Evaluation of neuroprotective role of ethanol extract of *Pterocarpus marsupium Roxb.* bark against monosodium glutamate-induced excitotoxicity model in SD rats

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IN

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OCTOBER – 2016

Certificates

EVALUATION CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"Evaluation of neuroprotective role of ethanol extract of *Pterocarpus marsupium Roxb.* bark against monosodium glutamate-induced excitotoxicity model in SD rats",** submitted to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment to the requirement for the award of Degree of **Master of Pharmacy** in **Pharmacology**, is a bonafide work carried out by **Mr. PRABAKARAN.K [Reg.No.261425227]**, during the academic year 2015-2016, under my guidance and direct supervision in the Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Kumarapalayam.

Internal Examiner **External Examiner**

CERTIFICATE

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DECLARATION

I hereby declare that the dissertation entitled **"Evaluation of neuroprotective role of ethanol extract of *Pterocarpus marsupium Roxb.* bark against monosodium glutamate-induced excitotoxicity model in SD rats",** has been carried out under the guidance and supervision of **Dr. R.SHANMUGA SUNDARAM, M.Pharm., Ph.D.,** Vice Principal, Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Kumarapalayam, in partial fulfillment of the requirements for the award of degree of **Master of Pharmacy** in **Pharmacology** during the academic year 2015-2016.

I further declare that, this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma associate ship and fellowship or any other similar title.

Place: Kumarapalayam **Mr. PRABAKARAN.K,**

Date: **Reg.No.261425227**

Dedicated to
Almighty,
My beloved family,
Teachers and
Friends.

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Mr. PRABAKARAN.K,
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1. Introduction

1.1 Neurodegeneration

Neurodegenerative disorders are a heterogeneous group of diseases of the nervous system. Due to the prevalence, morbidity, and mortality of the neurodegenerative diseases, they represent significant medical, social and financial burden on the society. Many of the Neurodegenerative disorders are hereditary, some are secondary to toxic metabolic processes, and others result from infections. These are including the brain, spinal cord and peripheral nerves that have many different etiologies. Neuropathologically, these are characterized by abnormalities of relatively specific regions of the brain and specific populations of neurons. The degenerating neuron clusters in the different diseases determine the clinical phenotype of that particular illness. Recent investigations in medical genetics have identified specific genes for various neurodegenerative disorders and specially bred animal models have begun to be used to study the etiological factors and underlying pathogenic mechanisms (*Singh et al., 2004*). Over the last decade there has been an incomprehensible increase in the number of neurodegenerative diseases, especially Alzheimer's disease (AD), Parkinson's disease (PD) and Amyotrophic lateral sclerosis (ALS), that appears to go beyond the normal ageing of the population (*Blaylock, 1998*).

Neurodegenerative diseases lead to nervous system dysfunction by the result of the gradual and progressive loss of neural cells. Neurodegenerative disorders are characterized by progressive and irreversible loss of neurons from specific regions of the brain. Prototypical neurodegenerative disorders include AD, PD, Huntington's disease (HD), and ALS. Even though the pathology and the pathogenesis are distinctly different, they share a common pathogenic mechanism in the process of neuronal cell death and degradation. The common mechanisms include: (a) neuronal injury resulting from the presence of excess glutamate in the brain; (b) energy, metabolism and ageing; (c) selective vulnerability, characterized by the exquisite specificity of the disease processes for particular types of neuron; (d) genetic predisposition, playing an important role in the etiology of neurodegenerative disease; infectious agents and environmental toxins have also been proposed as etiologic agents; (e) oxidative

stress, where neurons depend on oxidative metabolism for survival, a consequence of this process is the production of reactive compounds such as hydrogen peroxide and oxyradicals (*Standaert & Young, 2006*); and (f) Inflammation, due to increased levels of proinflammatory cytokines (Interleukins - IL-1, IL-2, IL-6), Interferon-γ (INF-γ), Proteases, Complement proteins, S100β, Tumor necrosis factor-α (TNF-α), etc. These agents other than the mediators can lead to DNA damage, peroxidation of membrane lipids and neuronal death. Stress, hypoxia, ischemia, metabolic alterations like atherosclerosis and diabetes and neurohumoral changes like hypertension are also implicated in neurodegeneration.

Neurodegenerative diseases comprise a wide range of diseases that share the common characteristic of progressive loss of structure or function of neurons and glial cells in the brain and spinal cord. Many neurodegenerative diseases are a result of neuronal loss, although glial cells are also involved (*Glass et al., 2010*).

Neurodegenerative diseases present a chronic and slowly progressive process. Neurons in neurodegenerative diseases are affected by neuronal dysfunction at the level of synaptic transmission, synaptic contacts, and axonal and dendritic degeneration. In different neurodegenerative diseases, neurite degeneration and cell loss of neurons are present within specific neurotransmitter populations. In addition, numbers of functional neurons in neurogenic regions, and adult neurogenesis are altered or decreased. Adult neurogenesis increases after several acute pathologic stimuli, including stroke, seizure and acute trauma (*Arvidsson et al., 2002; Rice et al., 2003; Parent, 2007*). Brain regions differ in their vulnerability to aging; some regions that are very sensitive to age-related neurodegenerative changes are the dentate gyrus (DG) of the hippocampus, subiculum (*Small, 2003*) and subventricular zone (SVZ) ⁄ olfactory bulb (OB) *(Braak et al., 2003*). The generation and cell death of newly generated cells have critical roles in brain development and maintenance in the embryonic and adult brain, and alterations in these processes are seen in neurodegenerative diseases.

1.2 Neurodegenerative disorders

Although neuronal degeneration predominantly affects or starts with specific neuronal populations [including dopaminergic neurons in PD, striatal medium spiny neurons in HD, motor neurons in amyotrophic lateral sclerosis, and cortical and hippocampal neurons in AD], there are many similarities between different neurodegenerative disorders. These include atypical protein assemblies and oligomerization as well as induced cell death. At a late disease stage, protein aggregation is no longer restricted to specific brain regions.

Interestingly, in PD and HD, the specific alterations in neurogenic areas such as the DG and SVZ/OB system parallel the early or premotor symptoms that are seen in the early stages of neurodegenerative disease, such as depression, anxiety or olfactory dysfunction. Therefore, it is intriguing that the mechanisms of neurodegenerative diseases are closely linked to brain plasticity. Brain plasticity in the adult, originally conceived of as changes at the level of synaptic transmission, synaptic contacts and gene expression (reviewed in *Buonomano & Merzenich, 1998*), became a more complicated concept.

Genes that are key players in neurodegenerative diseases [α-syn, presenilin (PSEN)1, tau, huntington] are also physiologically involved in modulating brain plasticity in the embryonic brain, specifically as membrane proteins and when concentrated in synapses. These proteins commonly show high conservation between species and are located close to membranes or are involved in microtubule transport. α-syn is a protein that is physiologically enriched in presynaptic termini (*Abeliovich et al., 2000*). Initially shown to be upregulated in a discrete population of presynaptic terminals of the song bird brain during a period of acquisition-related synaptic rearrangement (*George et al., 1995*), α-syn can interact with tubulin (*Alim et al., 2002*). In addition, it is involved in DA synthesis, metabolism and release, and slight changes in concentration can have vast effects on neurotransmitter release (*Nemani et al., 2010*).

1.3 Neurodegenerative disorders of interest

1.3.1 Alzheimer's disease (AD)

AD was first described by Alois Alzheimer more than a century ago in Germany, and it constitutes one of the most common causes of senile dementia. AD refers to a clinical syndrome that occurs in the elderly and is severe enough to interfere with social and occupational activities. At least two clinical

abnormalities are essential for diagnosis of the disease, namely, memory loss in an alert person and impairment of one or more of the following functions: language, attention, perception, judgment or problem solving (*Forstl & Kurz, 1999*).

The pathology of AD includes neuronal and synaptic loss, neurofibrillary tangles due to hyperphosphorylated tau proteins and deposition of amyloid-β (Ab) protein in senile plaques in the basal forebrain cholinergic neurons as well as in the cortex, hippocampus and amygdala (*Hardy & Selkoe, 2002*). Ab is the product of proteolysis of amyloid precursor protein (APP) by b- and c-secretase enzymes.

Amyloid β (Aβ) accumulates intracellularly in the neuronal endoplasmic reticulum but also extracellularly (*Trojanowski & Lee, 2000; Cuello, 2005*). Although Aβ plaques are the neuropathological hallmarks of AD, the small Aboligomeric species rather than its amyloid counterpart is thought to be the toxic culprit in the disease. Evidence for this assumption includes a correlation between oligomerization and memory deficit in both transgenic mice and humans, the presence of oligomers in the brains of transgenic mice, the toxicity of Aβ dimer and trimer measured by long-term potentiation, and lack of a good correlation between plaque amount and AD (at least in the early phase of the disease) (*Walsh et al., 2002; Walsh & Selkoe, 2007*). Patient deficits include olfactory deficits, memory impairment, cognitive and functional decline, and death. These symptoms can be partly related to regions and functions of adult neurogenesis.

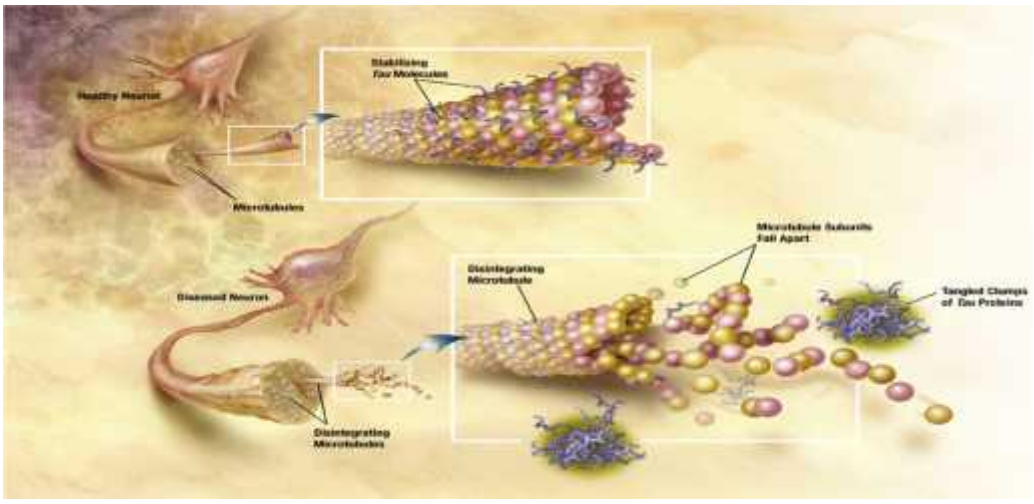


Fig.1 Changes in Tau protein lead to microtubule disintegration.

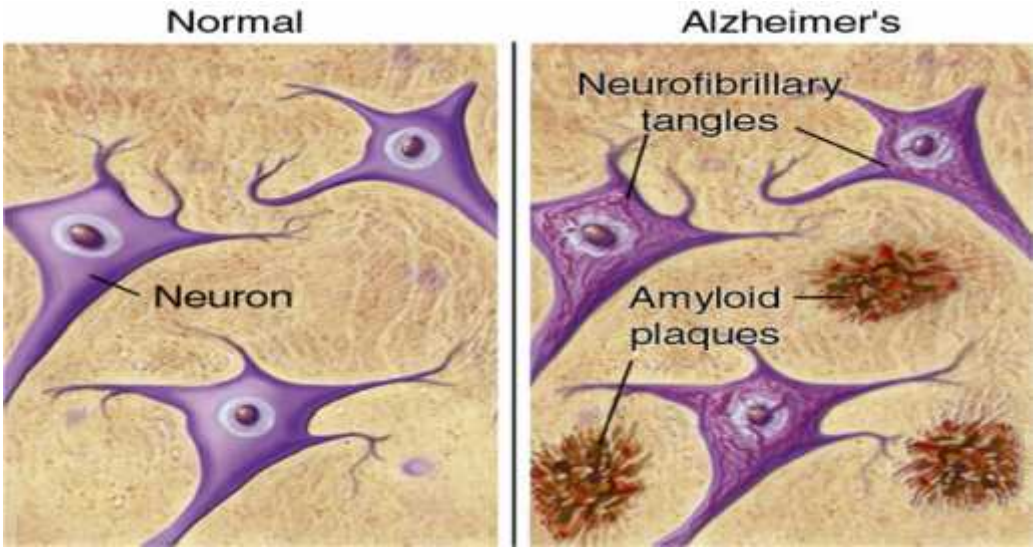
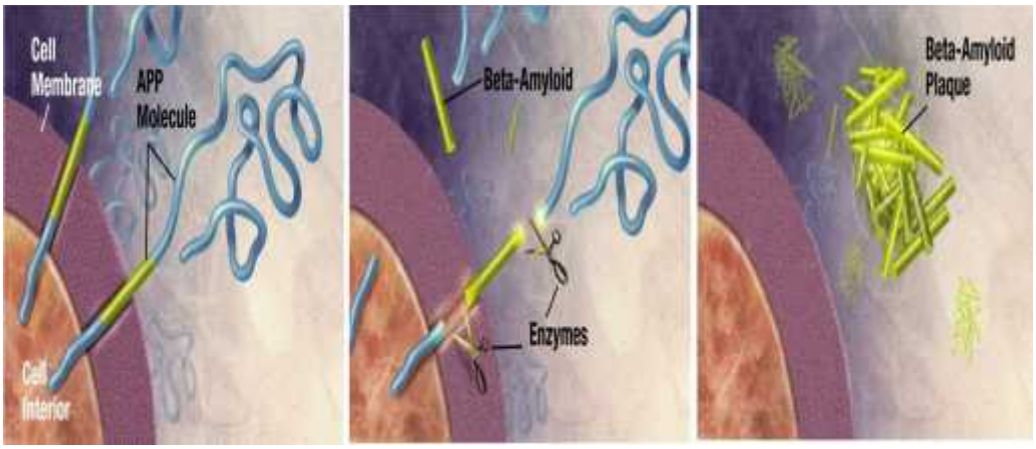


Fig.2 "Tangles" of a protein called "tau" occur in Alzheimer's patients' brains-causing neurons to lose their function and increasing memory loss.



[β amyloid] [Normal Neuron] [Amyloid Plague]

Fig.3 Enzymes act on the APP (amyloid precursor protein) and cut it into fragments. The beta-amyloid fragment is crucial in the formation of senile plaques in AD.

1.3.2 Parkinson's disease (PD)

PD is the second most common neurodegenerative disease, affecting about 1% of the population over 65 years of age. PD is characterized clinically by resting tremor, rigidity and bradykinesia, resulting from the progressive and selective loss of DA neurons in the substantianigra (SN) pars compacta, and histopathologically by the eosinophilic proteinaceous intracytoplasmic inclusion known as Lewy bodies (LBs) in surviving dopaminergic cells (*Forno, 1996*). The etiopathogenesis of PD is probably multifactorial, including both environmental and genetic factors (*Di Monte, 2003; Hardy et al., 2003*). As mentioned above, α-syn is the principal component of LBs (*Spillantini et al., 1997*). This was seen in molecular hereditary investigations that identified autosomal dominant missense mutations of α-synuclein (A53T) in heritable cases of PD (*Polymeropoulos et al., 1997*). Another missense mutation on the α-syn gene (A30P) was associated with familial PD (*Kruger et al., 1998*) and a new mutation (A46K) was found recently in a Spanish family, associated with PD, dementia of LBs (*Zarranz et al., 2004*).

It was suggested that α-synuclein aggregation is the key event that triggers neuronal damage and death (*Forloni et al., 2000; Volles & Lansbury, 2003*). In this context, α-syn mutations would speed up the protein aggregation (*Conway et al., 2000*). It is likely that the α-synuclein concentration is crucial to switch from a physiological to a pathological condition, and only when a threshold concentration is reached do the deleterious effects of aggregation become evident. Misregulation in the homeostasis of α-syn (caused by mitochondrial complex I inhibition, environmental toxins, oxidative stress or proteasome impairment) would be sufficient to trigger α-syn chemical modifications and aggregation in sporadic PD too (*Sherer et al., 2003; Norris et al., 2003*).

Loss of DAergic neurons in the SN of the midbrain and loss of other neurotransmitter phenotype neurons in other brain regions are characteristic neuropathological hallmarks (*Goedert, 2001*). Prominent clinical features of PD are motor symptoms (bradykinesia, tremor, rigidity and postural instability) and non-motorrelated PD symptoms (olfactory deficits, autonomic dysfunction, depression, cognitive deficits and sleep disorders). Non-DA brain regions that

are affected in PD have recently attracted increasing interest because the onsets of the non-motor symptoms linked to these neuropathological alterations are observed early in the course of the disease. They include rapid eye movement (REM) sleep behavior disorder, subtle cognitive deficits, depression, olfactory dysfunction and constipation (*Tolosa&Poewe, 2009*). As described above, a subset of these functions is connected to the stem and progenitor cell populations in the hippocampus and SVZ / OB system. Interestingly, several monogenetic forms of PD show a decreased gray matter volume in the hippocampal region (*Reetz et al., 2010*).

1.3.3 Huntington's disease (HD)

HD is an autosomal dominant neurodegenerative disorder characterized by motor dysfunction, cognitive impairment and psychosis (*Sharp et al., 1995*). The disease is caused by an *IT15* gene mutation on chromosome 4 (*Sharp et al., 1995*). This mutation produces a CAG / polyglutamate repeat expansion in the gene's protein product, huntington (*Sharp et al., 1995*). The DNA sequence CAG encodes the protein glutamate, an amino acid known primarily for its roles in metabolism and as a neurotransmitter (*Cotman & Monaghan 1986; Kandel, 2001*). Normal huntington protein contains 9-35 CAG repeats; however, its mutant form may contain up to 250 of these repeats (*Cattaneo et al., 2002*). Both forms of the protein are known to undergo caspase cleavage to generate smaller, truncated fragments; however, the mutant protein's fragments are distinct in their correlation with neurodegeneration (*Wellington et al., 2002*). Researchers are unsure why the mutant protein's CAG repeats cause HD; however, two theories exist to explain disease onset. One theory, the loss of function hypothesis, suggests that the CAG expansion disables huntington from carrying out its normal function (*Catteneo et al., 2002*). A second theory, the gain of function hypothesis, suggests that the *IT15* mutation produces a toxic huntington protein with a distinct conformation that enables it to stick to both itself and normal huntington (*Catteneo et al., 2002*).

1.3.4 Amyotrophic lateral sclerosis (ALS)

ALS is a progressive, degenerative disorder characterized by the selective loss of motor neurons in the brain and spinal cord leading to paralysis, muscle atrophy and eventually, death (*Pasinelli & Brown, 2006*). Two missense mutations in the gene encoding the human Vesicle-associated membrane

protein (VAMP)-Associated Protein B (hVAPB) causes a range of dominantly inherited motor neuron diseases including ALS8 (*Nishimura et al., 2004; Chen et al., 2010*). VAP family proteins are characterized by N-terminal major sperm protein (MSP) domain, a coiled-coil (CC) motif and a transmembrane (TM)-spanning region. They are implicated in several biological processes, including regulation of lipid transport, endoplasmic reticulum (ER) morphology and membrane trafficking (*Lev et al., 2008*). Drosophila Vap-33-1 (DVAP) hereafter, regulates synaptic structure, synaptic microtubule (MT) stability and the composition of postsynaptic glutamate receptors (*Pennetta et al., 2002; Chai et al., 2008*). MSP domains in DVAP are cleaved and secreted into the extracellular space where they bind Ephrin receptors (*Tsuda et al., 2008*). MSPs also bind postsynaptic Roundabout and Lar-like receptors to control muscle mitochondria morphology, localization and function (*Han et al., 2012*). Transgenic expression of the disease-linked alleles (DVAP-P58S and DVAP-T48I) in the larval motor system recapitulates major hallmarks of the human disease, including aggregate formation, locomotion defects and chaperone upregulation (*Chen et al.,2010;Chai et al.,2008;Ratnaparkhi et al.,2008*). Several studies have also implicated the ALS mutant allele in abnormal unfolded protein response (UPR) (*Chen et al., 2010; Kanekura et al., 2006; Langou et al., 2010; Suzuki et al., 2009; Gkogkas et al., 2008*) and in the disruption of the anterograde axonal transport of mitochondria (*Mo´rotz et al.,2012*). However, it is unclear how these diverse VAP functions are achieved and which mechanisms underlie the disease pathogenesis in humans. One way to address these questions is to search for DVAP-interacting.

