]
Rot + BMC	3.50 ± 0.31 [a, f]
Rot + 5-HT + BMC	1.14 ± 0.15 [c, e]
Rot + GABA + BMC	1.18 ± 0.16 [c, e]
Rot + 5-HT + GABA + BMC	0.75 ± 0.09 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 3
Real Time PCR amplification of Dopamine D_2 receptor mRNA in the substantia nigra *pars compacta* of control and experimental groups of rats

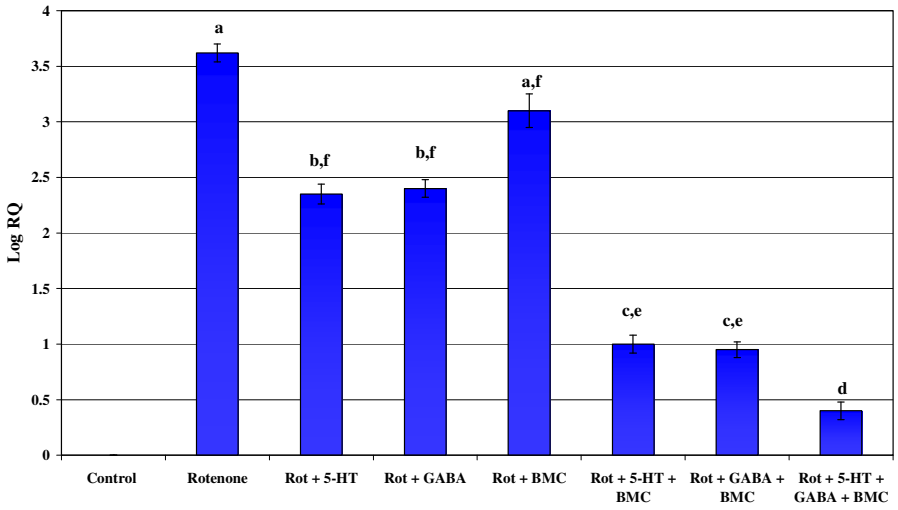


Table - 4
Real Time PCR amplification of Dopamine D_2 receptor mRNA in the substantia nigra *pars compacta* of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	3.62 ± 0.17 [a]
Rot + 5-HT	2.35 ± 0.18 [b, f]
Rot + GABA	2.40 ± 0.20 [b, f]
Rot + BMC	3.10 ± 0.16 [a, f]
Rot + 5-HT + BMC	1.00 ± 0.15 [c, e]
Rot + GABA + BMC	0.95 ± 0.16 [c, e]
Rot + 5-HT + GABA + BMC	0.40 ± 0.12 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 4
Real Time PCR amplification of Nestin mRNA in the substantia nigra *pars compacta* of control and experimental groups of rats

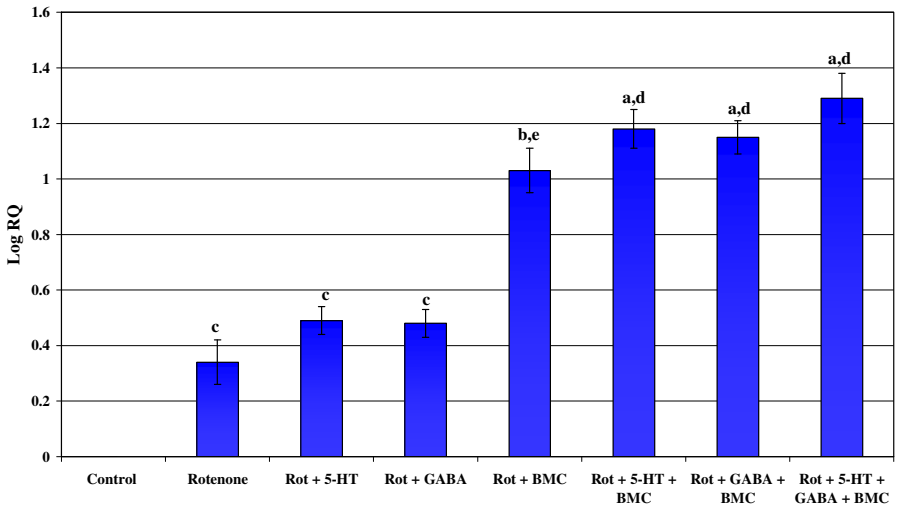


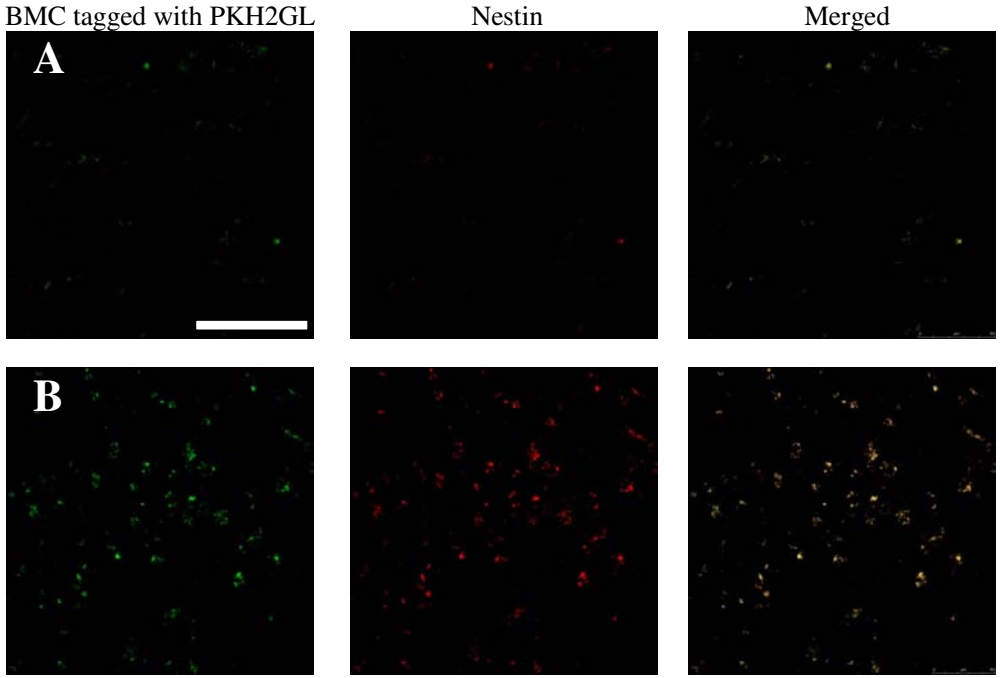
Table - 5
Real Time PCR amplification of Nestin mRNA in the substantia nigra *pars compacta* of Control and Experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	0.34 ± 0.08 [c]
Rot + 5-HT	0.49 ± 0.04 [c]
Rot + GABA	0.48 ± 0.03 [c]
Rot + BMC	1.03 ± 0.15 [b ,e]
Rot + 5-HT + BMC	1.18 ± 0.16 [a ,d]
Rot + GABA + BMC	1.15 ± 0.14 [a ,d]
Rot + 5-HT + GABA + BMC	1.29 ± 0.14 [a ,d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 5

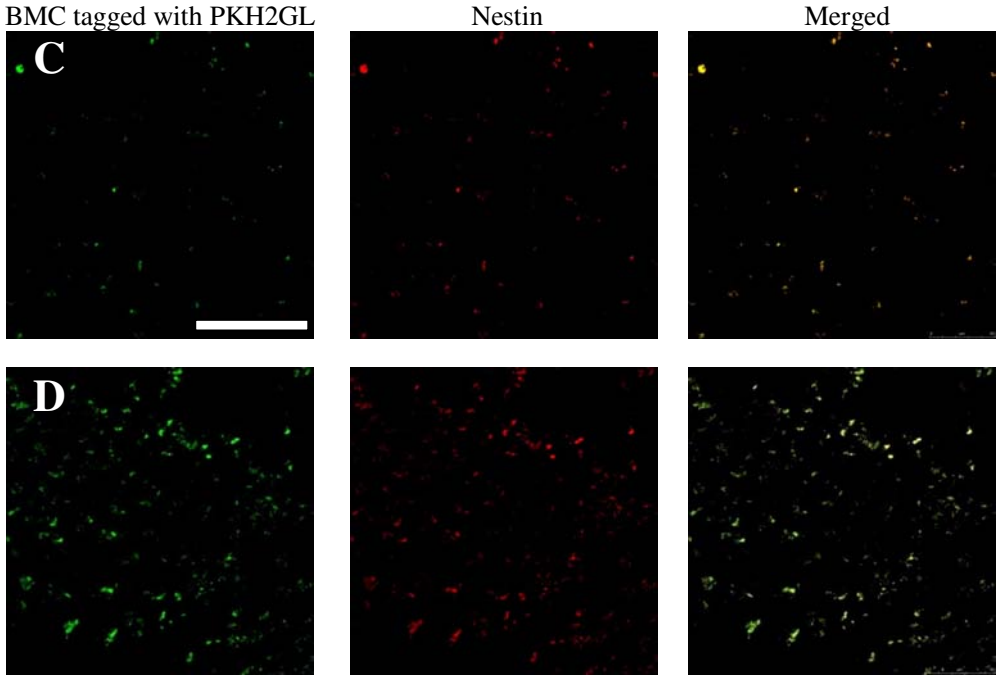
***In vivo* expression studies of bone marrow cells and Nestin in the substantia nigra *pars compacta* of experimental rats**



A - Rotenone infused treated with BMC; B - Rotenone infused treated with Serotonin and BMC. The scale bar represents 50 μm.

Figure - 6

***In vivo* expression studies of bone marrow cells and Nestin in the substantia nigra *pars compacta* of experimental rats**



C - Rotenone infused treated with GABA and BMC; D - Rotenone infused treated with Serotonin, GABA and BMC. The scale bar represents 50 µm.

Table - 6
***In vivo* expression studies of bone marrow cells and Nestin in the substantia nigra *pars compacta* of experimental rats**

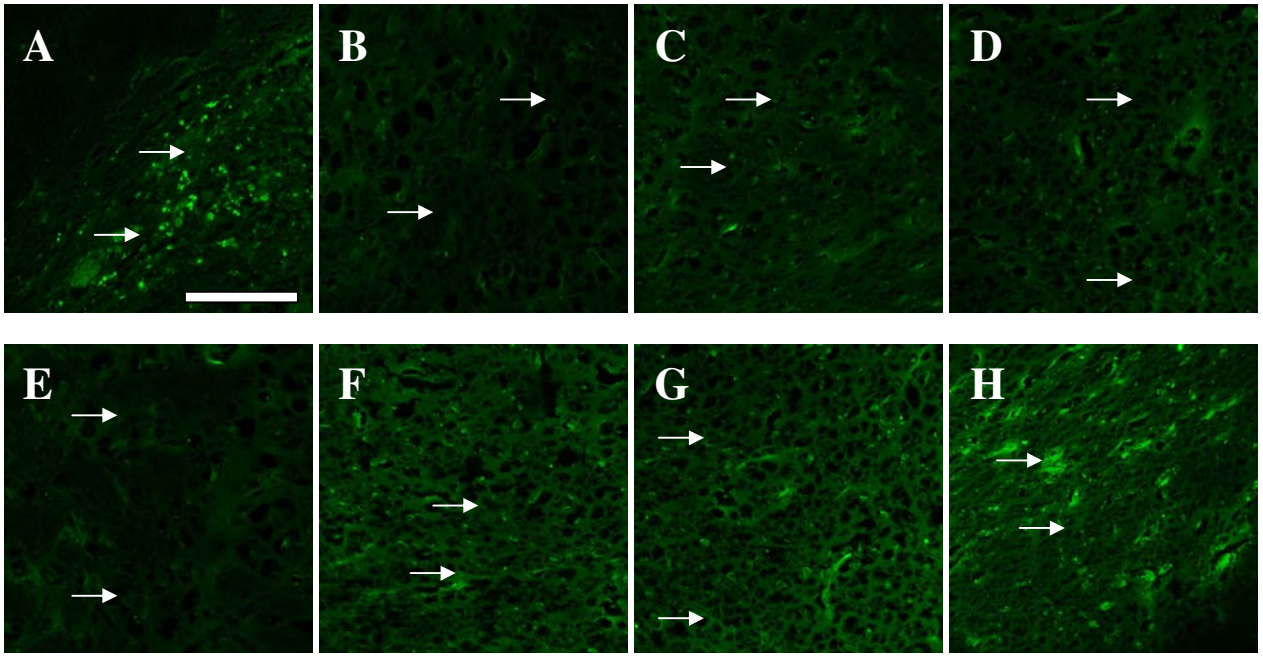
Experimental groups	Mean pixel intensity
Rot + BMC	36.42 ± 2.96
Rot + 5-HT + BMC	54.78 ± 4.99 [b]
Rot + GABA + BMC	57.96 ± 4.62 [b]
Rot + 5-HT + GABA + BMC	85.35 ± 6.61 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$ when compared to Rot + BMC group.

Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 7
Tyrosine hydroxylase expression in the substantia nigra *pars compacta* of control and experimental rats



A – Control, B – Rotenone infused, C – Rotenone infused treated with Serotonin, D – Rotenone infused treated with GABA, E – Rotenone infused treated with BMC, F - Rotenone infused treated with Serotonin and BMC, G - Rotenone infused treated with GABA and BMC, H - Rotenone infused treated with Serotonin, GABA and BMC. The scale bar represents 75 µm.

Table - 7
Tyrosine hydroxylase expression in the substantia nigra *pars compacta* of control and experimental rats

Experimental groups	Mean pixel intensity
Control	81.27 ± 7.03
Rotenone	24.13 ± 2.87 [a]
Rot + 5-HT	54.85 ± 5.99 [b, f]
Rot + GABA	51.89 ± 4.43 [b, f]
Rot + BMC	35.98 ± 4.12 [a]
Rot + 5-HT + BMC	68.63 ± 5.44 [c, e]
Rot + GABA + BMC	70.22 ± 6.34 [c, e]
Rot + 5-HT + GABA + BMC	78.71 ± 7.22 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Table - 8
Dopamine content in the corpus striatum of control and experimental groups of rats

Experimental groups	DA(nmoles/g wet wt.)
Control	34.87 ± 1.20
Rotenone	9.90 ± 0.90 [a]
Rot + 5-HT	16.67 ± 1.33 [b, f]
Rot + GABA	17.32 ± 1.33 [b, f]
Rot + BMC	12.33 ± 1.20 [a]
Rot + 5-HT + BMC	20.00 ± 1.58 [c, e]
Rot + GABA + BMC	21.00 ± 1.35 [c, e]
Rot + 5-HT + GABA + BMC	26.33 ± 1.88 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 8
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the corpus striatum of control and experimental groups of rats

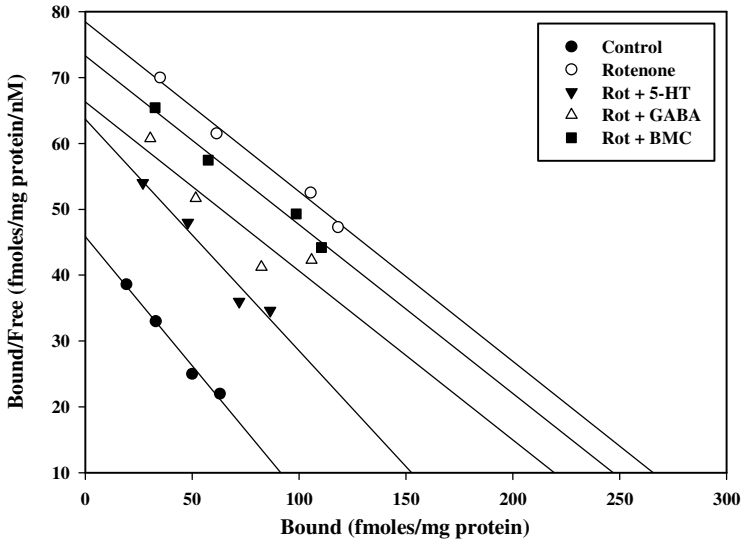


Table - 9
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the corpus striatum of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	89.13 ± 8.16	2.02 ± 0.26
Rotenone	264.56 ± 26.12 [a]	3.40 ± 0.22 [a]
Rot + 5-HT	152.53 ± 15.15 [b, f]	2.39 ± 0.33 [b, f]
Rot + GABA	207.07 ± 20.20 [b, f]	3.25 ± 0.31 [a]
Rot + BMC	246.31 ± 24.13 [a]	3.41 ± 0.26 [a]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 9
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the corpus striatum of control and experimental groups of rats

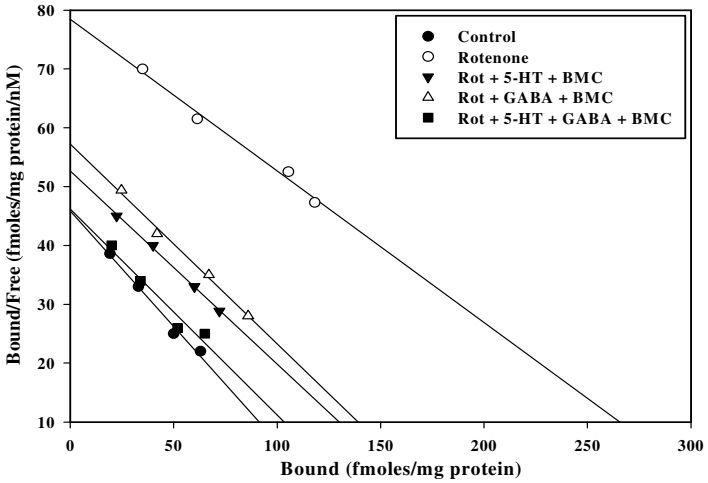


Table - 10
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the corpus striatum of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	89.13 ± 8.16	2.02 ± 0.26
Rotenone	264.56 ± 26.12 [a]	3.40 ± 0.22 [a]
Rot + 5-HT + BMC	128.73 ± 12.12 [b, e]	2.41 ± 0.22 [b, e]
Rot + GABA + BMC	136.07 ± 13.20 [b, e]	2.46 ± 0.22 [b, e]
Rot + 5-HT + GABA + BMC	101.49 ± 10.17 [c, d]	2.21 ± 0.23 [c, d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 10
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the corpus striatum of control and experimental groups of rats

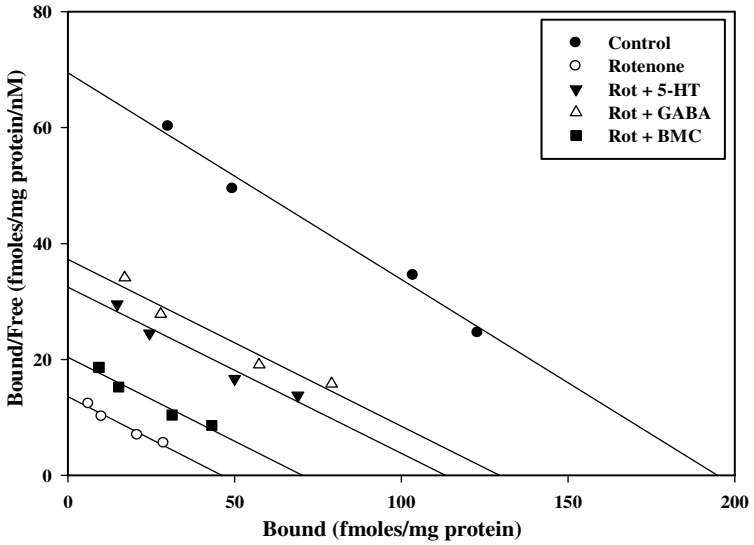


Table - 11
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the corpus striatum of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	183.51 ± 18.26	2.30 ± 0.26
Rotenone	40.67 ± 4.76 [a]	2.60 ± 0.16
Rot + 5-HT	127.46 ± 12.17 [b, f]	2.08 ± 0.13
Rot + GABA	140.15 ± 14.22 [b, f]	2.19 ± 0.26
Rot + BMC	68.32 ± 6.12 [a]	2.15 ± 0.37

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 11
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the corpus striatum of control and experimental groups of rats

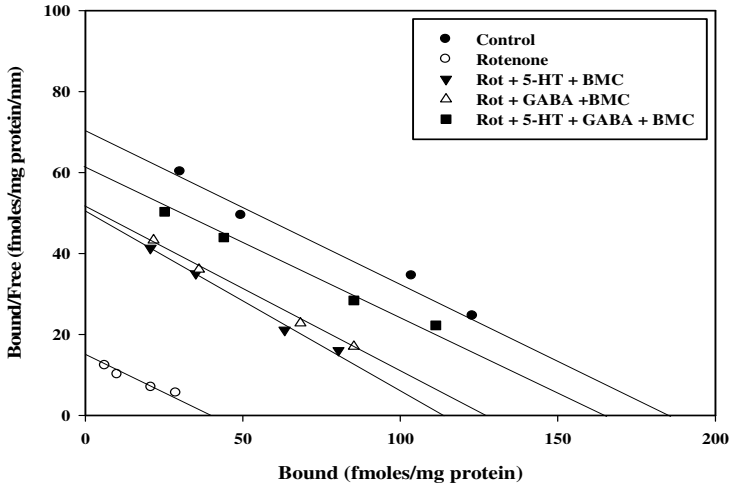


Table - 12
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the corpus striatum of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	183.51 ± 18.26	2.30 ± 0.26
Rotenone	40.67 ± 4.76 [a]	2.60 ± 0.16
Rot + 5-HT + BMC	163.73 ± 16.12 [b, e]	2.41 ± 0.28
Rot + GABA + BMC	126.07 ± 12.20 [b, e]	2.52 ± 0.22
Rot + 5-HT + GABA + BMC	113.39 ± 11.18 [c, d]	2.26 ± 0.26

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 12
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the corpus striatum of control and experimental groups of rats

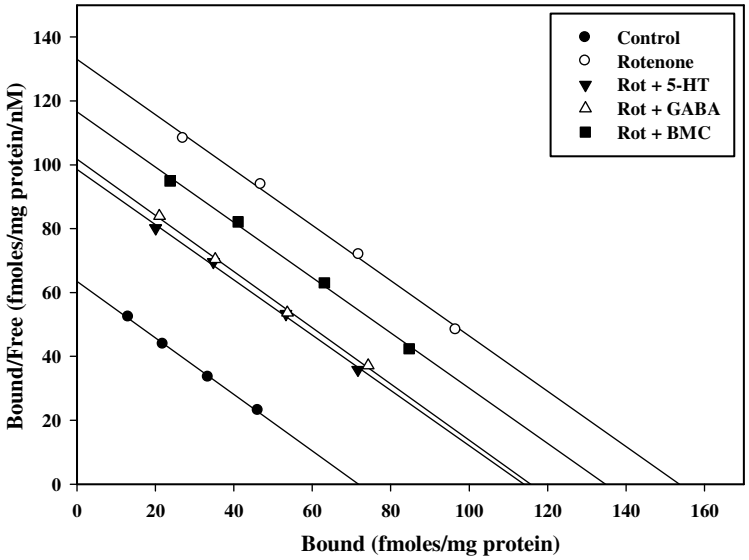


Table - 13
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the corpus striatum of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	69.49 ± 6.03	3.14 ± 0.22
Rotenone	155.49 ± 15.17 [a]	3.26 ± 0.24
Rot + 5-HT	107.41 ± 10.18 [b, f]	3.08 ± 0.22
Rot + GABA	103.18 ± 10.22 [b, f]	3.19 ± 0.21
Rot + BMC	134.37 ± 13.12 [a]	3.15 ± 0.35

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 13
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the corpus striatum of control and experimental groups of rats

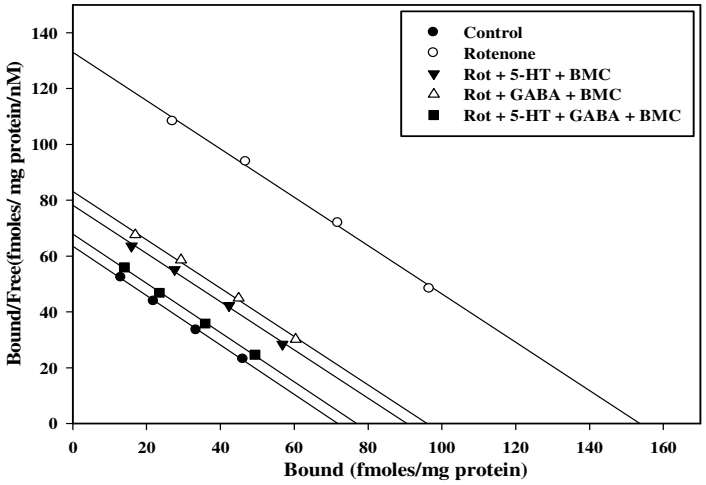


Table - 14
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the corpus striatum of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	69.49 ± 6.03	3.14 ± 0.22
Rotenone	155.49 ± 15.17 [a]	3.26 ± 0.24
Rot + 5-HT + BMC	90.03 ± 9.12 [b, e]	3.16 ± 0.21
Rot + GABA + BMC	94.62 ± 9.17 [b, e]	3.29 ± 0.28
Rot + 5-HT + GABA + BMC	73.46 ± 7.02 [c, d]	3.22 ± 0.17

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 14
Real Time PCR amplification of Dopamine D_1 receptor mRNA in the corpus striatum of control and experimental groups of rats

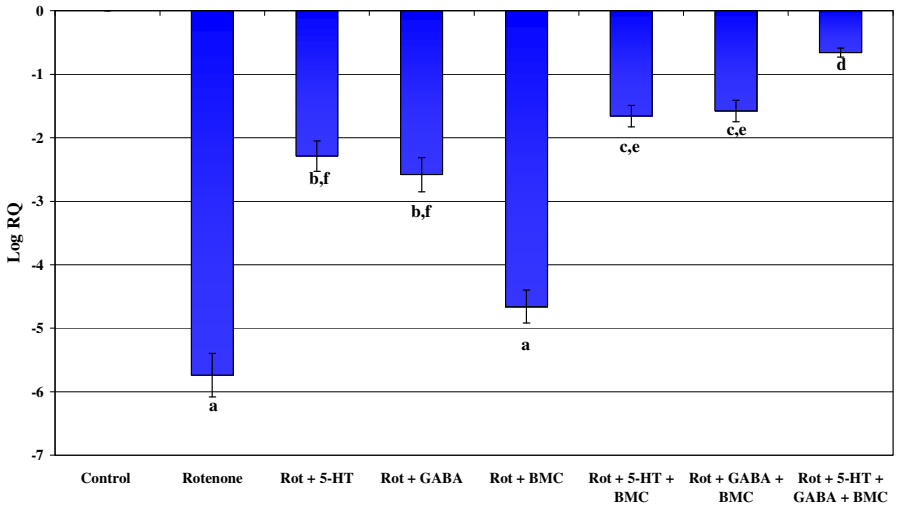


Table - 15
Real Time PCR amplification of Dopamine D_1 receptor mRNA in the corpus striatum of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	-5.74 ± 0.34 [a]
Rot + 5-HT	-2.29 ± 0.24 [b, f]
Rot + GABA	-2.58 ± 0.27 [b, f]
Rot + BMC	-4.66 ± 0.26 [a]
Rot + 5-HT + BMC	-1.66 ± 0.17 [c, e]
Rot + GABA + BMC	-1.58 ± 0.17 [c, e]
Rot + 5-HT + GABA + BMC	-0.66 ± 0.07 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 15
Real Time PCR amplification of Dopamine D_2 receptor mRNA in the corpus striatum of control and experimental groups of rats

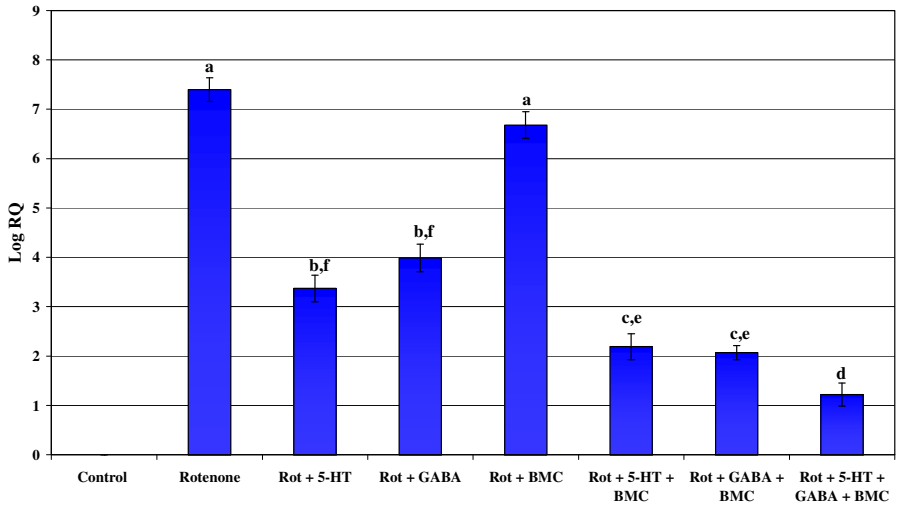
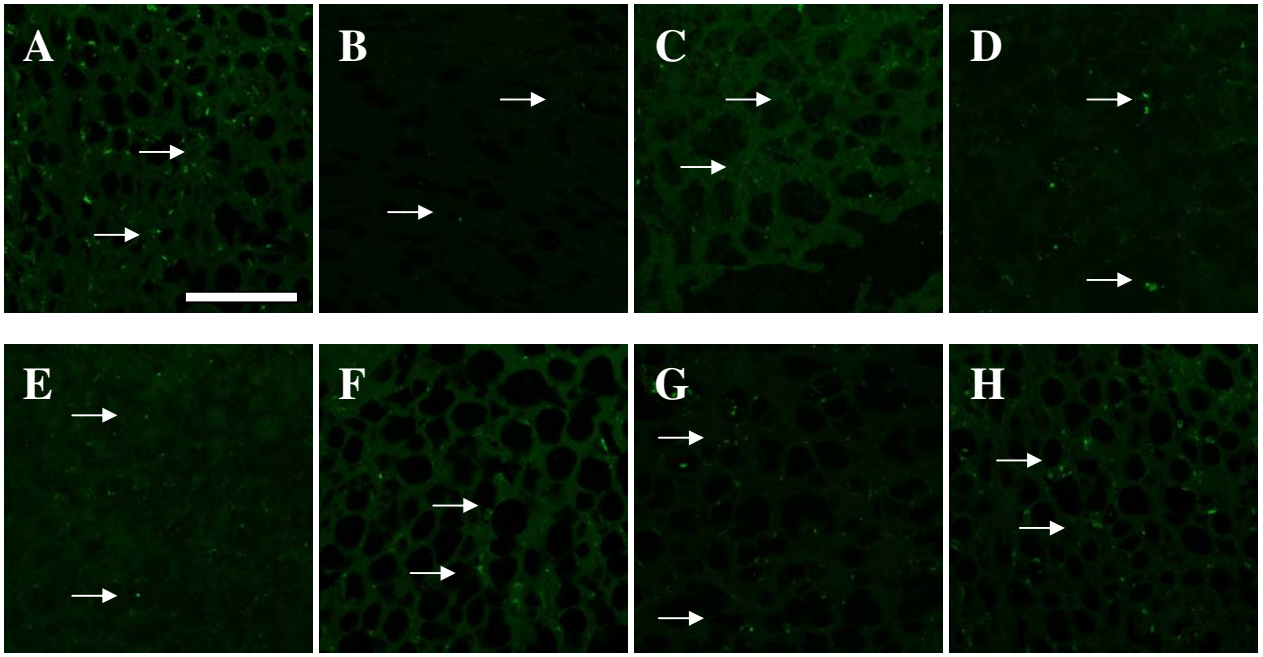


Table - 16
Real Time PCR amplification of Dopamine D_2 receptor mRNA in the corpus striatum of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	7.40 ± 0.24 [a]
Rot + 5-HT	3.37 ± 0.27 [b, f]
Rot + GABA	3.99 ± 0.28 [b, f]
Rot + BMC	6.68 ± 0.27 [a]
Rot + 5-HT + BMC	2.19 ± 0.26 [c, e]
Rot + GABA + BMC	2.07 ± 0.14 [c, e]
Rot + 5-HT + GABA + BMC	1.22 ± 0.23 [c, e]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 16
Dopamine D_1 receptor expression in the corpus striatum of control and experimental rats



A – Control, B – Rotenone infused, C – Rotenone infused treated with Serotonin, D – Rotenone infused treated with GABA, E – Rotenone infused treated with BMC, F - Rotenone infused treated with Serotonin and BMC, G - Rotenone infused treated with GABA and BMC, H - Rotenone infused treated with Serotonin, GABA and BMC. The scale bar represents 75 μm.