1.3.5 Multiple sclerosis (MS)

MS is a chronic idiopathic demyelinating and neurodegenerative disease of the CNS. As such, both the onset and exacerbation of MS are thought to be influenced by multiple factors, including infectious agents, genetic composition and environment (*Hauser et al, 2006*).A key defining feature of MS is that lesions are disseminated in both space and time, i.e., they occur at more than one site and develop on more than one occasion. Clinically, MS symptoms emerges between the ages of 20 and 40 years in approximately 70% of patients (*Weinshenker et al.,1989; Confavreux et al.,1980*) although changes visible on

MRI are much more common than clinical activity and may well precede the latter (*O'Riordan et al.,1998; Sailer et al.,1999; Brex et al.,2002*).

1.4 Common motifs in neurodegeneration

Neurodegenerative disorders such as AD and PD account for a significant and increasing proportion of morbidity and mortality in the developed world (*Hebert et al., 2001; Hebert et al., 2003*). Largely as a result of increased life expectancy and changing population demographics (i.e., the aging of baby boomers), neurodegenerative dementias and neurodegenerative movement disorders are becoming more common (*Brookmeyer et al., 1998; Samii et al., 2004*). As our population ages, an improved understanding of these diseases will be vital to developing more effective therapies and combating the staggering personal, social, and economic costs of these diseases (*Ernst et al., 1997*). Unifying theories of pathogenesis in neurodegenerative disease provide an avenue for developing therapeutic strategies with broad applicability for disease prevention and an opportunity for decreasing morbidity and mortality from these disorders in the elderly population (*Forman et al., 2004*). Converging lines of investigation have revealed a potential single common pathogenic mechanism underlying many diverse neurodegenerative disorders.

1.5 Mechanisms of neuronal death

Acute injury to cells causes them to undergo *necrosis*, recognized pathologically by cell swelling, vacuolization and lysis, and associated with calcium (Ca^{2+}) overload of the cells and membrane damage. Necrotic cells typically spill their contents into the surrounding tissue, evoking an inflammatory response. Cells can also die by apoptosis or programmed cell death (*Raff, 1998*), a slower process that occurs normally during development and is essential for many processes throughout life, for example development, immune regulation and tissue remodeling. Apoptosis, as well as necrosis, occurs in many neurodegenerative disorders including acute conditions such as stroke and head injury (*Bredesen, 1995*). The distinction between necrosis and apoptosis as processes leading to neurodegeneration is not absolute, for challenges such as excitotoxicity and oxidative stress may be enough to kill cells directly by necrosis, or, if less intense, may induce them to undergo apoptosis. Both processes, therefore, represent possible targets for putative neuroprotective drug therapy. Pharmacological interference with the apoptotic

pathway may become possible in the future, but for the present, most efforts are directed at the processes involved in cell necrosis, and at compensating pharmacologically for the neuronal loss.

In recent years there has been an increasing interest in the studies on neurodegeneration, including the physiological or programmed neuronal death and the cell disruption occurring as a consequence of necrosis. This interest has been greatly stimulated by the fact that precipitation and localization of neuronal destruction is a central event in the course of many acute and chronic disorders of the CNS. These disorders include stroke (anoxia-ischemia), hypoglycemia, cerebral trauma, epilepsy and several devastating neurodegenerative diseases, such as ALS, PD, AD and HD. Among the cellular mechanisms possibly involved in neuronal death in neurodegenerative disorders, three closely related factors seem to play important roles: (1) the generation of reactive oxygen species (ROS) or free radicals, (2) the over activation of synaptic excitatory amino acid (EAA) receptors, and (3) the increase in cytoplasmic free Ca^{2+} concentration and (4) infection. As shown in Fig. 1, the links between these factors are multiple and an initial event may lead, in a cascade manner, to the generation of further alterations (*Rang et al., 2006*). In addition to these, several other factors like selective vulnerability and exquisite specificity of the disease process for particular types of neurons; genetic predisposition; excitotoxicity; oxidative stress; environmental triggers – infectious agents, toxins, brain injury; aging and disruption in energy metabolism (*Standaert & Young, 2006*).

1.6 Monosodium L-glutamate (MSG)

MSG, a widely used food flavor enhancer, may cause serious adverse effects in some cases, such as allergy, retina injury, osteoblast dysfunction and cardiac tachyarrhythmias. These symptoms are described as “monosodium glutamate symptom complex” or “Chinese restaurant syndrome”. However, the underlying mechanisms are not clearly elucidated. MSG is water soluble and ionised into glutamate (Glu) and Na+ in the water at body temperature. MSG ingestion is considered safe in the general population, but the possibility remains that some individuals are sensitive to it: those with neurodegenerative diseases and heart diseases. The circulating level of glutamate is one source that

may give rise to adverse effects. Other sources of unusually high glutamate levels may take roles in certain local sites of a diseased organ, for example, in the extracellular spaces of an infarct heart due to “glutamate outflow”, or in an injured brain potentially caused by glutamate release from the glia or the dysfunction of glutamate transporters. These high local glutamate levels are correlated with organ injuries and dysfunction. Glutamate is also an important excitatory neurotransmitter in the mammalian CNS and its excessive activity may be a cause of excitotoxicity in the brain, whether gutamate induces excitotoxicity in peripheral tissues is not well documented. Virtually all members of the glutamate receptor family are involved in excitotoxicity. However, the ionotropic glutamate receptors (iGluRs) are recognized as the key receptors.

1.7 Glutamate and excitotoxicity

One of the key events in this chain of reactions resulting in neuronal damage is an excess excitatory synaptic neurotransmission mediated by EAA, mainly glutamate. Pioneer studies in the decade of the seventies showed that exposure of nervous tissue to high concentrations of glutamate and other EAA produced neuronal degeneration and death, and demonstrated that such effects are related to the ability of these neurotransmitters to depolarize the membrane and therefore to excite neurons (*Olney, 1971; Olney et al., 1971; Rothman et al., 1987*). Because of the capability of glutamate to produce both excitation and toxicity in neurons, the term “*excitotoxicity*” was proposed by *Olney (1978).*

For years the central effects of glutamate were thought to be exclusively mediated by ion channel mechanisms, however, glutamate receptors can now be categorized into two groups: ionotropic [i(GluR)] activated by glutamate and aspartate; and metabotropic [m(GluR)]. The i(GluR)s are ligand-gated ion channels, mainly localized postsynaptically, characterized by their selective affinity for the specific agonists: N-methyl-D-aspartate (NMDA), α-amino-2-hydroxy-4-methyl-3-isoxazolepropionate (AMPA); and kainic acid (KA). The second group is that of mGluR, which are frequently present in the presynaptic membrane and do not form ion channels but are associated to G proteins and coupled to the production of second intracellular messengers (*Hollmann & Heine-mann, 1994; Michaelis, 1998; Miller, 1994*). They are subdivided into 3

types by amino acid sequence, agonist sensitivity and signal transduction mechanisms. Group I (mGluR1-5) are coupled to phospholipase C-mediated polyphosphoinositide hydrolysis, while group II (mGluR2 and 3) and group III (mGluR4,6,7 and 8) are either negatively coupled to adenyl cyclase or linked to ion channels (*Hynd et al., 2004; Stone & Addae, 2002*).

EAA neurotoxicity is cellular selective and different EAAs produce distinctive degenerative patterns (*Coyle et al., 1981*); it is predominantly mediated by i(GluR) (*Olney, 1990*). Two deleterious processes can be distinguished by differences in time-dependence and ionic characteristics (*Choi, 1992*). The first involves acute swelling of cell bodies and dendrites via the opening of membrane cation channels, causing depolarization. The Na^+ influx, and passive influx of CI^- ions and water, precedes the cell volume expansion. Swelling occurs within minutes of glutamate exposure and is critically dependent on the extracellular concentrations of Na^+ and Cl^- ions (*Rothman, 1985*). The second, slowly evolving component is marked by delayed neuronal degeneration. *In vitro,* the accumulation of radiolabelled Ca^{2+} correlates closely with the degenerative process, suggesting that the toxicity is principally mediated by Ca^{2+} influx, probably via NMDA-receptor (NMDAR) (*Choi, 1992*). NMDARs exhibit a higher permeability for Ca^{2+} than do AMPA or KA receptors, and possess a higher capacity for inducing intracellular Ca^{2+} overload and initiating the degenerative cascade (*Choi, 1992*).

Although non-NMDA receptors were not originally thought to be permeable to Ca^{2+}, heteromeric complexes comprised of both AMPA- and KA-sensitive receptor subunits can actually form channels that sustain Ca^{2+} ion currents (*Wisden & Seeberg, 1993*). In addition, some variants of AMPA-kainate receptors are coupled to ion channels that are somewhat permeable to Ca^{2+} (*Hollmann et al., 1991*) and can thus contribute to excessive calcium entry. Furthermore, stimulation of any of the i(GluR)s results in membrane depolarization because of the influx of positively charged ions, and thus indirectly activate voltage-gated calcium ions channels further contributing to neurotoxicity (*Hynd et al., 2004*).

Under resting conditions, the NMDAR is not readily opened by the agonists, because its channel is blocked by magnesium (Mg^{2+}). However, because of the

voltage sensitivity of this receptor, when the membrane is depolarized Mg^{2+} is expelled out of the channel and the receptor is able to respond to its ligand. Consequently, when the non-NMDAR is activated and depolarization occurs due to the entrance of Na^{+} through its channel, the over activation of the NMDAR is facilitated. The hyperactivation of the NMDAR results in a massive entrance of Ca^{2+} (*Hartley et al., 1993; Randall & Thayer, 1992*), which may overcome the intracellular calcium (i[Ca^{2+}]) buffering mechanisms that, together with plasma membrane Ca^{2+}-ATPases and Na^{+}-Ca^{2+} exchangers, are responsible for maintaining a sub-micromolar intracellular concentration of the cation. The resulting accumulation of cytoplasmic Ca^{2+} eventually leads to neuronal death due to several factors, such as activation of proteases, lipases and endonucleases, membrane protein and lipid alterations, generation of toxic ROS, mitochondrial damage, disruption of energy metabolism, and membrane depolarization. These events potentiate each other in a cascade manner to produce membrane damage and consequently cell death. (*Mattson, 1994; Siesjo, 1994*).

Studies in tissue culture indicate that glutamate receptor (GR)-mediated neuronal degeneration can be separated into two distinct forms: acute and delayed form of neuronal degeneration. The acute form of neurotoxicity is characterized by neuronal swelling in the presence of agonist, which leads to osmotic lysis of the neurons, and can be prevented by eliminating from the culture medium sodium or chloride ions, two ions responsible for the massive influx of water when glutamate-gated cation channels are open. In contrast, delayed neuronal degeneration caused by NMDA and in most cases, KA agonists is calcium ion-dependent and transpires over several hours after a brief exposure to a high concentration of agonist or prolonged exposure to a low concentration of agonist. The Ca^{2+} mediated effects of GR activation leading to neuronal degeneration may involve a number of different pathways that cause oxidative stress and degeneration (*Choi et al., 1987; Kato et al., 1991; Rothman et al., 1987*).

NMDAR activation or neuronal increases in Ca^{2+} subsequent to sodium, or both can activate a series of enzymes, including protein kinases C (PKC), Phopholipases (PL), proteases, protein phosphatases, and nitric oxide synthase

(NOS) (*Choi, 1988; Dawson et al., 1992; Trout et al., 1993*). After PL_2 is activated, arachidonic acid (AA), its metabolites, and platelet activating factor (PAF) are generated. PAF increases neuronal calcium ions levels (Ca^{2+}), apparently by stimulating the release of glutamate (*Bito et al., 1992; Clark et al., 1992*). AA potentiates NMDA-evoked currents (*Miller et al., 1992*) and inhibits reuptake of glutamate into astrocytes and neurons (*Volterra et al., 1992*), further exacerbating the situation; ROS can be formed during AA metabolism (*Lafon-Cazal et al., 1993*), leading to further PLA_2 activation, which represents positive feedback (*Chan et al., 1985*). These processes can cause the neuron to digest itself by protein breakdown, free-radical formation, and lipid peroxidation. In addition, one might envisage that in cerebral ischemia, tissue reperfusion increases this damage by providing additional free radical in the form of superoxide anions (*Rosen et al., 1993*). When NMDARs are excessively stimulated, influx of Ca^{2+} activates the generation of NO and superoxide in increased quantities. Under these conditions, NO and superoxide ions may react to form a toxic substance called peroxynitrite ($ONOO^-$), resulting in neuronal death (*Dawson et al., 1991; Lipton et al., 1993*). In addition to these effects Ca^{2+} can activate nuclear enzyme (endonucleases) that result in condensation of nuclear chromatin and ultimately DNA fragmentation and nuclear breakdown, a pathological process known as apoptosis (*Kane et al., 1993*).

PLs capable of breaking down the cell membrane and liberating AA are activated by glutamate (*Hynd et al., 2004*). AA metabolism, by cellular oxidases, generates ROS, resulting in the degradation of lipid membranes (*Chan & Fishman, 1985*). Endonucleases that break down genomic DNA may be activated. Elevated cytosolic Ca^{2+} may act in concert with diacylglycerol to activate Ca^{2+}-sensitive protein kinases, resulting in the hyper-phosphorylation of cytoskeletal proteins including *tau* and *ubiquitin*, which are constituents of neurofibrillary tangles (*Mattson, 1994*). The influx of extracellular Ca^{2+}, augmented by release from intracellular i[Ca^{2+}] stores, may act via a positive feedback mechanism to enhance synaptic efficacy and neuronal excitability, causing further release of glutamate (*Choi, 1992*).

Studies have demonstrated that synaptic glutamate release and uptake are energy (ATP)-dependent, and any impairment or breakdown may lead to generation of ROS and inactivation of glutamate reuptake mechanism leading to excessive glutamate accumulation. If the circumstance continues unabated, there is excessive influx of Na^+, Cl^- and Ca^{2+}, via post-synaptic ion channels producing swelling and destruction of post synaptic elements not only in the immediate vicinity but also the entire neuron as well. Upon destruction of neurons by this mechanism, additional glutamate may be released further increasing the level of extracellular glutamate and thereby propagating the excitotoxicity and death of additional glutamate-sensitive neurons in the region of involvement (*Auer & Siesjo, 1988; Benveniste et al., 1984; Nicholis & Attwell, 1990; Novelli et al., 1988; Siesjo, 1984*).

In spite of its ubiquitous role as a neurotransmitter, glutamate is highly toxic to neurons, a phenomenon dubbed '*excitotoxicity'* (*Choi, 1988*). A low concentration of glutamate applied to neurons in culture kills the cells and the finding in the 1970s that glutamate given orally produces neurodegeneration *in vivo* caused considerable alarm, because of the widespread use of glutamate as a 'taste-enhancing' food additive. The 'Chinese restaurant syndrome'-an acute attack of neck stiffness and chest pain is well known, but so far the possibility of more serious neurotoxicity from dietary glutamate is only hypothetical. Local injection of KA is used experimentally to produce neurotoxic lesions. It acts by excitation of local glutamate-releasing neurons and the release of glutamate, acting on NMDA and also mGluR leading to neuronal death. Ca^{2+} overload is the essential factor in excitotoxicity. The mechanism by which this occurs leading to cell death is depicted in Figure 4, as following (*Rang et al., 2006*):

- Glutamate activates NMDA, AMPA and mGluR (sites1, 2 and 3 in Figure 4). Activation of AMPA receptors depolarises the cell, which unblocks the NMDA-channels, permitting Ca^{2+} entry. Depolarization also opens voltage-activated Ca^{2+} channels (site 4), releasing more glutamate. mGluR cause the release of $i[Ca^{2+}]$ from the endoplasmic reticulum. Sodium entry further contributes to Ca^{2+} entry by stimulating Ca^{2+}/Na^+ exchange (site 5).
- Depolarization inhibits or reverses glutamate uptake (site 6), thus increasing the extracellular glutamate concentration.

- The mechanisms that normally operate to counteract the rise in i[Ca^{2+}] include the Ca^{2+} efflux pump (site 7) and, indirectly, the Na^{+} pump (site 8).
- The mitochondria and endoplasmic reticulum act as capacious sinks for Ca^{2+} and normally keep $_{i}[Ca^{2+}]$ under control. Loading of the mitochondrial stores beyond a certain point, however, disrupts mitochondrial function, reducing ATP synthesis, thus reducing the energy available for the membrane pumps and for Ca^{2+} accumulation by the endoplasmic reticulum. Formation of ROS is also enhanced. This represents the danger point at which positive feedback exaggerates the process.
- Raised i[Ca^{2+}] affects many processes, the chief ones relevant to neurotoxicity being: (a) increased glutamate release (b) activation of proteases (calpains) and lipases, causing membrane damage (c) activation of NO, nitric oxide synthase (NOS); while low concentrations of NO are neuroprotective, high concentrations, in the presence of ROS, generate peroxynitrite and hydroxyl free radicals, which damage many important biomolecules, including membrane lipids, proteins and DNA (d) increased AA release, which increases free radical production and also inhibits glutamate uptake (site 6).

  Glutamate and Ca^{2+} are arguably the two most ubiquitous chemical signals, extracellular and intracellular respectively, underlying brain function, so it is disconcerting that such cytotoxic mayhem can be unleashed when they get out of control. Both are stored in dangerous amounts in subcellular organelles, like hand-grenades in an ammunition store. Defense against excitotoxicity is clearly essential if our brains are to have any chance of staying alive. Mitochondrial energy metabolism provides one line of defense, and impaired mitochondrial function, by rendering neurons vulnerable to excitotoxic damage, may be a factor in various neurodegenerative conditions, including PD. The role of excitotoxicity in ischemic brain damage is well established, and it is also believed to be a factor in other neurodegenerative diseases (*Lipton & Rosenberg, 1994*). There are several examples of neurodegenerative conditions caused by environmental toxins, acting as agonists on glutamate receptors (*Olney, 1990*).

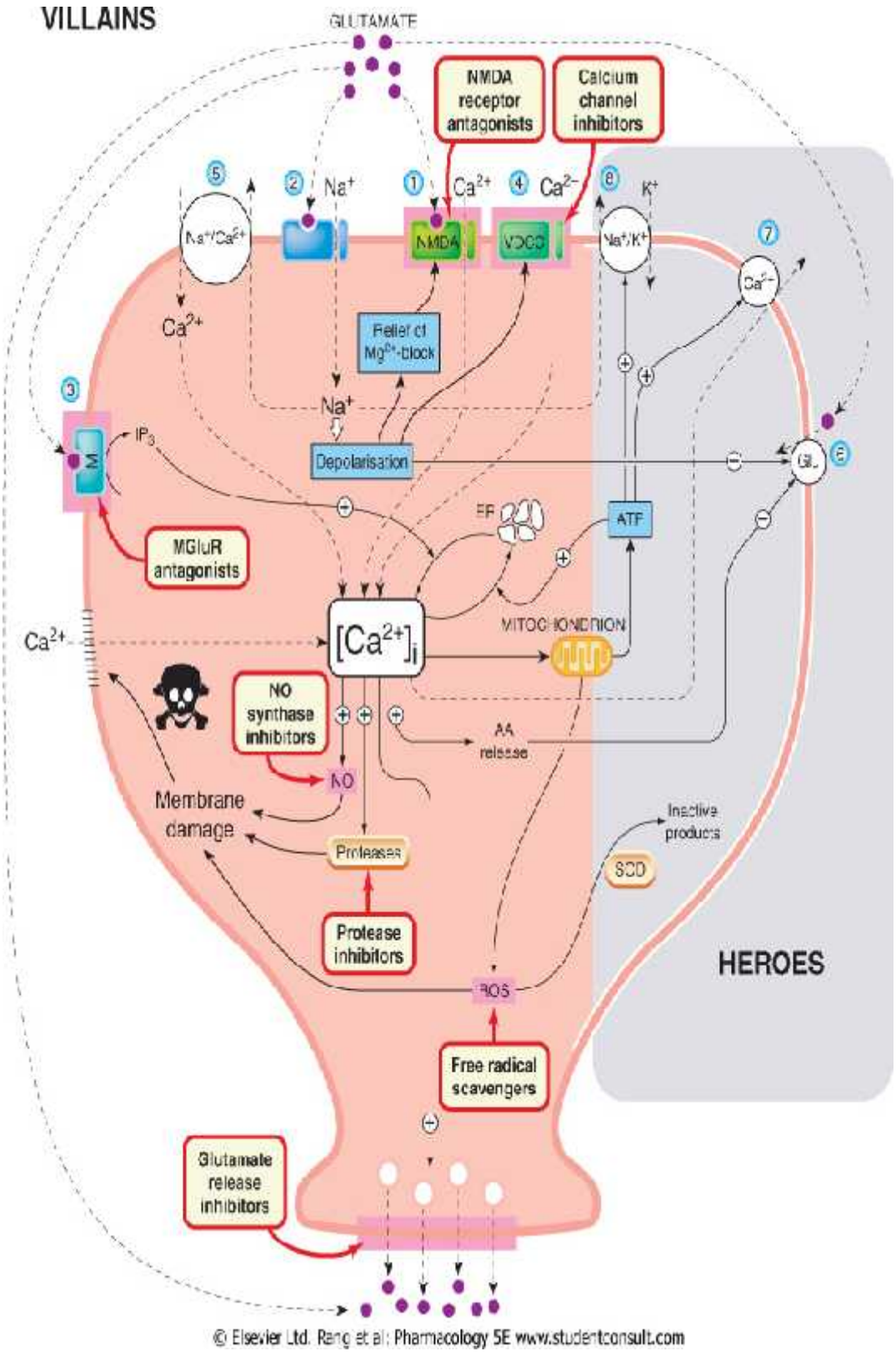


Figure 4. A detailed mechanism of glutamate-induced excitotoxicity

1.8.1 Glutamate and oxidative stress

The production of ROS is associated with many forms of apoptosis (*Suzuki et al., 1997*), as well as the cell death that occurs in stroke, ischemia, and many neurodegenerative diseases (*Halliwell, 1992; Ames et al., 1993; Coyle & Puttfarcken, 1993; Jenner, 1994; Shigenaga et al., 1994*). Glutamate toxicity is a major contributor to pathological cell death within the nervous system and appears to be mediated by ROS (*Coyle & Puttfarcken, 1993*). There are two forms of glutamate toxicity: receptor-initiated excitotoxicity (*Choi, 1988*) and non-receptor-mediated oxidative glutamate toxicity (*Murphy et al., 1990*). Oxidative glutamate toxicity is initiated by high concentration of extracellular glutamate that prevent cystine uptake into the cells, followed by the depletion of intracellular cystine and the loss of GSH. With a diminishing supply of GSH, there is an accumulation of excessive amounts of ROS and ultimately cell death. Understanding the relationship between GSH depletion and ROS production should lead to a better understanding of all forms of programmed cell death in which ROS play a central role.

Oxidative glutamate toxicity has been observed in primary neuronal cell cultures (*Murphy et al., 1990; Oka et al., 1993*), and tissue slices (*Vornov & Coyle, 1991*) and has been studied recently in the immortalized mouse hippocampal cell line, HT22 (*Davis & Maher, 1994; Maher & Davis, 1996; Li et al., 1997a,b; Sagara et al., 1998*). In HT22 cells, glutamate induces a form of programmed cell death with characteristics of both apoptosis and necrosis. The exposure of HT22 cells, cortical neurons and neuroblastoma cells to glutamate results in the rapid depletion of GSH followed by an increase in ROS. The assumption has been that the increase in ROS is a direct result of this GSH depletion, but the functional relationship between the two has not been defined. *Tan et al., (1998)* summarized that there are two phases of ROS formation after exposure to glutamate: an early 5-10 fold increase coupled to GSH depletion and a later 200-400 fold increase derived from mitochondria. Early gene activation and caspase activity are required for both maximal ROS production a subsequent cell death.

1.9 Current scenario

Neurodegeneration is a process involved in both neuropathological conditions and brain ageing. It is known that brain pathology in the form of cerebrovascular and neurodegenerative disease is a leading cause of death all over the world, with an incidence of about 2/1000 and an 8% total death rate (*Kolominsky Rabas et al., 1998*). Cognitive dysfunction, is a major health problem in the 21st century, and many neuropsychiatric disorders and neurodegenerative disorders, such as schizophrenia, depression, AD, dementia, cerebrovascular impairment, seizure disorders, head injury, Parkinsonism can be severely functionally debilitating in nature (*Commenges et al., 2000*).

A recent study (*Andlin-Sobocki et al., 2005*) has evaluated the total cost of brain diseases per year, including direct and indirect costs, in 28 countries in Europe at about 386 billion Euros for the year 2004. This represented 35% of the total burden of diseases affecting about 27% of the 465 million people who are suffering brain diseases. If mental disorders are excluded from the calculation the total cost of neurological diseases including dementia could be about 146 billion Euros per year and the total specific cost of the neurodegenerative diseases could be as much as 72 billion Euros. These diseases are found in about 5% of the total number of patients suffering brain diseases. They are characterized by more or less selective neuronal degenerations inducing neurological syndromes, and affect both sensory-motor areas and cognitive functions.

In industrial countries, PD has a prevalence of approximately 0.3% in the general population and affects about 1% of those older than 60 (*de Lau & Breteler, 2006*). This disease rarely occurs before the age of 50, and men are at higher risk than women. In Europe, PD affected 1.2 million people in 2010, resulting in costs per patient of EUR 5,626 for direct health care and EUR 4,417 for non-medical care. In 30 European countries, the total cost of all care for patients with PD in 2010 was EUR 13.9 billion (*de Lau & Breteler, 2006*).

According to a recent estimation, it is possible that almost 80% of individuals with dementia suffer from AD (*Jellinger & Attems, 2010*). AD is a severe progressive neurodegenerative brain disorder that affects approximately 5% of the population older than 65 years (*Shah et al., 2008*). According to the US

Centers for Disease Control and Prevention (2003), the number of people in the world who are over the age of 65 will increase to around 1 billion by 2030. It has also been projected that by 2050 the number of dementia cases will reach around 14 million in Europe (*Mura et al., 2010*) and 13.2 million in the United States (*Hebert et al., 2001*). Furthermore, it has been estimated that the annual incidence of AD in the United States will increase from the 337,000 cases recorded in 1995 to 959,000 cases in 2050 (*Hebert et al., 2001*). At the level of individuals, AD decreases the quality of life and shortens life expectancy. At the societal level, the long-term care of AD patients in nursing homes is an economic challenge in Western countries, as illustrated by a report in which *Olesen and colleagues (2012)* showed that in Europe the annual cost for patients with dementia was EUR 105.2 billion in 2010. The mentioned date certainly indicate the tremendous impact of AD in terms of the enormous number of patients with this disease, the pressure on their relatives, and the negative socioeconomic consequences. In short, it can be said that AD is one of the major public health problems in the world.

1.9.1 Current therapeutic approaches in neurodegeneration

Drugs to improve memory generally work by altering the balance of particular chemicals (neurotransmitters) in the brain that are involved in the initial learning of a memory or its subsequent reinforcement. Some of them along with their mechanism are listed in table 2. Some acts by selective enhancement of cerebral blood flow and metabolism, including enhanced glucose uptake, which may protect against the effects of hypoxia and ischemia. Reports from literature reveal that some medications currently available to patients with memory disorders may also increase performances in healthy people. Drugs designed for psychiatric disorders can also be used to enhance certain mental functions.

However, the long-term effects of these drugs are unknown. Drugs which act as cognition enhancer increase synaptic plasticity by, regulating release of neurotransmitter from the pre-synaptic terminal and increasing sensitivity and specificity of receptors and ion channels in the membranes of synapse to neurotransmitter signaling. Some of the agents also modulate the process at transcriptional and translational level.

1.10 Herbal medicines

In traditional practices of medicine, numerous plants have been used to treat cognitive disorders, including neurodegenerative diseases such as AD and other memory related disorders. Various studies have been undergone to identifying potential new drugs from plant sources, including those for memory disorders. There are numerous drugs available in market that have been isolated from plants, e.g. alkaloids from plant sources have been investigated for their potential in AD therapy, and are now in clinical use. Usually herbal preparations are well tolerated but they may have harmful side-effects, including interactions with pharmaceuticals (*Howes et al., 2003*). Herbal medicines, such as, *Ginkgo Biloba, Bacopa monnieri* (Bramhi) (*Das et al., 2002*), Shankh pushpi etc., has been found to increase memory power. Some of the herbal medicinal plants with potential cognitive enhancement activity are listed in table 3 **(***Howes & Houghton, 2003; Kennedy et al., 2003***)**.

The past decade has also witnessed an intense interest in herbal medicines in which phytochemical constituents can have long-term health promoting or medicinal qualities. In contrast, many medicinal plants exert specific medicinal actions without serving a nutritional role in the human diet and may be used in response to specific health problems over short- or long-term intervals. Phytochemicals present in vegetables and fruits are believed to reduce the risk of several major diseases including cardiovascular diseases, cancers as well as neurodegenerative disorders. Therefore people who consume higher vegetables and fruits may be at reduced risk for some of diseases caused by neuronal dysfunction (*Selvam, 2008; Lobo et al., 2010*). Herbal medicine has long been used to treat neural symptoms. Although the precise mechanisms of action of herbal drugs have yet to be determined, some of them have been shown to exert anti-inflammatory and/or antioxidant effects in a variety of peripheral systems. Now, as increasing evidence indicates that neuroglia-derived chronic inflammatory responses play a pathological role in the CNS, anti-inflammatory herbal medicine and its constituents are being proved to be a potent neuroprotector against various brain pathologies. Structural diversity of medicinal herbs makes them a valuable source of novel lead compounds against therapeutic targets that are newly discovered by genomics, proteomics, and high-throughput screening. This review will highlight the importance of

phytochemicals on neuroprotective function and other related disorders, in particular their mechanism of action and therapeutic potential (*Pueyo & Calvo, 2009*).

Table 1. Some putative cognitive enhancing plants

Acoruscalamas	*Embeliaribes*	*Nicotianatabacum*
Angelica archangelica	*Emblicaofficinalis*	*Paeoniaemodi*
Asparagus racemosus	*Eugenia caryophyllus*	*Panax ginseng*
Bacopamonniera	*Evodiarutaecarpa*	*Piper longum*
Biota orientalis	*Galanthusnivalis*	*Polygonummultiflorum*
Boerhaviadiffusa	*Ginkgo biloba*	*Polygala tenuifolia*
Celastruspaniculatus	*Glycyrrhizaglabra*	*Pongamiapinnata*
Centellaasiatica	*Huperziaserrata*	*Rosmarinusofficinalis*
Clitoriaternatea	*Hydrocotylasiatica*	*Salvia lavandulifolia*
Codonopsispilosula	*Lawsoniainermis*	*Salvia miltiorrhiza*
Convolvulus pluricaulis	*Lycoris radiate*	*Schizandrachinensis*
Coptischinensis	*Magnolia officinalis*	*Terminaliachebula*
Crocus sativus	*Melissa cordifolia*	*Tinosporacordifolia*
Curcuma longa	*Nardostachysjatamansi*	*Withaniasomnifera*

1.11. Phytochemicals in neuroprotection

There has been considerable public and scientific interest in the use of phytoconstituents for neuroprotection or to prevent neurodegenerative diseases. Many phytochemicals have been shown to exert neuroprotective actions in animal and cell culture models of example, a chalcone (safflor yellow B) can protect neurons against ischemic brain injury and piceatannol can protect cultured neurons against Aβ-induced death. Epidemiological studies of human populations, and experiments in animal models of neurodegenerative disorders, have provided evidence that phytochemicals in fruits and vegetables can protect the nervous system against disease (*Liu, 2003; de Rivera et al., 2005*). The vast majority of studies on health benefits of phytochemicals have focused on the fact that many of the active chemicals possess antioxidant activity. Neuroprotective effects of various phytochemicals are associated with reduced levels of oxidative stress. For example, resveratrol, quercetin and catechins

reduced oxidative stress and protected cultured hippocampal neurons against nitric oxide-mediated cell death.

Some of the neuroprotective herbs with their major bioactive compound and mode of action were shown in table 4. Hundreds of articles have been published reporting neuroprotective effects of compounds in natural products, including α-tocopherol, lycopene, resveratrol, *ginkgo biloba* and ginsenosides.

1.12 Flavonoids

Recently, there has been intense interest in the potential of flavonoids to modulate neuronal function and prevent age-related neurodegeneration. Dietary intervention studies in several mammalian species, including humans, using flavonoid rich plant or food extracts have indicated an ability to improve memory and learning, by protecting vulnerable neurons, enhancing existing neuronal function or by stimulating neuronal regeneration. Individual flavonoids such as the citrus flavanone tangeretin, have been observed to maintain nigro-striatal integrity and functionality following lesioning with 6-hydroxydopamine, suggesting that it may serve as a potential neuroprotective agent against the underlying pathology associated with Parkinson's disease (*Youdim et al., 2004*). In order for flavonoids to access the brain, they must first cross the blood brain barrier (BBB), which controls entry of xenobiotics into the brain.

Flavanones such as naringenin and their *in vivo* metabolites, along with some dietary anthocyanins, cyanidin-3-rutinoside and pelargonidin-3-glucoside, have been shown to traverse the BBB in relevant *in vitro* and *in situ* models (*Youdim et al., 2004; Youdim et al., 2002*). Anthocyanins can possibly cross the monolayer in blood-brain barrier models *in vitro*.Flavonoids and tannins are phenolic compounds that are a major group of compounds act as primary antioxidants or free radical scavengers.

1.12.1. The role of plant flavonoids in neurodegeneration

There has been a recent explosion of interest by research scientists in the flavonoid compounds, with a multitude of medically useful properties having been demonstrated in experimental, as well as, clinical studies of flavonoids. For instance, flavonoids have been shown to act as powerful free radical scavengers for a multitude of free radical species, even the powerful

peroxynitrite radical. In addition, several flavonoids have shown powerful metal-chelating properties, especially for iron and copper, two of the most potent-free radical catalysts (*Morel et al., 1998*). Of equal importance are several studies that have shown that flavonoids interact with cell membranes, improving their fluidity, thereby protecting them from lipid peroxidation (*Saija et al., 1995; Ratty & Das, 1988*). Along these same lines is the protection of micro vessels in the nervous system by specific flavonoids from free radical damage. This not only prevents leakage of such vessels, but has been shown to preserve the blood–brain barrier as well (*Robert et al., 1977*). There is also evidence that several of the flavonoids can inhibit platelet

Table 2. Nootropic herbs with their active constituents' that help in neuroprotection

Plant name	Active constituents	Phytochemical group	Activity
Acorus calamus	Asarone	Monoterpene	Sedaative and important medhya drug, capable of improving memory power and intellect
All cruciferous plants	Sulforaphane	Isothiocyanate	Decreased brain edema
Centella asiatica	Asiaticoside, centelloside, madecassoside, and asiatic acid	Triterpenoid saponins	Brain tonic, cognition and anti-anxiety
Corydalis ternata	Protropine	Alkaloid	Anti-cholinesterase and anti-amnesic properties
Curcuma longa	Curcumin	Phenols	Protects against synaptic dysfunction
Emblica officinalis	Vit-C, phyllemblin	Vitamin polyphenol	Anti cholinesterase activity
Evolvulus alsinoides	Betaine, sankhapushpine and evolvine	Alkaloids	Memory enhancing agent used in treating dementia
Ginkgo biloba	Bilobolide, ginkgolides, kaemferal, quercetin, isorhamneting sitosterol and stigmasterol	Terpenoids flavanoids steroids	Action an corticosterone produce neuronal atrophy and cell death in the hippocampus
Glycyrrhiza glabra	Glycyrrhizin	Triterpenoid saponins	Improved learning and memory on scopolamine induced nuerodisorders
Huperzia serrata	Huperzine A and B	Alkaloids	Action on neuromuscular systems related to cholinesterase activity.
Ilex paraguariensis	Chlorogenic acid caffeine, theophylline, and theobromine quercetin, kaemferol	Polyphenols xanthenes flavanoids	Memory enhancing activity on dementia
Magnolia officinalis	4-0-methyl honokiol, honokiol and magnolol	Phenols	For the treatment of neurosis, anxiety, stroke, dementia
Uncaria rhynchophylla	Rhynchophylline, corynoxeine, isorhynchophylline and isocorynoxeine	Alkaloids	Neuroprotective in particular against ischemia-induced neuronal loss
Zingiber officinale	Gingerol, shogaol and zingerone	Phenols	Brain acetyl cholinesterase inhibition activity
Ziziphus spinosa	Jujuboside A, and jujuboside B	Saponins	Anti-calmodulin action

thereby preventing strokes (*Tzeng et al., 1991*). Finally, some of the flavonoids

have the unique ability to inhibit certain enzymes, such as the COX-2 enzyme (*Kim et al., 1998*).

1.12.2 Flavonoids as free radical scavengers

The flavonoid compounds have two properties that make them especially useful as antioxidants. First, many are powerful, primary free radical scavengers against a wide variety of radicals, including singlet oxygen, superoxide, peroxyl, hydroxyl, and the peroxynitrite radicals (*Saija et al., 1995*). Second, several are known to be very effective metal chelators (*Duthie et al., 1997*). Most flavonoids are present in plants as glycosides. In the intestines, this moiety is cleaved off, leaving the aglycone form of the flavonoids (*Griffiths, 1982*). It is the aglycone form that is thought to have the highest antioxidant activity in biological systems. There is experimental evidence that hydrogen peroxide accumulation occurs during the process of catecholamine catabolism, making it especially important in PD (*Li et al., 1995*). Recent evidence also indicates that H_2O_2 plays an important role in the toxicity of Alzheimer's plaques. As we have seen, iron accumulation within neurons is characteristic of ageing of the nervous system, but is especially high in the case of neurodegeneration. A multitude of phytochemicals have specific properties that make them especially useful in combating neurodegeneration, and a list of nutrients that stimulate energy generation, primarily through the mitochondrial system.

2. Plant Profile

(Wealth of India, 2003 [Anon]; Devasagayam 2007; Wealth of India, CSIR, 1969; Indian Medicinl Plants [Kitikar & Basu] 1975, 1987, 1999; The Flora of Orissa; Saxsena & Brahmam, 1994; Sharma et al., 2005; Sharma 2003; Swain & Das 2007; Nadkarni 1976; Kapoor, 1989).

Botanical Name: *Pterocarpus marsupium* Roxb.
Botanical Source: The plant consists of the bark, leaves, heartwood of *Pterocarpus marsupium P. marsupium*

Order: Fabales

Family: Leguminosaa (Fabaceae)

Subfamily: Faboideae

Tribe: Dalbergieae

Genus: *Pterocarpus*

Species: *marsupium*

Authority: Roxb.

Vernacular names: (Sharma 2003; Swain & Das 2007)

1. Sanskrit: Pitasala, Bijaka, Murga
2. Hindi: Bijasal
3. English: Malabarkino; Indian Kino Tree
4. Bengali: Pitsal
5. Nepalese: Bijasar
6. Sinhalese: Gammalu
7. German: Malabarkino
8. Kannada: Honne
8. French: Pterocarp
9. Unani: Dammul-akhajan
10. Arabian: Dammul Akhwayn
11. Persian: Khoon-e-siyaun-shan
12. Tamil: Vengai
13. Telugu: Yegi
14. Malayalam: Venga

P. marsupium, also known as Malabar kino, (Gamble, 1935) Indian kino tree or vijayasar, is a medium to large, deciduous tree that can grow up to 30 metres tall. It is

native to India, Nepal, and Sri Lanka, where it occurs in parts of the Western Ghats in the Karnataka-Kerala region and also in the forests of Central India. Parts of the Indian kino (heartwood, leaves, and flowers) have long been believed to have medicinal properties in Ayurveda (The Flora of Orissa, Saxsena & Brahmam, 1994). In Karnataka the plant is known as honne or kempu honne. The Kannada people in India make a wooden tumbler from the heartwood of this herb tree (Saldanha, Flora of Karnataka, 1984).

Bark of *Pterocarpus marsupium marsupium*

Leaves of *Pterocarpus*

2.1 Habitat

A moderate to large deciduous tree about 90ft or more high, commonly found in hilly region of central and peninsular India (Andhra Pradesh, Bihar, Gujarat, Kerala, Madhya Pradesh, Maharashtra, Karnataka, Orissa, Tamilnadu, Uttar Pradesh); found at 3000 ft in Gujarat, Madhya Pradesh and Himalayan & sub Himalayan tracts-Nepal (Kapoor, 1989) and Sri Lanka. It grows on a variety of formation provided the drainage is good. It prefers a soil with a fair proportion of sand though it is often found on red loam with a certain amount of clay. The normal rainfall in its natural

habitat ranges from 75 to 200cm but it attains its largest size in parts of Mysore and Kerala, where the rainfall is even higher. It is a moderate light demander and the young seedlings are frost-tender (Wealth of India, CSIR, 1969).

Parts used: Bark, Leaves, Kino (gum)

2.2 Pharmacognostical Characteristics

Morphology

It is a moderate-sized to large deciduous tree. bark grey, longitudinally fissured and scaly. The older trees exude a blood red gum-resin.

Description

Leaves: compound; with 5 to 7 leaflets, 3 to 5 in long, oblong or elliptical with wavy margin or rounded or obtuse or retuse ends, glaucous beneath, secondary nerves close and parallel, over 12 cm each side.

Flowers: yellow, , up to 1.5 cm long, corolla papilionaceous, exserted beyond calyx, Stamen 10, split in 2 bundles , yellow, in very large, dense bunches.

Fruits: 2 to 5 cm long, roundish, winged, with one seed. Legume indehiscent, orbicular, compressed, broadly hardened winged around margin, usually single seeded, seeds subreniform, hilum small.

The Heartwood: is golden to yellowish brown with dark streaks staining yellow when damp and turning darker on exposure, strong and tough.