Table - 17
Dopamine D_1 receptor expression in the corpus striatum of control and experimental rats

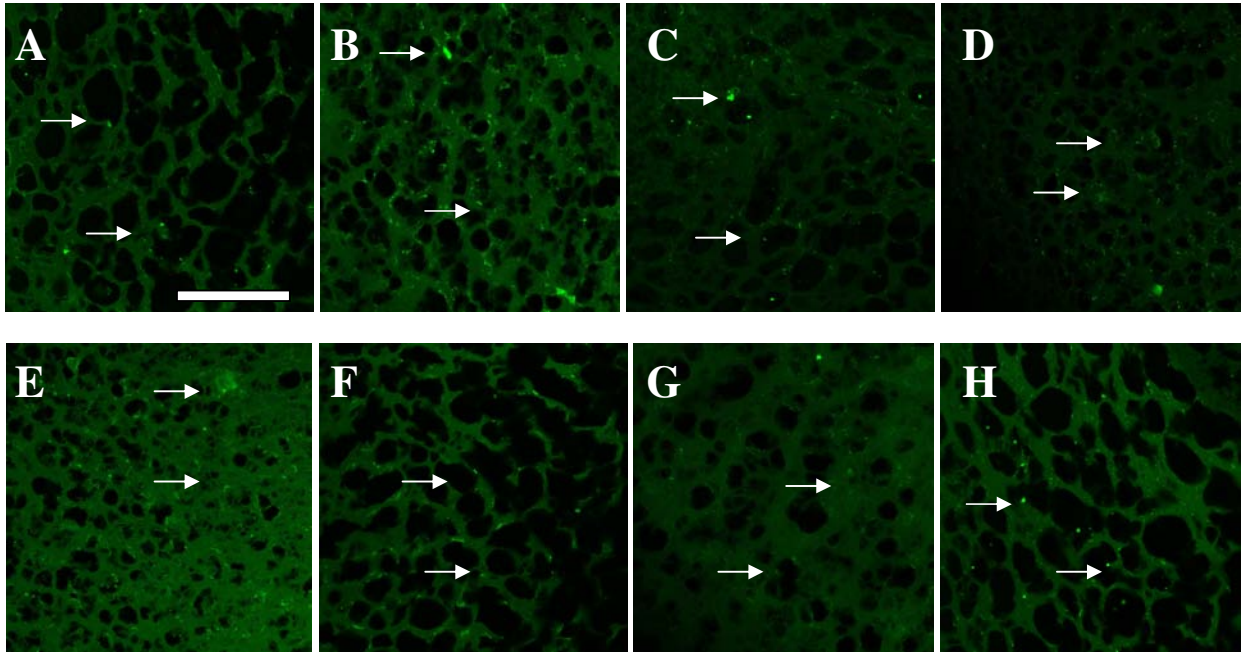
Experimental groups	Mean pixel intensity
Control	85.12 ± 3.50
Rotenone	25.25 ± 3.23 [a]
Rot + 5-HT	54.42 ± 2.50 [b, f]
Rot + GABA	58.77 ± 3.05 [b, f]
Rot + BMC	39.69 ± 7.55 [a]
Rot + 5-HT + BMC	69.12 ± 5.93 [c, e]
Rot + GABA + BMC	70.23 ± 5.56 [c, e]
Rot + 5-HT + GABA + BMC	77.33± 6.42 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 17
Dopamine D_2 receptor expression in the corpus striatum of control and experimental rats



A – Control, B – Rotenone infused, C – Rotenone infused treated with Serotonin, D – Rotenone infused treated with GABA, E – Rotenone infused treated with BMC, F - Rotenone infused treated with Serotonin and BMC, G - Rotenone infused treated with GABA and BMC, H - Rotenone infused treated with Serotonin, GABA and BMC. The scale bar represents 75 μm.

Table - 18
Dopamine D_2 receptor expression in the corpus striatum of control and experimental rats

Experimental groups	Mean pixel intensity
Control	30.55 ± 2.93
Rotenone	94.11 ± 6.42 [a]
Rot + 5-HT	57.34 ± 3.11 [b, f]
Rot + GABA	65.45 ± 4.56 [b, f]
Rot + BMC	88.43 ± 8.96 [a]
Rot + 5-HT + BMC	37.71 ± 4.99 [c, e]
Rot + GABA + BMC	36.93 ± 5.62 [c, e]
Rot + 5-HT + GABA + BMC	29.19 ± 3.61 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 18
IP3 content in the corpus striatum of control and experimental groups of rats

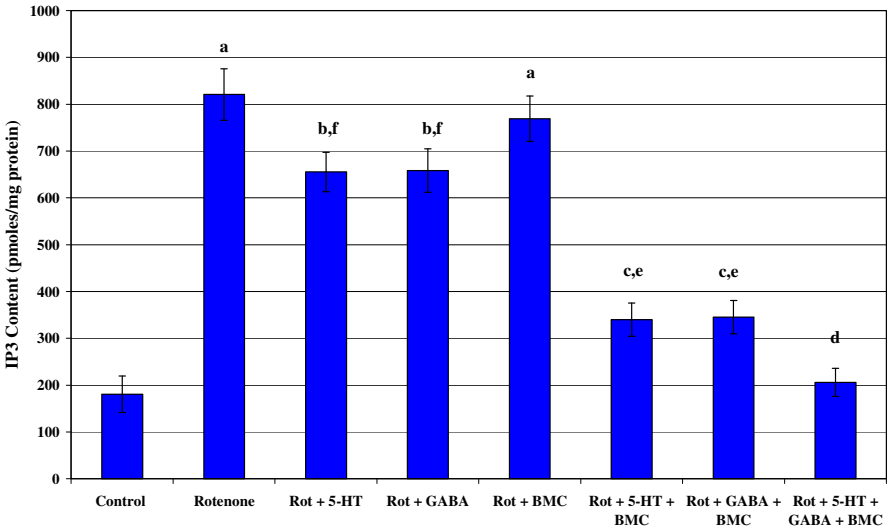


Table - 19
IP3 content in the corpus striatum of control and experimental groups of rats

Experimental groups	IP3 Content (pmoles/mg protein)
Control	180.51 ± 38.95
Rotenone	820.74 ± 55.23 [a]
Rot + 5-HT	655.23 ± 42.32 [b, f]
Rot + GABA	658.19 ± 46.23 [b, f]
Rot + BMC	768.12 ± 48.75 [a]
Rot + 5-HT + BMC	339.68 ± 35.62 [c, e]
Rot + GABA + BMC	345.23 ± 35.65 [c, e]
Rot + 5-HT + GABA + BMC	205.85 ± 30.12 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 19
cAMP content in the corpus striatum of control and experimental groups of rats

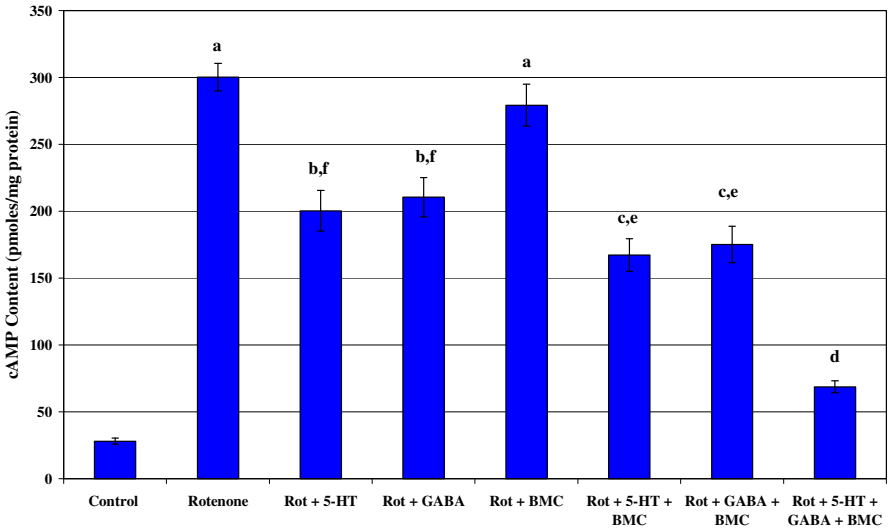


Table - 20
cAMP content in the corpus striatum of control and experimental groups of rats

Experimental groups	cAMP Content (pmoles/mg protein)
Control	28.36 ± 2.23
Rotenone	300.24 ± 10.25 [a]
Rot + 5-HT	200.30 ± 15.23 [b, f]
Rot + GABA	210.81 ± 14.65 [b, f]
Rot + BMC	279.53 ± 15.68 [a]
Rot + 5-HT + BMC	167.23 ± 12.23 [c, e]
Rot + GABA + BMC	175.44 ± 13.62 [c, e]
Rot + 5-HT + GABA + BMC	68.79 ± 4.32 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 20
cGMP content in the corpus striatum of control and experimental groups of rats

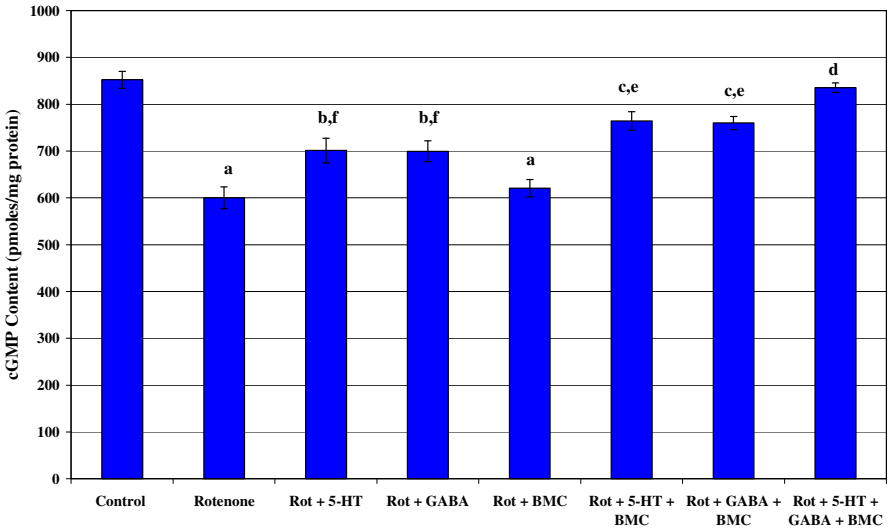


Table - 21
cGMP content in the corpus striatum of control and experimental groups of rats

Experimental groups	cGMP Content (pmoles/mg protein)
Control	852.01 ± 18.25
Rotenone	600.23 ± 23.25 [a]
Rot + 5-HT	701.23 ± 26.53 [b, f]
Rot + GABA	699.32 ± 22.12 [b, f]
Rot + BMC	621.01 ± 18.32 [a]
Rot + 5-HT + BMC	764.25 ± 19.65 [c, e]
Rot + GABA + BMC	759.85 ± 14.25 [c, e]
Rot + 5-HT + GABA + BMC	835.16 ± 10.23 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 21
Real Time PCR amplification of CREB mRNA in the corpus striatum of control and experimental groups of rats

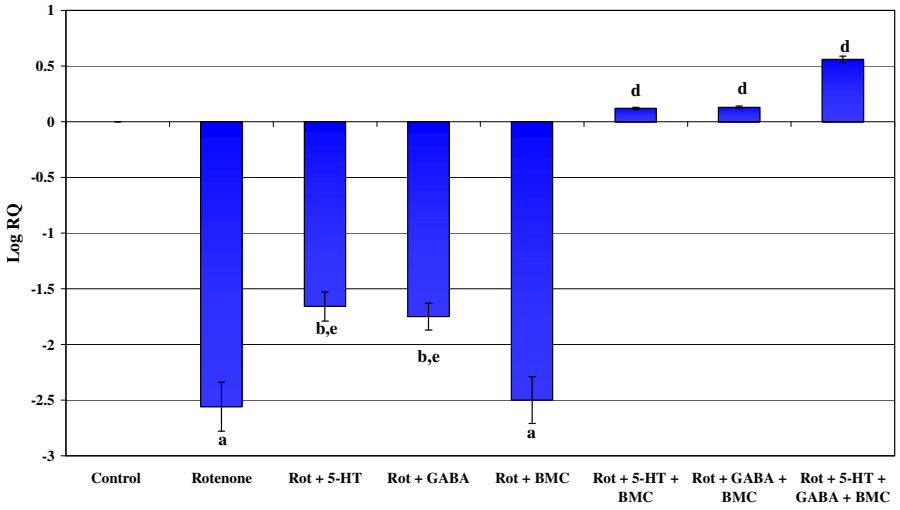


Table - 22
Real Time PCR amplification of CREB mRNA in the corpus striatum of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	-2.56 ± 0.22 [a]
Rot + 5-HT	-1.66 ± 0.13 [b, e]
Rot + GABA	-1.75 ± 0.12 [b, e]
Rot + BMC	-2.50 ± 0.21 [a]
Rot + 5-HT + BMC	0.12 ± 0.01 [d]
Rot + GABA + BMC	0.13 ± 0.01 [d]
Rot + 5-HT + GABA + BMC	0.56 ± 0.03 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 22
Real Time PCR amplification of Bax mRNA in the corpus striatum of control and experimental groups of rats

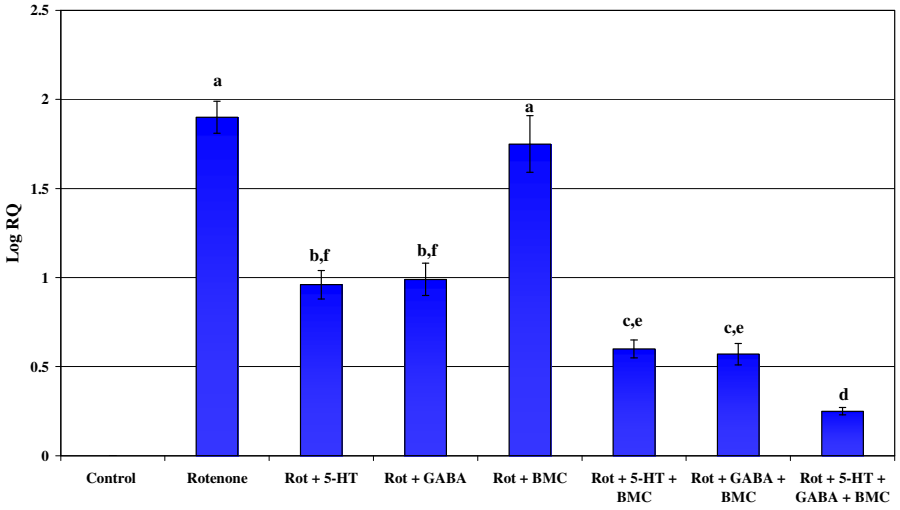


Table - 23
Real Time PCR amplification of Bax mRNA in the corpus striatum of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	1.90 ± 0.09 [a]
Rot + 5-HT	0.96 ± 0.08 [b, f]
Rot + GABA	0.99 ± 0.09 [b, f]
Rot + BMC	1.75 ± 0.16 [a]
Rot + 5-HT + BMC	0.60 ± 0.05 [c, e]
Rot + GABA + BMC	0.57 ± 0.06 [c, e]
Rot + 5-HT + GABA + BMC	0.25 ± 0.02 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 23
Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the corpus striatum of control and experimental groups of rats

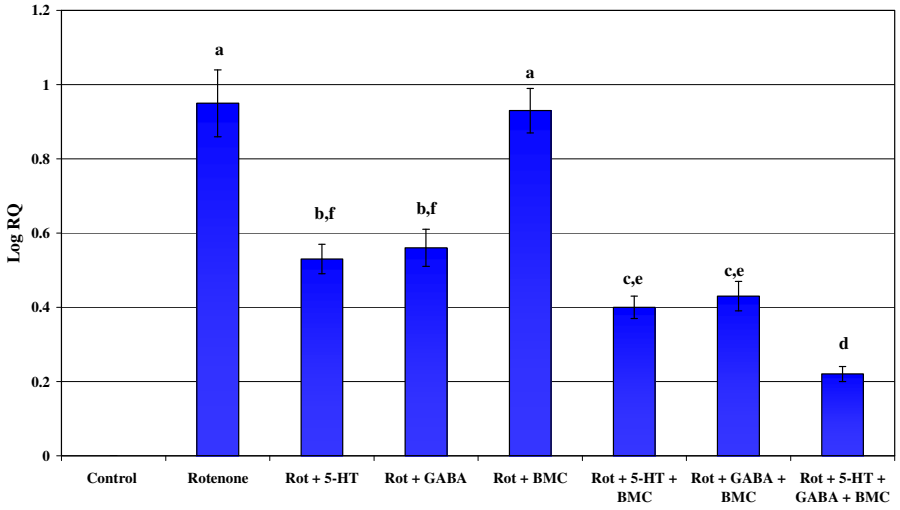


Table - 24
Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the corpus striatum of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	0.95 ± 0.09 [a]
Rot + 5-HT	0.53 ± 0.04 [b, f]
Rot + GABA	0.56 ± 0.05 [b, f]
Rot + BMC	0.93 ± 0.06 [a]
Rot + 5-HT + BMC	0.40 ± 0.03 [c, e]
Rot + GABA + BMC	0.43 ± 0.04 [c, e]
Rot + 5-HT + GABA + BMC	0.22 ± 0.02 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 24
Real Time PCR amplification of α-synuclein in the corpus striatum of control and experimental groups of rats

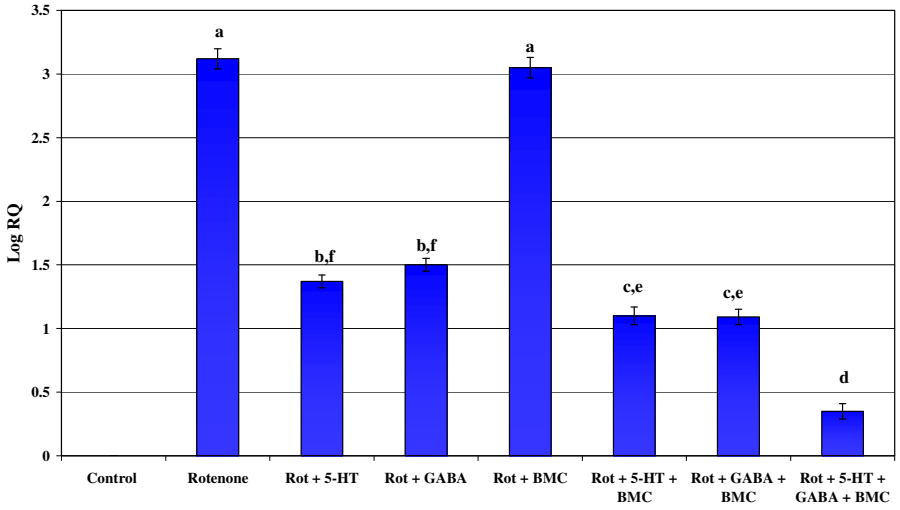


Table - 25
Real Time PCR amplification of α-synuclein in the corpus striatum of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	3.12 ± 0.08 [a]
Rot + 5-HT	1.37 ± 0.05 [b, f]
Rot + GABA	1.50 ± 0.05 [b, f]
Rot + BMC	3.05 ± 0.08 [a]
Rot + 5-HT + BMC	1.10 ± 0.07 [c, e]
Rot + GABA + BMC	1.09 ± 0.06 [c, e]
Rot + 5-HT + GABA + BMC	0.35 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] p<0.001, [b] p<0.01, [c] p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f] p<0.05 when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Table -26
Dopamine content in the cerebral cortex of control and experimental groups of rats

Experimental groups	DA(nmoles/g wet wt.)
Control	33.07 ± 3.20
Rotenone	10.90 ± 1.20 [a]
Rot + 5-HT	17.60 ± 1.33 [b, f]
Rot + GABA	18.52 ± 1.33 [b, f]
Rot + BMC	13.33 ± 1.20 [a]
Rot + 5-HT + BMC	22.80 ± 2.58 [c, e]
Rot + GABA + BMC	24.40 ± 2.58 [c, e]
Rot + 5-HT + GABA + BMC	28.13 ± 2.88 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 25
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the cerebral cortex of control and experimental groups of rats

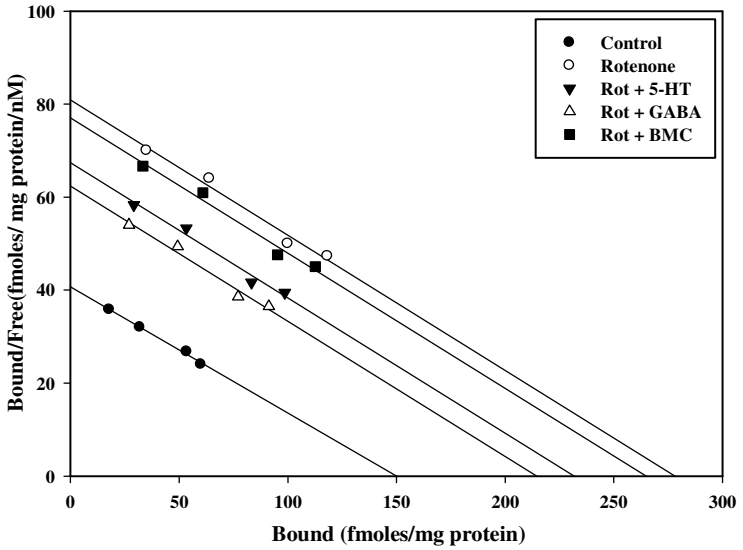


Table - 27
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the cerebral cortex of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	143.37 ± 14.86	3.30 ± 0.31
Rotenone	274.16 ± 27.36 [a]	3.31 ± 0.36
Rot + 5-HT	229.43 ± 22.16 [b, f]	3.44 ± 0.23
Rot + GABA	213.07 ± 21.20 [b, f]	3.43 ± 0.20
Rot + BMC	262.31 ± 26.13 [a]	3.41 ± 0.36

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 26
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the cerebral cortex of control and experimental groups of rats

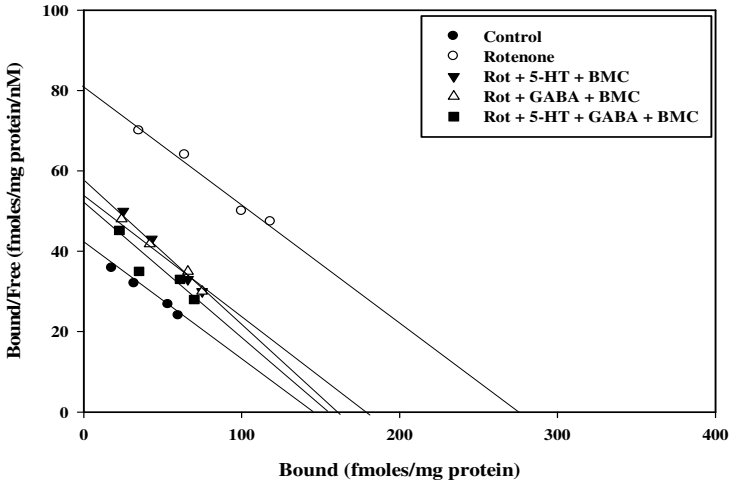


Table - 28
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the cerebral cortex of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	143.37 ± 14.86	3.30 ± 0.31
Rotenone	274.16 ± 27.36 [a]	3.31 ± 0.36
Rot + 5-HT + BMC	180.23 ± 18.12 [b, e]	3.01 ± 0.38
Rot + GABA + BMC	160.07 ± 16.10 [b, e]	3.25 ± 0.32
Rot + 5-HT + GABA + BMC	152.29 ± 15.08 [c, d]	3.22 ± 0.34

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 27
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the cerebral cortex of control and experimental groups of rats

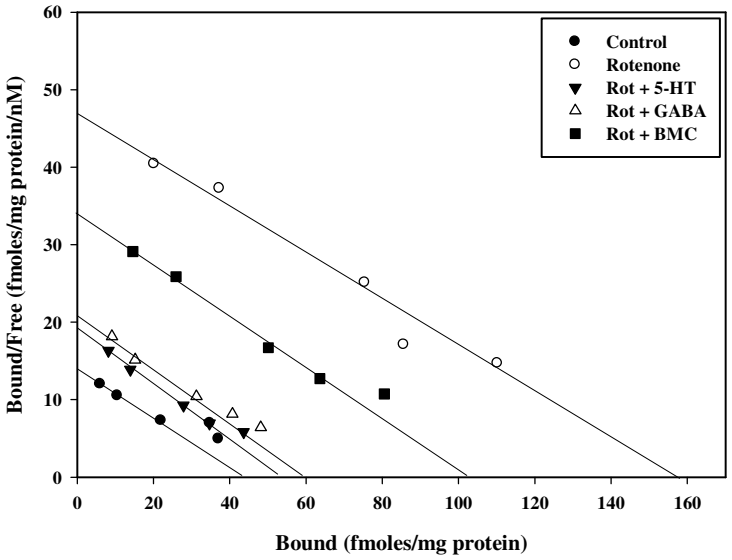


Table - 29
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the cerebral cortex of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	43.13 ± 4.14	3.45 ± 0.35
Rotenone	157.14 ± 15.13 [a]	3.08 ± 0.34
Rot + 5-HT	52.41 ± 5.16 [b, f]	3.08 ± 0.29
Rot + GABA	58.18 ± 5.22 [b, f]	3.29 ± 0.25
Rot + BMC	104.35 ± 10.13 [a]	3.47 ± 0.39

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 28
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the cerebral cortex of control and experimental groups of rats

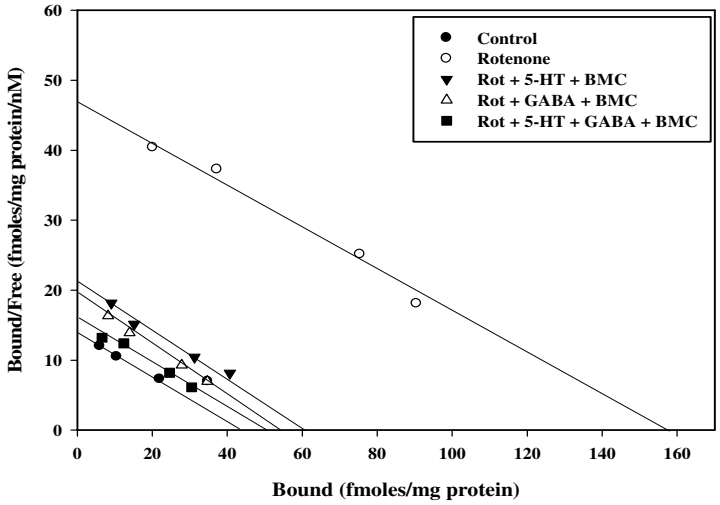


Table - 30
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the cerebral cortex of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	43.13 ± 4.14	3.45 ± 0.35
Rotenone	157.14 ± 15.13 [a]	3.08 ± 0.34
Rot + 5-HT + BMC	60.04 ± 6.12 [b, e]	3.26 ± 0.21
Rot + GABA + BMC	52.85 ± 5.14 [b, e]	3.27 ± 0.26
Rot + 5-HT + GABA + BMC	48.60 ± 4.08 [c, d]	3.22 ± 0.28

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 29
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the cerebral cortex of control and experimental groups of rats

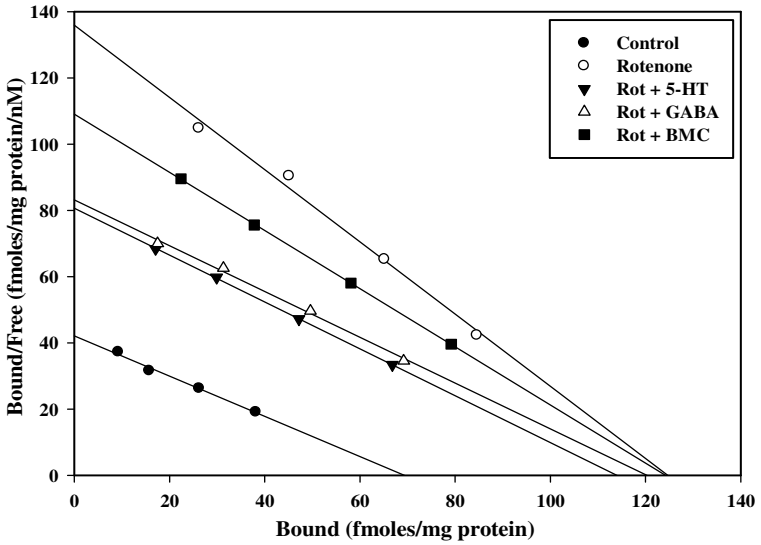


Table - 31
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the cerebral cortex of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	69.49 ± 6.03	1.65 ± 0.29
Rotenone	124.46 ± 12.13 [a]	1.12 ± 0.31
Rot + 5-HT	113.41 ± 10.18 [b, f]	1.41 ± 0.14
Rot + GABA	119.54 ± 10.22 [b, f]	1.43 ± 0.15
Rot + BMC	123.64 ± 12.13 [a]	1.13 ± 0.34

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 30
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the cerebral cortex of control and experimental groups of rats

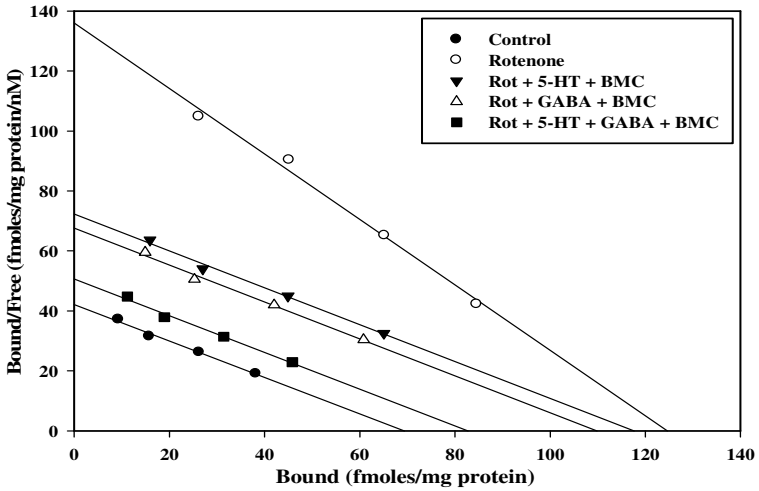


Table - 32
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the cerebral cortex of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	69.49 ± 6.03	1.65 ± 0.29
Rotenone	124.46 ± 12.13 [a]	1.12 ± 0.31
Rot + 5-HT + BMC	116.03 ± 11.12 [b, e]	1.61 ± 0.22
Rot + GABA + BMC	109.65 ± 10.11 [b, e]	1.63 ± 0.25
Rot + 5-HT + GABA + BMC	81.52 ± 8.06 [d]	1.62 ± 0.17

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure -31
Real Time PCR amplification of Dopamine D_1 receptor mRNA in the cerebral cortex of control and experimental groups of rats

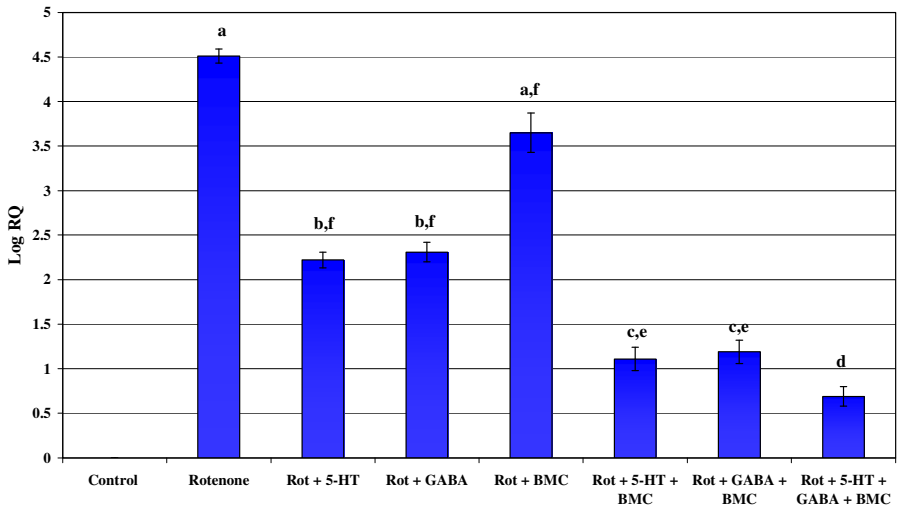


Table - 33
Real Time PCR amplification of Dopamine D_1 receptor mRNA in the cerebral cortex of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	4.51 ± 0.22 [a]
Rot + 5-HT	2.22 ± 0.26 [b, f]
Rot + GABA	2.31 ± 0.25 [b, f]
Rot + BMC	3.65 ± 0.26 [a]
Rot + 5-HT + BMC	1.11 ± 0.26 [c, e]
Rot + GABA + BMC	1.19 ± 0.15 [c, e]
Rot + 5-HT + GABA + BMC	0.69 ± 0.13 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure -32
Real Time PCR amplification of Dopamine D_2 receptor mRNA in the cerebral cortex of control and experimental groups of rats

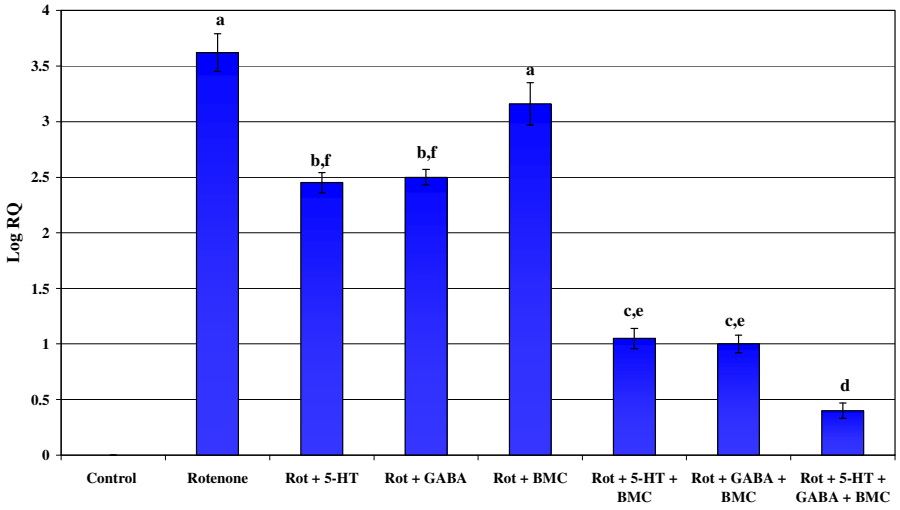
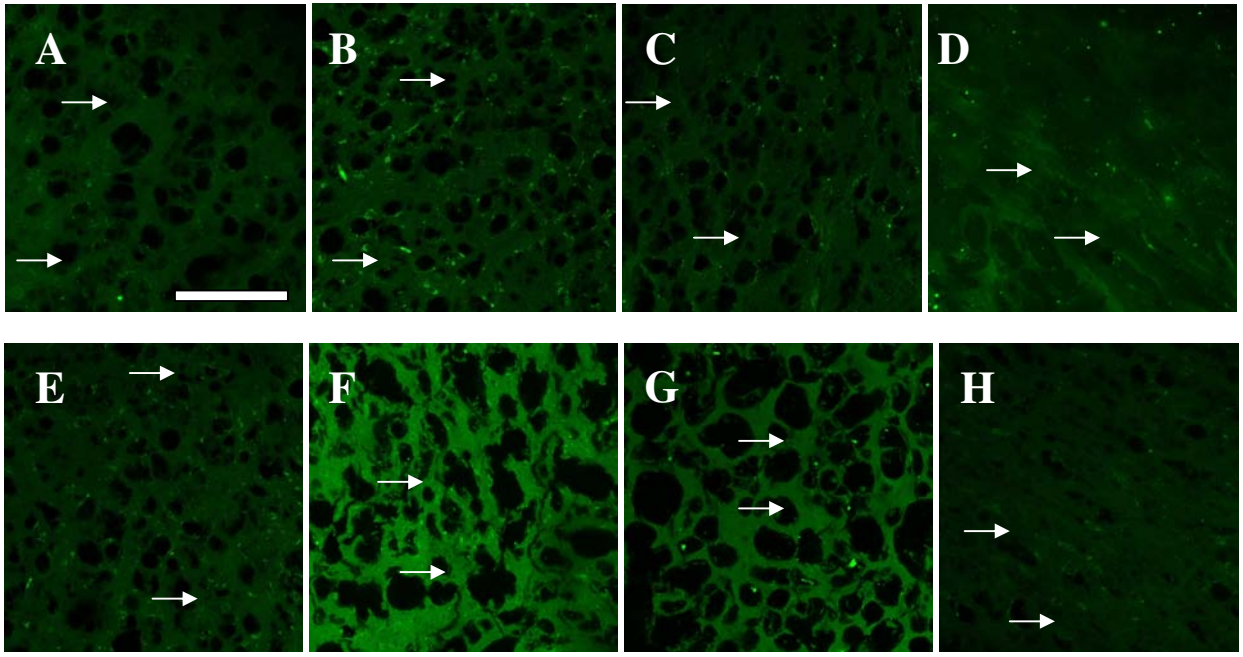


Table - 34
Real Time PCR amplification of Dopamine D_2 receptor mRNA in the cerebral cortex of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	3.62 ± 0.16 [a]
Rot + 5-HT	2.45 ± 0.17 [b, f]
Rot + GABA	2.50 ± 0.18 [b, f]
Rot + BMC	3.16 ± 0.19 [a]
Rot + 5-HT + BMC	1.05 ± 0.15 [c, e]
Rot + GABA + BMC	1.00 ± 0.12 [c, e]
Rot + 5-HT + GABA + BMC	0.40 ± 0.14 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 33
Dopamine D_1 receptor expression in the cerebral cortex of control and experimental rats



A – Control, B – Rotenone infused, C – Rotenone infused treated with Serotonin, D – Rotenone infused treated with GABA, E – Rotenone infused treated with BMC, F - Rotenone infused treated with Serotonin and BMC, G - Rotenone infused treated with GABA and BMC, H - Rotenone infused treated with Serotonin, GABA and BMC. The scale bar represents 75 µm.