2.3 Microscopic Characteristics

The wood consists of vessels, tracheids, fibre tracheids and wood parenchyma all the elements being lignified and filled with tannin. Vessels are medium sized drum shaped, scattered, leading to semiring-porous conditions, tyloses present. Tracheids are long, abundant, thick walled, with tapering ends and simple pits on the side walls. Xylem parenchyma is small, thick walled with blunt ends; rectangular simple pitted surrounding the vessel. A few crystal fibers are observed in tangential section of the wood. Tree bark yields a reddish gum known as Kino gum, which becomes brittle on hardening and is very astringent. Sclerenchyma diffused pores Red marks are resin canals 8 Stem hairs overlapping metaxylem and protoxylem.

2.4 Chemical Constituents

Researches in the past have established the genus *Pterocarpus* to be the rich sources of polyphenolic compounds. All active principles of *P. marsupium* are thermostable.

The primary chemical components of P. marsupium are pterosupin, pterostilbene, isoliquiritigenin, liquiritigenin, epicatechin, kinotannic acid, kinoin, kino-red beta-eudesmol,
marsupol, carpusin and marsupinol.

The plant contains pterostilbene 4- 5%, alkaloids 0.4%, tannins 5%, protein, pentosan, pterosupin, pseudobaptigenin, liquiritigenin, isoliquiritigenin, garbanzol, 5- de- oxykaempferol, P- hydroxybenzaldehyde, beudesmol, erythrodirol- 3- monoacetate, l- epicatechin, marsupol, carpusin, propterol, propterol B, marsupinol, irisolidone- 7- O- A- L- rhamnopyranoside, have been obtained mainly from the heartwood and root.
The gum kino from the bark provides non- glucosidal tannins - kinotannic acid, kinonin (C28H24O12), kino- red (C28H22O11), pyrocatechin, pyrocatechin acid & small quantities of resin, pectin and gallic acid.

Aqueous extract of the heartwood of *Pterocarpus marsupium* contains 5 new flavonoids C- glucosides namely 6- hydroxyl- 2- (4- hydroxybenzyl)- benzo- furan- 7C- â- D- glucopyr anoside, 3- (á - methoxy- 4- hydro xybenzylidene) - 6- hydroxybenzo- 2(3H)- furanone- 7- C- â- D- glucopyranoside, 2- glucopyranoside, 8- (C- â- D- glucopyranosyl)- 7,3,4- trihydroxyflavone and 1,2- bis (2,4- dihydroxy, 3- C- glucopyranosyl) – ethanedione and two known compounds C- â- D- glucopyranosyl- 2,6- dihydroxyl benzene and sesquiterpene were isolated. Ether extract of the roots of Pterocarpus marsupium consists of a new flavonol glycoside 6- hydroxy- 3,5,7,4- te tramethoxyflavone 6- O- rhamnopyranoside, 8- hydroxy-4’-m ethoxyisoflavone- 7- O- glucopyranoside.

A benzofuranone derivative 2,4’6-trihydroxy-4-methoxy benzofuran-3(2H)-one designated carpus in, 1,3- bis(4- hydroxyphenyl) propan- 2- ol designated propterol, 1- (2,4- di hydroxyphenyl)- 3- (4- hydroxyphenyl) pro-pan-2-ol designated propterol, 6- hydroxy- 7- O- methyl- 3- (3- hydroxy- 4- O- methyl benzyl) chroman-4-one. Ethyl acetate extract of root contains benzofuranone, marsupin, dihydrochalcone, pterosupin, stilbene, pterostilbene, aliquiritigenin, isoliquiritigenin.

Methanolic extract of heart wood c ontains an isoflavone 7- O- á- L- rham-no pyranosyloxy-4’-methoxy-5-hydroxy-isoflavone. Three new isoflavone glycosides viz retusin 7- glucoside, irisolidone 7- rhamnoside and 5,7- dihydroxy- 6- methoxy

isoflavone 7- rhamnoside have been isolated from the heartwood of Pterocarpus marsupium. 2,6-dihydroxy- 2- (P- hydroxybenzyl)- 3(2H)- benzofuran- 7- C- â- D- glucopyranoside (Maurya et al., 2004; Gairola et a., 2010; Yogesh et al., 2010; Tiwari & Khare 2015).

2.5 Ethnomedicinal Uses (Tiwari et al., 2015)

Useful parts of the herb are heartwood, leaves, flowers, gum. The genus is widely distributed on the Earth and the astringent drug from this genus is known as "Kino". The phloem of stem contains red astringent fluid present in secretory cell, which exudes after given incision. Kino is odourless but has astringent taste and sticks in the teeth, colouring the saliva red in colour. As astringent it is used in diarrhoea, dysentery etc.

Bruised leaves are applied on fractures, leprosy, leucoderma, skin diseases, sores and boils, Constipation, depurative, rectalgia, opthamology, hemorrhages and Rheumatoid arthritis. Marsupin and Pterostilbene significantly lower the blood glucose levels useful in NIDDM. Bark is used as diuretic in Gabon and fresh leaves are used as food in Nizeria. Also is used in the form of powder or decoction in diarrhoea, and decoction is very useful for diabetic patients.

Stem in the treatment of neurological problems.

Leaves are used in GIT disorders, wood, stem bark, seed and flours are used in African traditional medicine, especially in the Cameroonian pharmacopoeia, for treating various diseases including hypertension, diabetes, intestinal parasitizes, renal and cutaneous diseases. The leaf paste is used as an ointment to treat skin diseases, sores and boils.

Wood: The heartwood is used as an ointment to astringent, bitter, acrid, cooling, anti-inflammatory, union promoter, depurative, urinary astringent, haemostatic, asthelmintic, constipating, anodyne alterant and rejuvenation. It is also useful in elephantiasis, inflammations, fractures bruises, leprosy, skin disease, leucoderma, erysipelas urethrorrhoea, diabetes, rectalgia, rectitis, opthalmopathy, diarrhea, dysentery, cough, asthma, bronchitis and greyness of hair.

Flower: The flower is used as appetizing and febrifuge and also taken to treat anorexia and fever.

Gum-resin: The gum is taken to treat bitter, styptic, vulnerary, antipyretic, anthelmintic and liver tonic. It is useful in spasmodic gastralgia, boils, gleet, urethrorrhoea, odontalgia, diarrhea, psoriasis, wound and ulcers, helminthasis, fevers, hepatopathy and ophthalmia.

Some facts: *P. marsupium* is a plant drug belonging to a group called '*Rasayana*' in Ayurvedic system of medicine. These '*Rasayana*' drugs are immunomodulators and relieve stress in the body. In India, Kannada peoples are used to make a wooden tumbler from the heartwood. Water is left overnight in the wooden tumbler and is consumed in the next morning to cure diabetes. Kol tribes in Odisha pound a paste mixture of the bark of *P. marsupium* with the bark of *Mangifera indica, Shorea robusta & Spondias pinnata* to treat some dysentery illness. The gum resin of this plant is the only herbal product ever found that regenerate beta cells that produce insulin in pancreas.

2.6 Biological activity

Although a large number of compounds have been isolated from various parts of *P. marsupium*, few of them have been studied for biological activity as shown in Table 1. The structure of some of these bioactive compounds has been presented in Figure 1. The bark contains l-epicatechin and a reddish brown colouring matter. The bark is occasionally employed for dyeing. The heartwood yields liquiritigenin, isoliquirtigenin, a neutral unidentified component, alkaloid and resin. The wood also contains a yellow colouring matter and an essential oil and a semi-drying fixed oil.

The tree yields a gum-Kino which exudes when an incision is made through the bark up to the cambium. It is odourless and bitter with astringent taste and colours saliva pink when masticated. Kino contains a non-glucosidal tannin kinotannic acid, kinoin and Kino-red, small quantities of catechol, protocatechuic acid, resin, pectin and gallic acid. The therapeutic value of Kino is due to Kino is due to kinotannic acid. Kino is powerfully astringent and was formerly used widely in the treatment of diarrhea and dysentery. It is locally applied in leucorrhoea and in passive haemorrhages. It is also used for toothache. The bark is used as an astringent and in toothache. The flowers are said to be used in fever. The bruised leaves are considered useful as an external application for boils, sores and skin diseases. The aqueous

infusion of the wood is said to be of use in diabetes and water stored in vessels made of the wood is reputed to have antidiabetic qualities (Anon, Wealth of India, 2003).

2.7 Medicinal use of various parts of *P.marsupium*

Various parts of the *P. marsupium* tree have been used as traditional ayurvedic medicine in India from time immemorial. The medicinal utilities have been described, especially for leaf, fruit and bark. The bark is used for the treatment of stomachache, cholera, dysentery, urinary

complaints, tongue diseases and toothache. The gum exude 'kino', derived from this tree, is used as an astringent (Singh et al., 1965). The gum is bitter with a bad taste. However, it is antipyretic, anthelmintic and tonic to liver, useful in all diseases of body and styptic vulnerant and good for griping and biliousness, opthalmiya, boils and urinary discharges. The flowers are bitter, improve the appetite and cause flatulence (Indian Medicinal Plants 1999). *P. marsupium* has a long history of use in India as a treatment for diabetes. It is a drug that is believed to have some unique features such as beta cell protective and regenerative properties apart from blood glucose reduction (WHO 1980; Chakravarthy et al., 1981). Some of the medicinal attributes of various parts of *P. marsupium* have been summarized (Yogesh et al., 2010) in table 2.

3. LITERATURE REVIEW

3.1 Anti-diabetic and antioxidant activity

P. marsupium demonstrates unique pharmacological properties, which include beta cell protective and regenerative properties as well as blood glucose lowering activity. The animal studies conducted have used various species including rats, dogs, and rabbits with induced diabetes and subsequent treatment with various extracts of *P. marsupium*. In all of these studies, *P. marsupium* was found to reverse the damage to the beta cells and actually repopulate the islets, causing a nearly complete restoration of normal insulin secretion (Chakravarthy et al., 1981, 1982a,b; Manickam et al., 1997; Ahmad et al., 1991a,b; Pandey & Sharma 1976; Shah 1967).

In one study it was shown that aqueous extract of *P. marsupium* modulates the inflammatory cytokine TNF-alpha in type 2diabetic rats and this has an indirect effect on PPAR-Gamma expression. By decreasing TNF-α, drug can upregulate the PPAR-Gamma and in turn the glucose metabolism (Halagappa et al., 2010).

The bark of *P. marsupium* is traditionally used in the Indian Ayurvedic system of medicine as an anti-diabetic drug. The compound that is responsible for antidiabetic activity is (-) epicatechin, a member of the catechin group of compounds belonging to the class of flavonoids (Zaid et al., 2002).

It has been shown that *P. marsupium* works by the regeneration of the beta cells and increase proinsulin biosynthesis. Marsupin and Pterostilbene significantly lowered the blood glucose level of hyperglycemic rats, and the effect was comparable to that of 1,1-dimethyl biguanide (metformin) (Manickam et al., 1997).

Overnight water stored in water tumblers made out of the heartwood of *P. marsupium* is used as a traditional therapy for patients of Diabetes mellitus especially in the state of Madhya Pradesh (Maheswari et al., 1980).

Isolated compounds from *P. marsupium* have been shown to enhance the conversion of Pro-insulin to insulin and stimulate cAMP content in the islets of Langerhans (Ahmad et al., 1991a,b).

It is proposed that the flavonoid fraction of *P. marsupium* bark effectively reverses the alloxan induced changes in the blood sugar level and the beta cell population in the pancreas (Chakravarthy et al., 1980).

P. marsupium methanol extract has been found to cause normalization of serum protein and albumin levels, possibly through the increase in insulin mediated amino acid uptake, enhancement of protein synthesis and inhibition of protein degradation (Dice et al., 1978).

Administration of the bark extract to diabetic rats restored the levels of serum electrolytes, glycolytic enzymes and hepatic cytochrome p-450 dependent enzyme systems by inhibiting the formation of liver and kidney lipid peroxides (Gayathri & Kannabiran et al., 2010).

3.2 Cardiotonic activity

Cardiotonic activity was reported of the aqueous extract of heartwood of *P. marsupium*. This plant species contains 5,7,2,4-tetrahydroxy isoflavone 6-6 glucoside which are potent antioxidants and are believed to prevent cardiovascular diseases. The cardiotonic effect of the aqueous extract of heartwood of *P. marsupium* was studied by using the isolated frog heart perfusion technique. Calcium free Ringer solution was used as vehicle for administration of aqueous extract of *P.marsupium* as a test extract and digoxin as a standard (Mohire et al., 2007). Liquiritigenin and Pterosupin, the flavonoid constituents of *P. marsupium* are effective against reducing serum cholesterol levels, LDL cholesterol, and atherogenic index. Pterosupin being additionally effective in lowering serum triglycerides (Jahromi & Ray, 1993**).**

3.3 Hepatoprotective activity

Methanol extract of the stem barks of *P. marsupium* possesses significant hepatoprotective activity (Mankani et al., 2005).

3.4 Antioxidant activity

The whole aqueous extract of the stem bark of *P. marsupium* showed high anti-oxidant activity and protects the mitochondria against oxidative damage (Mohammadi et al., 2009).

Heartwood extracts of *P. marsupium* promotes wound healing in both normal and diabetic animals by topical application of the extracts (Singhal et al., 2013).

Ethanol extracts of the heartwood of *P. marsupium* is found to be useful in preventing allergic conditions and diseases such as asthma owing to its ability to decrease the increased eosinophilic , leucocytic count, prevention of mast cell degranulation (Suralkar et al., 2012).

Antidiarrhoeal activity of Ethanol heartwood extract of *Pterocarpus marsupium* was also studied (Dilpesh et al., 2011).

3.5 Antibacterial activity

Hexane, ethyl acetate and methanol extracts were tested against four selected Gram positive and Gram negative bacteria- *S. aureu*, *K. pneuminiae*, and *P. aeruginosa* (Sapha 1956; Gayathri & Kannibaran, 2010). *In vitro*, it inhibits *Pseudomonas aeruginosa*, *Streptococcus pyrogens* and *Staphylococcus aureus*. Ethyl and methanol extracts were more sensitive to the bacteria than extracts made out of hexane. Both the extracts exhibited concentration dependent variation in their anti-bacterial activity. Similar observations have been reported where it has been showed that ethanol extracts of *P. marsupium* exhibited significant anti-ulcer and antioxidant properties in rats (Nair et al., 2005; Patil & Gaikwad, 2011).

3.6 Anti-inflammatory activity

P. marsupium has also shown strong potential for its antiinflammatory activity. In this study, an extract of *P. marsupium* containing pterostilbene has been evaluated for its PGE2- inhibitory activity in LPS-stimulated PBMC. In addition, the COX-1/2 selective inhibitory activity of *P. marsupium* extract was investigated (Hougee et al., 2005; Salunkhe et al., 2005).

3.7 Central Nervous System

The methanol extracts of *P. marsupium* has potent nootropic activity (Chauhan & Chaudhary, 2012).

3.8 Other Studies

Anti-cataract activity of *P. marsupium* in diabetes was observed (Vats et al., 2004).

Lukewarm aqueous suspension of 2g gum with jaggery is given early in the morning for a week to treat asthma (Patil et al., 2008).

Bark is useful in vitiated condition of kapha, pitta, elephantiasis, erysipelas, urethrorrhea, rectalgia, opthalmopathy, hemorrhages, dysentry, cough, and grayness of hair (Patil & Gaikwad., 2011).

20g of the stem bark boiled with 1 litre of water till 200ml along with 7 black pepper dried seeds of *Piper nigrum*) taken orally cures spermaturia, spermatorrhea, leucorrhoea, amenorrhoea, dysmennorhoea, menorrhagia and impotency (Behera & Mishra, 2005).

Table 3. Primary chemical components from *Pterocarpus marsupium*

Neem compound	Source Biological	Reference
Liquiritigenin bark	Antidiabetic, Antihyperlipidemic effect	Jahromi & Ray 1993
Isoliquiritigenin bark	Antidiabetic	Jahromi & Ray 1993
Pterosupin	Antihyperlipidemic effect	Jahromi & Ray 1993
Epicatechin bark	Antidiabetic, Anthelmentic properties	
Pterostilbene bark	blood glucose levels, Anti-oxidant and anti tumor effects	Grover et al., 2005
Marsupinol bark	Antihyperlipidemic effect	Jahromi & Ray 1993

Table 4. Some medicinal uses of *Pterocarpus marsupium* as mentioned in Ayurveda

Parts	Medicinal use
Leaf	External application for boils, sores and skin diseases, stomach pain
Bark	Astringent, toothache
Flower	Fever
Gum-Kino	Diarrhea, dysentery, leucorrhoea, passive haemorrhages

3.9 Clinical studies and plausible medicinal applications

Although studies have been carried out on various biological activities of *P. marsupium* extracts and some of the isolated compounds in several animal models, a few reports are available on clinical studies with the extracts or the compounds and their medicinal applications (Anon, Wealth of India, 2003). *Pterocarpus marsupium* (Leguminnaceae family), commonly known as Bija, that has been recommended as

early as 1000 BC, by Sushruta for the treatment of diabetes. Various reports indicate the hypoglycemic activity of PMS both in experimental and clinical studies (Pandey & Sharma 1976; Remsberg et al., 2008; Manlio et al., 2005).

4. Scope of the work

As human life expectancy has increased, so too has the incidence of stress related neurodegenerative disorders such as AD, PD and HD. Plant extracts have a wide range of medicinal actions, and throughout history, they have been used to treat many different types of diseases. In the treatment of many diseases, antioxidant therapy plays a key role, so current research is now directed towards finding naturally occurring antioxidant of plant origin.

Although medicinal herbs has been found to share many medicinal properties with '*rasayans*' in Ayurveda, its neuroprotective effect in different experimental neurodegenerative models are still debatable. Actually, little is known about the feeding pattern and feeding rhythms that underly the hypophagia of the stress treated rats. The general behavior, anxiolytic, antidepressant and antioxidant enzyme examination of the rat whole brain, pattern of food and water intake are used for the first time, to assess the protective effect of ethanol extract of *Pterocarpus marsupium* bark (EEPM) against MSG-induced neurodegenerative injury. We anticipate that the behavioral tests used in the present study could contribute to the evaluation of potential drugs effective in the prevention of functional deficits induced by neurotoxic agent and may shed an insight into the mechanism of action. Hence, a special attention is focused to understand the treatment of neurodegenerative diseases by natural phenolic antioxidants from this plant.

There are, indeed, a multitude of paradigms assessing various aspects of the behavioral performance and cognitive abilities. Till now, some of the paradigms will be not used at all in the evaluation of *Pterocarpus marsupium* bark against behavioral consequences of adult rats in stress.

Hence, in order to contribute further to the knowledge on *Pterocarpus marsupium* bark, the objective of the present study has been designed to evaluate the possible neuroprotective effect of EEPM against MSG-induced excitotoxicity in adult rats.

4.1 SPECIFIC OBJECTIVE:

- To observe behavioral parameters in anxiety & depression following MSG induced excitotoxic neurodegeneration.
- To evaluate the neuroprotective role of EEPM bark against MSG-induced excitotoxicity model in SD rats, following pre-treatment with EEPM for 30 days,

and analyzing the possible effects on attenuating and normalizing the perturbations following intraperitoneal administration, to induce neurotoxic effects, using MSG for 07 days and thereafter , by studying:

- The behavior of animals in anxiety and depression models.
- The perturbations in the levels of antioxidant defense systems-SOD, CAT, GSH, and LPO in the whole brain of the rat.

5. Plan of work

Phase I:

5.1 Phytochemical studies

- Collection and authentication of plants.
- Extraction of plant material by using various solvent systems.
- Preliminary phytochemical study for the identification of plant secondary constituents.

Phase II:

5.2 Pharmacological Studies:

5.2.a. Pretreatment with ethanol extract of *Pterocarpus marsupium* (EEPM) Roxb. bark:

EEPM was administered orally for 30 days, to the animals in all the groups, except the control, MSG-only treated and diazepam-only treated groups, at two different dose levels (100 and 200mg/kg), prior to the administration of MSG on day 31.

5.2.b. Induction of neurotoxicity:

Following 30 days of pretreatment with EEPM, neurodegeneration was induced by intraperitoneal injection of 2g/kg of MSG for seven days, in all the groups except the control group.

5.3. Evaluation of general behavioral alterations in excitotoxicity-induced rats:

5.3.a. General parameters

i. Body weight
ii. Food intake and
iii. Water intake

5.3.b. Tests for anxiety

i. Actophotometer

5.3.b. Test for depression

i. Test for depression

Phase III:

5.4. Biochemical Analysis

5.4..a. Specimen Preparation

5.4.b. *In-vivo Studies*

Enzymatic and non-enzymatic antioxidants play a crucial role in neurodegeneration. Hence, the effects of EEPM and the following antioxidant defense elements in brain were also be measured: Biochemical Analysis of rat brain

- ***In vivo* antioxidant studies**
    i. Superoxide dismutase (SOD)
    ii. Catalase (CAT)
    iii. Glutathione (GSH) and
    iv. Lipid Peroxidation (LPO)

Phase IV:

5.5. Statistical analysis

The data's were presented as mean ± SEM and were subjected to statistical analysis by Dunnett's test followed by one way ANOVA. P-value less than 0.05 were considered statistically significant.