Table - 35
Dopamine D_1 receptor expression in the cerebral cortex of control and experimental rats

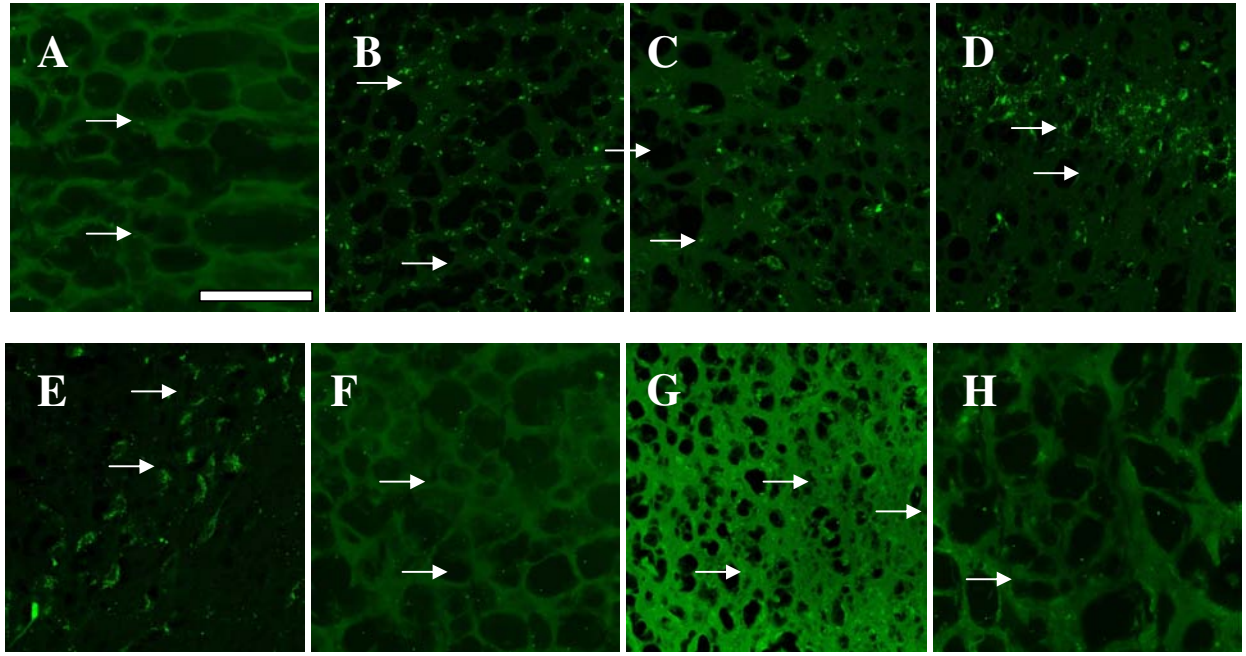
Experimental groups	Mean pixel intensity
Control	20.12 ± 2.03
Rotenone	99.74 ± 9.13 [a]
Rot + 5-HT	64.12 ± 5.19 [b, f]
Rot + GABA	65.87 ± 6.42 [b, f]
Rot + BMC	92.75 ± 9.87 [a]
Rot + 5-HT + BMC	49.12 ± 3.12 [c, e]
Rot + GABA + BMC	50.42 ± 4.11 [c, e]
Rot + 5-HT + GABA + BMC	29.76 ± 2.14 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 34
Dopamine D_2 receptor expression in the cerebral cortex of control and experimental rats



A – Control, B – Rotenone infused, C – Rotenone infused treated with Serotonin, D – Rotenone infused treated with GABA, E – Rotenone infused treated with BMC, F - Rotenone infused treated with Serotonin and BMC, G - Rotenone infused treated with GABA and BMC, H - Rotenone infused treated with Serotonin, GABA and BMC. The scale bar represents 75 µm.

Table - 36
Dopamine D_2 receptor expression in the cerebral cortex of control and experimental rats

Experimental groups	Mean pixel intensity
Control	25.12 ± 2.03
Rotenone	90.74 ± 9.13 [a]
Rot + 5-HT	62.12 ± 5.19 [b, f]
Rot + GABA	68.87 ± 6.42 [b, f]
Rot + BMC	86.75 ± 9.87 [a]
Rot + 5-HT + BMC	45.12 ± 3.12 [c, e]
Rot + GABA + BMC	58.42 ± 4.11 [c, e]
Rot + 5-HT + GABA + BMC	25.76 ± 2.14[d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 35
IP3 content in the cerebral cortex of control and experimental groups of rats

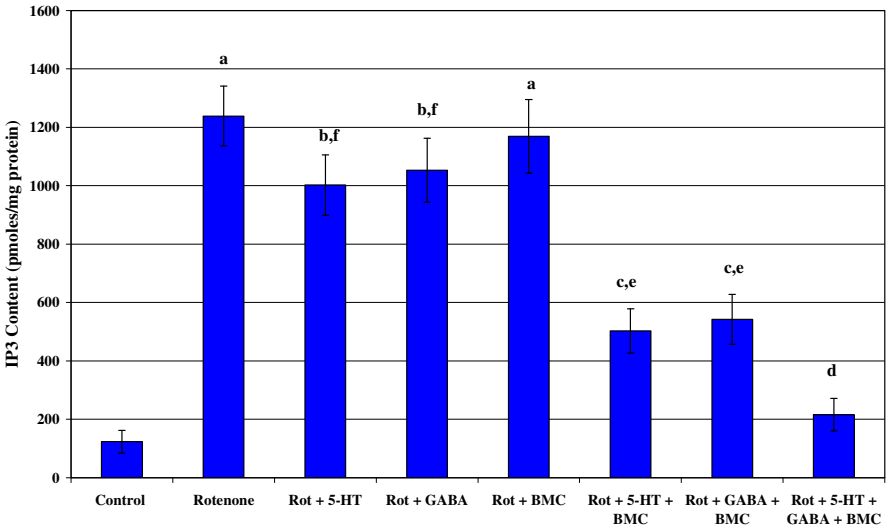


Table - 37
IP3 content in the cerebral cortex of control and experimental groups of rats

Experimental groups	IP3 Content (pmoles/mg protein)
Control	123.34 ± 39.11
Rotenone	1238.23 ± 57.12 [a]
Rot + 5-HT	1002.12 ± 44.25 [b, f]
Rot + GABA	1052.42 ± 49.12 [b, f]
Rot + BMC	1169.20 ± 39.23 [a]
Rot + 5-HT + BMC	502.85 ± 75.32 [c, e]
Rot + GABA + BMC	542.23 ± 85.32 [c, e]
Rot + 5-HT + GABA + BMC	215.35 ± 55.82 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 36
cAMP content in the cerebral cortex of control and experimental groups of rats

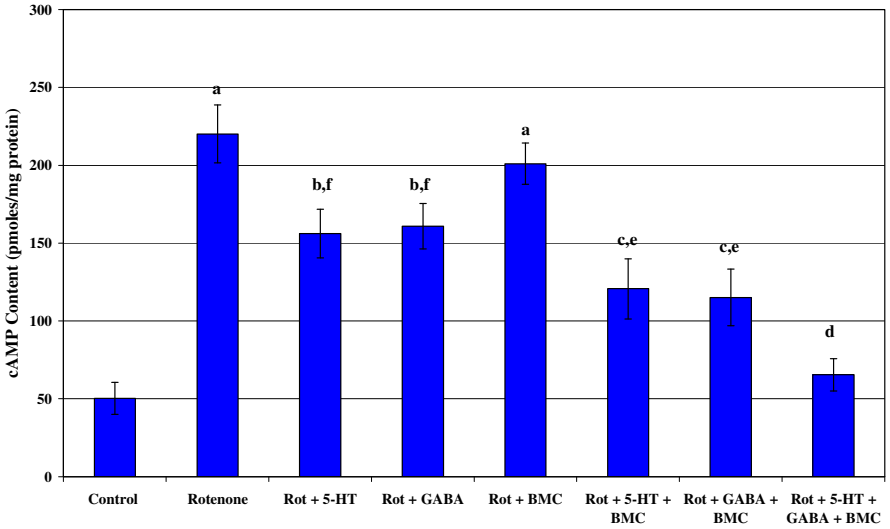


Table - 38
cAMP content in the cerebral cortex of control and experimental groups of rats

Experimental groups	cAMP Content (pmoles/mg protein)
Control	50.36 ± 5.23
Rotenone	220.24 ± 10.25 [a]
Rot + 5-HT	156.30 ± 15.23 [b, f]
Rot + GABA	160.81 ± 14.65 [b, f]
Rot + BMC	200.53 ± 15.68 [a]
Rot + 5-HT + BMC	120.65 ± 5.23 [c, e]
Rot + GABA + BMC	115.44 ± 5.62 [c, e]
Rot + 5-HT + GABA + BMC	65.20 ± 4.32 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 37
cGMP content in the cerebral cortex of control and experimental groups of rats

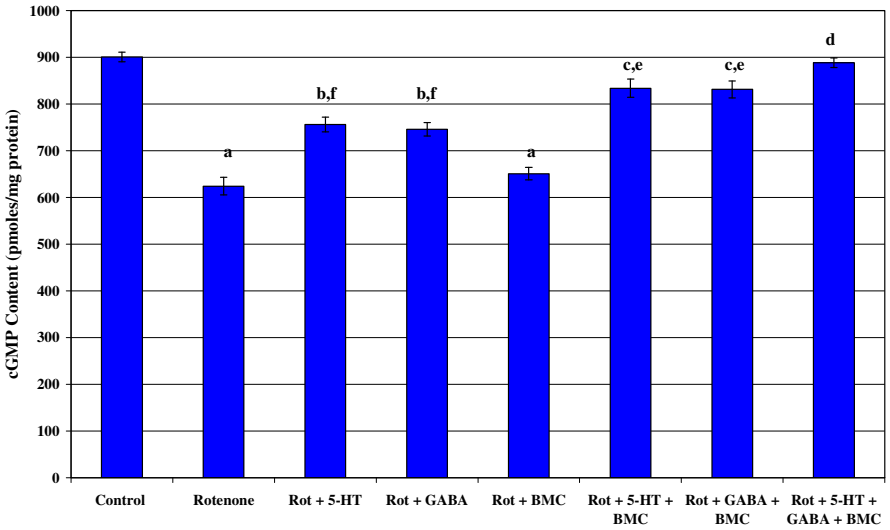


Table - 39
cGMP content in the cerebral cortex of control and experimental groups of rats

Experimental groups	cGMP Content (pmoles/mg protein)
Control	900.21 ± 16.21
Rotenone	624.12 ± 18.53 [a]
Rot + 5-HT	756.12 ± 18.42 [b, f]
Rot + GABA	745.85 ± 19.24 [b, f]
Rot + BMC	650.95 ± 22.02 [a]
Rot + 5-HT + BMC	833.67 ± 19.45 [c, e]
Rot + GABA + BMC	831.09 ± 20.12 [c, e]
Rot + 5-HT + GABA + BMC	888.42 ± 19.13 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 38
Real Time PCR amplification of CREB mRNA in the cerebral cortex of control and experimental groups of rats

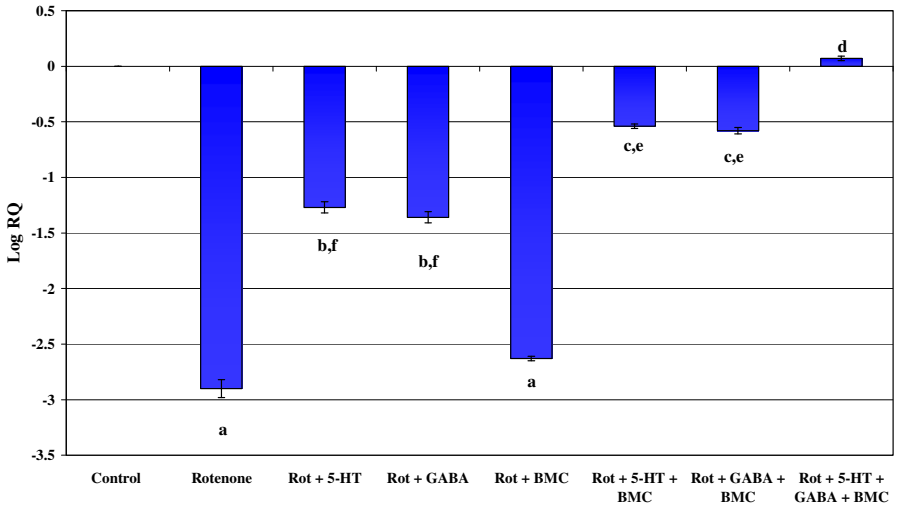


Table - 40
Real Time PCR amplification of CREB mRNA in the cerebral cortex of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	-2.90 ± 0.09 [a]
Rot + 5-HT	-1.27 ± 0.08 [b, f]
Rot + GABA	-1.36 ± 0.09 [b, f]
Rot + BMC	-2.63 ± 0.06 [a]
Rot + 5-HT + BMC	-0.54 ± 0.05 [c, e]
Rot + GABA + BMC	-0.58 ± 0.06 [c, e]
Rot + 5-HT + GABA + BMC	0.07 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 39
Real Time PCR amplification of Bax mRNA in the cerebral cortex of control and experimental groups of rats

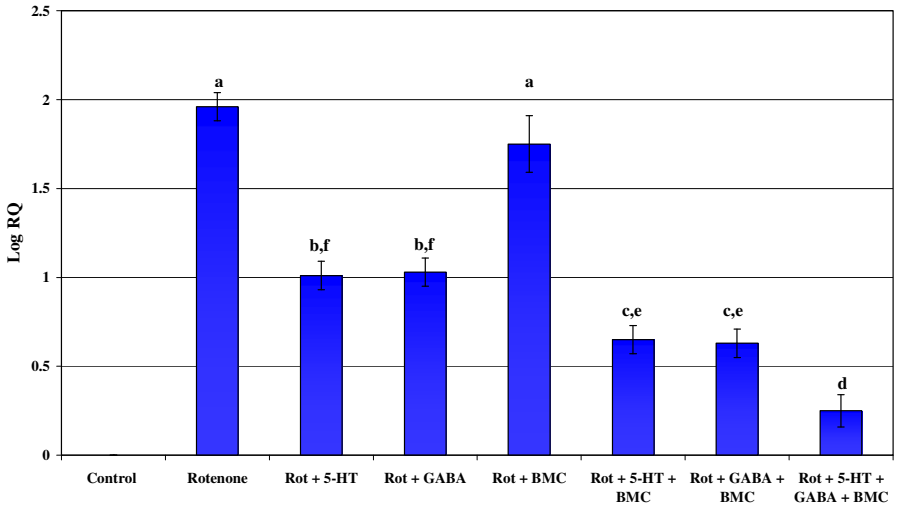


Table - 41
Real Time PCR amplification of Bax mRNA in the cerebral cortex of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	1.96 ± 0.18 [a]
Rot + 5-HT	1.01 ± 0.19 [b, f]
Rot + GABA	1.03 ± 0.11 [b, f]
Rot + BMC	1.75 ± 0.10 [a]
Rot + 5-HT + BMC	0.65 ± 0.10 [c, e]
Rot + GABA + BMC	0.63 ± 0.07 [c, e]
Rot + 5-HT + GABA + BMC	0.25 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 40
Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the cerebral cortex of control and experimental groups of rats

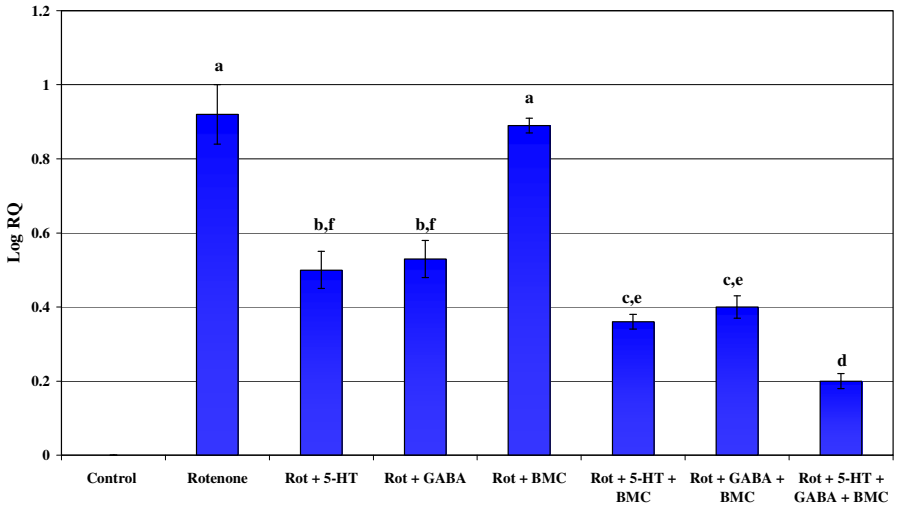


Table - 42
Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the cerebral cortex of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	0.92 ± 0.06 [a]
Rot + 5-HT	0.50 ± 0.05 [b, f]
Rot + GABA	0.53 ± 0.07 [b, f]
Rot + BMC	0.89 ± 0.04 [a]
Rot + 5-HT + BMC	0.36 ± 0.06 [c, e]
Rot + GABA + BMC	0.40 ± 0.04 [c, e]
Rot + 5-HT + GABA + BMC	0.20 ± 0.02 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 41
Real Time PCR amplification of α-synuclein in the cerebral cortex of control and experimental groups of rats

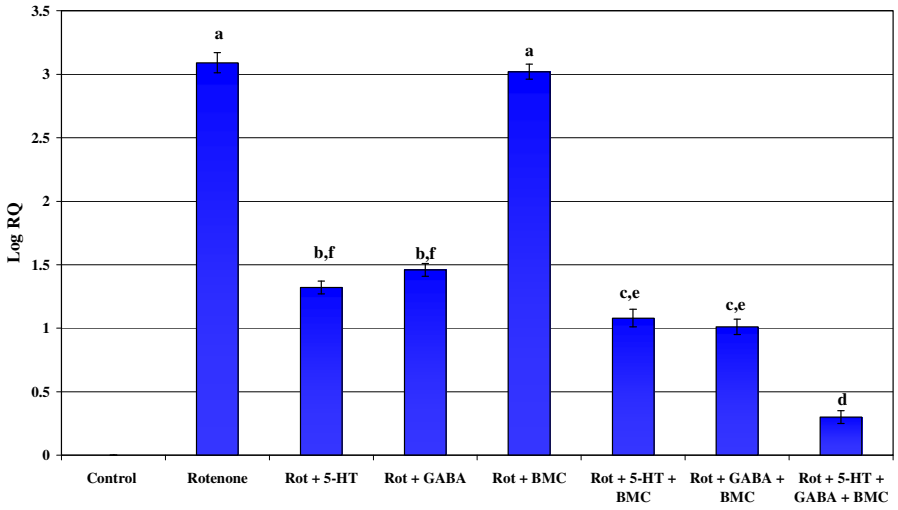


Table - 43
Real Time PCR amplification of α-synuclein in the cerebral cortex of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	3.09 ± 0.31 [a]
Rot + 5-HT	1.32 ± 0.29 [b, e]
Rot + GABA	1.46 ± 0.26 [b, e]
Rot + BMC	3.02 ± 0.24 [a]
Rot + 5-HT + BMC	1.08 ± 0.09 [d]
Rot + GABA + BMC	1.01 ± 0.12 [d]
Rot + 5-HT + GABA + BMC	0.30 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Table -44
Dopamine content in the cerebellum of control and experimental groups of rats

Experimental groups	DA(nmoles/g wet wt.)
Control	30.07 ± 3.20
Rotenone	8.90 ± 0.80 [a]
Rot + 5-HT	15.60 ± 1.33 [b, f]
Rot + GABA	17.52 ± 1.33 [b, f]
Rot + BMC	11.33 ± 1.20 [a]
Rot + 5-HT + BMC	21.80 ± 2.58 [c, e]
Rot + GABA + BMC	23.40 ± 2.58 [c, e]
Rot + 5-HT + GABA + BMC	26.13 ± 2.88 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 42
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the cerebellum of control and experimental groups of rats

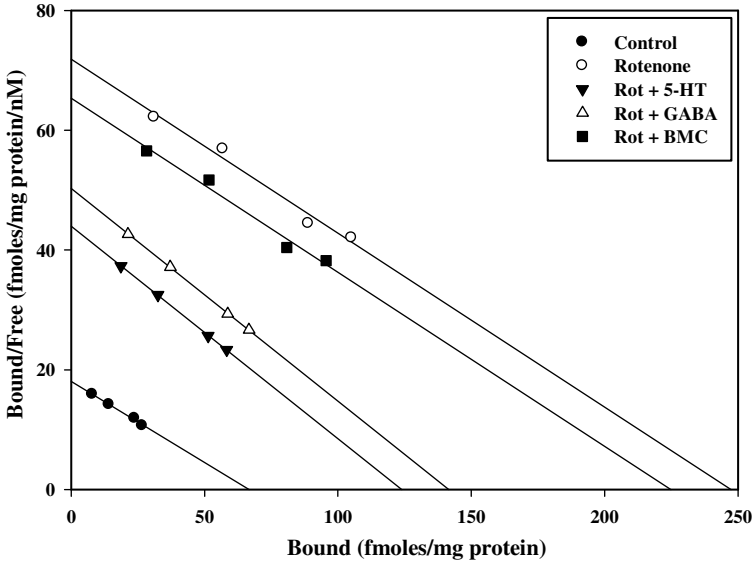


Table - 45
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the cerebellum of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	65.12 ± 6.12	3.41 ± 0.33
Rotenone	247.41 ± 24.13 [a]	3.21 ± 0.32
Rot + 5-HT	122.07 ± 12.22 [b, f]	2.83 ± 0.23
Rot + GABA	140.43 ± 14.15 [b, f]	2.80 ± 0.20
Rot + BMC	224.31 ± 22.17 [a]	3.38 ± 0.46

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 43
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the cerebellum of control and experimental groups of rats

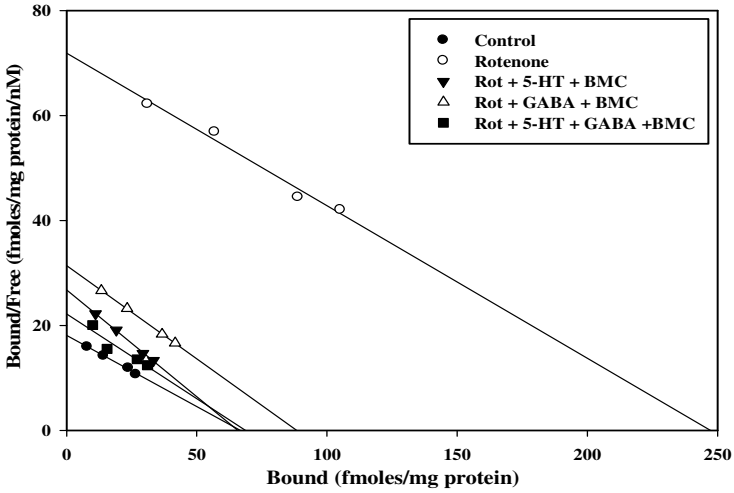


Table - 46
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the cerebellum of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	65.12 ± 6.12	3.41 ± 0.33
Rotenone	247.41 ± 24.13 [a]	3.21 ± 0.32
Rot + 5-HT + BMC	79.04 ± 7.12 [b, e]	3.36 ± 0.21
Rot + GABA + BMC	87.85 ± 8.14 [b, e]	3.17 ± 0.26
Rot + 5-HT + GABA + BMC	69.60 ± 6.08 [c, d]	3.26 ± 0.28

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 44
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the cerebellum of control and experimental groups of rats

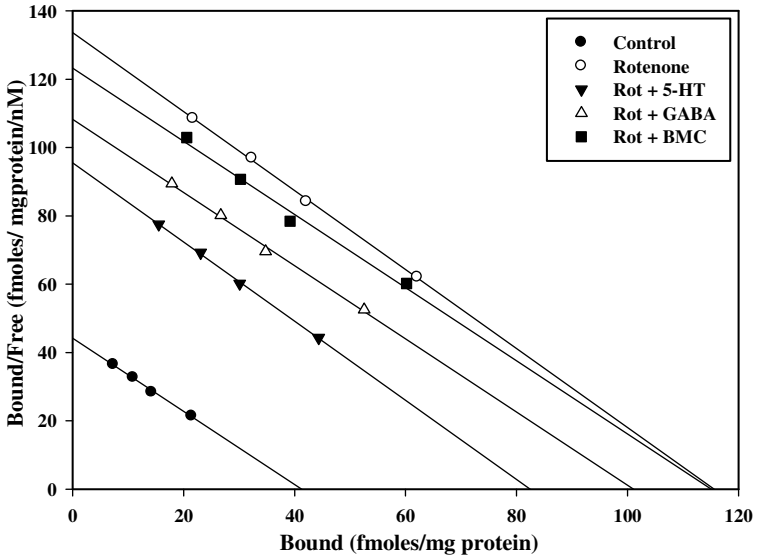


Table - 47
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the cerebellum of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	41.63 ± 4.17	0.91 ± 0.08
Rotenone	115.16 ± 11.12 [a]	0.86 ± 0.09
Rot + 5-HT	82.44 ± 8.16 [b, f]	0.92 ± 0.09
Rot + GABA	100.12 ± 10.23 [b, f]	0.86 ± 0.07
Rot + BMC	114.63 ± 11.12 [a]	0.89 ± 0.08

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 45
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the cerebellum of control and experimental groups of rats

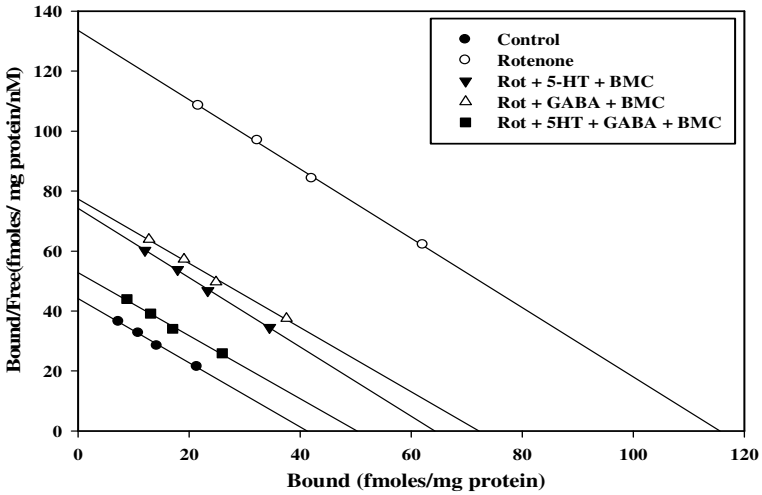


Table - 48
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the cerebellum of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	41.63 ± 4.17	0.91 ± 0.08
Rotenone	115.16 ± 11.12 [a]	0.86 ± 0.09
Rot + 5-HT + BMC	63.06 ± 6.13 [b, f]	0.86 ± 0.07
Rot + GABA + BMC	71.62 ± 7.18 [b, f]	0.93 ± 0.09
Rot + 5-HT + GABA + BMC	49.41 ± 4.06 [d]	0.96 ± 0.09

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 46
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the cerebellum of control and experimental groups of rats

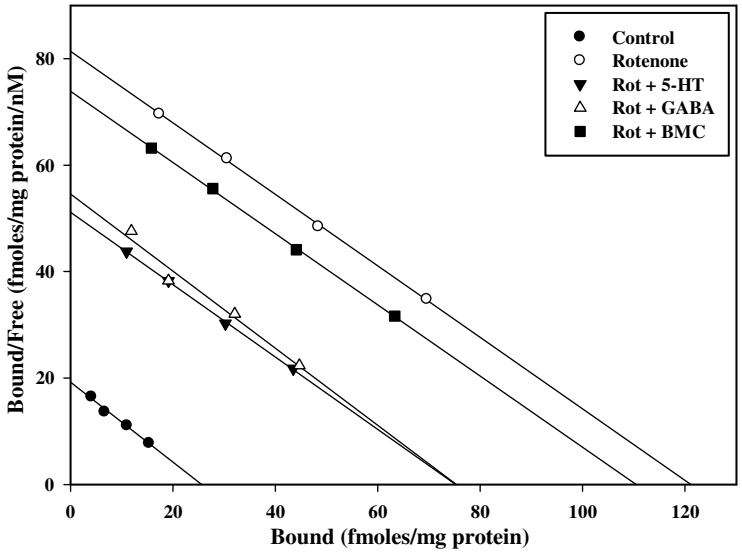


Table - 49
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the cerebellum of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	25.11 ± 2.14	1.32 ± 0.11
Rotenone	121.51 ± 12.13 [a]	1.49 ± 0.15
Rot + 5-HT	69.45 ± 6.16 [b, f]	1.45 ± 0.17
Rot + GABA	70.08 ± 7.22 [b, f]	1.39 ± 0.12
Rot + BMC	110.33 ± 11.13 [a]	1.51 ± 0.16

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 47
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the cerebellum of control and experimental groups of rats

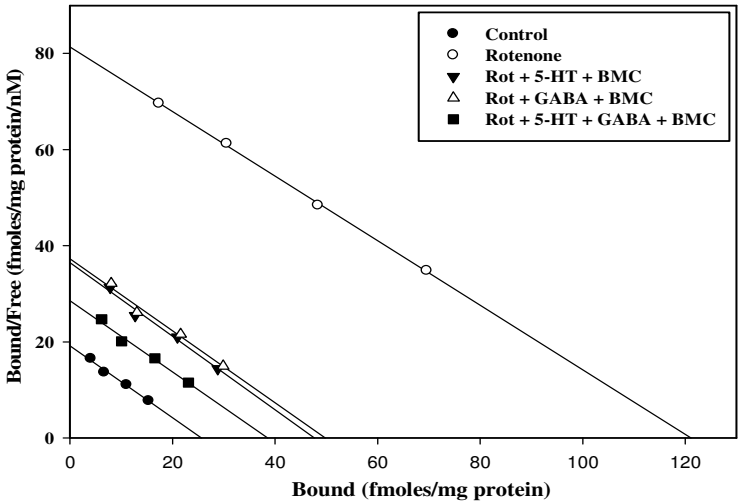


Table - 50
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the cerebellum of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	25.11 ± 2.14	1.32 ± 0.11
Rotenone	121.51 ± 12.13 [a]	1.49 ± 0.15
Rot + 5-HT + BMC	47.78 ± 4.19 [c, e]	1.34 ± 0.19
Rot + GABA + BMC	49.08 ± 4.15 [c, e]	1.36 ± 0.15
Rot + 5-HT + GABA + BMC	37.66 ± 3.05 [d]	1.36 ± 0.13

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure -48
Real Time PCR amplification of Dopamine D_1 receptor mRNA in the cerebellum of control and experimental groups of rats

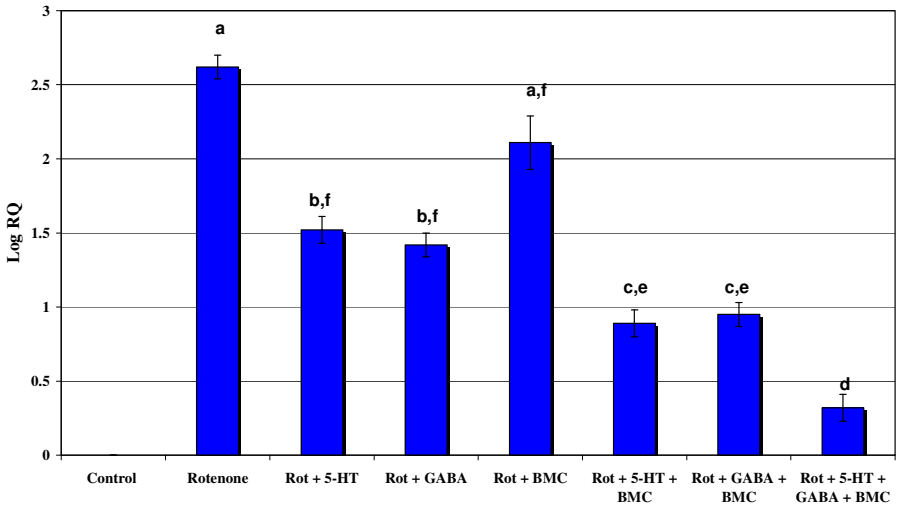


Table - 51
Real Time PCR amplification of Dopamine D_1 receptor mRNA in the cerebellum of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	2.62 ± 0.12 [a]
Rot + 5-HT	1.51 ± 0.15 [b, f]
Rot + GABA	1.42 ± 0.15 [b, f]
Rot + BMC	2.11 ± 0.13 [a]
Rot + 5-HT + BMC	0.89 ± 0.08 [c, e]
Rot + GABA + BMC	0.95 ± 0.09 [c, e]
Rot + 5-HT + GABA + BMC	0.32 ± 0.03 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure -49
Real Time PCR amplification of Dopamine D_2 receptor mRNA in the cerebellum of control and experimental groups of rats

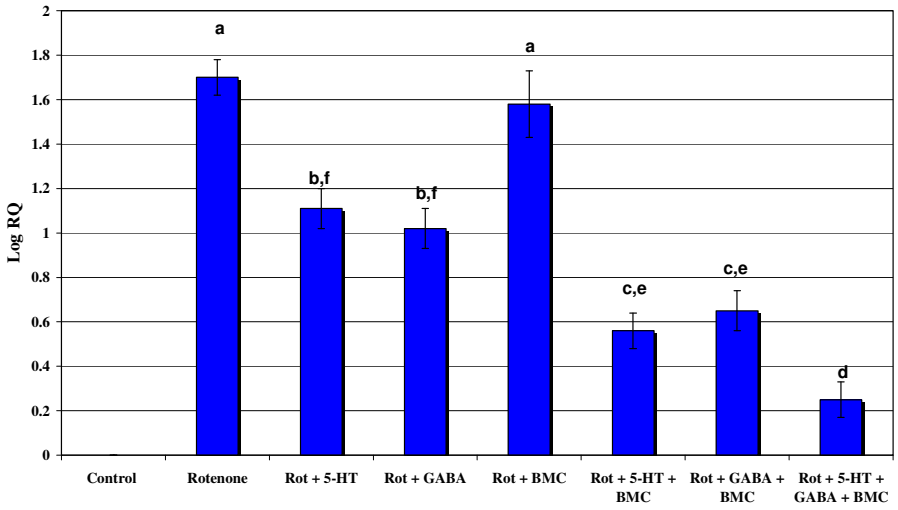
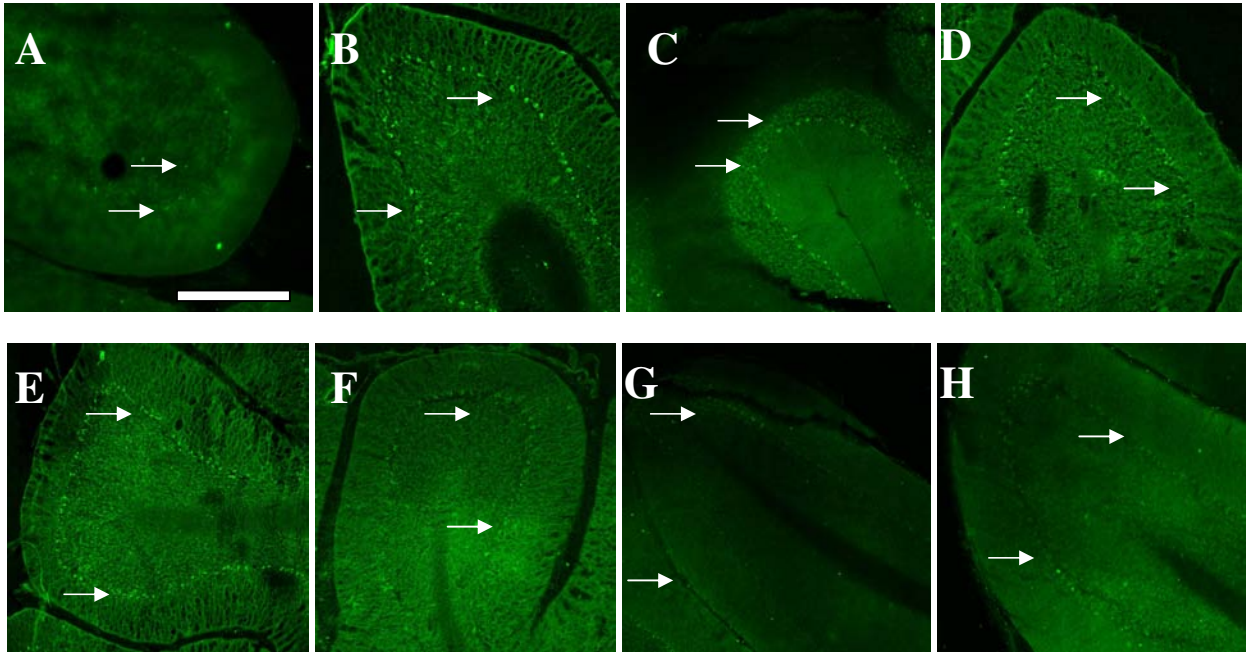


Table - 52
Real Time PCR amplification of Dopamine D_2 receptor mRNA in the cerebellum of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	1.75 ± 0.09 [a]
Rot + 5-HT	1.11 ± 0.08 [b, f]
Rot + GABA	1.02 ± 0.09 [b, f]
Rot + BMC	1.58 ± 0.06 [a]
Rot + 5-HT + BMC	0.56 ± 0.05 [c, e]
Rot + GABA + BMC	0.65 ± 0.06 [c, e]
Rot + 5-HT + GABA + BMC	0.25 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 50
Dopamine D_1 receptor expression in the cerebellum of control and experimental rats



A – Control, B – Rotenone infused, C – Rotenone infused treated with Serotonin, D – Rotenone infused treated with GABA, E – Rotenone infused treated with BMC, F - Rotenone infused treated with Serotonin and BMC, G - Rotenone infused treated with GABA and BMC, H - Rotenone infused treated with Serotonin, GABA and BMC. The scale bar represents 250 μm.

Table - 53
Dopamine D_1 receptor expression in the cerebellum of control and experimental rats

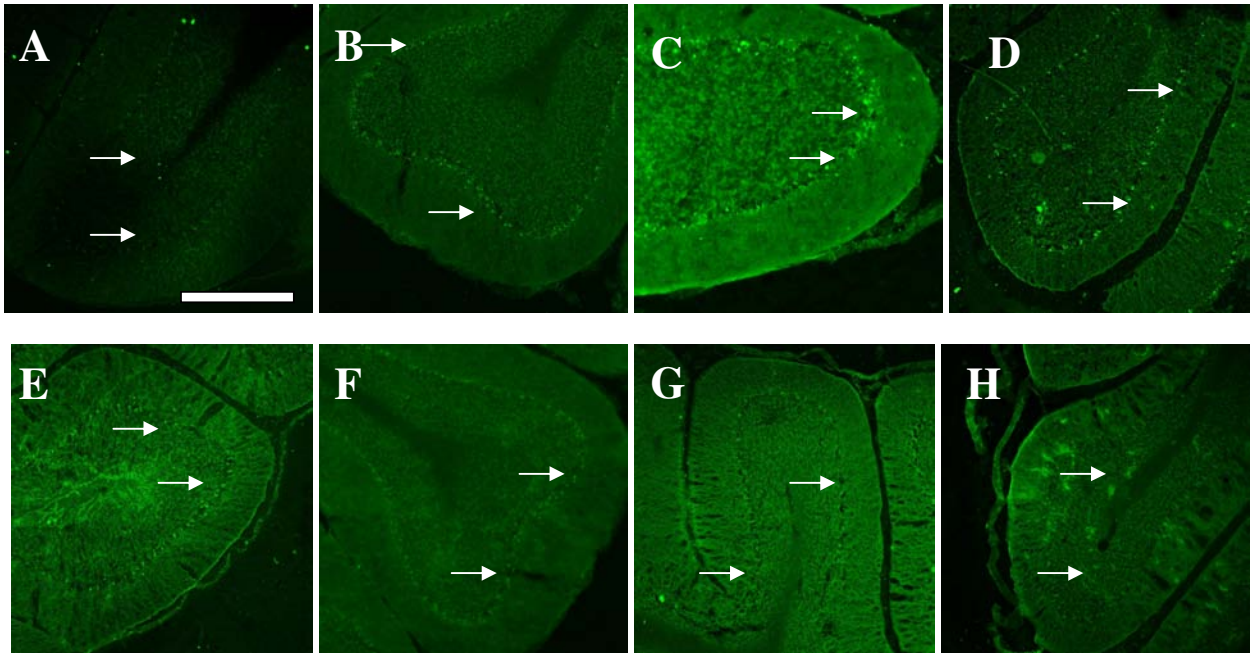
Experimental groups	Mean pixel intensity
Control	15.29 ± 1.54
Rotenone	75.13 ± 6.13[a]
Rot + 5-HT	60.12 ± 4.50 [b, f]
Rot + GABA	62.20 ± 5.05 [b, f]
Rot + BMC	70.30 ± 6.55 [a]
Rot + 5-HT + BMC	45.40 ± 3.97 [c, e]
Rot + GABA + BMC	49.65 ± 4.50 [c, e]
Rot + 5-HT + GABA + BMC	27.60 ± 1.45 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 51
Dopamine D_2 receptor expression in the cerebellum of control and experimental rats



A – Control, B – Rotenone infused, C – Rotenone infused treated with Serotonin, D – Rotenone infused treated with GABA, E – Rotenone infused treated with BMC, F - Rotenone infused treated with Serotonin and BMC, G - Rotenone infused treated with GABA and BMC, H - Rotenone infused treated with Serotonin, GABA and BMC. The scale bar represents 250 μm.

Table - 54
Dopamine D_2 receptor expression in the cerebellum of control and experimental rats

Experimental groups	Mean pixel intensity
Control	20.55 ± 1.93
Rotenone	82.16 ± 6.42 [a]
Rot + 5-HT	50.93 ± 4.11 [b, f]
Rot + GABA	53.77 ± 5.56 [b, f]
Rot + BMC	76.42 ± 5.96 [a]
Rot + 5-HT + BMC	31.78 ± 2.99 [c, e]
Rot + GABA + BMC	33.96 ± 3.62 [c, e]
Rot + 5-HT + GABA + BMC	25.35 ± 1.61 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 52
IP3 content in the cerebellum of control and experimental groups of rats

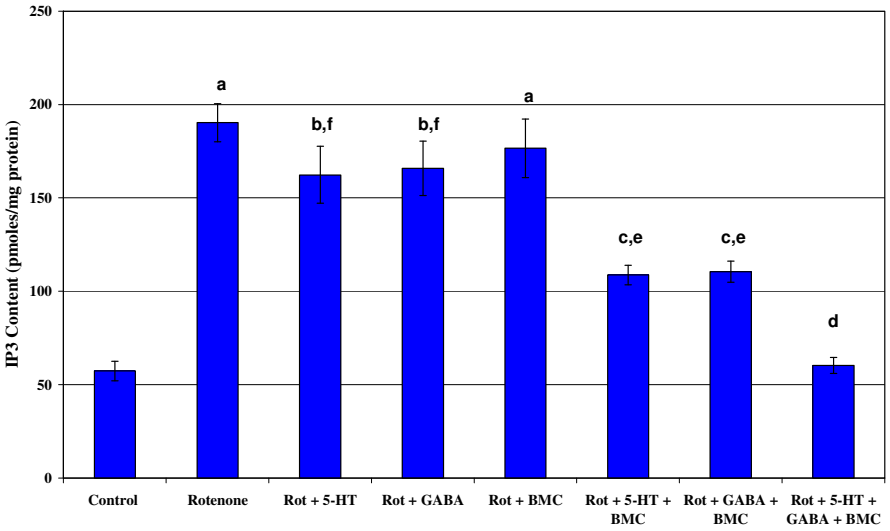


Table - 55
IP3 content in the cerebellum of control and experimental groups of rats

Experimental groups	IP3 Content (pmoles/mg protein)
Control	57.32 ± 5.23
Rotenone	190.25 ± 10.25 [a]
Rot + 5-HT	162.32 ± 15.23 [b, f]
Rot + GABA	165.85 ± 14.65 [b, f]
Rot + BMC	176.59 ± 15.68 [a]
Rot + 5-HT + BMC	108.65 ± 5.23 [c, e]
Rot + GABA + BMC	110.49 ± 5.62 [c, e]
Rot + 5-HT + GABA + BMC	60.23 ± 4.32 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 53
cAMP content in the cerebellum of control and experimental groups of rats

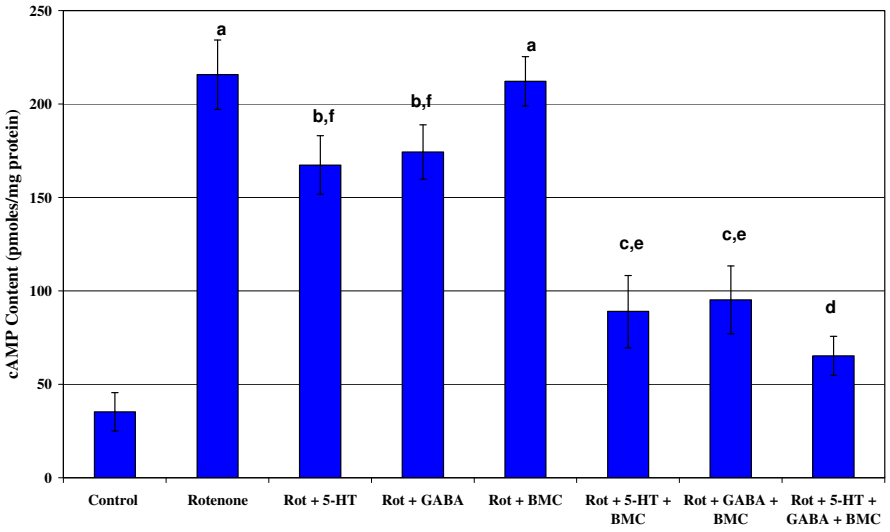


Table - 56
cAMP content in the cerebellum of control and experimental groups of rats

Experimental groups	cAMP Content (pmoles/mg protein)
Control	35.36 ± 5.23
Rotenone	215.24 ± 10.25 [a]
Rot + 5-HT	167.30 ± 15.23 [b, f]
Rot + GABA	174.81 ± 14.65 [b, f]
Rot + BMC	212.53 ± 15.68 [a]
Rot + 5-HT + BMC	88.65 ± 5.23 [c, e]
Rot + GABA + BMC	95.44 ± 5.62 [c, e]
Rot + 5-HT + GABA + BMC	65.20 ± 4.32 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 54
cGMP content in the cerebellum of control and experimental groups of rats

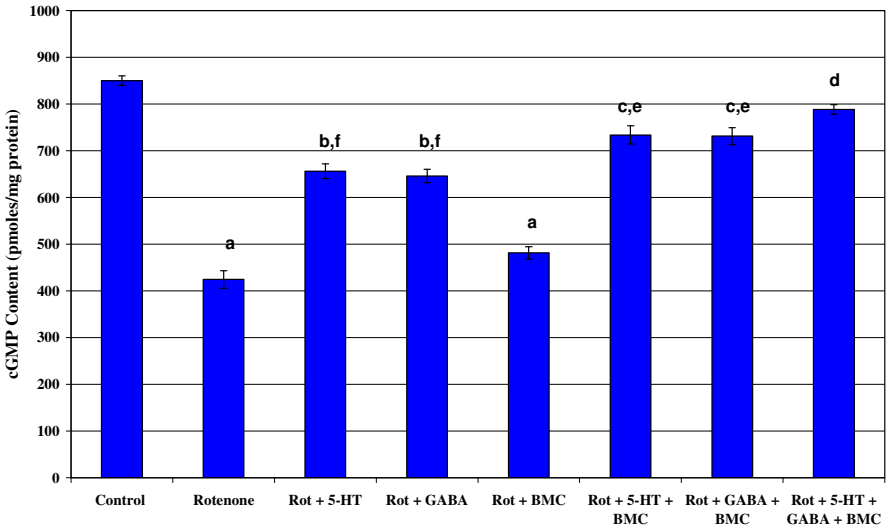


Table - 57
cGMP content in the cerebellum of control and experimental groups of rats

Experimental groups	cGMP Content (pmoles/mg protein)
Control	850.21 ± 16.21
Rotenone	424.12 ± 18.53 [a]
Rot + 5-HT	656.12 ± 18.42 [b, f]
Rot + GABA	645.85 ± 19.24 [b, f]
Rot + BMC	480.95 ± 22.02 [a]
Rot + 5-HT + BMC	733.67 ± 19.45 [c, e]
Rot + GABA + BMC	731.09 ± 20.12 [c, e]
Rot + 5-HT + GABA + BMC	788.42 ± 19.13 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 55
Real Time PCR amplification of CREB mRNA in the cerebellum of control and experimental groups of rats

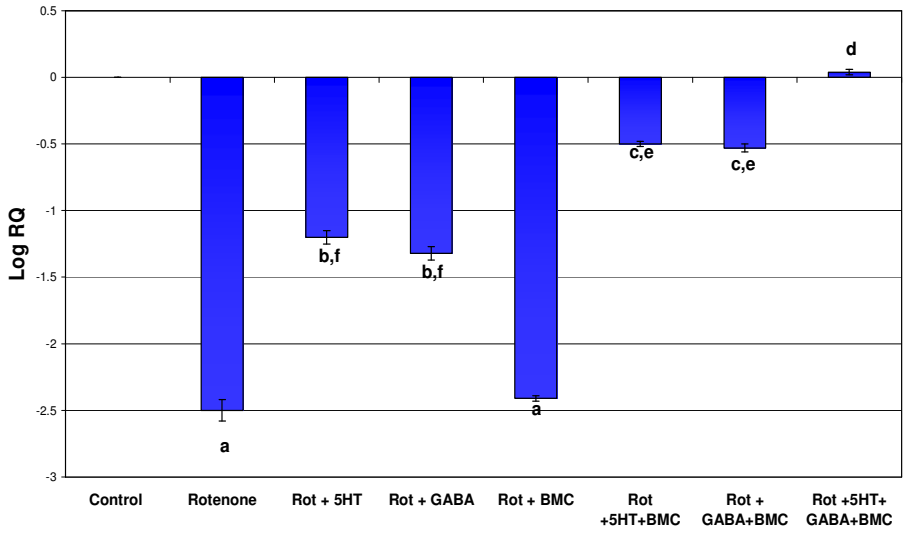


Table - 58
Real Time PCR amplification of CREB mRNA in the cerebellum of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	-2.50 ± 0.22 [a]
Rot + 5-HT	-1.22 ± 0.13 [b, e]
Rot + GABA	-1.32 ± 0.12 [b, e]
Rot + BMC	-2.41 ± 0.21 [a]
Rot + 5-HT + BMC	-0.50 ± 0.08 [d]
Rot + GABA + BMC	-0.53 ± 0.09 [d]
Rot + 5-HT + GABA + BMC	0.04 ± 0.03 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 56
Real Time PCR amplification of Bax mRNA in the cerebellum of control and experimental groups of rats

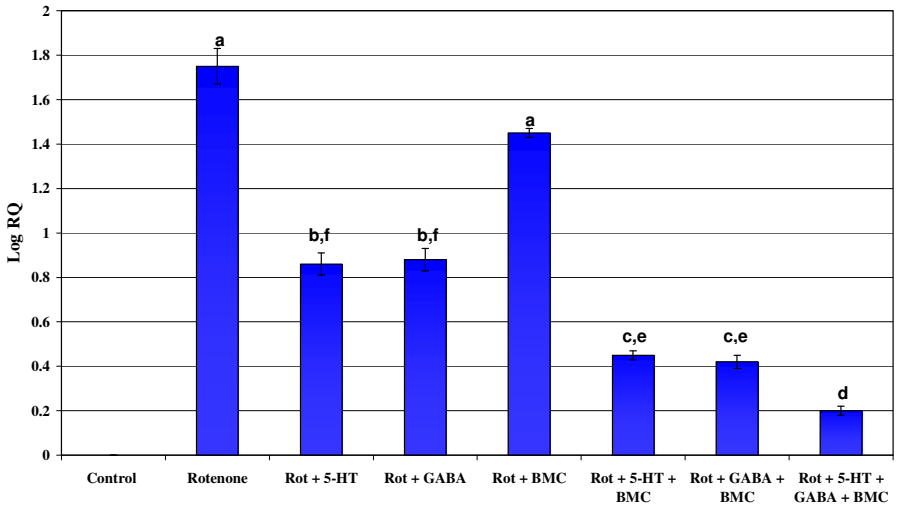


Table - 59
Real Time PCR amplification of Bax mRNA in the cerebellum of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	1.75 ± 0.18 [a]
Rot + 5-HT	0.86 ± 0.19 [b, f]
Rot + GABA	0.88 ± 0.11 [b, f]
Rot + BMC	1.45 ± 0.10 [a]
Rot + 5-HT + BMC	0.45 ± 0.10 [c, e]
Rot + GABA + BMC	0.42 ± 0.07 [c, e]
Rot + 5-HT + GABA + BMC	0.20 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 57
Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the cerebellum of control and experimental groups of rats

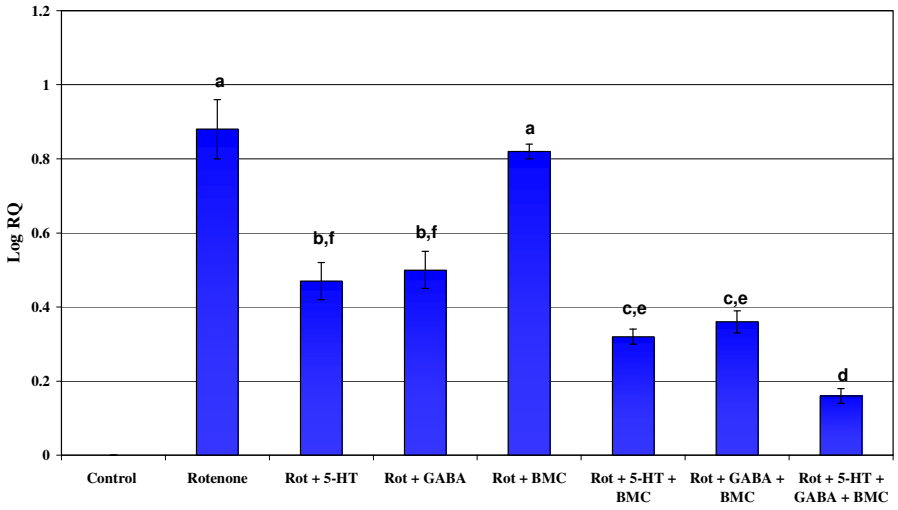


Table - 60
Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the cerebellum of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	0.88 ± 0.06 [a]
Rot + 5-HT	0.47 ± 0.05 [b, f]
Rot + GABA	0.50 ± 0.07 [b, f]
Rot + BMC	0.82 ± 0.04 [a]
Rot + 5-HT + BMC	0.32 ± 0.06 [c, e]
Rot + GABA + BMC	0.37 ± 0.04 [c, e]
Rot + 5-HT + GABA + BMC	0.16 ± 0.02 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 58
Real Time PCR amplification of α-synuclein in the cerebellum of control and experimental groups of rats

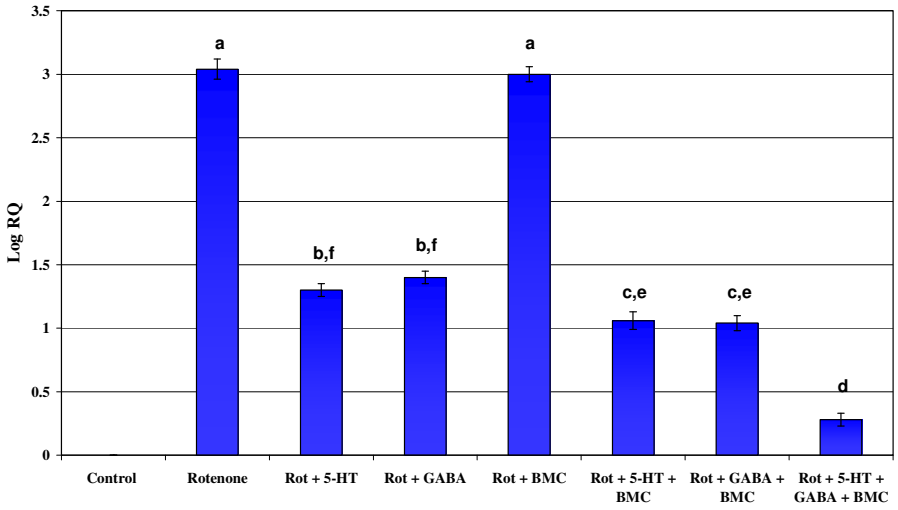


Table - 61
Real Time PCR amplification of α-synuclein in the cerebellum of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	3.04 ± 0.31 [a]
Rot + 5-HT	1.30 ± 0.29 [b, e]
Rot + GABA	1.40 ± 0.26 [b, e]
Rot + BMC	3.00 ± 0.24 [a]
Rot + 5-HT + BMC	1.06 ± 0.09 [c, e]
Rot + GABA + BMC	1.04 ± 0.12 [c, e]
Rot + 5-HT + GABA + BMC	0.27 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Table - 62
Dopamine content in the brain stem of control and experimental groups of rats

Experimental groups	DA(nmoles/g wet wt.)
Control	28.07 ± 3.20
Rotenone	6.90 ± 0.70 [a]
Rot + 5-HT	13.60 ± 1.33 [b, f]
Rot + GABA	15.52 ± 1.56 [b, f]
Rot + BMC	9.33 ± 1.20 [a]
Rot + 5-HT + BMC	19.80 ± 2.08 [c, e]
Rot + GABA + BMC	21.40 ± 2.58 [c, e]
Rot + 5-HT + GABA + BMC	24.13 ± 2.21 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 59
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the brain stem of control and experimental groups of rats

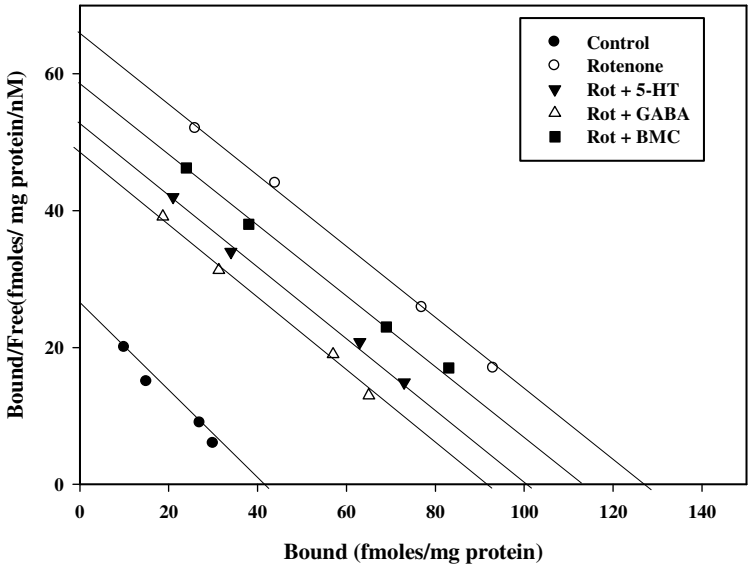


Table - 63
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the brain stem of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	41.24 ± 4.75	1.58 ± 0.14
Rotenone	126.74 ± 11.13 [a]	1.92 ± 0.18
Rot + 5-HT	100.71 ± 12.16 [b, f]	1.91 ± 0.20
Rot + GABA	91.38 ± 8.22 [b, f]	1.90 ± 0.20
Rot + BMC	112.56 ± 12.45 [a]	1.92 ± 0.17

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 60
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the brain stem of control and experimental groups of rats

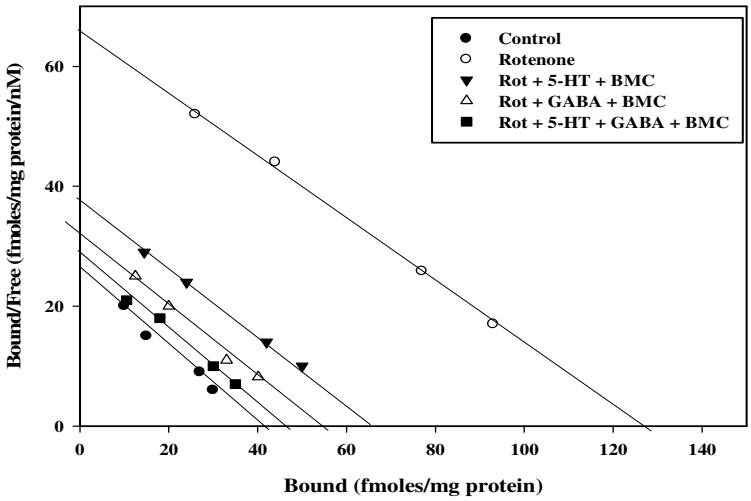


Table - 64
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the brain stem of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	41.24 ± 4.75	1.58 ± 0.14
Rotenone	126.74 ± 11.13 [a]	1.92 ± 0.18
Rot + 5-HT + BMC	65.80 ± 6.32 [b, f]	1.72 ± 0.17
Rot + GABA + BMC	55.01 ± 5.09 [b, f]	1.72 ± 0.19
Rot + 5-HT + GABA + BMC	45.93 ± 4.32 [d]	1.58 ± 0.15

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 61
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the brain stem of control and experimental groups of rats

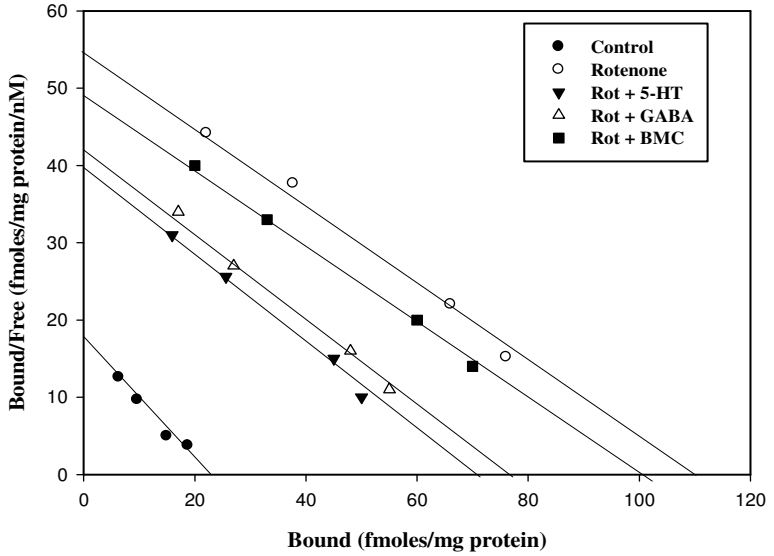


Table - 65
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the brain stem of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	22.84 ± 2.24	1.35 ± 0.34
Rotenone	105.35 ± 10.26 [a]	1.71 ± 0.45
Rot + 5-HT	70.79 ± 7.11 [b, f]	1.75 ± 0.52
Rot + GABA	76.21 ± 7.25 [b, f]	1.76 ± 0.48
Rot + BMC	100.24 ± 10.12 [a]	1.79 ± 0.51

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 62
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the brain stem of control and experimental groups of rats