6. Materials & methods

6.1. Chemicals used

Ferric chloride, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), quercetin, thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium nitroprusside, Griess reagent, Eggphospatidyl choline, n-butanol, copper(II)chloride, neocuprine, Ammonium acetate buffer, O-phenanthroline, Sodium nitrite, Aluminium chloride, Corticosterone, potassium ferricyanide and dextromethorphan were purchased from Sigma Aldrich Co., St Louis, USA. Reduced glutathione (GSH), 5, 5-dithiobis-2-nitrobenzoic acid (DTNB) were obtained from Sisco Research Laboratories Pvt Ltd., Mumbai, India. Gallic acid, Ascorbic acid, Tris–hydrochloride buffer, and methanol, ethanol, sodium bicarbonate, sodium carbonate, copper sulphate, sodium potassium tartarate, hydrochloric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, were obtained from S.D. Fine Chem Ltd., Biosar, India. Epinephrine, saline was purchased from Kovai Medical Center and Hospital. Folin's ciocalteau reagent, potassium hydroxide, Merck Ltd., Mumbai, India. Monosodium glutamate was purchased from Chemico laboratories. Acetonitrile (HPLC grade) was purchased from Qualigens Fine Chemicals, Mumbai, India. The water used in HPLC for sample preparation was purchased from Ranbaxy Laboratories Ltd., Mumbai, India. All other chemicals and reagents used were of analytical grade.

The present study was designed to assess the neuroprotective effect of standardized bark extract of *P. marsupium* in excitotoxicity-induced neurodegenerative models and the work was carried out using the methods described below:

6.3. Phase-I: Collection and authentication of bark extract of *P. marsupium*

6.4. Phase II: Pharmacological studies

6.4.1. Animal study

Adult female Sprague Dawley rats were used in the present study.

6.4.2. Housing and feeding condition

All the rats were kept at room temperature (22 ± 30°c). They were housed and treated as per the internationally accepted ethical guidelines for the care of

laboratory animals. Prior to the experiments, rats were fed with standard food, water *ad libitum* and were acclimatized to the standard laboratory conditions of temperature (22 ± 30°c) and maintained a 12:12 h light: dark cycle. All the experimental procedures were performed on the animals after approval from the ethics committee and in accordance with the recommendations for the proper care and use of laboratory animals.

6.4.3. Experimental Protocol

The animals were divided into 05 groups and the animals in all the groups, except group-I (Vehicle control), II (MSG-only) and III (MSG+memantine), all the other groups were pretreated with EEPM for 30 days. Then from day 31 to 37, the animals in group III, IV and V groups were administered with MSG (2g/kg, i.p.) for seven days to induce excitotoxicity, as mentioned below:

1. Pretreatment with EEPM (100 and 200mg/kg): 30 days
2. MSG treatment (2g/kg, ip.): 07 days
3. Observations on changes in general behavior: 07 days
    - Measurement of food intake
    - Measurements of water intake
    - Measurement of body weight
4. Tests for anxiety and depression on day 37
5. Analysis of brain anti-oxidant systems following behavioral tests on day 37

Grouping of animals:

Group- I, served as control group and received 0.9% normal saline.

Group- II, was administered only MSG

Group-III, served as positive control (memantine 20 mg/kg) + MSG

Group- IV, pretreated with EEPM (100 mg/kg) + MSG and

Group- V, pretreated with EEPM (200 mg/kg) + MSG.

6.4.4. Evaluation of general behavioral alterations in MSG-treated rats

The animals were subjected to the following behavioral procedures immediately following intraperitoneal administration of MSG to animals in all the groups, excepting the control group, which was not exposed to MSG. The following

studies were carried out from day 31 to day 37. During the entire period of study the animals were observed for any changes in behavior and suitably noted. On day 37, the rats were sacrificed and the brain was removed for estimation of antioxidant enzymes. All the measurements were made every day between 8.30am and 9.15am, starting from the day of MSG and drug treatment, and continued for 7 days thereafter.

a. Measurement of body weight

The body weight of the animals was monitored daily by weighing on an electrical balance with accuracy to ± 0.1 g. All measurements were made every day between 8.30 and 9.15 h, immediately before administration of stress. Changes in body weight were calculated by subtracting the weight of the animal obtained on last day of stress from that of the animal weight immediately before the first stressor.

b. Measurement of food intake

The measurement of food intake was studied by presenting pre-weighed food to the animals in all the groups' immediately following stress and drug treatment. The amount of food (Brook Bond, Lipton, India) consumed by the animals (food intake in g/g weight of rat) was evaluated by weighing the remaining amount of food, 24 h after food presentation with accuracy to ± 0.1 g. Spillage of food pellets was rare, but any obvious spillage was noted and those data excluded from the analysis. The food pellets were placed at a height accessible to the experimental animals (5 cm from the floor of the cage), so they did not need to rear up to reach water and food. Water and food intake evaluation started immediately after stress administration.

c. Measurements of water intake

The animals in all groups had free access to water during the entire duration of the study. The intake of water was studied by measuring the volume of water (water intake in ml / gram body weight of rat) consumed over a 24 h period, following induction of stress, in all the groups. Clean water was provided in graduated burettes with drinking spouts allowing direct volumetric measurements of intake to the nearest 0.1 ml. The drinking spouts were placed at a height accessible to the experimental animals (5 cm from the floor of the cage), so that they did not need to rear up to reach water.

6.4.6. Evaluation of behavioral studies

6.4.6.a. Test for anxiety studies

i. Actophotometer

The actimeter test was performed independently as a test to record the effects of the drugs on the spontaneous locomotor activity of rats using a photo-electric actimeter, 1 h after administration of all drugs. The apparatus consist of stainless steel box containing transparent cages (270+220+110) in which the animal's horizontal activity in measured by two light beams connected to a photoelectric cell. The total numbers of beam crossings are then recorded over a period of 5 min (*Ramanathan et al., 2007*).

6.4.6.b. Test for depression

i. Forced swim test

A modified forced swim test procedure consisting of an increase in water depth was used to enhance sensitivity for detecting putative antidepressant activity of drugs (*Porsolt et al., 1991*). Rats were placed into plastic buckets (19 cm diameter, 23 cm deep, filled with 23–25°C water) and videotaped for the entire session. As described previously by *Porsolt,* only the last 4 min. were scored for mobility duration.

6.5. Phase III: Biochemical studies

At the end of the behavioral studies, the animal models were anesthetized with mild chloroform and sacrificed by cervical dislocation; the whole brain were quickly removed, rinsed in ice-cold isotonic saline and packed in an aluminium foil for further use.

6.5.1. Test drug and chemicals

All chemicals used for the experiments were of analytical grade obtained from SD Fine Chemicals Mumbai, India.

6.5.2. Preparation of homogenate

The brain tissue were weighed and 10% tissue homogenate was prepared with 0.025M Tris-Hcl buffer, pH 7.5. After centrifugation at 10,000 x g for 10 min. the resulting supernatant was used for enzyme assays for the estimation of non-enzymatic and enzymatic antioxidants.

6.5.2.a. Estimation of Superoxide Dismutase (SOD)

The SOD activity in supernatant was measured by the method of *Misra and Fridovich (1967).* The supernatant (500 µl) was added to 0.800ml of carbonate buffer (100 mM, pH 10.2) and 100 µl of epinephrine (3 mM). The change in

absorbance of each sample was then recorded at 480 nm in spectrophotometer for 2 min. at an interval of 15 sec. Parallel blank and standard were run for determination SOD activity. One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto oxidation.

Reagents	**(Standard)**	**(Sample)**	**Blank**
Carbonate buffer	0.900 ml	0.800 ml	1.0 ml
Supernatant	……	0.1 ml	……
Epinephrine	0.1 ml	0.1 ml	……

The reaction mixtures are diluted 1/10 just before taking the readings in a spectophotometer.

Calculation

% Inhibition= [X A_{480nm}/min. Uninhibited – X A_{480nm} /min. inhibited X 100] / [X A_{480nm} /min. Uninhibited – X A_{480nm} /min. Blank]

Units/ml enzyme = [% Inhibition X V_t] / [(50%) X V_s]

Units/mg protein = [Units/ml enzyme] / [mg protein/ml enzyme]

6.5.2.b. Estimation of Catalase (CAT)

Catalase activity was measured by the method of *Aebi*. 0.1 ml of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/mg protein. A unit is defined as the velocity constant per second.

Reagents	**Sample**	**Blank**
Phosphate buffer solution	1.9 ml	2..9 ml
Supernatant	0.1 ml	0.1 ml
H_2O_2	1 ml	……

The reaction occurs immediately after the addition of H_2O_2. Solutions are mixed well and the first absorbance (A_1) is read after 15 seconds (t_1) and the second absorbance (A_2) after 30 seconds (t_2).The absorbance is read at wave length 240 nm.

Calculation

$K= [(V_t/ V_s) \times (2.3 \times \Delta_t) \times (\text{Log } A_1/ A_2) \times 60]$

where,

K = Rate constant of the reaction; $\Delta_t = (t_2 - t_1)$ =15 seconds; A_1= absorbance after 15 seconds; A_2= absorbance after 30 seconds; V_t = total volume (3 ml); V_s = volume of the sample (0.1ml);

6.5.2.c. Estimation of reduced glutathione (GSH)

Reduced glutathione (GSH) was measured by the method of *Ellman et al., (1959)*. The PMS of rat brain (720 µl) and 5% TCA were mixed to precipitate the protein content of the supernatant. After centrifugation at 10,000 x g for 5 min. the supernatant was taken. DTNB (5,5'-dithio-bis(2)-nitrobenzoic acid) Ellman's reagent was added to it and the absorbance was measured at 412 nm. A standard graph was drawn using different concentration of standard GSH solution. GSH contents were calculated in the rat brain.

6.5.2.d. Estimation of Lipid peroxidation (LPO)

Ohkawa et al., (1979) method was used to estimate the total amount of lipid peroxidation (LPO) product. LPO was estimated in terms of TBARS and malondialdehyde (MDA) was taken to represent the TBARS. The incubation mixture consisting of 0.5ml of supernatant brain homogenate, 0.2 ml of 8% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid solution (adjusted to pH 3.5 with 1NaOH / 0.1N HCl) and 1.5 ml of 0.9% aqueous solution of thiobarbituric acid (adjusted to pH 7.4 with 1N NaOH / 0.1N HCl) was made up to 5.0 ml with double distilled water and then heated in boiling water bath for 30 minutes. After cooling, the red chromogen was extracted into 5 ml of the mixture of n-butanol and pyridine (15.1v/v) centrifuged at 4000 rpm for 10 minutes. The absorbance of organic layer was measured at 532 nm. 1, 2, 3, 3-tetraethoxypropane (TEP) was used as an external standard and the levels of lipid peroxide was expressed as *µmoles of MDA / g* protein. The calibration

curve of TEP was prepared by the above procedure taking 80-240 nmoles of TEP as standard over which, linearity was obtained.

7. Results

7.1.1. Effect of EEPM and memantine on general behavior parameters in MSG treated rats

7.1.1a. General behavior

Following the administration of MSG, (for the induction of exitoxicity), the animals were observed for general behavior up to 45 min, on all the 07 days. MSG may result in psychological and physiological changes in behavior, attributable to excitotoxicity. The observations made on general behavior for a period of 45 minutes following administration of MSG, memantine and pretreatment with EEPM, is described below:

Rats treated with MSG exhibited a prolonged aggressive behavior in comparison to the control rats. Pretreatment with EEPM for 30 days, resulted in profound calmness. Memantine-treated animals exhibited little or no obviously observable behavior effects.

The MSG-treated group exhibited a score of 4.1 ± 0.21, and the relative scores of other groups of animals are EEPM 100 and 200 mg/kg were observed to be 3.35 ± 0.36 and 2.65± 0.66, respectively. Memantine (2.41 ± 0.25) rats exhibited similar scores, in comparison to the control animals (2.32 ± 0.28).

7.2.2b. Feed intake

The cumulative measurement of food intake is shown in table 3 and figure X. In comparison to control group (0.165 g/g b.w), MSG-treated rats consumed significantly less food (0.122 g/g b.w). The suppression of food intake was found to be highest with the MSG-treated group, in comparison to all other groups. This diminishment in food intake was associated with an increase in time spent in eating and in meal duration. The food intake of animals pre-treated with EEPM was measured to be 0.126 g/g b.w, (100 mg/kg) and 0.152 g/g b.w. (200mg/kg). Memantine- treated group was found to be 0.179 g/g b.w of food.

The suppression of food intake in rats pre-treated EEPM at different dose level was found to be significantly antagonized in comparison to MSG-treated rats.

The suppression of food intake was found to be minimal with memantine-treated rats.

Table 6.Effect of MSG, Memantine and EEPM on feed intake in MSG-treated rats

Groups	Feed intake (in g/g b.w)
Control	0.165±0.05
MSG only	0.122±0.01
MSG+Memantine	0.179±0.05
MSG+EEPM 100mg/Kg	0.126±0.02
MSG+EEPM 200mg/Kg	0.152±0.04

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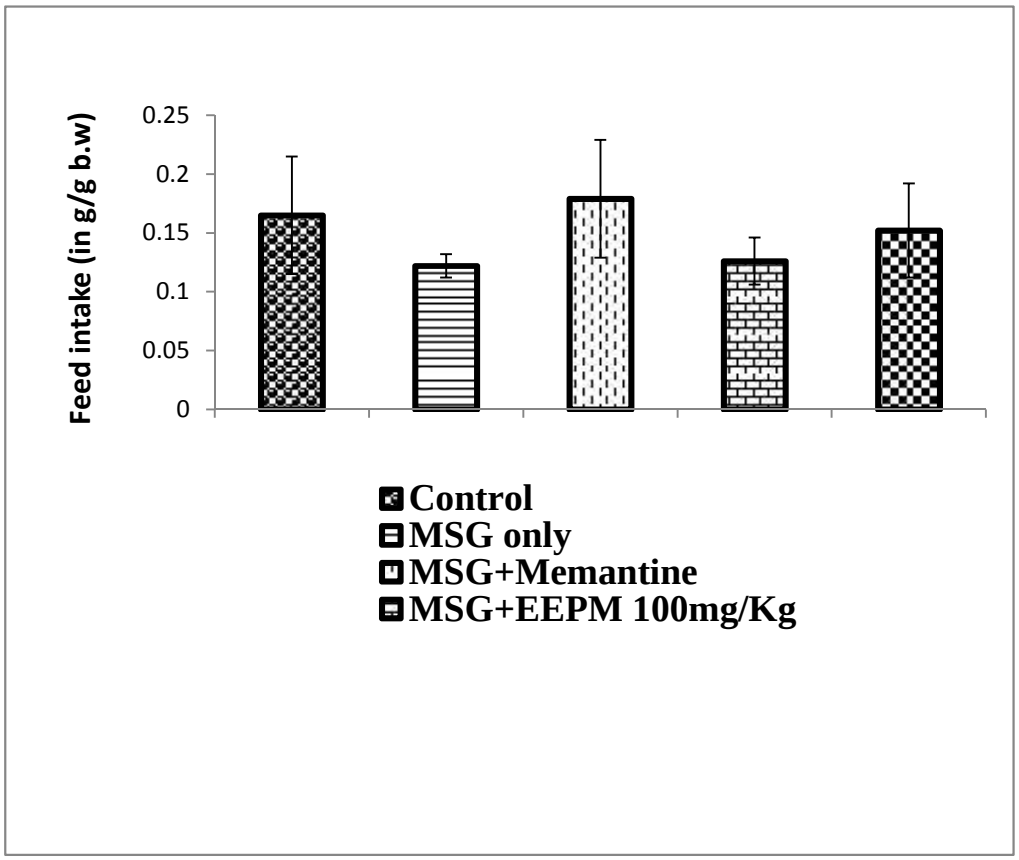


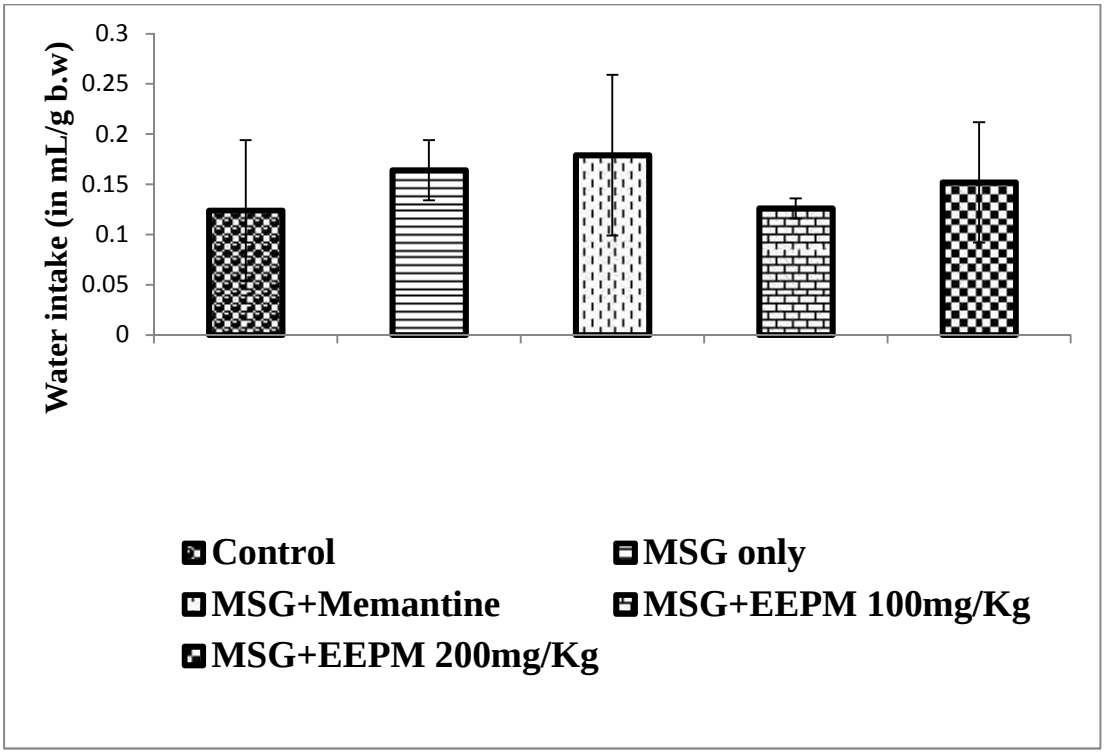
(Values are mean ± SE from 6 observations in each group)
[a]p<0.05, [b]p<0.01, [c]p<0.001– compared with control group
[p]p<0.05, [q]p<0.01, [r]p<0.001 – compared with MSG group

7.2.1c. Water intake

The results on cumulative intake of water in all the groups of animals studied are shown in table 4 and figure. In comparison to control group (0.124±0.07 ml/g b.w); MSG-treated rats consumed significantly more water (0.164±0.03 ml/g b.w). The intake of water in EEPM pre-treated rats (100 and 200 mg/kg) was found to be significantly less (0.116±0.01 and 0.103±0.06 ml/g b.w; $p<0.01$, respectively), in comparison to control and MSG-treated group. Similarly memantine-treated rats had consumed 0.123±0.08 ml/g b.w of water ($p<0.01$) in comparison to MSG-treated group. The results indicate that administration of MSG had increased consumption of water and this effect was antagonized by pre-treatment with EEPM and memantine.