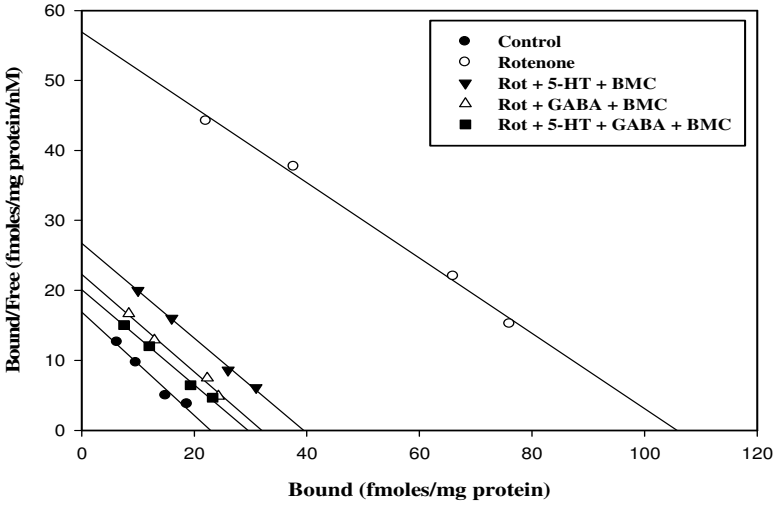


Table - 66
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the brain stem of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	22.84 ± 2.24	1.35 ± 0.34
Rotenone	105.35 ± 10.26 [a]	1.71 ± 0.45
Rot + 5-HT + BMC	39.89 ± 3.65 [b, f]	1.47 ± 0.53
Rot + GABA + BMC	31.77 ± 3.24 [b, f]	1.44 ± 0.42
Rot + 5-HT + GABA + BMC	29.33 ± 2.81 [d]	1.45 ± 0.58

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 63
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the brain stem of control and experimental groups of rats

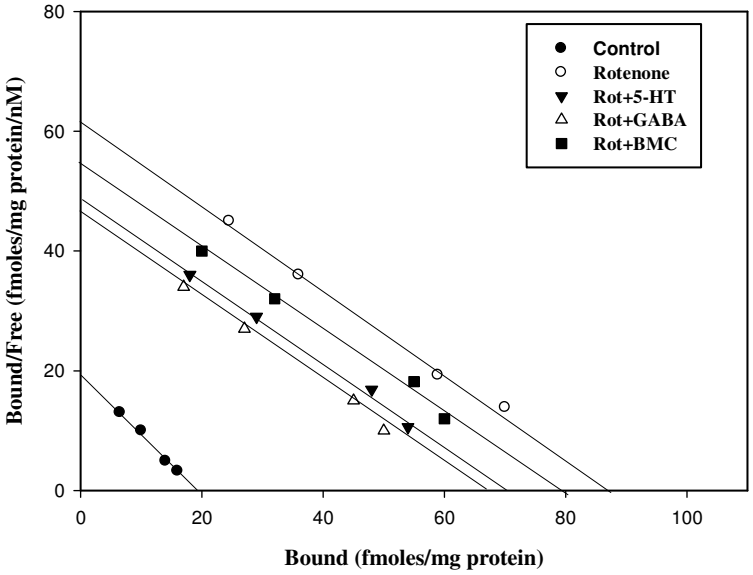


Table - 67
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the brain stem of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	18.36 ± 1.82	1.14 ± 0.23
Rotenone	88.52 ± 8.84 [a]	1.44 ± 0.25
Rot + 5-HT	69.54 ± 6.98 [b, f]	1.40 ± 0.31
Rot + GABA	66.35 ± 6.61 [b, f]	1.52 ±0.29
Rot + BMC	78.19 ± 7.25 [a]	1.43 ± 0.31

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 64
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the brain stem of control and experimental groups of rats

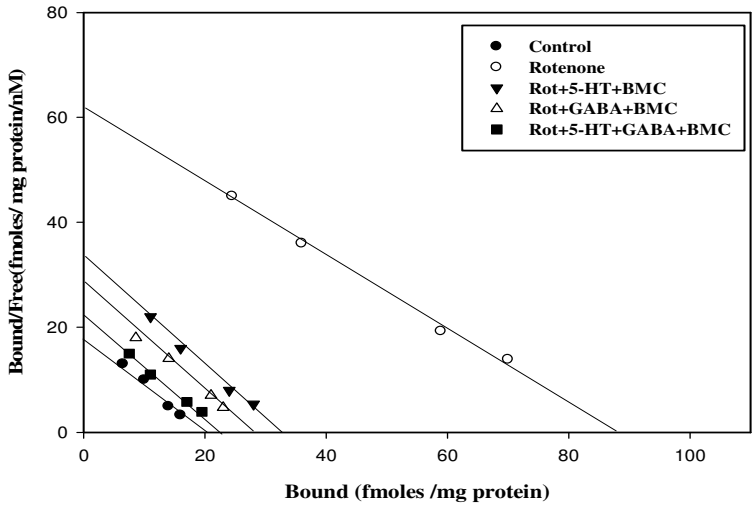


Table - 68
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the brain stem of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	18.36 ± 1.82	1.14 ± 0.23
Rotenone	88.52 ± 8.84 [a]	1.44 ± 0.25
Rot + 5-HT + BMC	32.25 ± 3.22 [b, f]	1.16 ± 0.31
Rot + GABA + BMC	26.53 ± 2.62 [b, f]	1.23 ± 0.22
Rot + 5-HT + GABA + BMC	23.25 ± 2.29 [d]	1.09 ± 0.39

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure -65
Real Time PCR amplification of Dopamine D_1 receptor mRNA in the brain stem of control and experimental groups of rats

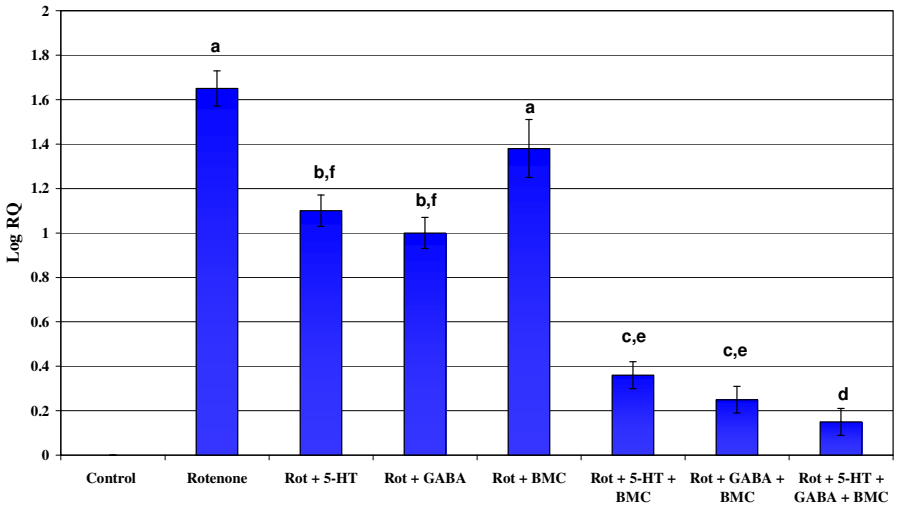


Table - 69
Real Time PCR amplification of Dopamine D_1 receptor mRNA in the brain stem of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	1.65 ± 0.09 [a]
Rot + 5-HT	1.10 ± 0.08 [b, f]
Rot + GABA	1.00 ± 0.09 [b, f]
Rot + BMC	1.38 ± 0.06 [a]
Rot + 5-HT + BMC	0.36 ± 0.05 [c, e]
Rot + GABA + BMC	0.25 ± 0.06 [c, e]
Rot + 5-HT + GABA + BMC	0.15 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure -66
Real Time PCR amplification of Dopamine D_2 receptor mRNA in the brain stem of control and experimental groups of rats

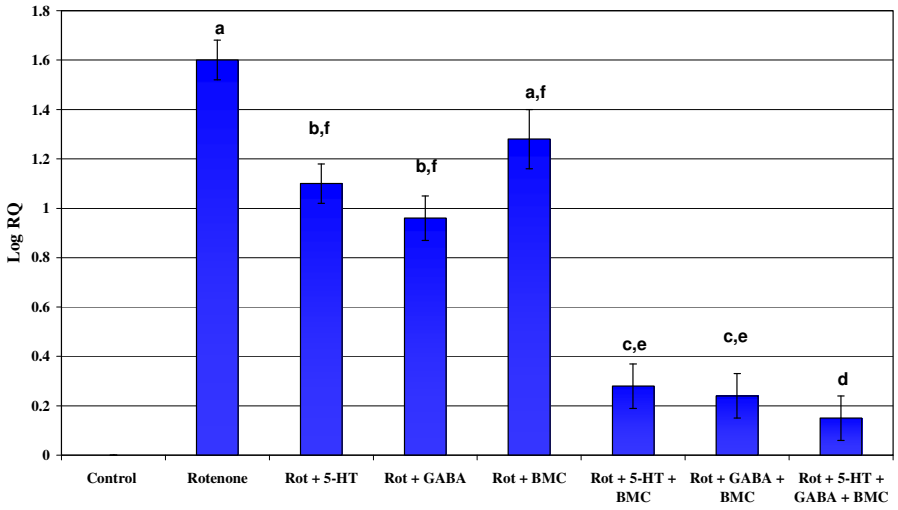
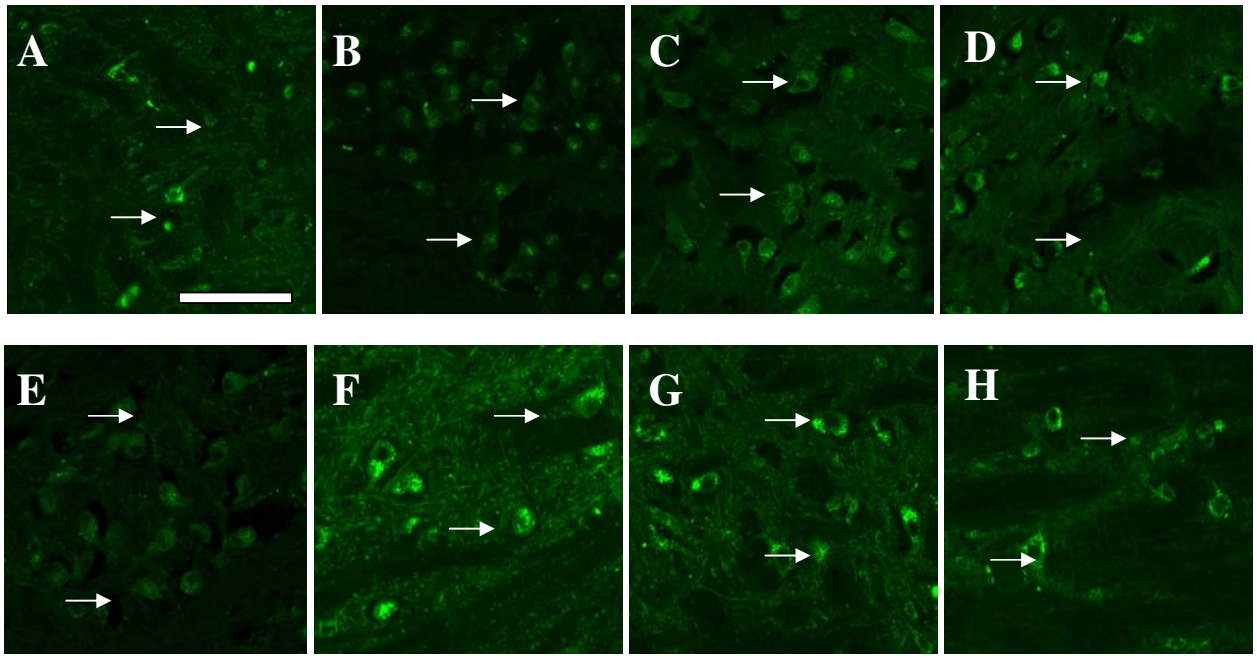


Table - 70
Real Time PCR amplification of Dopamine D_2 receptor mRNA in the brain stem of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	1.60 ± 0.09 [a]
Rot + 5-HT	1.10 ± 0.08 [b, f]
Rot + GABA	0.96 ± 0.09 [b, f]
Rot + BMC	1.28 ± 0.06 [a]
Rot + 5-HT + BMC	0.28 ± 0.05 [c, e]
Rot + GABA + BMC	0.24 ± 0.06 [c, e]
Rot + 5-HT + GABA + BMC	0.15 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 67
Dopamine D_1 receptor expression in the brain stem of control and experimental rats



A – Control, B – Rotenone infused, C – Rotenone infused treated with Serotonin, D – Rotenone infused treated with GABA, E – Rotenone infused treated with BMC, F - Rotenone infused treated with Serotonin and BMC, G - Rotenone infused treated with GABA and BMC, H - Rotenone infused treated with Serotonin, GABA and BMC. The scale bar represents 75 µm.

Table - 71
Dopamine D_1 receptor expression in the brain stem of control and experimental rats

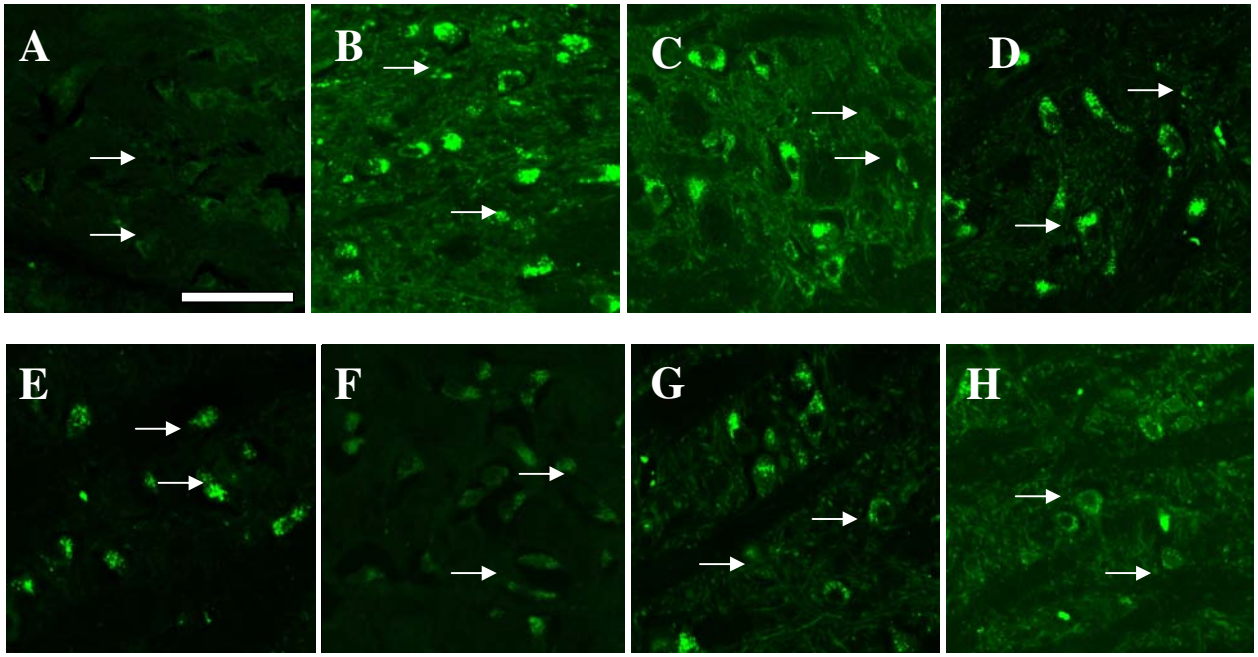
Experimental groups	Mean pixel intensity
Control	23.77 ± 2.19
Rotenone	87.12 ± 8.65 [a]
Rot + 5-HT	66.74 ± 5.88 [b, f]
Rot + GABA	67.23 ± 6.21 [b, f]
Rot + BMC	85.32 ± 9.41 [a]
Rot + 5-HT + BMC	41.54 ± 3.98 [c, e]
Rot + GABA + BMC	43.82 ± 4.45 [c, e]
Rot + 5-HT + GABA + BMC	27.13 ± 2.55 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 68
Dopamine D_2 receptor expression in the brain stem of control and experimental rats



A – Control, B – Rotenone infused, C – Rotenone infused treated with Serotonin, D – Rotenone infused treated with GABA, E – Rotenone infused treated with BMC, F - Rotenone infused treated with Serotonin and BMC, G - Rotenone infused treated with GABA and BMC, H - Rotenone infused treated with Serotonin, GABA and BMC. The scale bar represents 75 µm.

Table - 72
Dopamine D_2 receptor expression in the brain stem of control and experimental rats

Experimental groups	Mean pixel intensity
Control	19.35 ± 1.91
Rotenone	92.47 ± 9.58 [a]
Rot + 5-HT	58.84 ± 5.66 [b, f]
Rot + GABA	57.25 ± 5.63 [b, f]
Rot + BMC	83.22 ± 9.14 [a]
Rot + 5-HT + BMC	38.86 ± 3.47 [c, e]
Rot + GABA + BMC	41.67 ± 4.31 [c, e]
Rot + 5-HT + GABA + BMC	22.61 ± 2.11 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 69
IP3 content in the brain stem of control and experimental groups of rats

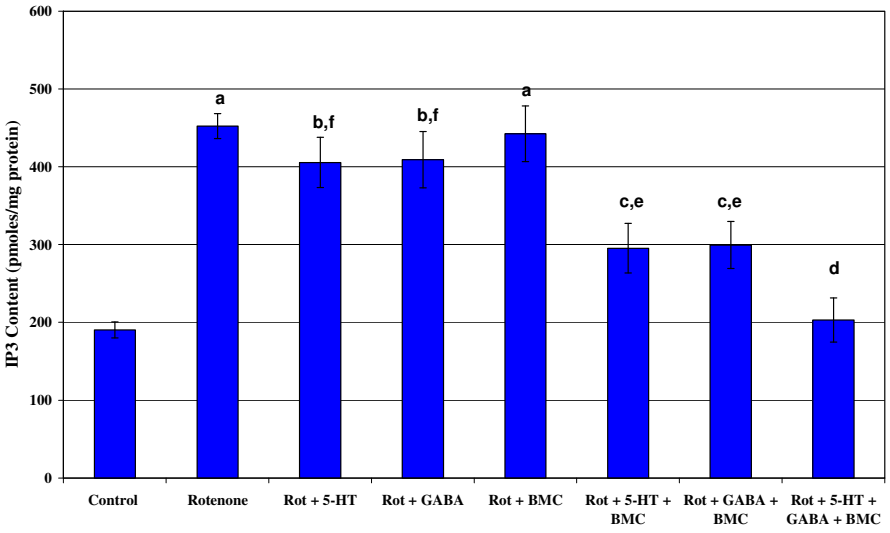


Table - 73
IP3 content in the brain stem of control and experimental groups of rats

Experimental groups	IP3 Content (pmoles/mg protein)
Control	190.30 ± 5.23
Rotenone	452.29 ± 10.25 [a]
Rot + 5-HT	405.31 ± 15.23 [b, f]
Rot + GABA	409.83 ± 14.65 [b, f]
Rot + BMC	442.56 ± 15.68 [a]
Rot + 5-HT + BMC	295.68 ± 5.23 [c, e]
Rot + GABA + BMC	299.46 ± 5.62 [c, e]
Rot + 5-HT + GABA + BMC	203.22 ± 4.32 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 70
cAMP content in the brain stem of control and experimental groups of rats

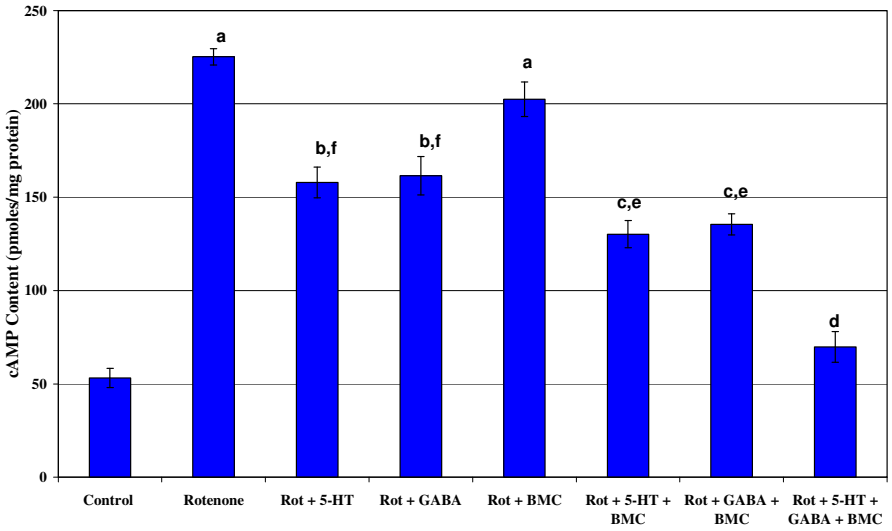


Table - 74
cAMP content in the brain stem of control and experimental groups of rats

Experimental groups	cAMP Content (pmoles/mg protein)
Control	53.36 ± 5.23
Rotenone	225.24 ± 10.25 [a]
Rot + 5-HT	157.30 ± 15.23 [b, f]
Rot + GABA	161.81 ± 14.65 [b, f]
Rot + BMC	202.53 ± 15.68 [a]
Rot + 5-HT + BMC	130.65 ± 5.23 [c, e]
Rot + GABA + BMC	135.44 ± 5.62 [c, e]
Rot + 5-HT + GABA + BMC	69.20 ± 4.32 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] p<0.001, [b] p<0.01, [c] p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f] p<0.05 when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 71
cGMP content in the brain stem of control and experimental groups of rats

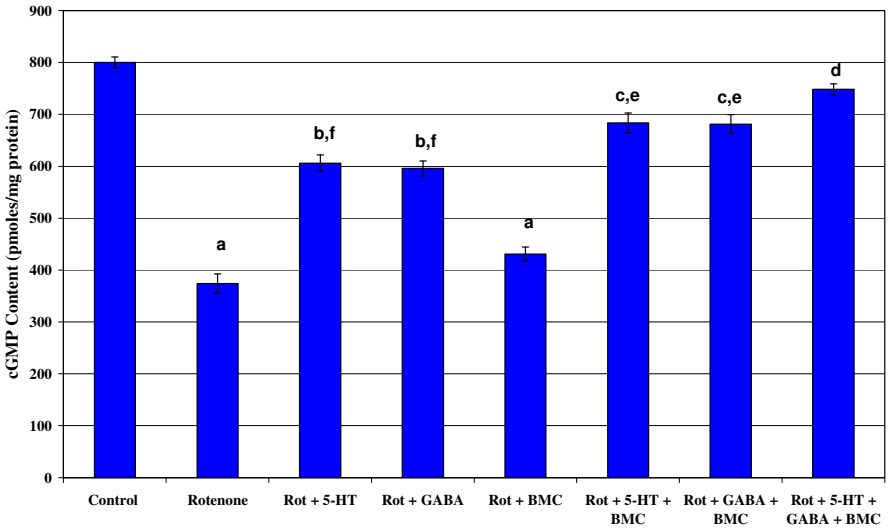


Table - 75
cGMP content in the brain stem of control and experimental groups of rats

Experimental groups	cGMP Content (pmoles/mg protein)
Control	800.21 ± 16.21
Rotenone	374.12 ± 18.53 [a]
Rot + 5-HT	606.12 ± 18.42 [b, f]
Rot + GABA	595.85 ± 19.24 [b, f]
Rot + BMC	430.95 ± 22.02 [a]
Rot + 5-HT + BMC	683.67 ± 19.45 [c, e]
Rot + GABA + BMC	681.09 ± 20.12 [c, e]
Rot + 5-HT + GABA + BMC	748.42 ± 19.13 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 72
Real Time PCR amplification of CREB mRNA in the brain stem of control and experimental groups of rats

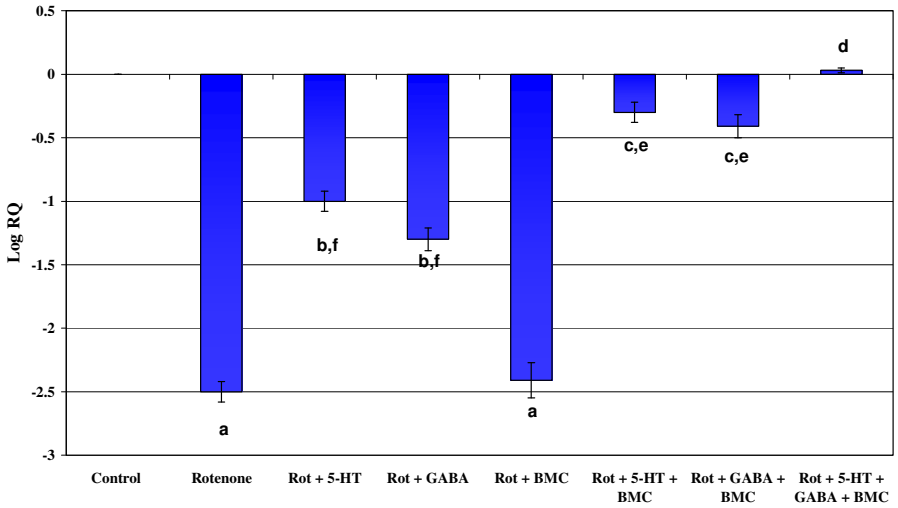


Table - 76
Real Time PCR amplification of CREB mRNA in the brain stem of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	-2.50 ± 0.22[a]
Rot + 5-HT	-1.00 ± 0.13[b,e]
Rot + GABA	-1.30 ± 0.12[b,e]
Rot + BMC	-2.41 ± 0.21[a]
Rot + 5-HT + BMC	0.30 ± 0.08[c, e]
Rot + GABA + BMC	0.41 ± 0.09[c, e]
Rot + 5-HT + GABA + BMC	0.03 ± 0.03[d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 73
Real Time PCR amplification of Bax mRNA in the brain stem of control and experimental groups of rats

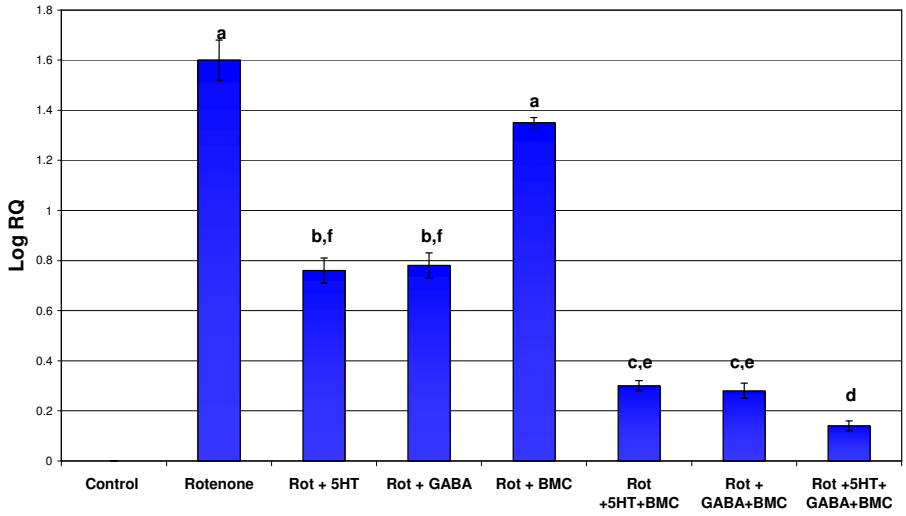


Table - 77
Real Time PCR amplification of Bax mRNA in the brain stem of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	1.60 ± 0.18 [a]
Rot + 5-HT	0.76 ± 0.19 [b, f]
Rot + GABA	0.78 ± 0.11 [b, f]
Rot + BMC	1.35 ± 0.10 [a]
Rot + 5-HT + BMC	0.30 ± 0.10 [c, e]
Rot + GABA + BMC	0.28 ± 0.07 [c, e]
Rot + 5-HT + GABA + BMC	0.14 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 74
Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the brain stem of control and experimental groups of rats

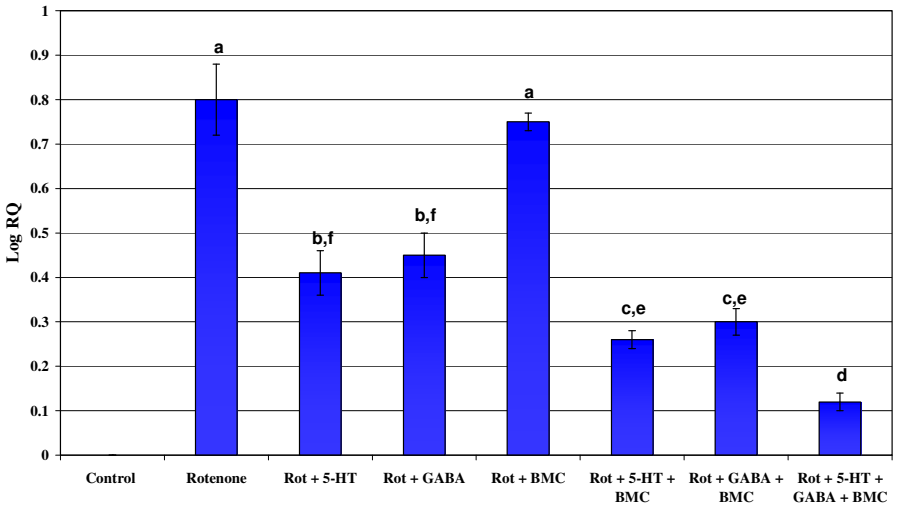


Table - 78
Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the brain stem of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	0.80 ± 0.06 [a]
Rot + 5-HT	0.41 ± 0.05 [b, f]
Rot + GABA	0.45 ± 0.07 [b, f]
Rot + BMC	0.75 ± 0.04 [a]
Rot + 5-HT + BMC	0.26 ± 0.06 [c, e]
Rot + GABA + BMC	0.30 ± 0.04 [c, e]
Rot + 5-HT + GABA + BMC	0.12 ± 0.02 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 75
Real Time PCR amplification of α-synuclein in the brain stem of control and experimental groups of rats

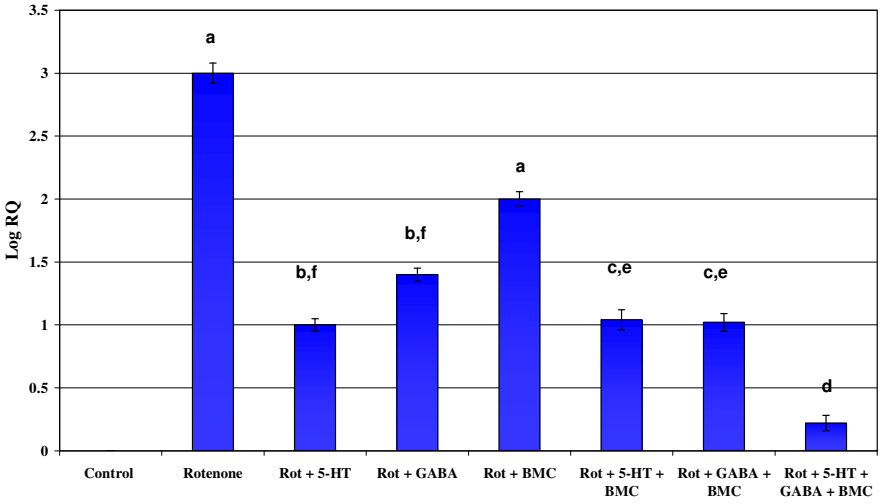


Table - 79
Real Time PCR amplification of α-synuclein in the brain stem of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	3.00 ± 0.31 [a]
Rot + 5-HT	1.00 ± 0.29 [b, e]
Rot + GABA	1.42 ± 0.26 [b, e]
Rot + BMC	2.01 ± 0.24 [a]
Rot + 5-HT + BMC	1.04 ± 0.09 [c, e]
Rot + GABA + BMC	1.02 ± 0.12 [c, e]
Rot + 5-HT + GABA + BMC	0.22 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Table - 80
Dopamine content in the hippocampus of control and experimental groups of rats

Experimental groups	DA(nmoles/g wet wt.)
Control	31.07 ± 3.20
Rotenone	10.90 ± 1.20 [a]
Rot + 5-HT	16.60 ± 1.33 [b, f]
Rot + GABA	18.52 ± 1.33 [b, f]
Rot + BMC	13.33 ± 1.20 [a]
Rot + 5-HT + BMC	22.80 ± 2.58 [c, e]
Rot + GABA + BMC	23.40 ± 2.58 [c, e]
Rot + 5-HT + GABA + BMC	25.13 ± 2.88 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 76
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the hippocampus of control and experimental groups of rats

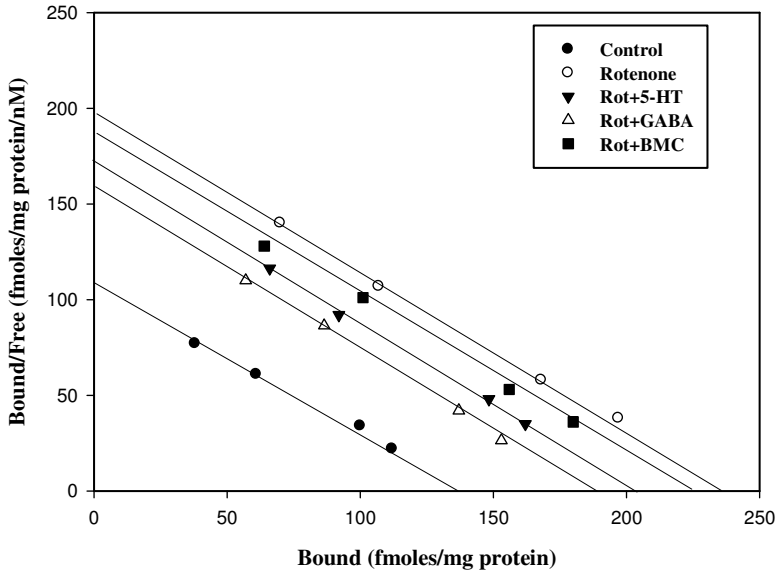


Table - 81
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the hippocampus of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	136.52 ± 13.14	1.27 ± 0.12
Rotenone	235.12 ± 21.35 [a]	1.18 ± 0.14
Rot + 5-HT	202.45 ± 19.23 [b, f]	1.28 ± 0.21
Rot + GABA	188.63 ± 18.85 [b, f]	1.19 ± 0.17
Rot + BMC	224.36 ± 22.41 [a]	1.24 ± 0.22