Table 7. Effect of MSG, Memantine and EEPM on water intake in MSG-treated rats

Groups	Waterintake (in mL/g b.w)
Control	0.124±0.07
MSG only	0.164±0.03
MSG+Memantine	0.123±0.08
MSG+EEPM 100mg/Kg	0.116±0.01
MSG+EEPM 200mg/Kg	0.103±0.06



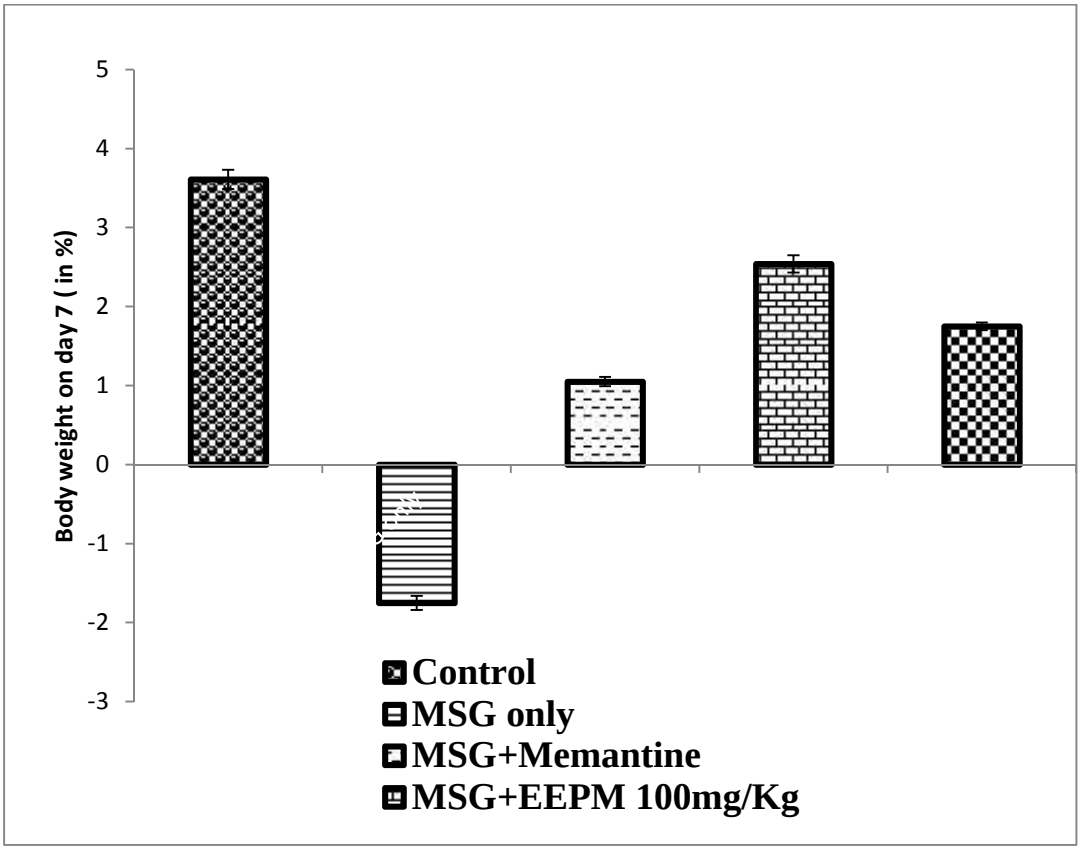
(Values are mean ± SE from 6 observations in each group)
[a]p<0.05, [b]p<0.01, [c]p<0.001– compared with control group
[p]p<0.05, [q]p<0.01, [r]p<0.001 – compared with MSG group

7.2.2c. Body weight

The results of body mass measured from day31 to day 37 are shown in table 5 and figure. In comparison to the control group animals, the MSG-treated animals were found to be decreasing in body weight from day 3 onwards and had significantly lower body weights on day 4 (-1.24%) ($p<0.05$) and, day 5 (-1.39%) ($p<0.05$), day 6 (-1.48 %) ($p<0.05$) and, day 7 (-1.57%) ($p<0.05$), in comparison to control group which gained +1.64 % g, +3.54 % g weight on day 4, and day 5, +2.61 % g on day 6 and, +3.49 % g weight on day 7 respectively. Pre-treatment with EEPM for 7 days, prevented the suppression of rat body growth induced by MSG insult. A reversal in the loss of body weight in animals was observed following treatment with different doses: EEPM 100 mg/kg gained +1.72 % and + 2.39 % on day 6 and day 7, respectively; EEPM 200 mg/kg gained +0.92 % and + 2.31 % on day 6 and day 7, respectively, in comparison to animals of the respective group on day 1. EEPM pre-treatment was found to improve the body weight of the animals. Memantine-treated animals were found to be undisturbed in the rate of growth of body weight mass, in comparison to control and MSG-treated animals.

Table 8. Effect of MSG, Memantine and EEPM on body weight in MSG-treated rats

Groups	Body weight on day 7 (in %)
Control	+3.61±0.12
MSG only	-1.75±0.09
MSG+Memantine	+1.05±0.06
MSG+EEPM 100mg/Kg	+ 2.54±0.11
MSG+EEPM 200mg/Kg	+ 1.75±0.05



(Values are mean ± SE from 6 observations in each group)
[a]p<0.05, [b]p<0.01, [c]p<0.001– compared with control group
[p]p<0.05, [q]p<0.01, [r]p<0.001 – compared with MSG gr

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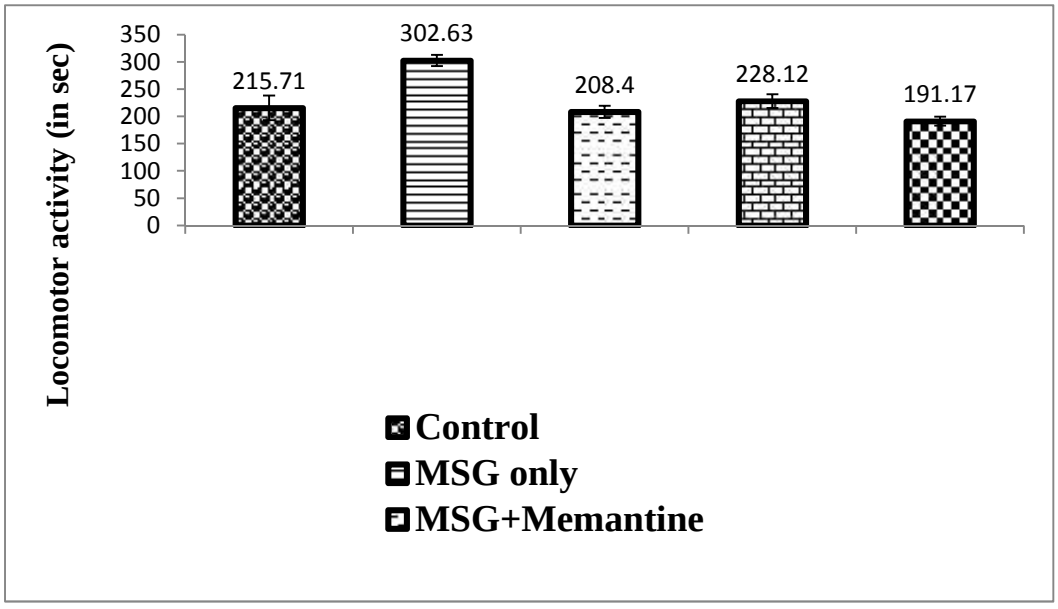
7.2.1b. Effect of EEPM and MSG on Locomotor Activity in MSG-Treated Rats Using Actophotometer

Locomotor activity of MSG-treated animals was determined with an actophotometer and the results are shown in figure and table 6. When tested in an actophotometer, a significant increase in movement levels were seen in MSG-treated rats (302.63±10.26), when compared with control animals (215.71±22.5). A significant reduction in movement activity was seen in MSG-treated animals pretreated different doses of EEPM (100 mg/kg: 228.12±12.6; 200 mg/kg: 191.17±08.14). The locomotor activity of rats treated with memantine (208.4±11.25) was found to be significantly better.

Table 9.Effect of MSG, Memantine and EEPM on locomotor activity

Groups	Locomotor activity (in sec)
Control	215.71±22.5
MSG only	302.63±10.26
MSG+Memantine	208.4±11.25
MSG+EEPM 100mg/Kg	228.12±12.6
MSG+EEPM 200mg/Kg	191.17±08.14



(Values are mean ± SE from 6 observations in each group)
[a]p<0.05, [b]p<0.01, [c]p<0.001– compared with control group
[p]p<0.05, [q]p<0.01, [r]p<0.001 – compared with MSG group

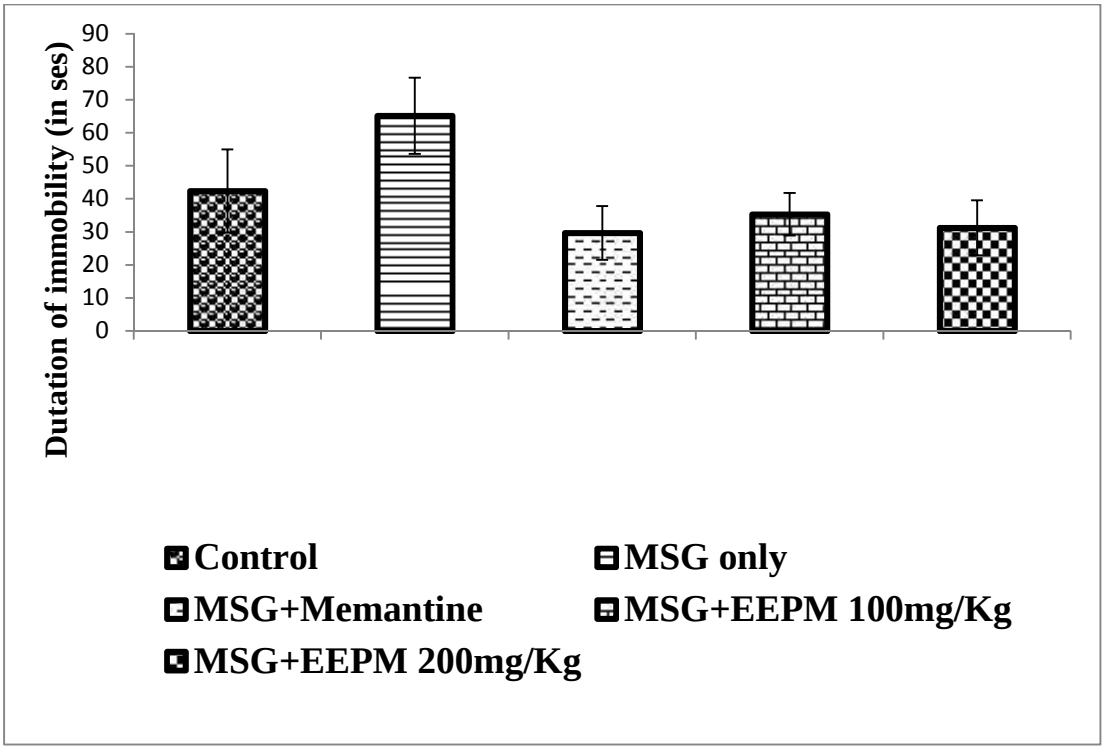
7.2.1c. Effect of EEPM and memantine on FST in MSG treated rats

The effect of MSG, EEPM and memantine on the duration of immobility of rats in the FST is shown in figure and table 9. Pretreatment with EEPM 200mg/kg (31.25±8.32; P<0.01) was found to attenuate the duration of immobility significantly compared to other EEPM-treated groups [EEPM 100mg/kg: 35.31±6.43 (P<0.01) and, in comparison to control [42.38±12.6] group.

Analysis of the results of the experiment, which examined the effect of MSG [65.14±11.5 (P<0.0001)], following pretreatment for day 30with EEPM on FST revealed that changes in the freezing time displayed by the rats were less apparent when compared with stressed group. The duration of immobility was found to be 29.67±8.14 (P<0.0001) for rats treated with memantine displaying antidepressant activity.

Table 10.Effect of MSG, Memantine and EEPM on forced swim test in MSG-treated rats

Groups	Duration of Immobility (in sec)
Control	42.38±12.6
MSG only	65.14±11.5
MSG+Memantine	29.67±8.14
MSG+EEPM 100mg/Kg	35.31±6.43
MSG+EEPM 200mg/Kg	31.25±8.32



(Values are mean ± SE from 6 observations in each group)
[a]p<0.05, [b]p<0.01, [c]p<0.001– compared with control group
[p]p<0.05, [q]p<0.01, [r]p<0.001 – compared with MSG group

7.3 Phase III: Biochemical analysis of brain antioxidants

7.3.1 Effect of EEPM and memantine on rat brain antioxidant system in MSG-treated rats

Table 11.Effect of MSG, Memantine and EEPM on the rat brain anti-oxidant system in MSG-treated rats

Groups	SOD *(U/mg protein)*	CAT *(U/mg protein)*	GSH *(mg/g protein)*	LPO *(µmoles of MDA/g protein)*
Control	39.19±8.51	102.14±12.4	35.25±5.12	1.32±0.05
MSG Only	22.92±6.58	45.21±5.91	22.12±6.54	1.88±0.02
MSG+Memantine	35.12±6.12	93.85±12.05	34.24±4.54	1.41±0.09
MSG+EEPM 100mg/Kg	25.17±7.62	71.55±9.45	27.19±3.60	1.58±0.05
MSG+EEPM 200mg/Kg	31.18±5.18	94.54±7.32	31.23±2.82	1.43±0.01

7.3.1a. Effect of EEPM on SOD (*U/mg protein*) levels in MSG-treated rat brain

The SOD profile in the rat brain is depicted in table 11 and figure. In comparison to control rats (39.19±8.51), MSG administration, resulted in significant reduction of SOD levels in brain (22.92±6.58). Pre-treatment with EEPM (100mg/kg: 25.17±7.62 and 200mg/kg: 31.18±5.18) for 30 days significantly improve the SOD activity in comparison to stress-treated rats in the brain studied. Among all the groups, a marked increase in SOD status was observed with EEPM 200mg/kg treatment (18.23±27.56).

The results suggest a significant dose dependent increase SOD status following EEPM pre-treatment for 30 days. Memantine (35.12±6.12) significantly attenuated the stress effect on SOD status and increased the antioxidant level in all the regions.

7.3.1b. Effect of EEPM on CAT (*U/mg protein*) levels in MSG-treated rat brain

Table 11 and figure shows the alteration of CAT levels in various brain regions studied. In comparison to control rats (102.14±12.4), MSG induction resulted in significant reduction of CAT level of brain (45.21±5.91; $P<0.0001$). Pre-treatment with EEPM (100mg/kg: 71.55±9.45 ($P<0.001$) and 200mg/kg: 94.54±7.32 ($P<0.001$) for 30 days significantly improve the CAT activity in comparison to stress-treated rats in the brain studied.

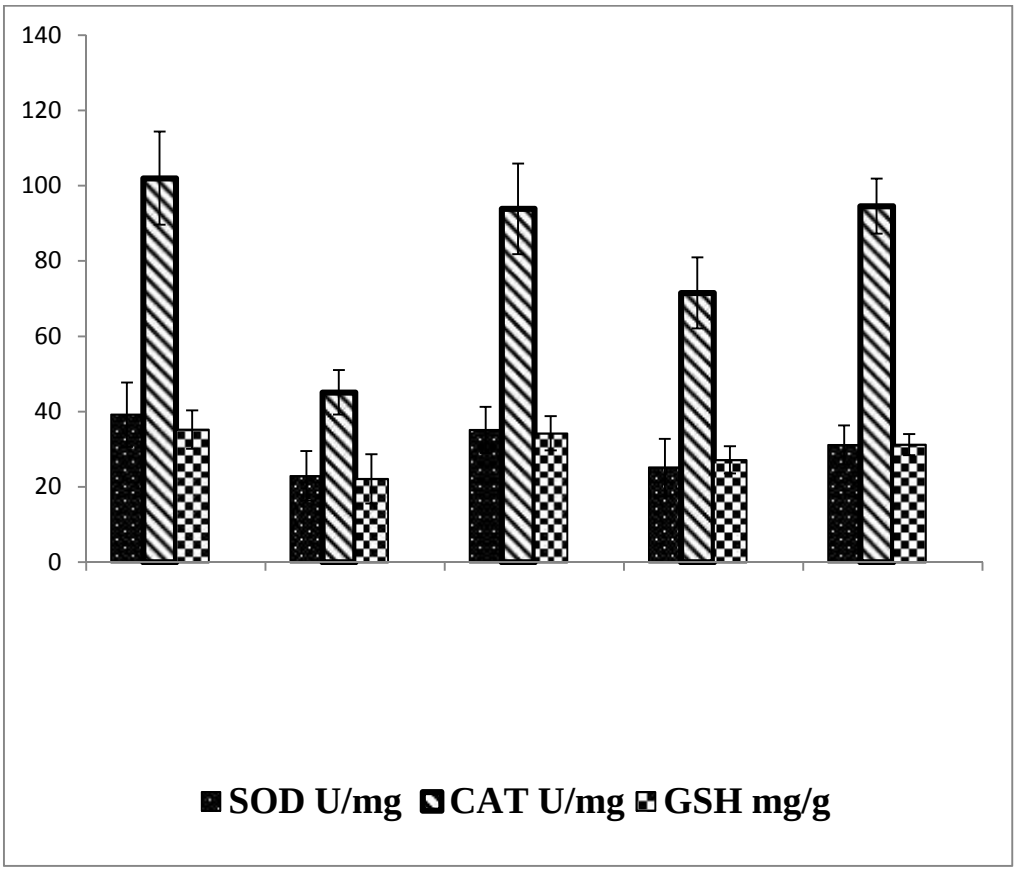
Among all the groups, a marked increase in CAT status was observed with control groups (106.0±3.41). The results suggest a significant dose dependent increase CAT status following EEPM pre-treatment for 30 days. Memantine (93.85±12.05; $P<0.0001$) significantly elevated the stress effect on CAT status and increased the antioxidant level in all the regions studied.

7.3.1c. Effect of EEPM on GSH (*mg/g protein*) levels in MSG-treated rat brain

The effect of drugs employed on GSH levels are summarized in figure 11 and table. In comparison to control rats (35.25±5.12), MSG stress resulted in significant decrease of GSH level of brain (22.12±6.54). Pre-treatment with EEPM (100mg/kg: 27.19±3.60; and 200mg/kg: 31.23±2.82, $P<0.0001$) for 30

days significantly increase the GSH activity in comparison to stress-treated rats in the brain studied.

The results suggest a significant dose dependent increase GSH status following EEPM pre-treatment for 30 days. Memantine (34.24±4.54, P<0.0001) significantly diminished the stress effect on GSH status and decreased the antioxidant level in all the regions studied.

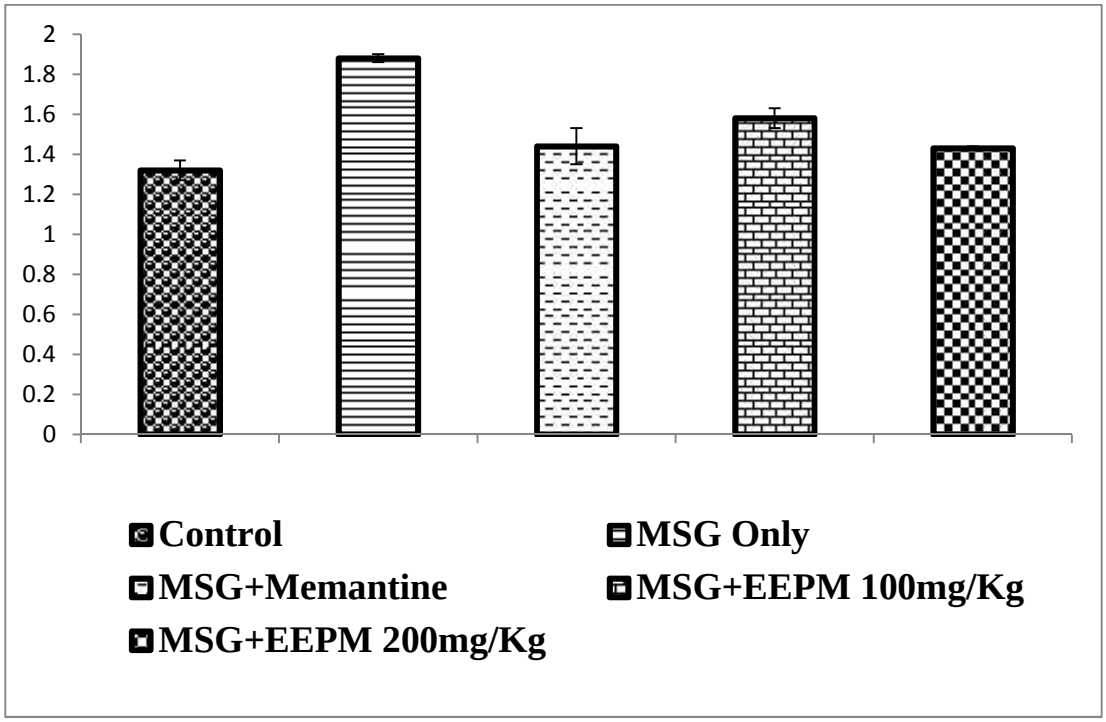


7.3.1d. Effect of EEPM on LPO (*μ Moles of MDA/mg protein*) levels in MSG-treated rat brain

The effect of drugs employed on LPO levels are summarized in figure 11 and table. In comparison to control rats (1.32±0.05), MSG resulted in significant increase of LPO level of brain (1.88±0.02). Pre-treatment with EEPM (50mg/kg: 0.072±0.01, P<0.0001; 100mg/kg: 1.58±0.05; and 200mg/kg: 1.43±0.01, P<0.0001) for 30 days significantly decrease the LPO activity in comparison to stress-treated groups in the brain studied.

Among all the groups, a marked decrease in LPO status was observed with control rats. The results suggest a significant dose dependent decrease LPO status following EEPM pre-treatment for 30 days. Memantine (1.41±0.09,

P<0.0001) significantly diminished the stress effect on LPO status and increased the antioxidant level in all the regions studied.



8. Discussion

The present study was designed to evaluate the possible neuroprotective effect of ethanol extract of *Pterocarpus marsupium* Roxb. bark (EEPM) pre-treatment against the excitotoxic effect of MSG-induced neurodegeneration in SD rats. The neuroprotective effect was assessed using a series of general behavior (feed intake, water intake and body weight), anxiety and depression behavioral tests and biochemical analysis of the brain antioxidant elements in rat brain.

Excitotoxicity was induced by intraperitoneal injection of MSG (2 g/kg) for seven days which resulted in glutamate receptor activation and Ca^{2+} overload (via a number of different mechanisms). To substantiate the neuroprotective effect of EEPM, the implications on general behavior, body weight (BW), food intake (FI) and water intake (WI) were also analyzed, as these behavior could be disturbed in depression and anxiety models that were employed in this study.

Also there may be perturbations in the levels of brain antioxidant enzyme systems in stressful states. Hence, the effects of EEPM on the levels of superoxide dismutase (SOD), Catalase (CAT), glutathione (GSH), and lipid peroxidation (LPO) were studied in the whole rat brain.

8.1 Behavior tests with MSG-induced excitotoxicity

- The effects of EEPM on the perturbations produced in behavioral paradigms like anxiety and depression were studied following pre-treatment with EEPM at two different dose levels (100 and 200 mg/ kg) by oral route for 30 days.
- The results indicated that administration of MSG induced anxiety and depression. The locomotor activity was found to have increased significantly following MSG and was found to be normalized due to pre-treatment with EEPM, when tested in an actophotometer cage. Depression was manifested as increased freezing time in FST with MSG only treated group and significant attenuation of the same in the EEPM and memantine treated groups.
- Administration of EEPM for 30 days significantly attenuated the MSG-induced behavioral alterations, at a higher dose level of 200 mg/kg body weight. The results on behavioral alterations are comparable with the NMDAR antagonist memantine (20 mg/kg). As our herb exhibited anxiolytic behavior, it may be assumed that EEPM may mediate its activity through

controlling the glutamate induced excitation in the CNS, among a number of other mechanisms as well. The result showed that stress-induced anxiety associated with MSG treatment is attenuated by EEPM pre-treatment as evident from locomotor activity.

- This study reveals that MSG-induced neurotoxicity produces behavioral changes in adult rats (*Ramanathan et al., 2007*). Significant differences in behavioral performance between the controls and MSG treated rats were found in all paradigms tested. Measurements in an activity cage showed that MSG treated rats had decreased spontaneous motor activity than the controls when tested. A similar reduction of locomotion has been reported in rats treated with 5 g/kg of MSG (*Pradhan et al., 1972*) but not in those given lower doses (*Ali et al., 2000; Pradhan et al., 1972*). The findings suggest that a distinction in locomotor activity between the control and MSG treated animals becomes evident in adult rats as well. This study also supports the findings that locomotor activity becomes distinctly disturbed between control and MSG treated animals with the progression of age. (*Zdenek Hlnak et al., 2005*).
- The locomotor activity is closely related to the hippocampus and its cholinergic input *(Carlton, 1968; Fibiger, 1991;* exploratory rearing behavior relates evidently to the hippocampal glutamatergic mechanisms (*Cerbone & Sadile, 1994*). It is possible to consider that a relative imbalance in the impairment of these two mechanisms may be decisive factor in behavioral output of animal. This suggests that different relationships between cholinergic and /or glutamatergic mechanisms might explain differences observed in MSG treated rats.
- Considerable evidence suggests that pharmacological agents which block NMDAR may possess therapeutic properties for the treatment of anxiety, pain, epilepsy and stroke.
- Exploratory rearing behavior is related evidently to the hippocampal glutamatergic mechanisms (*Cerbone & Sadile, 1994*). It is possible to consider that a relative imbalance in the impairment of these two mechanisms may be decisive factor in behavior output of animals.

- Recent reports suggested that alteration in the levels of biogenic amines (NE, E, DA, 5-HT, 5-HIAA, ACh and AChE) in rat brain following reperfusion and noise-stress was attenuated by EEPM pre-treatment, which may be correlated to anxiolytic and antidepressant activity.
- EEPM pre-treatment also attenuated the MSG induced depression in forced swim test.
- The present data support the view that a series of tests and not a single test should be used to characterize animal behavior. The testing paradigms used here are reproducible in the evaluation of potential drugs effective in the consequent prevention of long-term behavioral functioning induced by MSG treatment. Pre-treatment with EEPM in a dose dependent manner attenuated the depressive effects on feeding pattern and suppression in body weight gain.
- The detailed mechanism involved in the antidepressive-like properties of EEPM is not yet clear. Rats forced to swim in a restricted area assume an immobile posture after initial attempts to escape. In a subsequent immersion, the beginning of the immobility is faster and marked. *Porsolt et al., (1978)* named this phenomenon "behavior despair" and attributed the animals' response to the development of a depression process. Treatment with anti-depressive medicines reduces the immobility time during the swimming test. The authors presented it as a model of animal depression. In the forced swimming test, which evaluates the depressive behavior doted by *Porsolt*, a significant decrease of the immobility time was observed after the administration of different doses of EEPM to rats. These results suggest that EEPM induces an anti-depressive, of stimulant activity on the CNS, in a dose-dependent manner.

8.2 Other behavioral studies

The effect of EEPM in MSG induced changes on food intake, water intake and body weight were also studied. The results on these parameters indicated that MSG treated animals had significantly lower body weights than the control. The significant suppression of body weight in MSG treated rats coincides with a number of previous findings (*Klingberg et al., 1987; Pradhan et al., 1972; Squibb et al., 1981*). Considering that MSG treated animals develop obesity at later age (*Redding et al., 1971; Takasaki et al., 1979*), the growth curves, must

be followed for several months to see this effect. Unfortunately, this was not the essence of the present study.

A significant suppression in the consumption of food and increased intake of water was noted with MSG treated rats and these effects were attenuated by treatment with EEPM.

The present experiment has showed that MSG treated rats were characterized by disturbances in feeding pattern and feeding rhythms. MSG rats consumed less food and more water than normal rats mainly by decreasing meal size and eating rate. This agrees with many previous experiments recording gross food intake only (*Dawson & Lorden, 1981; Dawson & Annau , 1983; Dawson et al., 1989; Lorden & Caudle,1986; Beck et al., 1997*).

Among the brain transmitter systems that play a role in food intake and might support the behavior syndrome of the MSG rats, the intrahypothalamic arcuate-paraventricular neuropeptide Y (NPY) network is a good contender. There are major augment in its favor. The first one comes from central injection studies. When continuously infused in cerebral ventricles, NPY induces a disruption of dark/light rhythms of food intake independently of its orexigenic properties (*Beck et al., 1990*). Its stimulation properties in the paraventricular nucleus (PVN) vary along the daily cycle, being more effective in the first portion of the dark phase. When injected into the suprachasmatic nucleus, the circadian clock, it phase-shifts the activity of the hamsters (*Albers & Ferris, 1984*). The second one comes from biochemical studies which show the existence of daily rhythms of endogenous NPY levels within hypothalamic areas. In the parvocellular part of the PVN, it increases the onset of the dark period; while in the ARC levels reach peaks at the two phase shifts (*Jhanwar-Uniyal et al., 1990*). These endogenous peaks are in phase with those observed in food intake in the control animals of this experiment. The third one comes from studies showing that the activity of the hypothalamic NPY-containing pathway is higher in obese Zucker rats (*Stricker-Krongrad et al., 1994; Beck et al., 1993; McCarthy et al., 1991; Sanacora et al.,(1990*), fasted rats (*Beck et al., 1990; Frankish et al., 1993; Sahu et al., 1988; White & Kershaw,1990*) and diabetic rats (*Frankish et al., 1993; Williams et al., 1989*); all of which are characterized by having increased meal sizes (*Alingh-Prins et al., 1986; Thomas et al.,1976; Levitsky, 1970*). The

fourth one comes from studies showing that hypothalamic NPY is depleted in MSG rats (*Dawson et al., 1989; Stricker-Krongrad et al., 1996; Beck et al., 1997; Abe et al., 1990*). Recent evidence also suggests that leptin, the *ob* gene product, might contribute in association with NPY to the day-night feeding alterations observed in MSG+ stress treated rats (*Dawson et al., 1989*). These results describe profound eating behavior disturbances that are induced by MSG induced lesions of the arcuate nucleus. In our study the decrease in body weight of MSG treated rats could be attributed to suppression in food consumption.

In glutamate toxicity, the neuronal death is linked closely to glutamate-evoked excitotoxicity. Glutamate plays a central role in neurodegeneration and increases extracellular glutamate concentrations from 30 to 200 µM in ischemic brain. A characteristic response to glutamate challenge is the increase in the cytosolic Ca^{2+} level, which is due to either influx from the extracellular space through the activation of NMDA or non-NMDA or metabotropic receptor activation leading to release from the intracellular stores. In this excitotoxic condition the survival of a cell depends largely on functioning of the mitochondria. The mitochondrial potential, the driving force necessary to satisfy the cellular energy demands, is also involved in the generation of ROS, which in turn are suspected to cause cell death if unabated. The interplay between mitochondrial potential and ROS generation is not yet fully understood. Since EEPM could control the neurodegeneration and suppression of elevated antioxidant enzymes, it can be stated that EEPM produced antioxidant effect in this animal model of excitatory response through controlling the glutamate induced radical generation.

Recent work suggests that different types of stressful events may sometimes produce qualitatively different patterns of effects in both behavior and physiology. Adequate regulation of food intake under stress is important for survival. Therefore, it is not surprising that the HPA axis is not only the ‘conductor’ of an appropriate stress response, but is also tightly intertwined with endocrine parameters that regulate appetitive behaviours. In addition to regulation by the circadian rhythm, characterized by increased cortisol concentrations in the morning, low concentrations in the evening and fast feedback under stress activation (*Rosmond 2003*), glucocorticoid release is also

food-entrainable (*Shiraishi et al., 1984*). Studies suggest feedback loops between glucocorticoids, leptin, insulin and NPY under acute HPA activation (*Cavagnini et al., 2000*). The interactions between these hormones facilitate storage, distribution and release of energy according to needs and contribute to initiation and termination of a meal.

NPY is an anxiolytic peptide, leading to decreased anxiety. It is known to play an important role in the response to stress and in psychiatric disorders (*Yehuda et al., 2006*), thus, potentially an important mediator of what is anecdotally described as 'emotional eating'. Low NPY concentrations have been observed in subjects with posttraumatic stress disorder and depression (*Rasmusson et al., 2000*) -psychiatric conditions classically associated with a loss of appetite. Increased NPY is associated with stress resilience in subjects exposed to traumatic experience (*Yehuda et al., 2006*). NPY increases in response to stress may be one biochemical signal underlying stress eating.

Several researchers also have provided evidence that palatable food can cause endogenous opioid dependence (*Rada et al., 2005, Colantuoni et al., 2002*). Opioid dependence was tested by using naloxone, an opioid antagonist, and defined as naloxone-induced withdrawal after sucrose exposure (*Colantuoni et al., 2002*). Activation of the HPA axis elicits–among other neurotransmitter systems–the release of endogenous opioids (*O'Hare et al., 2004*). There is strong evidence suggesting that opioid release is part of an organisms' powerful defense mechanism against the detrimental effects of stress (*Drolet et al., 2001*). Opioids decrease activity of the HPA axis on different levels in order to terminate and attenuate the stress response, providing a negative feedback control mechanism (*Kreek and Koob 1998*). Opioid release increases palatable food intake and palatable food sustains opioid release. Thus, food intake resembles a powerful tool to shut down stress-induced HPA axis activation. If stress becomes chronic and eating is learned to be effective coping behavior, highly palatable food may appear to be 'addictive' via the neurobiological adaptions mentioned earlier.

Rats living in a stressful milieu may lose weight and regain weight in recovery, leaving them fattier than before. Continual bouts of minor daily stressors may keep the stress arousal system in chronically activated state. Indeed, cortisol

tends to be higher on working days than weekend days (*Kunz-Ebrecht et al., 2004, Schlotz et al., 2004*). This low but chronic level of stress may modulate appetite and food intake in ways that are only loosely related to true caloric need (*Epel et al., 2007*). The more intense the stressor and the longer the duration, the greater reduction in food intake and body weight in the rats. The studies done on rats conclude that stress invokes a reduction in food intake in the animals. The energy intake was significantly greater on the examination day, when compared to stress-free days.

8.3 Biochemical studies with MSG

NMDA receptor activation or neuronal increases in Ca^{2+} subsequent to Na^{+}, or both can activate a series of Ca^{2+} dependent enzymes, including protein kinases C (PKC), Phopholipases (PL), proteases, protein phosphatases, and nitric oxide synthase (NOS) (Choi, *1988; Dawson et al., 1992; Trout et al., 1993*). After PL_2 is activated, arachidonic acid (AA), its metabolites, and platelet activating factor (PAF) are generated. PAF increases neuronal Ca^{2+} levels, apparently by stimulating the release of glutamate (*Clark et al., 1992*); *Bito et al., 1992*). AA potentiates NMDA-evoked currents (*Miller et al., 1992*) and inhibits reuptake of glutamate into astrocytes and neurons (*Volterra et al., 1992*), further exacerbating the situation; reactive oxygen species (ROS) can be formed during AA metabolism (*Lafon-Cazal et al., 1993*), leading to further PLA_2 activation, which represents positive feedback (*Chan et al., 1985*). These processes can cause the neuron to digest itself by protein breakdown, free-radical formation, and lipid peroxidation. In addition, one might envisage that in cerebral ischemia, tissue reperfusion increases this damage by providing additional free radical in the form of superoxide anions (*Rosen et al., 1993*).When NMDA receptors are excessively stimulated influx of Ca^{2+} ions activates the generation of NO and superoxides in increased quantities. Under these conditions, NO and superoxide ions may react to form a toxic substance called peroxynitrite (ONOO•), resulting in neuronal death (*Lipton et al., 1993; Dawson & Dawson, 1991*). In addition to these effects Ca^{2+} can activate nuclear enzyme (endonucleases) that result in condensation of nuclear chromatin and ultimately DNA fragmentation and nuclear breakdown, a pathological process known as apoptosis (*Kane et al., 1993*).

Studies have demonstrated that synaptic glutamate release and uptake are energy-(ATP)-dependent, and any impairment or breakdown may lead to generation of ROS and inactivation of glutamate reuptake mechanism leading to excessive glutamate accumulation. If the circumstance continues unabated, there is excessive influx of Na^+, Cl^- and Ca^{2+} via post-synaptic ion channels producing swelling and destruction of post synaptic elements not only in the immediate vicinity but also the entire neuron as well. Upon destruction of neurons by this mechanism, additional glutamate may be released further increasing the level of extracellular glutamate and thereby propagating the excitotoxicity and death of additional glutamate-sensitive neurons in the region of involvement (*Seisjo 1984; Benveniste et al., 1984, Novelli et al., 1988; Auer & Siesjo 1988; Nicholis & Attwell 1990*).

Convincing evidence of the role of free radicals in non-NMDA receptor-mediated (AMPA and KA) neurotoxicity leading to the accumulation of lipid peroxidation products and attenuation by treatment with antioxidants is also mounting.

In addition to its action on the ionotropic receptors (NMDA and non-NMDA), glutamate also acts on a variety of metabotropic receptors, and has shown to modify Ca^{2+} levels either at postsynaptic or presynaptic sites and there is now clear agreement that they are able to modulate neuronal damage and death and, correspondingly antagonists protect against these effects (*Stone & Addae, 2002*).

The depletion in the levels of SOD, CAT, GSH, and increased LPO in the rat brain, following seven days administration of MSG is consistent with a previous study in our laboratory. These results clearly show that glutamate can lead to excitation and oxidative stress leading to neurodegeneration. Treatment with EEPM, containing among a number of phenolic compounds, fixed and volatile oils, significantly attenuated the glutamate-induced excitation and oxidative stress.

Memantine increased SOD, CAT and decreased TBAR levels in all the regions studied. These effects could be attributed to the antagonizing activity at

NMDAR, leading to controlling the glutamate excitotoxicity resulting in the preservation of brain antioxidant system.

Another interesting observation made in the present study was depletion of glutathione levels with glutamate treatment. Though the treatment of EEPM and memantine increased few or all antioxidant enzymes in brain, they all failed to show any effect on glutathione levels. It has been stated that synthesis of GSH in Astrocytes involves glutamate, cysteine and glycine. Excess of glutamate will inhibit the transport of cysteine there by blocking the GSH formation. Further, it is also reported that the oxidative pathway involves the breakdown of the glutamate-cysteine antiporter leading to decrease in GSH levels that allows for aberrant formation of free radicals, which are neurotoxic. In the present study, administration of MSG (2 g/kg) could have blocked the synthesis of GSH and resulted invariably in the depletion of GSH in all the groups.

9. Summary and Conclusion

The study was undertaken to evaluate the probable neuroprotective effect of pre-treatment of ethanol extract of *Pterocarpus marsupium* Roxb. bark (EEPM) against monosodium glutamate-induced excitotoxicity model in SD rats. The neuroprotective effect was assessed by measuring the changes in water intake, feed intake and body weight changes in addition to using a sequence of behavioral tests (anxiety and depression) and biochemical analysis (SOD, CAT, GSH and LPO) of the rat brain.

The protective effect of EEPM was evaluated for depression using forced swim test and anxiety using actophotometer and elevated plus-maze apparatus in MSG induced models.

To substantiate the neuroprotective effect of EEPM and its implications on general behaviors *viz.,* feed intake (FI), water intake (WI) and body weight (BW) were also undertaken in MSG induced models, as these behavior could be profoundly disturbed in anxiety, depression and may impair memory and cognitive abilities.

Also there may be perturbations in the levels of brain antioxidant enzyme system in stressful states. Hence, the effects of EEPM on the levels of SOD, CAT, GSH and LPO were studied in the rat brains.

Administration of MSG for 7 days resulted in elevated levels of anxiety and depression. Pretreatment with EEPM for 30 days prevented the effect of MSG in rats.

The anxiety and depression parameters were attenuated significantly in MSG treated rats. Moreover, the changes in food intake, water intake and body weight were also minimizes in MSG treated rats.

The perturbations in the levels of SOD, CAT, GSH & LPO were also found to be attenuated in EEPM groups.

10. References

Abe M, Saito M, Shimuzu T. Neuropeptide Y in the specific hypothalamic nuclei of rats neonatally treated with monosodium glutamate. Brain Res Bull, 1990; 24:289-291.

Abeliovich A, Schmitz Y, Farinas I, Choi-Lundberg D, Ho WH, Castillo PE et al., Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron*, 2000; 25:239–252.

Ahmad F, Khalid P, Khan MM, Chaaubey M, Rastogi AK , Kidwai JR. Hypoglycemic activity of *Pterocarpus marsupium* wood. J. Ethnopharmacol , 1991a; 35:71-75.

Ahmad F, Khan MM, Rastogi AK, Chaubey M, Kidwai JR. Effect of (-)-epicatechin on cAMP content, insulin release and conversion of proinsulin to insulin in immature and mature rat islets *in vitro*. Indian J Exp Biol, 1991b; 29:516-520.

Albers HE, Ferris CF. Neuropeptide Y: role in light-dark cycle entrainment of hamster circadian rhythms. *Neurosci Lett*, 1984; 50: 163-168.

Alim MA, Hossain MS, Arima K, Takeda K, Izumiyama Y, Naka mura M et al. Tubulin seeds alpha-synuclein fibril formation. J Biol Chem, 2002; 277:2112–2117.

Alingh Prins A, de Jong-Nagelsmit A, Keijser J, Strubbe JH. Daily rhythms of feeding in the genetically obese and lean Zucker rats. Physiol Behav, 1986; 38:423-426.

Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. Proc Natl Acad Sci, 1993; 90:7915-7922.

Andlin-Sobocki P, Jonson B, Wittchen H-U, Olesen J. Costs of disorders of the brain in Europe. Eur J Neurol, 2005; 12:1–27.

Anon. In: Wealth of India, 2003, vol.VIII, pp.302–305.

Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O. Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med, 2002; 8: 963–970.

Auer RN, Siesjö BK. Biological differences between ischemia, hypoglycemia and epilepsy. Ann Neurol, 1988; 24:699-707.