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 77
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the hippocampus of control and experimental groups of rats

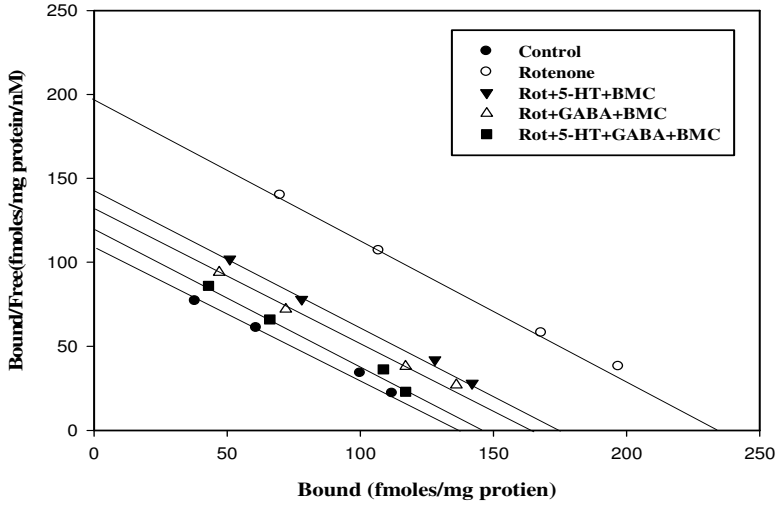


Table - 82
Scatchard analysis of total Dopamine receptors using [^{3}H]Dopamine binding against Dopamine in the hippocampus of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	136.52 ± 13.14	1.27 ± 0.12
Rotenone	235.12 ± 21.35 [a]	1.18 ± 0.14
Rot + 5-HT + BMC	172.25 ± 17.25 [b, e]	1.22 ± 0.17
Rot + GABA + BMC	161.25 ± 15.95 [b, e]	1.26 ± 0.21
Rot + 5-HT + GABA + BMC	142.25 ± 14.36 [d]	1.20 ± 0.19

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 78
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the hippocampus of control and experimental groups of rats

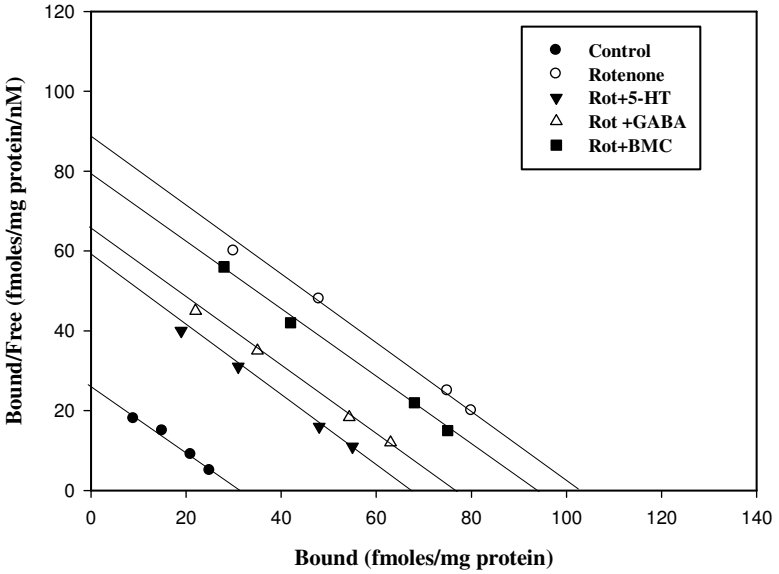


Table - 83
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the hippocampus of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	30.41 ± 3.14	1.23 ± 0.24
Rotenone	101.26 ± 10.13 [a]	1.18 ± 0.26
Rot + 5-HT	66.54 ± 6.54 [b, e]	1.13 ± 0.11
Rot + GABA	76.36 ± 7.36 [b, e]	1.16 ± 0.25
Rot + BMC	92.68 ± 9.64 [a]	1.19 ± 0.14

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 79
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the hippocampus of control and experimental groups of rats

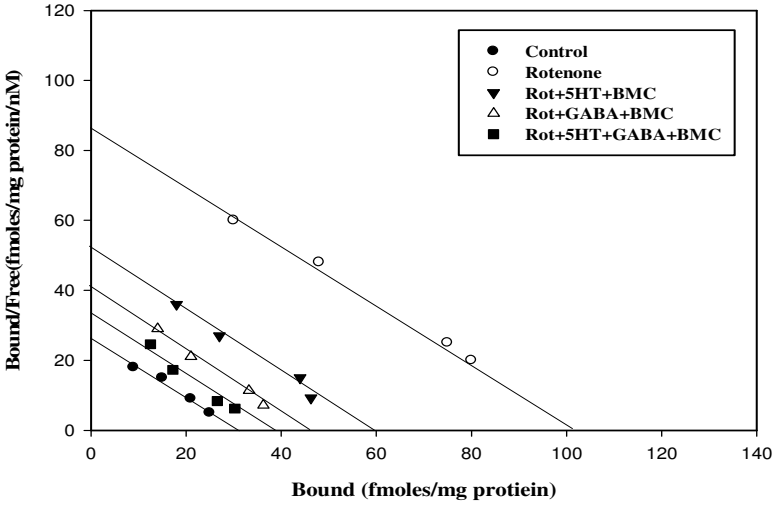


Table - 84
Scatchard analysis of Dopamine D_1 receptor using [^{3}H]SCH 23390 binding against SCH 23390 in the hippocampus of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	30.41 ± 3.14	1.23 ± 0.24
Rotenone	101.26 ± 10.13 [a]	1.18 ± 0.26
Rot + 5-HT + BMC	60.25 ± 6.25 [b, e]	1.15 ± 0.19
Rot + GABA + BMC	45.71 ± 4.52 [b, f]	1.12 ± 0.28
Rot + 5-HT + GABA + BMC	38.25 ± 3.25 [d]	1.15 ± 0.17

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 80
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the hippocampus of control and experimental groups of rats

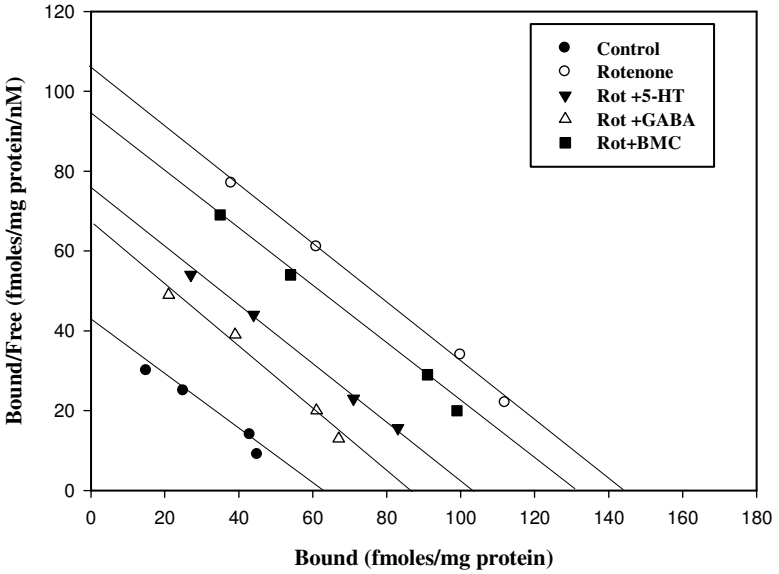


Table - 85
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the hippocampus of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	61.25 ± 6.25	1.48 ± 0.24
Rotenone	144.35 ± 14.22 [a]	1.38 ± 0.14
Rot + 5-HT	101.52 ± 10.24 [b, e]	1.34 ± 0.11
Rot + GABA	85.36 ± 8.25 [b, e]	1.30 ± 0.21
Rot + BMC	130.52 ± 14.25 [a]	1.38 ± 0.15

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC.

Figure - 81
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the hippocampus of control and experimental groups of rats

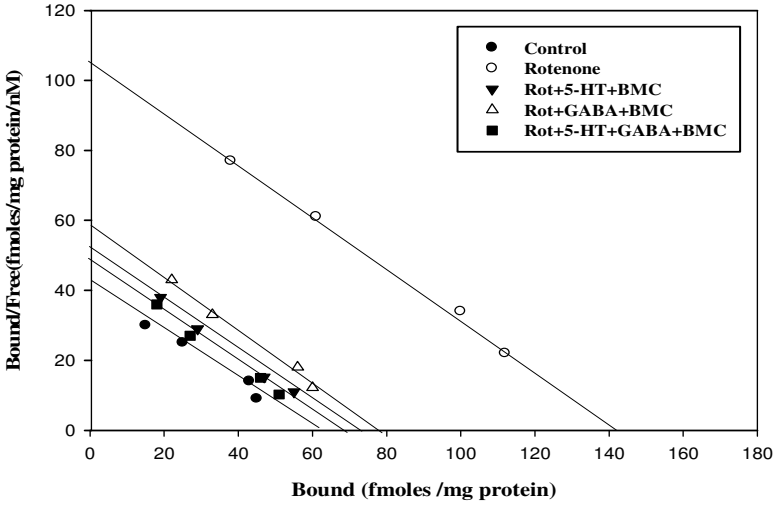


Table - 86
Scatchard analysis of Dopamine D_2 receptor using [^{3}H]YM-09151-2 binding against Sulpiride in the hippocampus of control and experimental groups of rats

Experimental groups	B_{max} (fmoles/mg protein)	K_d (nM)
Control	61.25 ± 6.25	1.48 ± 0.24
Rotenone	144.35 ± 14.22 [a]	1.38 ± 0.14
Rot + 5-HT + BMC	71.69 ± 7.62 [b, e]	1.39 ± 0.13
Rot + GABA + BMC	77.25 ± 7.14 [b, e]	1.35 ± 0.18
Rot + 5-HT + GABA + BMC	67.36 ± 6.72 [d]	1.36 ± 0.17

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
B_{max} – Maximal binding; K_d – Dissociation constant.

[a] $p<0.001$, [b] $p<0.01$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure -82
Real Time PCR amplification of Dopamine D_1 receptor mRNA in the hippocampus of control and experimental groups of rats

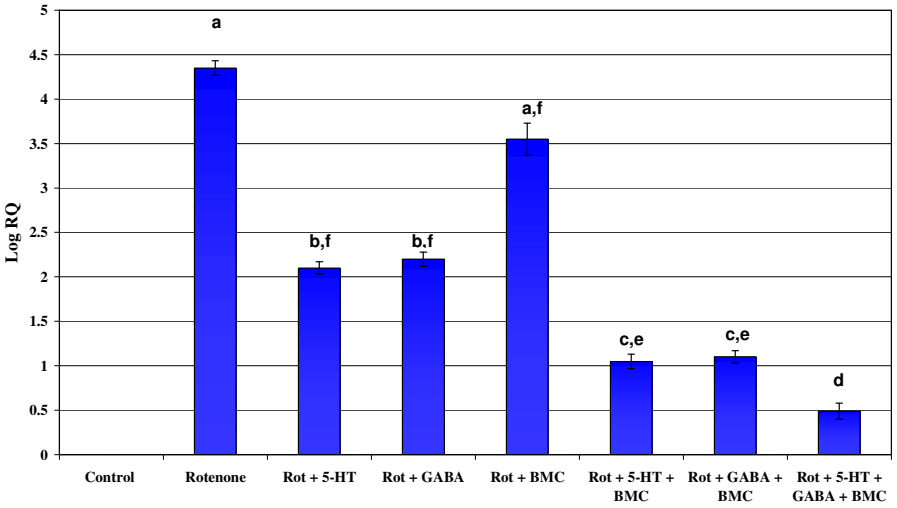


Table - 87
Real Time PCR amplification of Dopamine D_1 receptor mRNA in the hippocampus of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	4.35 ± 0.09 [a]
Rot + 5-HT	2.10 ± 0.08 [b, f]
Rot + GABA	2.20 ± 0.09 [b, f]
Rot + BMC	3.55 ± 0.06 [a]
Rot + 5-HT + BMC	1.05 ± 0.05 [c, e]
Rot + GABA + BMC	1.10 ± 0.06 [c, e]
Rot + 5-HT + GABA + BMC	0.49 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure -83
Real Time PCR amplification of Dopamine D_2 receptor mRNA in the hippocampus of control and experimental groups of rats

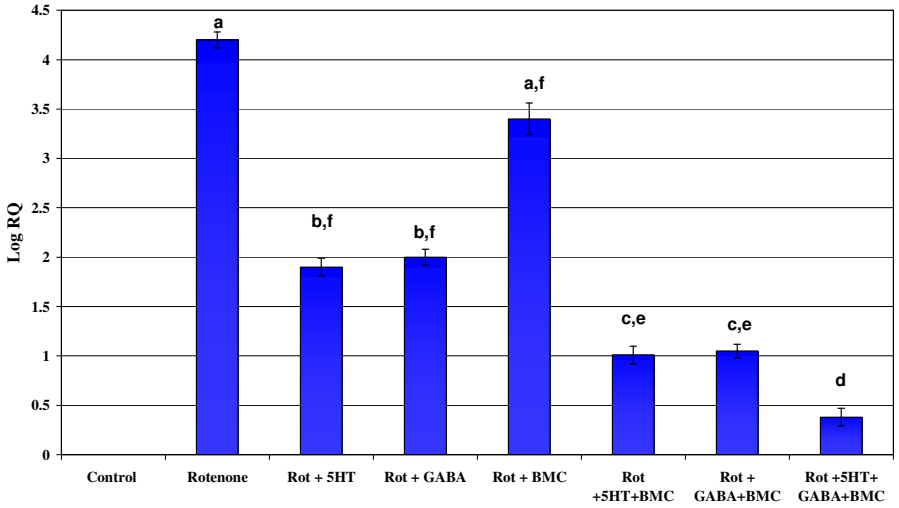
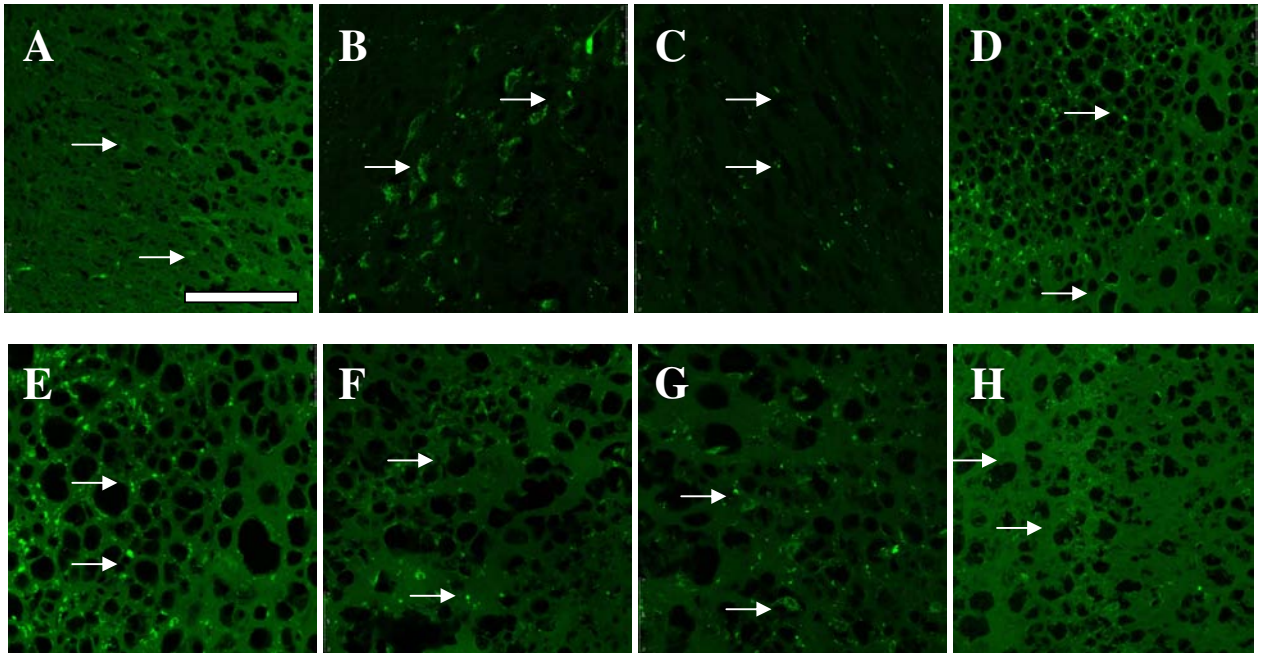


Table - 88
Real Time PCR amplification of Dopamine D_2 receptor mRNA in the hippocampus of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	4.20 ± 0.09 [a]
Rot + 5-HT	1.90 ± 0.08 [b, f]
Rot + GABA	2.00 ± 0.09 [b, f]
Rot + BMC	3.40 ± 0.06 [a, f]
Rot + 5-HT + BMC	1.10 ± 0.05 [c, e]
Rot + GABA + BMC	1.05 ± 0.06 [c, e]
Rot + 5-HT + GABA + BMC	0.38 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 84
Dopamine D_1 receptor expression in the hippocampus of control and experimental rats



A – Control, B – Rotenone infused, C – Rotenone infused treated with Serotonin, D – Rotenone infused treated with GABA, E – Rotenone infused treated with BMC, F - Rotenone infused treated with Serotonin and BMC, G - Rotenone infused treated with GABA and BMC, H - Rotenone infused treated with Serotonin, GABA and BMC. The scale bar represents 75 µm.

Table - 89
Dopamine D_1 receptor expression in the hippocampus of control and experimental rats

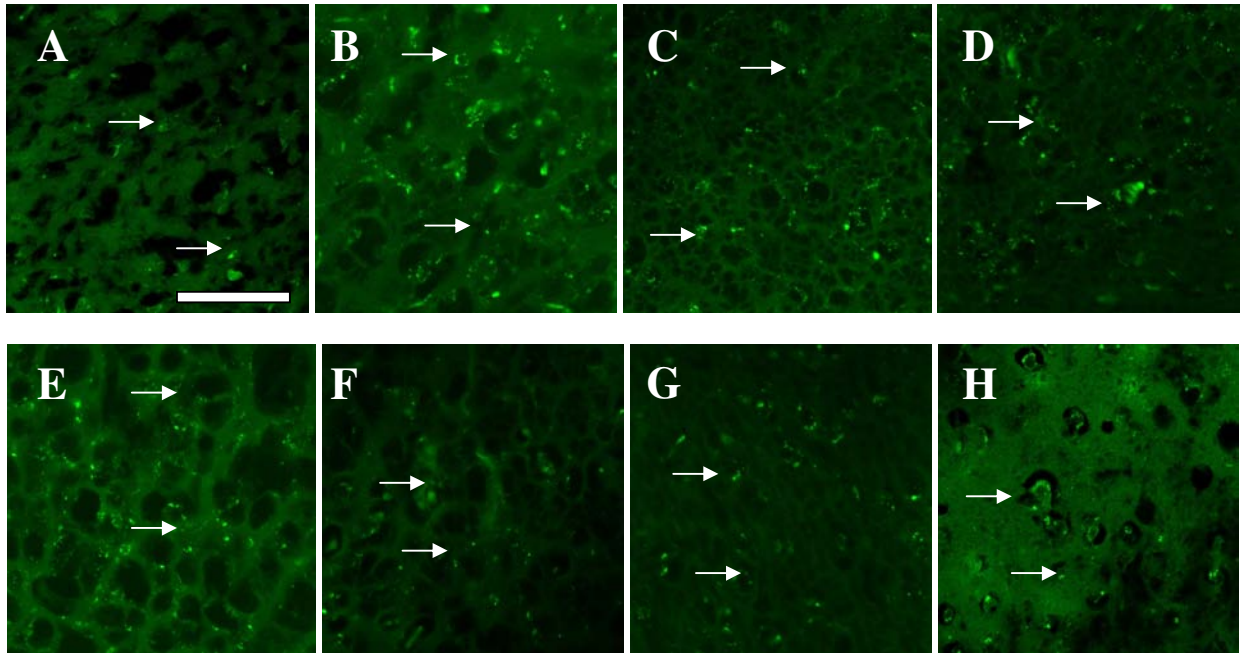
Experimental groups	Mean pixel intensity
Control	23.25 ± 2.50
Rotenone	81.16 ± 8.23 [a]
Rot + 5-HT	65.18 ± 6.50 [b, f]
Rot + GABA	71.25 ± 7.05 [b, f]
Rot + BMC	77.36 ± 7.55 [a]
Rot + 5-HT + BMC	52.44 ± 5.93 [c, e]
Rot + GABA + BMC	55.78 ± 5.56 [c, e]
Rot + 5-HT + GABA + BMC	36.65 ± 3.42 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 85
Dopamine D_2 receptor expression in the hippocampus of control and experimental rats



A – Control, B – Rotenone infused, C – Rotenone infused treated with Serotonin, D – Rotenone infused treated with GABA, E – Rotenone infused treated with BMC, F - Rotenone infused treated with Serotonin and BMC, G - Rotenone infused treated with GABA and BMC, H - Rotenone infused treated with Serotonin, GABA and BMC. The scale bar represents 75 µm.

Table - 90
Dopamine D_2 receptor expression in the hippocampus of control and experimental rats

Experimental groups	Mean pixel intensity
Control	20.51 ± 2.93
Rotenone	82.10 ± 8.42 [a]
Rot + 5-HT	50.34 ± 3.11 [b, f]
Rot + GABA	55.47 ± 4.56 [b, f]
Rot + BMC	80.43 ± 8.96 [a]
Rot + 5-HT + BMC	30.71 ± 4.99 [c, e]
Rot + GABA + BMC	32.90 ± 5.62 [c, e]
Rot + 5-HT + GABA + BMC	24.13 ± 3.61 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.

C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 86
IP3 content in the hippocampus of control and experimental groups of rats

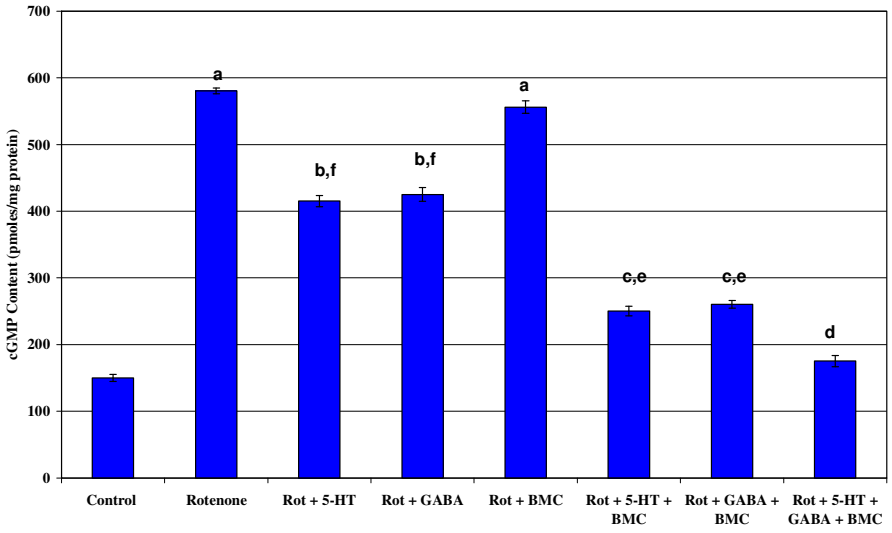


Table - 91
IP3 content in the hippocampus of control and experimental groups of rats

Experimental groups	IP3 Content (pmoles/mg protein)
Control	150.30 ± 5.23
Rotenone	580.29 ± 10.25 [a]
Rot + 5-HT	415.31 ± 15.23 [b, f]
Rot + GABA	425.83 ± 14.65 [b, f]
Rot + BMC	556.56 ± 15.68 [a]
Rot + 5-HT + BMC	250.68 ± 5.23 [c, e]
Rot + GABA + BMC	260.46 ± 5.62 [c, e]
Rot + 5-HT + GABA + BMC	175.22 ± 4.32 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 87
cAMP content in the hippocampus of control and experimental groups of rats

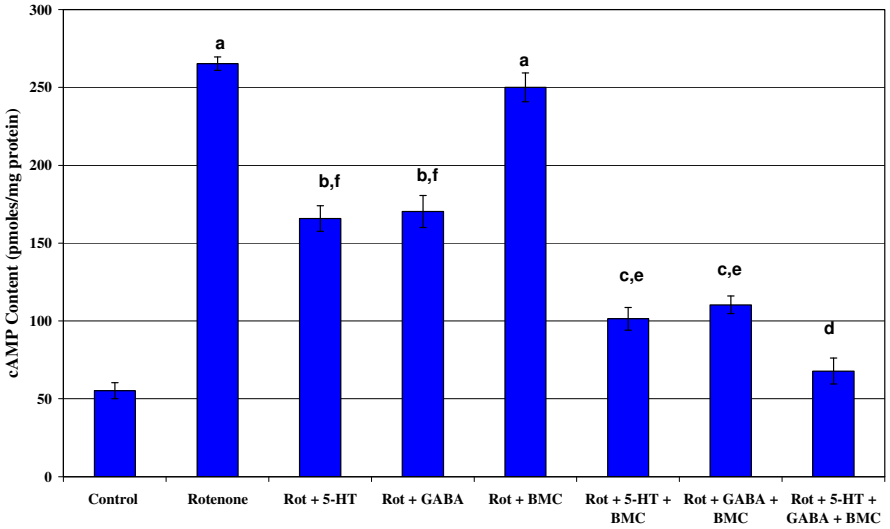


Table - 92
cAMP content in the hippocampus of control and experimental groups of rats

Experimental groups	cAMP Content (pmoles/mg protein)
Control	55.37 ± 5.23
Rotenone	265.23 ± 10.25 [a]
Rot + 5-HT	165.39 ± 15.23 [b, f]
Rot + GABA	170.80 ± 14.65 [b, f]
Rot + BMC	250.51 ± 15.68 [a]
Rot + 5-HT + BMC	101.68 ± 5.23 [c, e]
Rot + GABA + BMC	110.49 ± 5.62 [c, e]
Rot + 5-HT + GABA + BMC	67.26 ± 4.32 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 88
cGMP content in the hippocampus of control and experimental groups of rats

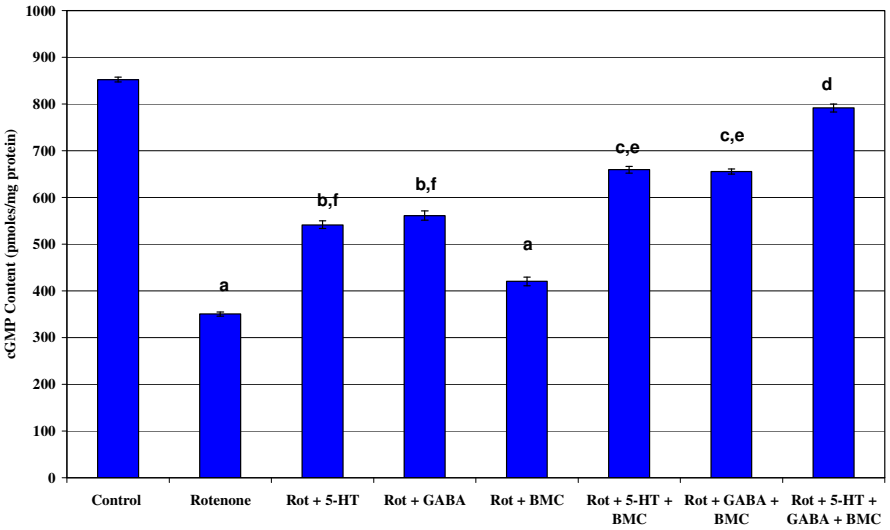


Table - 93
cGMP content in the hippocampus of control and experimental groups of rats

Experimental groups	cGMP Content (pmoles/mg protein)
Control	852.52 ± 24.12
Rotenone	350.82 ± 21.25 [a]
Rot + 5-HT	541.21 ± 23.62 [b, f]
Rot + GABA	561.12 ± 25.14 [b, f]
Rot + BMC	420.75 ± 28.21 [a]
Rot + 5-HT + BMC	659.12 ± 24.53 [c, e]
Rot + GABA + BMC	655.65 ± 26.13 [c, e]
Rot + 5-HT + GABA + BMC	791.12 ± 25.68 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 89
Real Time PCR amplification of CREB mRNA in the hippocampus of control and experimental groups of rats

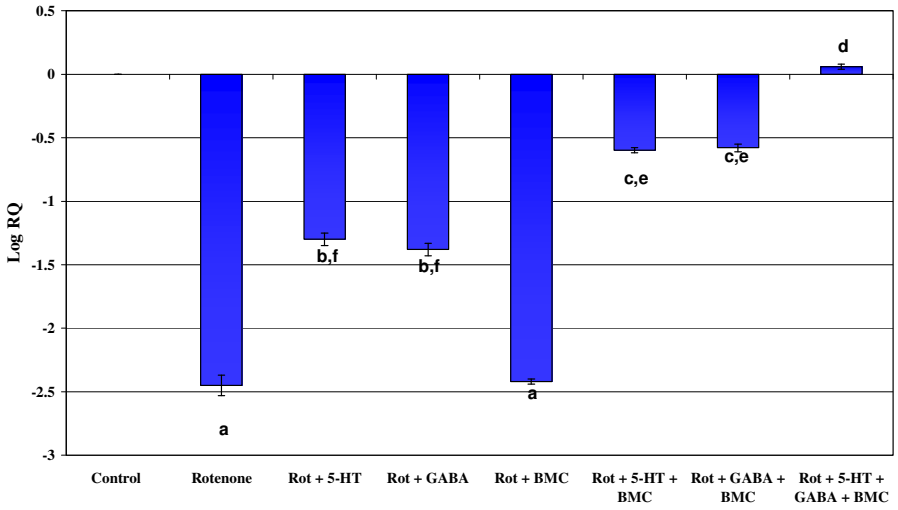


Table - 94
Real Time PCR amplification of CREB mRNA in the hippocampus of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	-2.45 ± 0.22 [a]
Rot + 5-HT	-1.30 ± 0.13 [b, f]
Rot + GABA	-1.38 ± 0.12 [b, f]
Rot + BMC	-2.43 ± 0.21 [a]
Rot + 5-HT + BMC	-0.60 ± 0.08 [c, e]
Rot + GABA + BMC	-0.58 ± 0.09 [c, e]
Rot + 5-HT + GABA + BMC	0.06 ± 0.03 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 90
Real Time PCR amplification of Bax mRNA in the hippocampus of control and experimental groups of rats

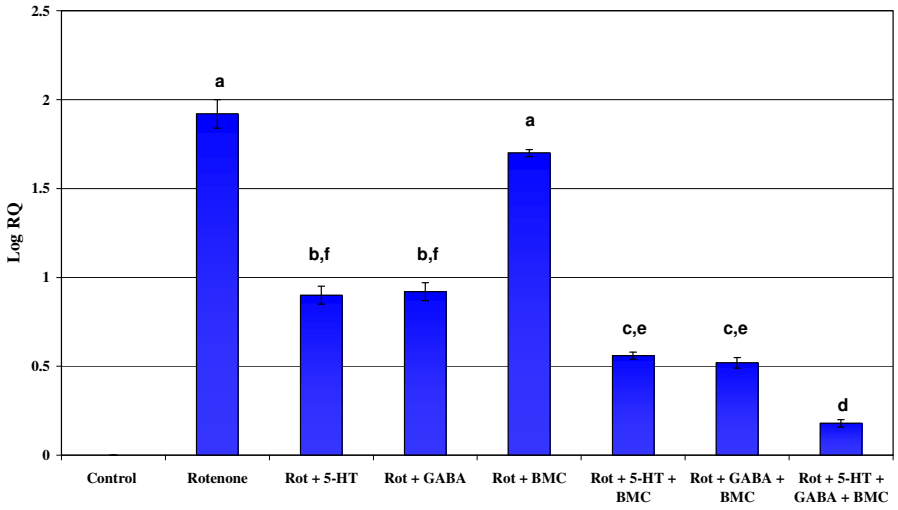


Table - 95
Real Time PCR amplification of Bax mRNA in the hippocampus of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	1.92 ± 0.18 [a]
Rot + 5-HT	0.90 ± 0.19 [b, f]
Rot + GABA	0.92 ± 0.11 [b, f]
Rot + BMC	1.70 ± 0.10 [a]
Rot + 5-HT + BMC	0.56 ± 0.10 [c, e]
Rot + GABA + BMC	0.52 ± 0.07 [c, e]
Rot + 5-HT + GABA + BMC	0.18 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 91

Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the hippocampus of control and experimental groups of rats

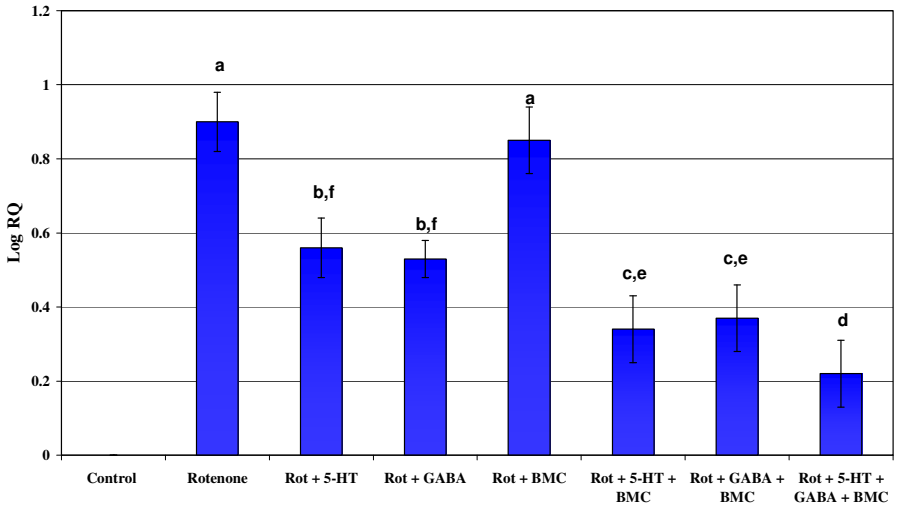


Table - 96

Real Time PCR amplification of ubiquitin carboxy-terminal hydrolase mRNA in the hippocampus of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	0.90 ± 0.06 [a]
Rot + 5-HT	0.56 ± 0.05 [b, f]
Rot + GABA	0.53 ± 0.07 [b, f]
Rot + BMC	0.85 ± 0.04 [a]
Rot + 5-HT + BMC	0.34 ± 0.06 [c, e]
Rot + GABA + BMC	0.37 ± 0.04 [c, e]
Rot + 5-HT + GABA + BMC	0.22 ± 0.02 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 92
Real Time PCR amplification of α-synuclein in the hippocampus of control and experimental groups of rats

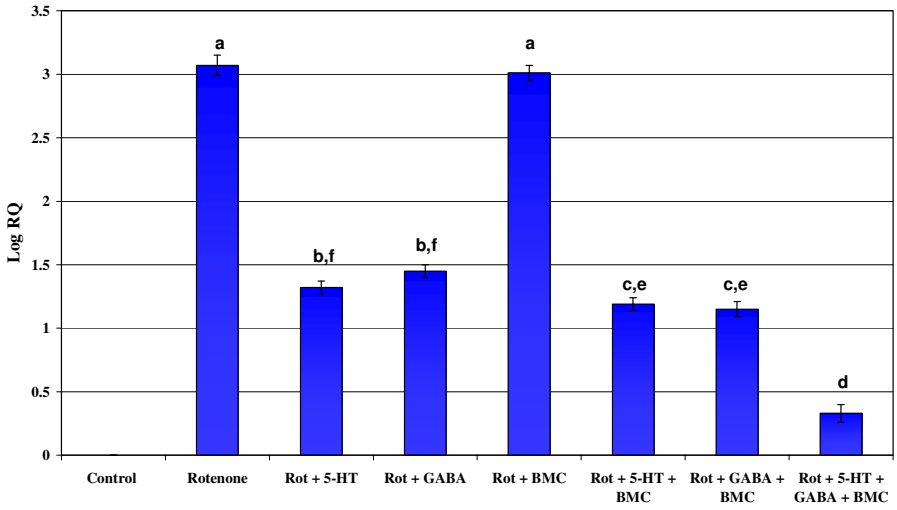


Table - 97
Real Time PCR amplification of α-synuclein in the hippocampus of control and experimental groups of rats

Experimental groups	Log RQ
Control	0
Rotenone	3.07 ± 0.31 [a]
Rot + 5-HT	1.32 ± 0.29 [b, e]
Rot + GABA	1.45 ± 0.26 [b, e]
Rot + BMC	3.01 ± 0.24 [a]
Rot + 5-HT + BMC	1.19 ± 0.09 [c, e]
Rot + GABA + BMC	1.15 ± 0.12 [c, e]
Rot + 5-HT + GABA + BMC	0.33 ± 0.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 93
Behavioural response on Rotarod Test of control and experimental groups of rats

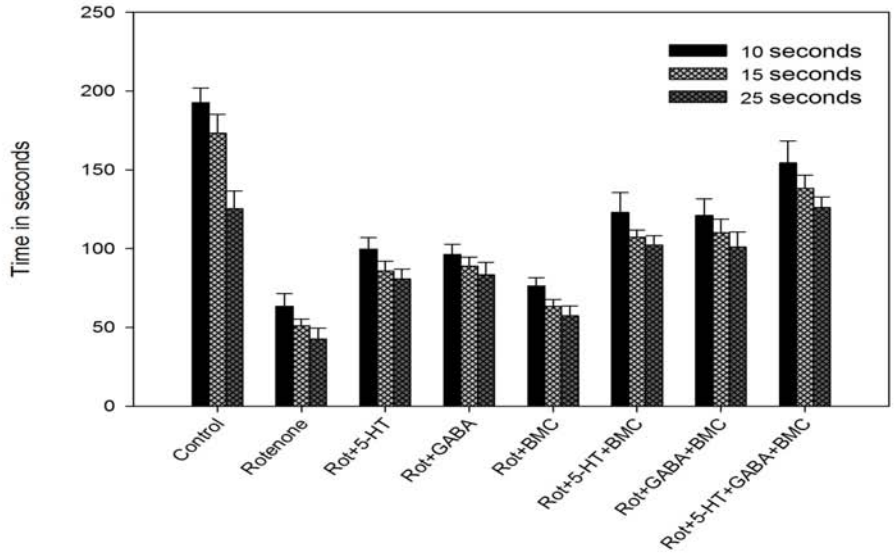


Table - 98
Behavioural response on Rotarod Test of control and experimental groups of rats

	Time in seconds		
Experimental groups	**10rpm**	**15rpm**	**25rpm**
Control	192.67 ± 15.53	173.33 ± 11.76	125.33 ± 10.20
Rotenone	63.33 ± 14.20 [a]	51.00 ± 2.22 [a]	42.67 ± 5.68 [a]
Rot + 5-HT	99.67 ± 9.33 [b,f]	85.67 ± 5.34 [b,f]	80.67 ± 5.33 [b,f]
Rot + GABA	96.32 ± 8.35 [b,f]	89.00±16.65 [b,f]	83.33 ± 6.88 [b,f]
Rot + BMC	76.33 ± 5.20 [a]	63.33 ± 1.20 [a]	57.33 ± 4.20 [a]
Rot + 5-HT + BMC	123.00 ±10.58 [c,e]	107.33±2.33 [c,e]	102.33±6.88 [c,e]
Rot + GABA + BMC	121.00 ± 10.58 [c,e]	110.00±10.58 [c,e]	101.00±7.58 [c,e]
Rot + 5-HT + GABA + BMC	154.33 ± 14.88 [d]	138.33± 1.20 [d]	126.00±11.73 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] p<0.001, [b] p<0.01, [c] p<0.05 when compared to Control.
[d] p<0.001, [e] p<0.01, [f] p<0.05 when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 94
Behavioural response on Grid Walk Test of control and experimental groups of rats

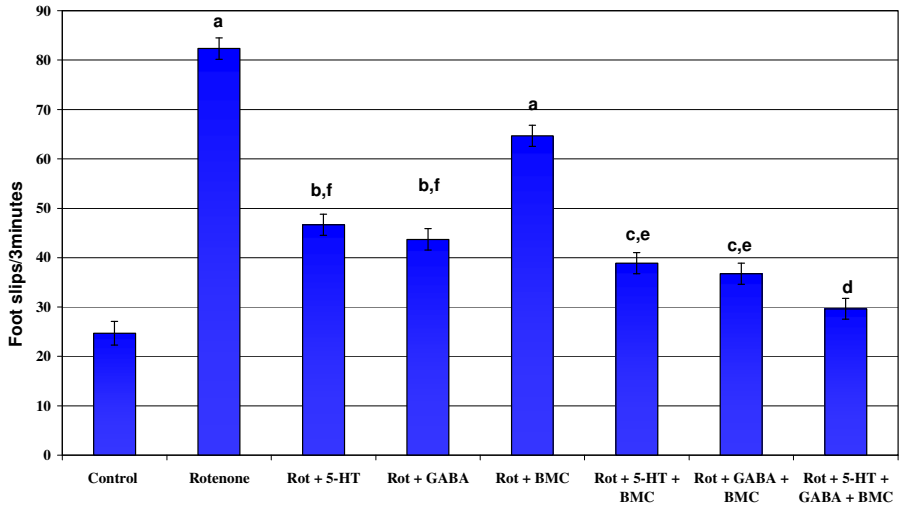


Table - 99
Behavioural response on Grid Walk Test of control and experimental groups of rats

Experimental groups	Foot slips/3 mins
Control	22.67 ± 2.66
Rotenone	82.92 ± 4.18 [a]
Rot + 5-HT	46.90 ± 4.19 [b, f]
Rot + GABA	43.92 ± 3.11 [b, f]
Rot + BMC	65.70 ± 5.10 [a]
Rot + 5-HT + BMC	38.56 ± 3.10 [c, e]
Rot + GABA + BMC	36.52 ± 2.07 [c, e]
Rot + 5-HT + GABA + BMC	29.18 ± 2.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 95
Behavioural response on Narrow Beam Test of control and experimental groups of rats

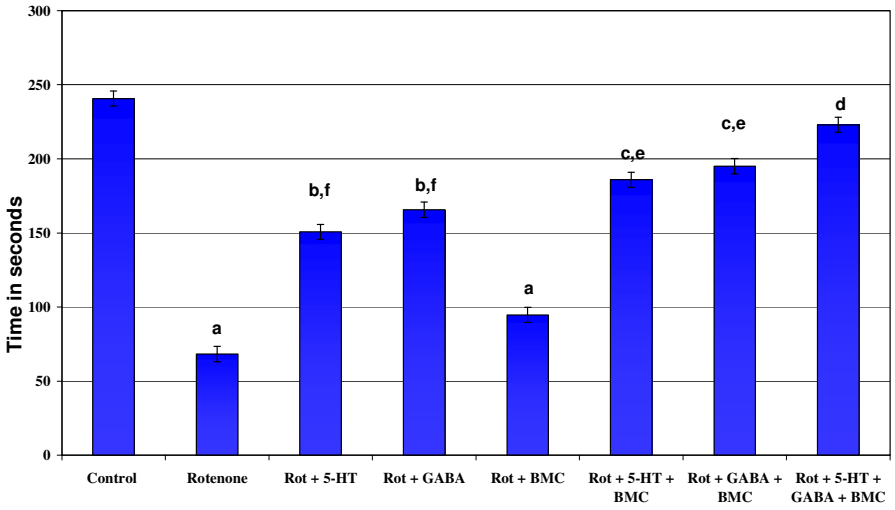


Table - 100
Behavioural response on Narrow Beam Test of control and experimental groups of rats

Experimental groups	Balance Retension Time (secs)
Control	240.67 ± 24.76
Rotenone	68.35 ± 5.09 [a]
Rot + 5-HT	150.10 ± 14.08 [b, f]
Rot + GABA	165.20 ± 15.09 [b, f]
Rot + BMC	94.55 ± 8.06 [a]
Rot + 5-HT + BMC	185.05 ± 14.05 [c, e]
Rot + GABA + BMC	195.10 ± 15.06 [c, e]
Rot + 5-HT + GABA + BMC	223.49 ± 18.06 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Table - 101
Behavioural response on Social Interaction Test of control and experimental groups of rats

Experimental groups	Allogrooming	Sniffing the partner	Aggressive attacks	Following
Control	18.33 ± 1.22	56.25±4.53	0	29.25±2.50
Rot	6.33 ± 0.26[a]	21.50±3.38[a]	0	1.50±1.01[a]
Rot+5-HT	12.66±1.61[b,f]	34.66±4.07[b,f]	0	7.25±1.43[b,f]
Rot+GABA	11.33±1.56[b,f]	36.00±3.04[b,f]	0	8.75±1.57[b,f]
Rot+BMC	8.33±0.21[a]	27.50±2.38[a]	0	13.75±1.67[a]
Rot+5-HT+BMC	14.66±1.43[c,e]	42.00±0.33[c,e]	0	0.75±1.45[c,e]
Rot+GABA+BMC	13.66±1.24[c,e]	43.00±0.25[c,e]	0	21.75±1.62[c,e]
Rot+5-HT +GABA+BMC	16.66±1.54[d]	47.00±0.49[d]	0	5.75±1.50[d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Figure - 96
Behavioural response on Elevated Plus-Maze Test of control and experimental groups of rats

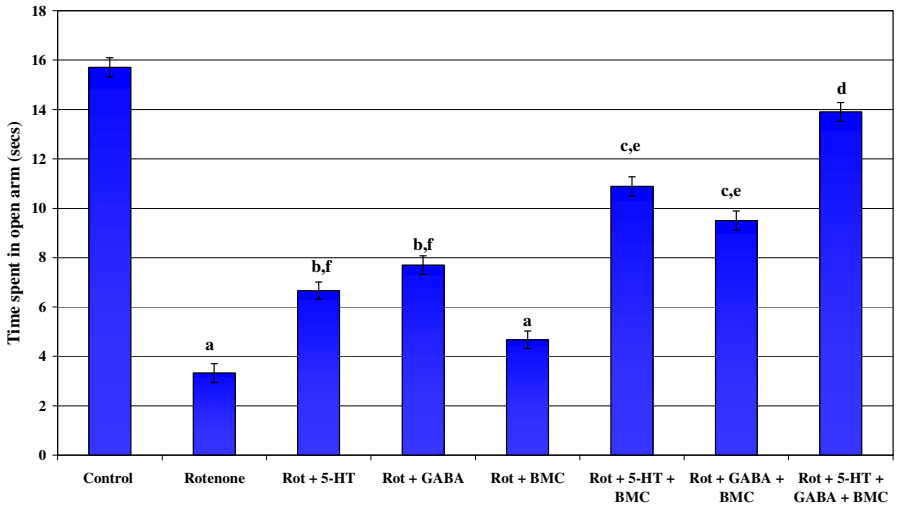


Table – 102
Behavioural response on Elevated Plus-Maze Test of control and experimental groups of rats

Experimental groups	Time spent in the open arm (secs)
Control	15.09 ± 0.94
Rotenone	3.20 ± 0.09 [a]
Rot + 5-HT	6.90 ± 0.38 [b,f]
Rot + GABA	7.0 ± 0.49 [b,f]
Rot + BMC	4.40 ± 0.16 [a]
Rot + 5-HT + BMC	10.10 ± 0.65 [c,e]
Rot + GABA + BMC	9.05 ± 0.56 [c,e]
Rot + 5-HT + GABA + BMC	13.38 ± 0.36 [d]

Values are Mean ± S.E.M of 4-6 separate experiments. Each group consist 6-8 rats.
[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$ when compared to Control.
[d] $p<0.001$, [e] $p<0.01$, [f] $p<0.05$ when compared to Rotenone group.
C – Control, Rotenone – Rotenone infused, Rot + 5-HT – Rotenone infused treated with Serotonin, Rot + GABA – Rotenone infused treated with GABA, Rot + BMC – Rotenone infused treated with BMC, Rot + 5-HT + BMC – Rotenone infused treated with Serotonin and BMC, Rot + GABA + BMC – Rotenone infused treated with GABA and BMC, Rot + 5-HT + GABA + BMC – Rotenone infused treated with Serotonin, GABA and BMC.

Discussion

Parkinson's disease (PD) is a progressive motor system disorder characterized by selective degeneration of dopaminergic neurons in substantia nigra *pars compacta* (SNpc) leading to marked reduction of dopamine (DA) levels in the striatum. Mitochondrial dysfunction in the brain leads to oxidative stress within neurons and cause conditions conducive to their degeneration, culminating in neurodegenerative diseases such as PD. Studies on post-mortem brain substantia nigra *pars compacta* (SNpc) region and platelets from the patients have suggested a general, significant impairment of mitochondrial function in PD (Mizuno, *et al.,* 1989; Schapira, *et al.,* 1989; Sheehan, *et al.,* 1997). Administration of rotenone, a well-characterized, high-affinity, specific inhibitor of complex-I of the inner mitochondrial membrane involved in oxidative phosphorylation (Schuler & Casida, 2001; Venda *et al*., 2011) has been demonstrated convincingly to produce nigrostriatal dopaminergic neurodegeneration, as well as behavioural and neuropathological hallmarks of PD in rodents. Intrajugular and subcutaneous infusion of rotenone resulted in the selective destruction of the nigrostriatal dopaminergic system, formation of cytoplasmic inclusions in nigral neurons and induction of hypokinesia and rigidity in rats (Betarbet *et al.,* 2000; Sherer *et al.,* 2003). Rotenone-induced selective toxicity to dopaminergic neurons in SN has been attributed to the inhibition of the complex-I activity in the mitochondrial respiratory chain and the unique vulnerability of dopaminergic neurons to oxidative damage as a result of mitochondrial enzyme inhibition (Greenamyre *et al.,* 1999; Jenner, 2001).

Chronic intraperitoneal injections and acute intranigral or median forebrain bundle infusion of rotenone reproduced most of the neurochemical, neuropathological and behavioural features of PD in rats (Heikkila *et al.,* 1985; Alam & Schmidt, 2002; Alam *et al.,* 2004; Sindhu *et al.,* 2005; Saravanan *et al.,*

2005). Studies have shown that rotenone can affect other basal ganglia structures in addition to striatal neurons (Ferrante *et al.,* 1997; Hoglinger *et al.,* 2003). This model reproduced several of the neurochemical and neuropathological symptoms of PD and indicated that there is involvement of oxidative stress in the specific dopaminergic neuronal degeneration. ROS production following rotenone exposure has been demonstrated in cell lines and organotypic cultures (Li *et al.,* 2003; Sherer *et al.,* 2003a; Testa *et al.,* 2005). Chronic rotenone administration shown to increase nitric oxide levels and lipid peroxidation (Bashkatova *et al.,* 2004). The potent parkinsonian neurotoxin, MPP+ has also been shown to inhibit complex-I (Nicklas *et al.,* 1985; Ramsay *et al.,* 1986; Gluck *et al.,* 1994), generating free radicals including superoxide anions, nitric oxide and •OH (Zang & Mishra, 1992; Jenner *et al.,* 1992; Wu *et al.,* 1993; Mohanakumar & Steinbusch, 1998; Fabre *et al.,* 1999; Cassarino and Bennett, 1999; Mohanakumar *et al.,* 2002) and damage the endogenous antioxidant machinery in the brain (Ferraro *et al.,* 1986; Yong *et al.,* 1986; Muralikrishnan & Mohanakumar, 1998; Thomas & Mohanakumar, 2003).

Studies on post-mortem brains of PD patients suggest that dopaminergic neurons in SNpc region experience a delicate state of oxidative stress, as indicated by an increase in the content of oxidized proteins, DNA and lipids (Dexter *et al.,* 1991; Alam *et al.,* 1997). The inhibition in complex-I activity has been shown to cause marked increases in reactive oxygen species (ROS) production (Turrens & Boveris, 1980; Meloni & Vasak *et al*., 2011), which is responsible for the oxidative damage generated in dopamine (DA) metabolism or oxidative phosphorylation (Lotharius & O'Malley, 2000).

The classic anti-parkinsonian drugs L-deprenyl and melatonin has been demonstrated to provide neuroprotection against rotenone-induced neuronal damage in rat by means of its •OH scavenging action (Saravanan *et al*., 2006; Saravanan *et al*., 2007). 5-HT and L-DOPA have been reported to exhibit protective effects on oxidative tissue damages (Ham *et al.,* 1999; Xu *et al*., 2011).

5-HT depresses lipid peroxidation of microsomes by Fe^{3+} ADP and NADPH system (Tse *et al.,* 1991). N-Acetylserotonin decreases the peroxidation of linoleic acid induced by 2, 2' -azobis (2-amidinopropane) (Longoni *et al.,* 1997). 5-HT is reported to scavenge superoxide anion and hypochlorous acid (HOCl). L-DOPA is currently used in symptomatic treatment of Parkinson's disease. However, it induces apoptosis in cultured postmitotic chick sympathetic neurons (Ziv *et al.,* 1997). Additionally, 5-HT metabolises to the potential neuroprotective antioxidants, normelatonin and melatonin, which also helps to prevent oxidative damage caused as a result of rotenone administration. N-acetyl-serotonin (normelatonin) and melatonin protect neurons against oxidative challenges and suppress the activity of the transcription factor NF-kappaB (Lezoualc'h *et al.,* 1998).

Dopamine Content

In PD, the central pathologic process is a rather selective degeneration of the dopaminergic neurons in the pars compacta of the substantia nigra, leading to anterograde loss of the ascending nigrostriatal projections and their nerve endings resulting in the depletion of dopamine (DA) in the other regions of central nervous system (CNS) (Sang *et al.,* 2003; Lew *et al*., 2007). Substantia nigra appears darker than neighbouring areas due to high levels of melanin in dopaminergic neurons. Parkinson's disease is caused by the death of dopaminergic neurons in the substantia nigra pars compacta. The substantia nigra consists of two parts with different connections and functions, the *pars compacta* and *pars reticulata*. The *pars compacta* serves mainly as an input to the basal ganglia circuit, supplying the striatum with dopamine. The *pars reticulata*, on the other hand, serves mainly as an output, conveying signals from the basal ganglia to numerous other brain structures.

The substantia nigra sends out fibers to the corpus striatum, grey and white bands of tissue in the caudate nucleus and putamen where the dopamine is

released. The transmission of dopamine and its release into the corpus striatum is necessary for smooth, coordinated muscle movement (Richard, 2009).

The basal ganglia represent parts of corticosubcortical circuits involved in a large variety of motor as well as non motor functions. Parkinsonism emerges as a complex disorder in which striatal dopamine depletion results in an increased and disordered discharge in motor areas of basal ganglia. Parkinsonian motor signs are caused by distinct abnormalities in the basal ganglia discharge and by involvement of subcircuits related to distinct cortical targets.

The main input to the SNr derives from the striatum. It comes by two routes, known as the *direct* and *indirect* pathways. The direct pathway consists of axons from medium spiny cells in the striatum which project directly to nigra. The indirect pathway consists of three links, first a projection from striatal medium spiny cells to the external part of the globus pallidus (GPe); second a GABAergic projection from GPe to the subthalamic nucleus (STN); third a glutamergic projection from STN to SNr. (Nauta & Cole 1978; Huang *et al*., 2010). Thus, striatal activity exerts an excitatory (or rather disinhibitory) effect on SNr neurons *via* the direct pathway, but an inhibitory effect *via* the indirect pathway. The direct and indirect pathways originate from different subsets of striatal medium spiny cells: they are tightly intermingled but express different types of dopamine receptors, as well as showing other neurochemical differences. There are significant projections to the thalamus (ventral lateral and ventral anterior nuclei), superior colliculus and other caudal nuclei from the *pars reticulata* (the nigrothalamic pathway). (Carpenter *et al.,* 1972) These neurons use GABA as their neurotransmitter. In addition, these neurons form up to five collaterals which branch within both the *pars compacta* and *pars reticulata*, likely modulating dopaminergic activity in the *pars compacta* (Deniau *et al.,* 1992).

Substantia nigra *pars compacta*

The SN has a well defined system of dopamine neurones giving rise to the nigrostriatal pathway and is known to have an important role in motor behaviour in animals and extra-pyramidal disorders such as Parkinsonism. One of the major outputs from the striatum appears to project to the SN through the striatonigral pathway. The cell bodies of this nigrothalamic pathway predominantly in the lateral and central regions of the zona reticulata of the SN and their axons project to the ventromedial nucleus (VM) and to a lesser extent to the Centromedianum-Parafascicular nuclear complex (CM-PF) of the ipsilateral thalamus in the rat (Clavier, 1976; Faull, 1978; Gjedde & Geday, 2009).

DA produced by neurons of the SNpc plays a key role in the regulation of GABA neurotransmission in the basal ganglia, including the striatum and substantia nigra pars reticulata. The destruction of these neurons leads to a downstream deficiency in GABA signaling in areas of the brain that regulate movement. GABA controls the activity of the DA containing cells of the substantia nigra and loss of GABA and its synthesizing enzyme glutamic acid decarboxylase have been observed in the basal ganglia of patients dying from Parkinson's disease (Precht & Yoshida 1971). As GABA helps "quiet" excessive neuronal firing and has been deficient in patients in the advanced stages of Parkinson's disease directly targeting GABA production rather than dopamine replacement is a more effective way of improving brain function in late-stage Parkinson's disease while also avoiding the known therapeutic limitations and complications associated with the over-production of dopamine.

Alterations in the brain monoamines DA and 5-HT have been implicated in the etiology and/or pharmacotherapy of multiple mental disorders. Most of the effects of 5-HT on DA neurons is indirect, mediated *via* actions on complex neuronal circuitry, rather than direct effects on DA terminals (Poewe *et al.,* 2009). Since the different 5-HT receptor subtypes are differently distributed in dopaminergic brain regions, it is possible to specifically "target" individual brain

regions with serotonergic ligands and thereby affect dopaminergic function selectively in these areas. Dopamine released from 5-HT neurons is responsible for L-DOPA-induced dyskinesia in rotenone-lesioned rats (Munoz *et al*., 2008). This is important therapeutically, since an individual patient has a range of symptoms that reflect dopaminergic dysfunctions in some brain areas but not others. Thus, the clinical efficacy of psychotherapeutic drugs that act on 5-HT systems is due in part to their effects on DA systems.

Endogenous progenitor cells are harnessed to replace neurons lost in neurodegenerative diseases but require the development of methods to stimulate their proliferation and differentiation. Researchers are also exploring a process called trans-differentiation -"tricking" cells of the bone marrow to produce brain cells or muscle cells.

5-HT and GABA as therapeutic agents for cell proliferation and differentiation is a novel approach. Our earlier studies showed that 5HT and GABA acting through specific receptor subtypes $5HT_2$ (Sudha & Paulose, 1998) and $GABA_B$ (Biju *et al.,* 2001) respectively, control cell proliferation and act as a co-mitogens. Also, it plays a major role in spinal cord regeneration and functional recovery by re-establishing the connections along with BMC (Paulose *et al.,* 2009). Our experiments on PD done using different neurotransmitters – 5-HT and GABA with and without pluripotent bone marrow cells extracted from the same individual given to the site of damage re-established the connection and the functional recovery was observed.

5-HT and GABA has a functional role in the corpous stritum this leads to reversal of DA receptors in the substantia nigra region of PD rats (Jes *et al.,* 2010; Nandhu *et al.,* 2009). In our present study we demonstrated using specific fluorescent dyes - PKH2GL to bone marrow cells and nestin to premature neurons -the autologous differentiation of bone marrow cells to neurons (O'Sullivan *et al*., 2010). Our results proved that BMC differentiate to neuronal cells when autologous combination treatment was given to SNpc, they differentiated to both

neuronal and glial cell types. PKH2GL tagged BMC when injected into the brain it started expressing both nestin and GFAP. The BMC division and differentiation was observed with 5-HT and GABA.

In the present work, the effects of 5-HT, GABA and bone marrow cell supplementation intranigrally to the substantia nigra and individually on unilateral Rotenone infused PD rats were analyzed. Real-time polymerase chain reaction work showed significant down-regulation in gene expression of Dopamine D_1 and Dopamine D_2 in the substantia nigra of Parkinsonism induced rats and these have been confirmed using immunofluorescent antibodies specific to Dopamine D_1 and Dopamine D_2 receptors. 5-HT, GABA and Bone Marrow Cells in combination functionally reversed in dopamine receptors in rotenone induced Hemi-parkinsonism rats.

5-HT and GABA could potentially regulate the function of DA neurons through actions on midbrain DA cell bodies and/or DA terminals. Several subtypes of 5-HT receptors, including the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_3 and 5-HT_4 receptors, act to facilitate DA release (Giovanni *et al.,* 2001; Matteo *et al.,* 2001). Dysfunction of DA, 5-HT and GABA neurotransmission underlie the pathophysiology of most of the neuropsychiatric disorders, including PD (Esposito *et al.,* 2008). Our results confirmed the 5-HT and GABA comitogenic effect in proliferation and differentiation of the BMC to neurons in the brain by confocal studies using PKH2GL and Nestin. 5-HT and GABA are involved in a variety of cellular processes involved in regulating metabolism, proliferation and morphology (Efrain, 2001). The fine integration of these dynamic events appears to involve multiple receptor action. Gene expression of DA D_1 and DA D_2 showed significantly increased expression on rotenone infused rats compared to control. This increase in activity is due to the damage of dopaminergic neurons which produces dopamine in the normal individuals. Absence of dopamine resulted in decresed number of DA receptors subtypes in the region. However, the

antioxidant and comitogenic property of 5-HT and GABA resulted in a reversal to near control value in the combinational treatment groups.

Corpus Striatum

Serotonergic terminals have been reported to make synaptic contacts with both DA containing and non-DA containing GABA interneurones in the SNc, SNr, striatum and ventral tegmental area (VTA) (Moukhels *et al.,* 1997; Azmita and Segal 1978). These brain areas contain the highest concentration of 5-HT, with the SNr receiving the greatest input. Raphe´ projections also innervate terminal areas to which the SNc and VTA project to, the striatum and nucleus accumbens.

Striatum is anatomically the most prominent nucleus of the basal ganglia and many of the proposed functions of the basal ganglia have been linked to synaptic processing among cells in the striatum (Mallet *et al.,* 2005; Ouyang *et al.,* 2007). All classes of striatal neurons receive prominent inhibitory GABAergic inputs. These inhibitory interactions are likely to be essential for striatal processing (Fujiyama,*et al.,* 2000; Waiddvogel *et al.,* 2004).

Bone marrow stromal cells (BMSC) normally give rise to bone, cartilage, and mesenchymal cells. Recently, bone marrow cells have been shown to have the capacity to differentiate into myocytes, hepatocytes and glial cells (Sanchez-Ramos *et al.,* 2000). The central finding is that cells with major characteristics of NSCs can be efficiently generated. Bone marrow stromal cells (MSC), the non-hematopoietic precursor cells (i.e. mesenchymal stem and progenitor cells) in bone marrow, offer an alternative source of cells for treatment of neurodegenerative diseases and central nervous system (CNS) injury. These cells normally differentiate into bone, cartilage and adipose tissue (Pittenger MF, 1999) but can be experimentally induced to differentiate into cells with surface markers characteristic of neurons (Woodbury *et al.,* 2000).

The dopaminergic neurons in the SNpc extend its arm to the CS and hence are directly affected in PD. CS is mainly associated with motor coordination. We investigated Parkinson's disease damage to dopaminergic function in CS of control and experimental groups of rats. In the CS, DA D_1 and D_2 receptor showed significantly decreased activity in Rotenone-infused rats and on supplementation of BMC compared to control. This decrease in activity is due to the damage of dopaminergic neurons which produces dopamine in normal individuals. Damage of dopaminergic neurons resulted in less number of DA D_1 and D_2 receptors in the region. However, the 5-HT and GABA-supplemented group resulted in a reversal to near control value. Real-time PCR studies confirm the data obtained in receptor-binding studies and these have been confirmed using immunofluorescent antibodies specific to DA D_1 and D_2 receptors in our study.

Our experimental findings also demonstrate an increase in intracellular IP3 and cAMP content in the Parkinson induced rats. This will trigger the release of Ca^{2+} from the endoplasmic reticulum. IP3-mediated Ca^{2+} release can in turn increase mitochondrial Ca^{2+} and consequently, increase respiration and ATP production (Hajnoczky *et al.,* 2000). Excessive stimulation of dopamine receptor/ion channel complexes triggers Ca^{2+} flooding and a cascade of intracellular events that results in apoptosis (Johnston, 2005). Up regulation of pro-apoptotic Bax protein expression in the corpus striatum indicates the mitochondria mediated apoptosis in Rotenone infused rats which in turn indicates the ROS mediated neurodegeneration in the striatum. Bax, one of the major pro-apoptotic family members, exerts its effects by compromising the membrane integrity leading to leakage of apoptogenic factors such as cytochrome C into the cytosol, resulting in caspase-3 activation and demise of the cell (Shacka & Roth, 2005). 5-HT, GABA and Bone marrow cell in combination reversed the increased DA receptors and thus the IP3, cAMP content and pro-apoptotic Bax protein was down regulated when compared to the Rotenone infused rats.

α-Synuclien is localized in neuronal mitochondria. α-synuclein is highly expressed in the mitochondria in olfactory bulb, hippocampus, striatum and thalamus. (Zhang *et al.,* 2008; Liu *et al.,* 2009) The hypothesis generated is that over expression of wild-type α -synuclein protein is sufficient to cause Parkinsonism. The true function of α –synuclein protein remains elusive although a number of putative roles have been postulated in vesicle dynamics, through phospholipase D_2 and tyrosine hydroxylase inhibition in Parkinson's induced rats. (Farrer, 2006). Up regulation of neuronal protein α –synuclein expression in the corpus striatum indicates the mitochondria mediated cell death in Rotenone infused rats. CREB-dependent gene expression has been reported to play a role in such diverse processes as cell survival, plasticity, growth and development and most recently, cell death (Walton & Dragunow 2000, Finkbeiner 2000; Shimamura *et al.,* 2000). CREB is controlling neuronal survival, in part, by controlling transcription of neuroprotective genes. For example, the promoter regions for both Brain Derived Neurotrophic Factor (BDNF) and the anti-apoptotic protein, Bcl_2, each contain CRE sites (Mayr & Montminy 2001) and both of these gene products have been shown to play an important role in neuronal survival. In the present study the gene expression of CREB was down regulated in corpus striatum of Rotenone rats compared to control. cGMP signal transduction pathway is triggered by Dopamine production. Decreased dopamine in Parkinsonism leads to the reduction in cGMP content and neuronal survival. cGMP activates a protein-kinase, modulating diverse biochemical events through the phosphorylation of specific substrate protein (Scott, 1991). It has been suggested that the state of phosphorylation of the protein DARPP-32, mediated by the nitric oxide/cGMP pathway, represents an important mechanism of information arriving at striatonigral neurons (Tsou *et al.,* 1993). In addition, the nitric oxide/cGMP pathway modulates striatal release of several neurotransmitters, including DA and excitatory amino acids (Guevara-Guzmán *et al.,* 1994).

PD is characterized by a gradual degeneration of midbrain dopaminergic neurons and the accumulation of ubiquitin in cytoplasmic inclusions (Lewy bodies) (Dauer & Przedborski 2003; Hardy & Gwinn-Hardy 1998). The role of the Lewy bodies for the pathological manifestations of PD remains enigmatic. Major components of Lewy bodies are ubiquitin and ubiquitinated proteins. (Alves-Rodrigues *et al.,* 1998) In general, ubiquitination of proteins is critical for protein degradation. Proteins that are conjugated with a chain of ubiquitin moieties are targeted to the ubiquitin-proteasome system (UPS) complex, where they undergo proteolytic degradation. Polyubiquitinated proteins are enzymatically degraded to peptides, and the ubiquitin moieties released intact. (Hendil & Hartmann-Petersen, 2004). The high levels of ubiquitin and ubiquitinated proteins in Lewy bodies therefore indicate that protein degradation is impaired in PD. Our experimental findings also demonstrate an increase in ubiquitin carboxy-terminal hydroxylase in the Parkinson induced rats. Our treatment showed that 5-HT and GABA along with BMC antagonized these effects maximally which have immense clinical significance in the management of PD.

Our results confirmed the 5-HT and GABA comitogenic effect in proliferation and differentiation of the BMC to neurons in the brain. 5-HT and GABA are involved in a variety of cellular processes involved in regulating metabolism, proliferation and morphology (Azmitia 2001).

Cerebral Cortex

The cerebral cortex is critical to speech, emotion, reasoning, memory, movement and integration of information. However, dopaminergic and glutamergic pathways play a leading role in the structural and functional organization of the cortico-basocortical loops involved in PD (Hirsch *et al.,* 2000). Changes in personality and moderate or mild cognitive debilitation are found in PD. Cerebral glucose metabolism is reduced in the cerebral cortex in PD patients suffering from cognitive impairment (Yong 2007). Metabolic and neuroimaging

observations have recently documented decreased prefrontal and parietal 18F-fluorodeoxyglycose uptake in PD cases with mild cognitive deficits (Huang *et al.,* 2007; Huang et al 2008). Recent observations have demonstrated complex I deficiency (Parker, 2008) and abnormal ATP synthase and inner protein membrane prohibition expression levels (Ferrer 2007) in the frontal cortex in PD. Several reports have highlighted the need of dopamine–glutamate coactivation for a number of cortical functions (Gurden 1999; Baldwin 2002).

Parkinson's disease (PD) has been considered a paradigm of degenerative diseases of the nervous system characterized by motor impairment (Parkinsonism) due to malfunction and loss of dopaminergic neurons of the substantia nigra *pars compacta*. However, PD is a systemic disease of the nervous system with variegated clinical symptoms appearing before Parkinsonism and due to the involvement of selected nuclei of the medulla oblongata, pons, autonomic nervous system and olfactory structures, among others. Furthermore, recent clinical data have shown modification in behaviour, personality changes and cognitive impairment leading to dementia. Lewy pathology, hallmark of PD, in the cerebral cortex does not correlate with cognitive impairment. However, recent studies have shown abnormal mitochondria content and function and increased oxidative stress and oxidative responses in the cerebral cortex in PD (Ferrer, 2009). It has been previously reported that disorders in PD largely occur due to the imbalance of inhibitory and excitatory processes in cortical and subcortical neuronal circuits (Elena 2010).

In our studies we observed an elevated cAMP and IP3 level in the cerebral cortex of Rotenone infused rats. The elevated IP3 level causes extra cellular release of Ca^{2+}, which in turn enhance metabolic stress on mitochondria that leads to excessive oxidative phosphorylation and increased production of reactive oxygen species. If the matrix Ca^{2+} level rises too high, then deleterious changes in mitochondrial structure occur. In particular, mitochondria can swell and rupture or undergo permeability transition, thereby releasing several pro-apoptotic factors

into the cytoplasm, such as cytochrome C, second mitochondrial activator of caspases (SMAC/Diablo) or apoptosis-inducing factor (AIF) (Orrenius *et al.,* 2003). Our study showed an increased activity of Bax gene expression in the cerebral cortex of the Rotenone infused rats which indicated the ROS mediated neurodegeneration in the cerebral cortex. Apoptosis whether caspase-dependent or caspase-independent, has been implicated as one of the important mechanisms leading to the death of dopaminergic neurons in the substantia nigra of Parkinson's disease (Schulz 2006). CREB is a transcription factor that plays an important role in neuronal survival, in part by controlling the transcription of neuroprotective genes (Finkbeiner 2000). The promoter regions of the genes for brain-derived neurotrophic factor (BDNF) and the pro-survival protein Bcl-2 contain cAMP response elements (CREs) (Mayr 2001). Rotenone administration causes a decrease in transactivation of the CRE promotor, resulting in reduced expression of downstream CREB-regulated genes (Chalovich 2006). In the present study the gene expression of CREB was down regulated in cerebral cortex of Rotenone compared to control. Even though cAMP level was increased, the CREB expression was decreased. Enhanced activation of the DA receptors leads to the production of second messengers. But its acute and prolonged action triggers the cell death pathways by activating pro apoptotic genes like Bax, bad and destabilizing jun- fos complex. The activation of apoptotic pathways down regulates the CREB expression thereby blocking the cAMP signalling cascade in PD rats. Down regulation of CREB is a consequence of apoptotic pathway activation and down regulation of DA receptor function. These findings suggest that decreased CREB expression is the result of cell loss. BMC administration along with the 5-HT and GABA reversed the expression of Bax and CREB to near control.

PD increased oxidative damage, abnormal α-synuclein solubility and aggregation and increased α-synuclein nitration in the cortex (Gomez & Ferrer 2010). Normally an unstructured soluble protein, alpha-synuclein aggregates in

the form of Lewy bodies and Lewy neurites in the frontal cortex in PD (Ferrer 2007; Arima *et al.,* 1998). High concentrations of Rotenone results in neuronal death accompanied by a decrease of the monomeric form of α-synuclein, leading to both decreased synthesis of the protein and its increased mono-ubiquitination accompanied by nuclear translocation (Monti *et al.,* 2007). Studies by Pierson *et al.,* (2007) showed an increased level of unconjugated ubiquitin in the dorsal striatum of the dopamine depleted hemisphere. Normal α-synuclein expression is essential for the viability of primary neurons. Gene expression studies of alpha-synuclein in the cerebral cortex showed a significant down regulation in the Rotenone induced rats compared to control. This indicates the reduced expression of normal α-synuclein in the PD rats. Up regulation of ubiquitin carboxy-terminal hydrolase gene expression in cerebral cortex confirmed the increased level of unconjugated ubiquitin in the Rotenone infused rats. 5HT, GABA and BMC combinational treatment significantly reversed these changes back to control.

We investigated the Parkinson's disease damage to dopaminergic functional regulation in CC of control and experimental groups of rats. The increased expression of DA D_1 and D_2 receptors in the cerebral cortex due to the supersensitivity of the DA D_1 and D_2 receptor to DA owing to loss of dopaminergic neurons in the substantia nigra as a result of administration of rotenone. Absence of dopamine results in less number of DA receptors in the region. Receptor supersensitvity, leading to imbalance between the direct and indirect striatal output pathways, is believed to underlie some of the motor complications that occur following chronic treatment with L-DOPA or DA agonists (Obeso *et al.,* 2000). In the absence of consistent alterations in the levels of receptor expression, altered functional responses of DA D_1 and D_2 receptors result from changes in signaling mechanisms (Corvol *et al.,* 2004). However the antioxidant and co-mitogenic property of 5-HT and GABA resulted in a reversal to near control value in the 5-HT, GABA and Bone marrow cells supplemented groups. Real-time polymerase chain reaction (PCR) results showed decrease in the

gene expression of DA D_1 and DA D_2 receptors in the CC of Parkinsonism induced rats and these have been confirmed using immunofluorescent antibodies specific to DA D_1 and DA D_2 receptors in our study.

Autologous BMC to treat neurological disorders offers several unique advantages over other cell replacement therapies. For one, immunological reactions are avoided and it also bypasses many of the ethical issues that surround the use of embryonic cells. Our study demonstrated that BMC administration alone cannot reverse the above said molecular changes occurring during PD. 5-HT, GABA and BMC in combination potentiates a restorative effect by reversing the alterations in DA receptor binding and gene expression that occur during Parkinson's disease.

Cerebellum

The cerebellum is the battery of the brain. Parkinson's disease is a progressive neurodegenerative disorder characterized by selective degeneration of dopaminergic neurons in substantia nigra *pars compacta* leading to marked reduction of dopamine levels in the cerebellum.

5-HT is widely expressed within the central nervous system, where it is thought to play a major role in the regulation of neuronal network excitability. In rats, 5HT-containing neurons originating from the dorsal and median raphe nuclei innervate forebrain dopamine-containing areas. However, this interaction between brain DA and 5HT-containing neuronal systems is complex, and the effect produced appears to dependent on the relative activity of each system (Jenner *et al.,* 1983; Berg *et al.,* 2008).

In the cerebellum, DA receptor sub types showed significantly increased activity in PD rats compared to control rats whereas its activity reversed to near control in the 5-HT, GABA and Bone marrow cells supplemented groups. Real time PCR studies were conducted to evaluate the DA functional regulation at the mRNA level during PD and supplementation with 5-HT, GABA and Bone

marrow cells. We obtained an up regulation in the DA receptor subtypes mRNA during PD. 5-HT, GABA and Bone marrow cells supplemented groups reversed the DA to near control.

Our experimental findings also demonstrate an increase in intracellular IP3 and cAMP content in the cerebral cortex of Parkinsonism induced rats. Inositol phosphates are known to regulate membrane trafficking, glucose metabolism, cytoskeletal organisation and intracellular Ca^{2+} homeostasis-particularly the release of stored Ca^{2+} *via* IP3 receptors. Excessive Ca^{2+} overload in cells have been reported to cause apoptosis. Boehning and co-workers (2003) demonstrated a small amount of cytochrome C released from mitochondria can bind to and promote Ca^{2+} conductance through IP3 in the endoplamic reticulum membrane. The released Ca^{2+} further triggers mass exodus of cytochrome C from all mitochondria in the cell and thus activating the caspase and nuclease enzyme of the apoptotic process.

The 5-HT and GABA facilitates neural differentiation and regenerative processes of the neurons (Nandhu *et al.,* 2011; Jes *et al.,* 2010), causing the DA neurons to secrete DA although not efficient as in the control group, thus leading to a reversal of the receptor gene expression to control level owing to decreased super sensitivity of the DA receptor subtypes in the cerebellum. The increased expression of DA receptor subtypes in the cerebellum of PD is due to the supersensitivity of the DA receptor subtypes. It is because of the loss of dopaminergic neurons in the substantia nigra as a result of rotenone administration. The Bone marrow cells alone treated group did not show any reversal compared to the other groups.

Our experimental results thus showed that GABA and 5-HT play important role in the differentiation of bone marrow cells in to neurons re-establishes the connections and functional recovery of Parkinson's disease. All our studies including behavioural and Real time PCR support the above statement. We conclude from our studies that 5-HT and GABA treatment potentiates a

therapeutic effect by reversing the alterations in DA receptor subtypes binding and gene expression that occur in cerebellum during Parkinson's disease.

Brain stem

The destruction of dopaminergic neurons in the substantia nigra constitutes an intermediate step in a broader neurodegenerative process rather than a unique feature of Parkinson's disease, as a consistent pattern of progression would exist, originating from the medulla oblongata/pontine tegmentum. However, if such a regular neurodegenerative pattern were to exist, consistent damages would be found in the brain stem (Thomas 2009).

The present work was carried out to study the changes in DA receptor subunits gene expression in the brain stem of control and Parkinsonism induced rats and to evaluate the role of 5-HT, GABA and Bone marrow cells supplementation. 5-HT and GABA as therapeutic agents for cell proliferation and differentiation is a novel approach. 5-HT and GABA acting through specific receptor subtypes 5-HT_2 and $GABA_B$ respectively, control cell proliferation and act as co-mitogens. In our present study we demonstrated the autologous differentiation of BMC to neurons using combinations of 5-HT and GABA. DA receptors activity in rotenone infused unilateral Parkinsonism induced rats. But the BMC treated group of our studies did not show significant change as compared to the other groups which is due to slow division and differentiation of BMC when it is administrated alone.

An increased production of IP3 and cAMP in rotenone infused rats which are mediated through the enhanced DA receptors. This will trigger the release of Ca^{2+} from the endoplasmic reticulum. IP3-mediated Ca^{2+} release can in turn increase mitochondrial Ca^{2+} and consequently, increase respiration and ATP production (Hajnoczky *et al.,* 2000). Excessive stimulation of DA receptor/ion channel complexes triggers Ca^{2+} flooding and a cascade of intracellular events that results in apoptosis (Johnston 2005). Up regulation of pro-apoptotic Bax protein

expression in the brain stem indicates the mitochondria mediated apoptosis in rotenone infused rats. 5-HT, GABA and BMC in combination reversed the increased DA receptors compared to the rotenone infused rats.

Hippocampus

In rats, 5-HT neurons in the Hippocampus raphe are among the first neurons to differentiate in the brain and play a key role in regulating neurogenesis (Kligman & Marshak 1985) 5-HT and GABA has a direct role in neuronal maturation and accelerate its differentiation (Marois & Croll 1992; Rodriguez 1994). 5-HT and GABA could potentially regulate the function of DA neurons through actions on midbrain DA cell bodies and/or DA terminals. 5-HT and GABA has a direct role in neuronal maturation and accelerate its differentiation (Rodriguez 1994). The present study focused on the hippocampal dysfunction occurring in the PD and the effectiveness of 5-HT, GABA and autologous bone marrow cells transplantation in these alterations.

Up regulation in the IP3 activity increased the intracellular Ca^{2+} which caused enhanced metabolic stress on mitochondria that leads to excessive oxidative phosphorylation and increased production of reactive oxygen species. Our study showed an increased activity of Bax gene expression in the hippocampus of the rotenone infused rats which indicated the ROS mediated neurodegeneration in the hippocampus. Bax, one of the major pro-apoptotic family members, exerts its effects by compromising the membrane integrity leading to leakage of apoptogenic factors such as cytochrome C into the cytosol, resulting in caspase-3 activation and demise of the cell (Shacka & Roth, 2005).

CREB is a transcription factor that plays an important role in neuronal survival, in part by controlling the transcription of neuroprotective genes (Finkbeiner, 2000). Agents that disrupt the activity of CREB specifically block the formation of long-term memory, whereas agents that increase the amount or activity of the transcription factors accelerate the process (Alcino *et al.,* 1998).

The study of the DA receptors expression in relation with CREB phosphorylation in PD is an important step towards elucidating the relationship between molecular adaptations and behavioural consequences. Our findings showed a significant down regulation of CREB in the hippocampus of rotenone infused rats. Even though cAMP level was increased, the CREB expression was decreased. Enhanced activation of the DA receptors leads to the production of second messengers. The activation of apoptotic pathways down regulates the CREB expression thereby blocking the cAMP signaling cascade in PD rats. These findings suggest that decreased CREB expression is the result of cell loss.

High concentrations of rotenone results in neuronal death accompanied by a decrease of the monomeric form of α-synuclein, leading to both decreased synthesis of the protein and its increased mono-ubiquitination accompanied by nuclear translocation (Monti *et al.,* 2007). α- Synuclien and β Synuclien positive lesions predominantly localized to abnormal aggregates in the mossy fiber terminals that synapse on hilar neurons, these abnormal processes impair synaptic transmission in hippocampal perforant pathway projections critical to memory and behavior (Galvin et al. 1999). Normal α-synuclein expression is essential for the viability of primary neurons. Gene expression studies of α-synuclein in the hippocampus showed a significant down regulation in the rotenone induced rats compared to control. This indicates the reduced expression of normal α-synuclein in the PD rats. Most of the effects of 5-HT and GABA on DA neurons are indirect, mediated *via* actions on complex neuronal circuitry, rather than direct effects on DA terminals (Poewe 2009). Since 5-HT and GABA receptor subtypes are differently distributed in dopaminergic brain regions, it is possible to specifically "target" individual brain regions with serotonergic ligands and thereby affecting dopaminergic function selectively in these areas.

We conclude from our studies that 5-HT and GABA along with BMC potentiates a restorative effect by reversing the alterations in DA receptors binding and gene expression that occur during Parkinson's disease. Thus, it is evident that

5-HT and GABA along with BMC to Rotenone infused rats renders protection against oxidative, related motor and cognitive deficits which makes them clinically significant for functional reestablishment and recovering from PD symptoms.

Behavioural Deficits in Parkinsonism induced Rats

We have evaluated the behavioural response of control, Parkinsonism induced and experimental groups of rats which includes rotaroad test, social interaction test, narrow beam test and grid walk test. The rotarod experiment demonstrated the impairment in the motor function and co-ordination in the Parkinsonism induced rats. Parkinsonism induced showed lower fall off time from the rotating rod when compared to control suggesting impairment in their ability to integrate sensory input with appropriate motor commands to balance their posture and at the same time adjust their limb movements on the metallic rod which is indicative of cerebellar dysfunction. Many other brain regions have been associated with timing tasks including the dorsal lateral premotor cortex, inferior parietal lobe, supplementary motor area, superior temporal gyrus, caudal putamen, ventrolateral thalamus and inferior frontal gyrus (Rao *et al.,* 1997; Jancke *et al.,* 2000; Lewis & Miall 2003). Abnormalities of some of these areas, such as the inferior frontal gyrus and superior temporal gyrus (Abell *et al.,* 1999; Castelli *et al.,* 2002) have been reported in autistic subjects rendering it difficult to isolate the cerebellum in this task. However, increased timing variance has been observed in patients with cerebellar disorders (Ivry *et al.,* 1988). Loss of coordination of motor movement, inability to judge distance and timing, incapacity to perform rapid alternating movements and hypotonia have been reported during cerebellar damage (Gowen & Miall, 2005). This study demonstrates the treatment of 5-HT, GABA and bone marrow cells, has modulating effect on the Parkinson's associated motor defects. The treatment of 5-HT, GABA and Bone marrow cells

to Parkinsonism induced rats increased the fall off time from the rod when compared to control rats.

Behaviour in rodents is determined by the conflict between the drive to explore the unknown area/object and the motivation to avoid potential danger. In Elevated plus Maze Test, the Parkinsonism induced rats exhibit significant alterations in its behavioural response due to damage to the cortical neurons. The PD rats remained for longer period in closed arms of elevated plus-maze which is characteristic to anxio-depressive traits. It has been demonstrated that the preference shown for the closed arms reflects an aversion toward the open arms, caused by fear or anxiety induced by the open space in the elevated plus maze test (de Souza *et al.,* 2007). The head dipping attempt, stretched attend posture and grooming attempts were also greatly reduced supporting the anxiogenic condition as a result of Parkinson's disease stress. The 5-HT, GABA and bone marrow cells treatment during PD rats reversed the behavioural abnormalities to control. 5-HT, GABA and Bone Marrow Cells in combination treatment to PD rats reversed anxiety effects, as indicated by increase of the time spent in the open arm and increase in head dipping attempt, stretched attend posture and grooming attempts compared to PD rats.

In the social interaction test, PD rats spent less time in active interactions in the novel environment. Attempts at allogrooming, sniffing the partner, following were reduced when compared to control rats and 5-HT, GABA and BMC in combination treatment to control rats. Administration of combinational treatment to Parkinson's rats resulted in an increase in the time spent in social interaction to near control values.

PD rats showed increased number of foot slips in grid walk test and decreased time spent in narrow beam test compared to control. This indicated the motor dysfunction in the PD rats. Moreover 5-HT, GABA and BMC in combination treatment improved the motor performance of the PD rats.

In the present work, the effects of 5-HT, GABA and BMC supplementation intranigrally to substantia nigra as treatment individually and in combination on rotenone induced Hemi-parkinson's disease in rats were analyzed. Dopaminergic binding parameters investigated its role in the regulation of dopamine receptor subtypes in the brain regions of the experimental rats. Gene expression analysis of receptor specific probes for DA D_1, DA D_2, pro apoptotic protein Bax, transcription factor – CREB, α-synuclien and ubiquitin in the brain regions of PD rats and treatment groups were studied. Confocal studies with specific antibodies in brain slices were done to confirm the binding studies and gene expression analysis using specific probes. Behavioural response in rotaroad, social interaction, Elevated plus maze, Grid walking, Narrow beam test was carried out to assess the motor learning and cognition deficit in rotenone induced PD rats.

We conclude from our studies that 5-HT and GABA along with BMC potentiates a restorative effect by reversing the alterations in DA receptors binding and gene expression that occur during Parkinson's disease. Thus, it is evident that 5-HT and GABA along with BMC to rotenone infused rats renders protection against oxidative, related motor and cognitive deficits which makes functional re-establishment and recovery from PD symptoms. Our results confirmed the 5-HT and GABA co-mitogenic effect in proliferation and differentiation of BMC to neurons which has immense therapeutic significance in the management of PD.

Conclusion

Parkinson's disease is characterized by progressive cell death in the substantia nigra *pars compacta*, which leads to dopamine depletion in the striatum and indirectly to cortical dysfunction. In Parkinson's disease dopamine transmission is inhibited in the substantia nigra, basal ganglia and corpus striatum of the brain. Molecular details showed oxidatively-damaged synuclein, forms Lewy bodies. These aggregates are thought to alter intracellular dynamics and cause apoptotic cell death of dopaminergic cells**.**

In the present study, the effects of 5-HT, GABA and Bone Marrow Cells infused intranigrally to substantia nigra individually and in combinations on unilateral rotenone infused Parkinsonism induced rats. Scatchard analysis of DA, DA D_1 and D_2 receptors in the corpus striatum, cerebral cortex, cerebellum, brain stem and hippocampus showed a significant increase in the Brain regions of rotenone infused rat compared to control. Real Time PCR amplification of DA D_1, D_2, Bax and ubiquitin carboxy-terminal hydrolase were up regulated in the brain regions of rotenone infused rats compared to control. Gene expression studies of ά-Synuclien, cGMP and Cyclic AMP response element-binding protein showed a significant down regulation in Rotenone infused rats compared to control. Behavioural studies were carried out to confirm the biochemical and molecular studies.

Our study demonstrated that BMC administration alone cannot reverse the above said molecular changes occurring in PD rat. 5-HT and GABA acting through their specific receptors in combination with bone marrow cells play a crucial role in the functional recovery of PD rats. 5-HT, GABA and Bone marrow cells treated PD rats showed significant reversal to control in DA receptor binding and gene expression. 5-HT and GABA have co-mitogenic property. Proliferation and differentiation of cells re-establishing the connections in Parkinson's disease

facilitates the functional recovery. Thus, it is evident that 5-HT and GABA along with BMC to rotenone infused rats renders protection against oxidative, related motor and cognitive deficits which makes them clinically significant for cell-based therapy. The BMC transformed to neurons when co-transplanted with 5-HT and GABA which was confirmed with PKH2GL and nestin. These newly formed neurons have functional significance in the therapeutic recovery of Parkinson's disease.

Summary

1) The present work was carried out to study the changes in dopamine receptor subtypes gene expression in the substantia nigra, corpus striatum, cerebral cortex, cerebellum, hippocampus, and brain stem of control and Parkinsonism induced rats and to evaluate the Role of 5-HT, GABA and Bone Marrow Cells supplementation individually and in combinations.

2) Rotenone induced Hemi-parkinsonism model of rats were used for the study of the dopamine receptor subtypes and their functional regulation by 5-HT, GABA and Bone Marrow Cells in Parkinson's disease.

3) A prominent change in dopamine receptor subtypes function was observed in the brain regions of Parkinsonism induced rats which contribute to reduced motor and non motor functions. 5-HT, GABA and Bone Marrow Cells in combinations functionally reversed the changes in dopamine receptor subtypes to near control. There was no significant reversal in BMC alone treated rats.

4) The expression of pro-apoptotic gene – Bax was up regulated in Parkinsonism induced rats indicating apoptotic cell death in the brain regions. 5-HT, GABA and Bone Marrow Cells in combinations functionally reversed the alteration in pro-apoptotic gene to near control. BMC alone treated group did not show any significant reversal to control.

5) Dopamine content was decreased in the brain regions - substantia nigra, corpus striatum, cerebral cortex, cerebellum, brain stem and hippocampus of rotenone induced PD rats. Treatment with 5-HT, GABA and Bone Marrow Cells individually and in combinations significantly reversed the dopamine content to near control. There was no significant reversal in BMC alone treated rats.

6) In Parkinsonism induced rats, general dopamine, DA D_1 and D_2 receptors were up regulated in the brain regions. 5-HT, GABA and Bone Marrow Cells in combinations functionally reversed the alteration in Dopamine receptors to near control. BMC alone treated rats did not show any significant reversal to control.

7) Gene expression analysis of receptor specific probes for DA D_1 and D_2 receptors were up regulated in the brain regions. 5-HT, GABA and Bone Marrow Cells in combinations functionally reversed the alteration in dopamine receptors to near control. BMC alone treated rats did not show any significant reversal to control.

8) The dopamine receptor changes in the brain regions were confirmed by confocal studies using receptor specific antibodies in the brain slices. Treatment with 5-HT, GABA and Bone marrow cells individually and in combinations significantly reversed to near control. BMC alone treated rats did not show any significant reversal to control.

9) In our present study we demonstrated using specific fluorescent cell tracking dye - PKH2GL to bone marrow cells and Nestin to premature neurons – the autologous differentiation of bone marrow cells to neurons. Nestin expression study confirmed that 5-HT and GABA induce the differentiation and proliferation of the bone marrow cells to neurons in the substantia nigra of rats. Also, the treatment in combinations of 5-HT, GABA and Bone marrow cells showed reversal of dopamine receptors and behaviour abnormality shown in the Parkinsonism induced rat model. Thus our results demonstrate for the bone marrow cells of the same individual differentiate to neurons in the presence of co-mitogenic 5-HT and GABA in the substantia nigra. The therapeutic significance in Parkinson's disease is of prominence.

10) Second messengers- cAMP and IP3 contents were increased and cGMP content was decreased in Parkinsonism induced rats. Enhanced cAMP and IP3 levels lead to increased intracellular Ca^{2+} and in turn triggers the apoptotic cascade. 5-HT, GABA and Bone Marrow Cells in combinations functionally reversed the alteration in Second messengers to near control. BMC alone treated group did not show any significant reversal to control.

11) cGMP signal transduction pathway is triggered by dopamine production. Decreased DA levels in Parkinsonism lead to the reduction in cGMP content and neuronal survival. 5-HT, GABA and Bone Marrow Cells in combinations functionally reversed the alteration in second messengers to near control. There was no significant reversal in BMC alone treated rats.

12) Cyclic AMP response element binding protein (CREB) showed decreased expression in Parkinsonism induced rats compared to control. Down regulated CREB was the result of neuronal cell death in PD rats. 5-HT, GABA and Bone Marrow Cells in combinations functionally reversed the alteration in transcription factors to near control. BMC alone treated rats did not show any significant reversal to control.

13) Behavioral studies showed deficits in motor learning in rotenone induced hemi-Parkinson's rat. Grid Walk and Rotarod test showed deficit in motor co-ordination. Narrow Beam Test showed deficit in motor control. Behavioral alterations in PD rats were reversed significantly by 5-HT, GABA and Bone Marrow Cells in combinations to near control. There was no significant reversal in BMC alone treated rats.

Thus our results showed significant changes in the DA D_1 and D_2 receptors, gene expression of DA receptors subtypes, transcription factor CREB, pro-apoptotic protein Bax, regulatory protein ubiquitin carboxy-terminal hydrolase, neural protein of α-synuclein and second messengers – IP3, cAMP, cGMP contents in brain regions of PD rats. 5-HT, GABA and Bone marrow cells supplemented in combinations reversed the changes to control. Also, the behavioural studies showed reversal of motor learnig deficits in PD rats to control. 5-HT, GABA and Bone Marrow Cells individually and in combinations functionally reversed in DA receptors in rotenone induced Hemi-parkinsonism induced rats. The BMC transformed to neurons when co-transplanted with 5-HT and GABA which was confirmed with PKH2GL and nestin. These newly formed neurons have functional significance in the therapeutic recovery of Parkinson's disease.

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List of Publications

1) Jes Paul, Nandhu. M. S, Korah P Kuruvilla and C S Paulose. Dopamine D_1 and D_2 receptor subtype's functional regulation in corpus striatum of unilateral rotenone lesioned Parkinson's rat model: effect of serotonin, dopamine and norepinephrine. Neurological Research 2010 (in press).

2) Jes Paul, Korah P Kuruvilla, Peeyush Kumar T and C S Paulose (2010). Dopamine D_1 and D_2 receptor subtypes functional regulation in the cerebral cortex of rotenone induced Parkinson's disease rats: Effect of dopamine, serotonin and nor epinephrine. Parkison's and related disorders. (Accepted) PARKRELDIS-D-10-00158R1. (2010)

3) Anju T R, **Jes P**, Nandhu M S, Paulose C S. BAX mediated apoptosis and behavioural deficits in neonatal rats exposed to hypoxic stress: Protective role of glucose, oxygen and epinephrine. Journal of Brain and cognition (Accepted)

4) Akash K George, **Jes Paul**, Sankara B. Kaimal and C.S. Paulose. Decreased Cerebral Cortex and Liver 5-HT 2A Receptor Gene Expression and enhanced ALDH Activity in Ethanol Treated Rats and Hepatocyte Cultures. Journal of Neurological Research.2009 NER 1723 (In press)

5) Jobin Mathew, **Jes Paul,** Nandu MS. and C. S. Paulose. Increased Excitability and Metabolism in Pilocarpine Induced Epileptic Rats: Effect of Bacopa monnieri. Fitoterapia doi:10.1016/j.fitote.2010.01.017 (In Press).

6) Jobin Mathew, **Jes Paul**, M.S. Nandu, C.S. Paulose (2009). Bacopa monnieri and Bacoside -A for ameliorating epilepsy associated behavioral deficits, Fitoterapia, doi:10.1016/j.fitote.2009.11.005 (In Press).

7) M.S. Nandhu **Jes Paul** Jobin Mathew T. Peeyush Kumar C.S. Paulose (2009) GYKI-52466: A potential therapeutic agent for glutamate-mediated excitotoxic injury in Cerebral Palsy, Medical Hypotheses, doi:10.1016/j.mehy.2009.10.033

8) Jobin Mathew**, Jes Paul**, Naijil George and C. S. Paulose Special learning Deficit and Hippocampal Dysfunction in Temporal Lobe Epilepsy: Effect of Bacopa monnieri and Bacoside A. Pharmacology biochemistry and behaviour, Manuscript Number: PBB-D-10-00061 (In Press).

9) Pretty Mary Abraham, **Jes Paul** & C. S. Paulose. Down regulation of cerebellar 5-HT_{2A} receptor in streptozotocin induced Diabetic Rats: Antagonism by pyridoxine and *Aegle marmelose*. Brain Research Bulliten. 10.1016/j.brainresbull.2010.02.005 (in press) (2010).

10) Anju T. R, Nandhu M S, **Jes Paul**, Paulose C. S. Endocrine regulation of neonatal hypoxia: Role of glucose, oxygen and epinephrine supplementation. Journal of Fetal and pediatric pathology (Accepted)

11) Anitha M, Nandhu M S, Anju T R, **Jes Paul** and C S Paulose. Targeting Glutamate mediated excitotoxicity in Huntington's disease: Neural progenitors and partial Glutamate antagonist, a new therapeutic strategy. Med Hypotheses.(In Press)

12) Sherin Antony, Peeyush Kumar T, **Jes Paul**, Jayanarayanan S and C S Paulose. Effect of Insulin Induced Hypoglycaemia on Hippocampal Cholinergic Receptor

Function in Diabetic and Control rats. Canadian journal of physiology and pharmacology (Accepted).

13) Peeyush Kumar T, Savitha Balakrishnan, Sherin Antony, Anju T R, and **Jes paul**. Cholinergic, Dopaminergic and Insulin Receptors Gene Expression in the Cerebellum of Streptozotocin Induced Diabetic Rats: Functional Regulation with Vitamin D3 Supplementation. Pharmacology Biochemistry and behavior 2010 (doi: 10.1016/j.pbb.2010.01.008).

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16) Nandhu M S, **Jes Paul,** Korah P Kuruvilla, Anitha Malat, Chinthu Romeo and C. S. Paulose. Enhanced glutamate, IP3 and cAMP activity in the cerebral cortex of Unilateral 6-hydroxydopamine induced Parkinson's rats: Effect of 5-HT, GABA and bone marrow cell supplementation. Journal of Biomedical Sciences (Accepted).

17) Peeyush Kumar, **Jes Paul,** Sherin Antony, Anu Joseph, and C.S. Paulose. Functional Regulation of Cholinergic, Insulin, and Vitamin D3 Supplimentation in the

Brain Stem of Steptozotocin Induced Diabetic Rats. Journal of Neuro chemistry International (Accepted).

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16) **Jes Paul**, Pretty Mary Abraham, Sherin Antony, Anju T.R. and C.S.Paulose. Dopamine D1 and D2 Receptor Subtypes Functional Regulation in Cerebellum of Unilateral Rotenone Lesioned Parkinson's Rat Model: Effect of Serotonin, Dopamine and Norepinephrine. Journal of International Journal of Developmental Neuroscience. (Communicated).

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22) Nandhu M S, **Jes Paul,** Korah P Kuruvila, Pretty M Abraham, Sherin Antony and C. S. Paulose. Cerebellar dysfunction leads to impaired motor coordination in unilateral 6-hydroxydopamine lessioned Parkinson's rat. Journal of Brain Research bulletin. (Communicated).