Beck B, Burlet A, Bazin R, Nicolas JP, Burlet C. Elevated neuropeptides Y in the arcuate nucleus of young obese Zucker rats may contribute to the development of their overeating. J Nutr, 1993; 123:1168-1172.

Beck B, Jhanwar-Uniyal M, Burlet A, Nicolas JP, Burlet C. Rapid and localized alterations of Neuropeptides Y in discrete hypothalamic nuclei with feeding status. Brain Res, 1990a; 528:245-249.

Beck B, Stricker-Krongrad A, Burlet A, Musse N, Nicolas JP, Burlet C. Dietary performance in monosodium glutamate-lesioned rats: age-variable influence of hypothalamic neuropeptide Y. Neurosci Letters, 1997; 225:153-156.

Behera SK, Mishra MK. Indigenous phytotherapy for genito-urinary diseases used by the Kandha tribe of Orissa. Indian J Ethnopharmacol, 2005; 102:319-325.

Benavente-Garia O, Castillo J, Marin, FR, Ortuno A, del Río JA. Use and properties of citrus flavonoids. J Agric Food Chem, 1997; 45:4505-4507.

Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J Neurochem, 1984; 43:1369-1374.

Berhow M. Survey of phenolic compounds produced in citrus. USDA Tech Bull, 1856.

Bito H, Nakamura M, Honda Z, Izumi T, Iwatsubo T, Seyama Y, Ogura A, Kudo Y, Shimizu Y. Platelet-activating factor receptor in rat brain: PAF mobilizes intracellular calcium in hippocampal neurons. Neuron, 1992; 9:285-294.

Blaylock RL. Neurodegeneration and aging of the central nervous system: prevention and treatment by phytochemicals and metabolic nutrients. Integ Med, 1998; 1:117–133.

Bone RC. Towards a theory regarding the pathogenesis of the systemic inflammatory response syndrome: what we do and do not know about cytokine regulation. Crit Care Med, 1996; 24:163-172.

Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging, 2003; 24:197–211.

Bredesen DE. Neural apoptosis. Ann Neurol, 1995; 38:839-851.

Brex P, Ciccarelli O, O'Riordan J, Sailer M, Thompson A, Miller D. A longitudinal study of abnormalities on MRI and disability from multiple sclerosis. N Engl J Med, 2002; 346:158-164.

Brookmeyer R, Gray S, Kawas C. Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. Am J Public Health, 1998; 88:1337-1342.

Buonomano DV, Merzenich MM. Cortical plasticity: from synapses to maps. Annu Rev Neuroscis, 1998; 21:149–186.

Carlton PL. Brain acetylcholine and habituation. Prog Brain Res, 1968; 28:48-60.

Cattaneo, E et al. Huntington's disease. Scientific American, 2002; 287:93- 97.

Wellington CL et al. Caspase cleavage of mutant huntington precedes neurodegeneration in Huntington's disease. J Neurosci, 2002; 22:7862-7872.

Cavagnini F, Croci M, Putignano P, Petroni ML, Invitti C. Glucocorticoids and neuroendocrine function. Int J Obes Relat Metab Disord, 2000; 24:S77–79.

Cerbone A, Sadile AG. Behavioral habituation to spatial novelty: interference and noninterference studies. *Neurosci Biobehav Rev,* 1994; 18:497-518.

Chakravarth BK, Gupta S, Gambhir SS, Gode KD. The prophylactic action of (-)-epicatechin against alloxan-induced diabetes in rats. Life Sci, 1981; 29:2043-2047.

Chakravarthy B, Gupta S, Gambhir SS, Gode KD. Pancreatic beta cell regeneration. A novel antidiabetic mechanism of *Pterocarpus marsupium* Rox. Indian J Pharmacol, 1980; 12(2):123-127.

Chakravarthy BK, Gupta S, Gode KD. Antidiabetic effect of (-)-Epicatechin. Lancet, 1982a; 2:272-273.

Chakravarthy BK, Gupta S, Gode KD. Functional beta cell regeneration in the islets of pancreas in alloxan induced diabetic rats by (-)-Epicatechin. Life Sci, 1982b; 31:2693-2697.

Chambliss KL, Yuhanna IS, Mineo C, Liu P, German Z, Sherman TS et al. Estrogen receptor alpha and endothelial nitric oxide synthase are organized into a functional signalling module in caveolae. Circ Res, 2000; 87(11):E44-5

Chan AC, Fragiskos B, Douglas CE & Choy PC. Increased arachidonate incorporation in perfused heart phospholipids from vitamin E-deficient rats. Lipids 1985; 20:328-330.

Chan PH & Fishman RA. Free fatty acids, oxygen free radicals, and membrane alterations in brain ischemia and injury. In: Cerebrovascular diseases. Plum F, Pulsinelli W, eds., Raven Press, New York (1985) pp.161-71.

Chauhan B, Chaudhary AK. Memory enhancing activity of methanolic extract of *Pterocarpus marsupium* Roxb. Phytopharma, 2012; 2(1):72-80.

Chen HJ, Anagnostou G, Chai A, Withers J, Morris A, Adhikaree J, Pennetta G et al., Characterization of the properties of a novel mutation in VAPB in familial amyotrophic lateral sclerosis. J Biol Chem, 2010; 285:40266-81

Choi DW, Maulucci-Gedde C, Kriegstein AR. Glutamate neurotoxicity in cortical cell culture. J Neurosci, 1987; 7:357-368.

Choi DW. Excitotoxic cell death. J Neurobiol, 1992; 23:1261–1276.

Choi DW. Glutamate neurotoxicity and diseases of the nervous system. Neuron, 1988; 1:623-634.

Clark GD, Happel LT, Zorumski CF, Bazan NG. Enhancement of hippocampal excitatory synaptic transmission by platelet activating factor. Neuron, 1992;9:1211-1216.

Commenges D, Rondeau V, Jacqmin-Gadda H, Dartigues JF. Relation between aluminum concentrations in drinking water and Alzheimer's disease: an 8-year follow-up study. Am J Epidemiol, 2000; 152:59-66.

Conway KA, Lee SJ, Rochet JC, Ding TT, Williamson RE, Lansbury PT Jr. Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early onset Parkinson's disease: implications for pathogenesis and therapy. Proc Natl Acad Sci USA, 2000; 97:571-576.

Cotman CW, Monaghan DT, Anatomical organization of excitatory amino acid receptors and their properties. Adv Exp Med Biol, 1986; 203:237–252.

Council of Scientific and Industrial Research. A dictionary of Indian Raw Materials and Industrial Products, The Wealth of India, 1969; 8:303-305.

Coyle JT, Puttfarcken P. Oxidative stress, glutamate and neurodegenerative disorders. Sci, 1993; 262:689-695.

Coyle JT, Bird SJ, Evans RH, Gulley RL, Nadler JV, Nicklas WJ, Olney, JW. Excitatory amino acid neurotoxins: selectivity, specificity, and mechanisms of action. Neurosci Res Prog Bull, 1981; 19:1-427.

Crozier A, Jaganath IB, Clifford MN. Dietary phenolics: chemistry, bioavailability and effects on health. Nat Prod Rep, 2009; 26(8):1001-43.

Dai Y, Hang BQ, Li PZ, Tan LW. Effects of oleanolic acid on immune system and type-I allergic reaction. Acta Pharmacologica Sinica, 1989b; 10:381-384.

Das A, Shanker G, Nath C et al. Anticholinesterase and cognitive enhancing activities. Pharmacol Biochem Behav, 2002; 73:893-900.

Davis JB, Maher P. Protein kinase C activation inhibits glutamate-induced cytotoxicity in a neuronal cell line. Brain Res, 1994; 652:169-173.

Dawson R, Annau Z. MSG-induced convulsions in rats: influence of route of administration, temperature and age. Neurobehav Toxicol Teratol, 1983; 5:399-406.

Dawson R, Lorden JF. Behavioral and neurochemical effect of neonatal administration of monosodium L-glutamate in mice. J Comp Physiol Psychol, 1981; 95:71-84.

Dawson R, Wallace DR, Gabriel SM. A pharmacological analysis of food intake regulation in rats treated neonatally with monosodium L-glutamate (MSG). Pharmacol Biochem Behav, 1989; 32:391-398.

Dawson TM, Dawson VL, Synder SH. A novel neuronal messenger molecule in brain: the free radical, nitric oxide. Ann Neurol, 1992; 32:297-311.

Dawson VL, Dawson TM, London ED, Bredt DS, Synder SH. Nitric oxide mediates glutamate neurotoxicity in primary cortical cultures. Proc Natl Acad Sci, 1991; 88:6368-6371.

De Beers D, Schulze AE, Joubert E, de Villiers A, Malherbe CJ, Stander MA. Food ingredient extracts of *Cyclopia subternata* (Honeybush): variation in phenolic composition and antioxidant capacity. *Molecules,* 2012; 17(12):14602-24

De Lau LM, Breteler MM. Epidemiology of Parkinson's disease. Lancet Neurol, 2006; 5:525-535.

de Rivera C, Shukitt-Hale B, Joseph JA, Mendelson JR. The effects of antioxidants in the senescent auditory cortex. Neurobiol Aging, 2006; 27:1035-1044.

Deng W, Fang WI, Wu J. Flavonoids function as antioxidants: By scavenging reactive oxygen species or by chelating iron. Radiat Phys Chem, 1997; 50:271-76

Devasagayam A. Indian Herbs and Herbal drugs used for the treatment of diabetes. J Clin Biochem Nutr, 2007; 40(3):163- 173.

Di monte DA. The environment and Parkinson's disease: is the nigrostriatal system preferentially targeted by neurotoxins? Lancet Neurol, 2003; 2:531-538.

Dice JW, Walker CD, Byrne B, Cardiel A. General characteristics of protein degradation in diabetes and starvation. Proc Natl Acad Sci USA, 1978; 75:2093-2097.

Dilpesh J, Patel I, Rahul S. Antidiarrhoeal activity of ethanolic heartwood extract of *Pterocarpus marsupium*. Pharmacol online, 2011; 1:552- 559.

Drolet G, Dumont EC, Gosselin I, Kinkead R, Laforest S, Trottier JF. Role of endogenous opioid system in the regulation of the stress response. Prog Neuropsychopharmacol Biol Psychiatry, 2001; 25:729–741.

Duthie SJ, Collins AR, Duthie GG, Dobsoin VL. Quercetin and myricetin protect against hydrogen peroxide-induced DNA damage (strand breaks and oxidized pyrimidines) in human lymphocytes. Mutat Res, 1997; 393(3):223-31

Epel ES, Adam TC. Stress, eating and the reward system. Physiol Behav, 2007; 91:449-458.

Ernst RL, Hay JW, Fenn C, Tinklenberg J, Yesavage JA. Cognitive function and the costs of Alzheimer disease. An exploratory study. Arch Neurol, 1997; 54:687–693

Fibiger HC, Damsma G, Day JC. Behavioral pharmacology and biochemistry of central cholinergic neurotransmission. Adv Exp Med Biol, 1991; 295:399-414.

Filho CB, Del Fabbro L, de Gomes MG, Goes AT, Souza LC, de Gomes MG et al. Alpha-synuclein and Parkinson's disease: selective neurodegenerative effect of alpha-synuclein fragment on dopaminergic neurons *in vitro* and *in vivo*. Ann Neurol, 2000; 47:632-640.

Forman MS, Trojanowski JQ, Lee VM. Neurodegenerative diseases: a decade of discoveries paves the way for therapeutic breakthroughs. Nat. Med, 2004; 10:1055–1063.

Forno LS. Neuropathology of Parkinson's disease. J Neuropathol Exp Neurol, 1996; 55:259-272.

Forstl H, Kurz A. Clinical features of Alzheimer's disease. *Eur Arch Psychiatry Clin Neurosci*, 1999; 249:288-290.

Fraga CG, Martino VS, Ferraro GE, Coussio JD, Boveris A. Flavonoids as antioxidants evaluated by *in vitro* and *in situ* liver chemiluminescence. Biochem Pharmacol, 1987; 36:717-20.

Frankish HM, McCarthy HD, Dryden S, Kilpatrick A, Williams G. Neuropeptide Y receptor numbers are reduced in the hypothalamus of streptozotocin-diabetic and food-deprived rats: further evidence of increased activity of hypothalamic NPY-containing pathways. Peptides, 1993; 14:941-948.

Gairola S, Gupta V, Singh Baljindar, Maithani Mukesh, Bansal Parveen. Phytochemistry and pharmacological activities of *Pterocarpus_marsupium*-a review. Int Res J Pharmacy, 2010; 1(1):100-104.

Gamble JS. Flora of the Presidency of Madras. Adlard and Sons Ltd, London, UK, 1935.

Gayathri M, Kannabiran K. Antimicrobial activity of *Hemidesmus indicus, Ficus bengalensis* and *Pterocarpus marsupium* Roxb. Indian J Pharm Sci, 2009; 71:578-581.

Gayathri M, Kannabiran K. Studies on the ameliorative potential of aqueous extract of bark of *Pterocarpus marsupium* Roxb in streptozotocin-induced diabetic rats. J Nat Remedies, 2010; 10(1):36-43.

George JM, Jin H, Woods WS, Clayton DF. Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. Neuron, 1995; 15: 361-372.

Gkogkas C, Middleton S, Kremer AM, Wardrope C, Hannah M, Gillingwater TH et al. VAPB interacts with and modulates the activity of ATF6. Hum Mol Genet, 2008; 17:1517-1526.

Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. Cell, 2010; 140:918-934.

Goedert M. Alpha-synuclein and neurodegenerative diseases. Nat Rev Neurosci, 2001; 7:492-501.

Griffiths LA. Mammalian metabolism of flavonoids. In: Harborne JB, Mabry IJ, editors. The flavonoids: advances in research. London: Chapman and Hall; 1982: pp.681

Grover JK, Vats V, Yadav SS. *Pterocarpus marsupium* extract (Vijayasar) prevented the alteration in metabolic patterns induced in the normal rat by feeding an adequate diet containing fructose as sole carbohydrate. Diabetes Obes Metab, 2005; 7:414-420.

Halagappa K, Girish HN, Srinivasan BP. The study of aqueous extract *Pterocarpus marsupium* Roxb on cytokine TNF-α in type 2 Diabetic rats. Indian J Pharmacol, 2010*;* 42(6):392-396.

Halliwell B. Reactive oxygen species and the central nervous system. J Neurochem, 1992; 59:1609-1623.

Han J, Lee JD, Bibbs L, Ulevitch RJ. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. Sci, 1994; 265:808-811.

Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Sci, 2002; 297:353–356.

Hardy J, Cookson MR, Singleton A. Genes and Parkinsonism. Lancet Neurol, 2003; 2:221-228.

Harold S. Deficiency of Vitamin C and Vitamin P in man. Lancet, 1940; 236(6117): 644-47.

Hartley DM, Kurth MC, Bjerkness L, Weiss JH, Choi DW. Glutamate receptor-induced 45Ca2+-accumulation in cortical cell culture correlates with subsequent neuronal degeneration. J Neurosci, 1993; 13:1993-2000.

Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. Neuron, 2006; 52:61–76.

Hebert LE, Beckett LA, Scherr PA, Evans DA. Annual incidence of Alzheimer disease in the United States projected to the years 2000 through 2050. Alzheimer Dis Assoc Disord, 2001; 15:169–173.

Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch Neurol, 2003; 60:1119–1122.

Herrera-Ruiz M, Zamilpa A, González-Cortazar M, Reyes-Chilpa R, León E, García MP et al. Antidepressant effect and pharmacological evaluation of standardized extract of flavonoids from *Byrsonima crassifolia*. Phytomed, 2011; 18(14):1255-61.

Hlinak Z, Krejci I. Concurrent administration of sub effective doses of scopolamine and MK-801 produces a short-term amnesia for the elevated plus-maze in mice. Behav Brain Res, 1998; 91:83-89.

Hollmann M, Heinemann S. Cloned glutamate receptors. Annu Rev Neurosci, 1994; 17:31-108.

Hollmann M, Hartley M, Heinemann S. Ca2+ permeability of KA-AMPA-gated glutamate receptor channels depends on subunit composition. Science, 1991; 252:851-853.

Hougee S, Faber J, Sanders A, Hoijer MA, Smit HF. Selective COX-2 inhibition by a *Pterocarpus marsupium* extract characterized by pterostilbene and its activity in healthy human volunteers. Planta Med, 2005; 71:387-392.

Howes MJ, Houghton PJ. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. Pharmacol Biochem Behav, 2003; 75:513-27.

Hynd MR, Scott HL, Dodd PR. Glutamate-mediated excitotoxicity and neurodegeneration in AD. Neurochem Int, 2004; 45:583-595.

Jahromi MAF, Ray AB. Antihyperlipidemic effect of Flavonoids from *Pterocarpus marsupium*. J Nat Prod, 1993; 56(7):989-994.

Jellinger KA, Attems J. Prevalence of dementia disorders in the oldest-old: an autopsy study. Acta neuropathologica, 2010; 119:421-433.

Jenner P. Oxidative damage in neurodegenerative diseases. Lancet, 1994; 344:796-798.

Jhanwar-Uniyal M, Beck B, Burlet C, Leibowitz SF. Diurnal rhythm of neuropeptide Y-like immunoreactivity in the suprachiasmatic, arcuate and paraventricular nuclei and other hypothalamic sites. Brain Res, 1990; 536:331-334.

Jovanovic SV, Steeden S, Tosic M, Marjanovic B, Simic MG: Flavonoids as antioxidants. J Am Chem Soc, 1994; 116:4846-51

Julia JP, Johanna TD, Gary RB, Seema AB, Susan EG, David BH, Joanne MH. Flavanones in oranges, tangerines (mandarins), tangors, and tangelos: a compilation and review of the data from the analytical literature. J Food Compos Anal, 2006; 19:S66-S73

Kandel ER. The molecular biology of membrane storage: a dialogue between genes and synapses. Sci, 2001; 294:1030-1038.

Kanekura K, Nishimoto I, Aiso S, and Matsuoka M. Characterization of amyotrophic lateral sclerosis-linked P56S mutation of vesicle-associated membrane protein-associated protein B (VAPB/ALS8). J Biol Chem, 2006; 281:30223–30233.

Kapoor LD. Hand book of Ayurvedic Medicinal Plants. Council of Scientific and Industrial Research, 1989; pp.276-277.

Kato K, Puttfarcken PS, Lyons WE & Coyle JT. Developmental time course and ionic dependence of kainate-mediated toxicity in rat cerebellar granule cell cultures. J Pharmacol Exp Ther, 1991; 256:402-411.

Katoch O, Kaushik S, Kumar MS, Agrawala PK, Misra K. Radioprotective property of an aqueous extract from *Valeriana wallichii*. J Pharm Bioallied Sci, 2012; 4(4):327-332.

Kennedy DO, Scholey AB. Ginseng: potential for the enhancement of cognitive performance and mood. Pharmacol Biochem Behav, 2003; 75:687-700.

Kitikar KR, Basu BD. Indian Medicinal Plants. Scientific Publishers, India, 1999; pp.1.

Klingberg H, Brankack J, Klingberg F. Long-term effects on behavior after postnatal treatment with monosodium-l-glutamate. Biomed Biochem Acta, 1987; 46:705-711.

Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A et al. Flavonoid intake and risk of chronic diseases. Am J Clin Nutr, 2002; 76(3):560-68

Kreek MJ, Koob GF. Drug dependence: stress and dysregulation of brain reward pathways. Drug Alcohol Depend, 1998; 51:23–47.

Kruger R, Riess O, Jakes R. Genetic dissection of familial Parkinson's disease. Mol Med Today, 1998; 4:438-444.

Kunz-Ebrecht SR, Kirschbaum C, Marmot M, Steptoe A. Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. Psycho neuro endocrinol, 2004; 29:516–528.

Laferrere B, Abraham C, Russell CD, Bowers CY. Growth hormone releasing peptide-2 (GHRP-2), like ghrelin, increases food intake in healthy men. J Clin Endocrinol Metab, 2005; 90(2):611-14.

Lafon-Cazal M, Pietri S, Culcasi M, Bockaert J. NMDA-dependent superoxide production and neurotoxicity. Nature, 1993; 364:535-537.

Langou K, Moumen A, Pellegrino C, Aebischer J, Medina I, Aebischer P et al. AAV-mediated expression of wild-type and ALS-linked mutant VAPB selectively triggers death of motoneurons through a Ca2+-dependent ER-associated pathway. J Neurochem, 2010; 114:795–809.

Lev S, Ben Halevy D, Peretti D, Dahan, N. The VAP protein family: from cellular functions to motor neuron disease. Trends Cell Biol, 2008; 18:282–290.

Levitsky DA. Feeding patterns of rats in response to fasts and changes in environmental conditions. Physiol Behav, 1970; 5:291-300.

Li Y, Maher P, Schubert D. A role for 12-lipoxygenase in nerve cell death caused by glutathione depletion. Neuron, 1997a; 19:453-463.

Li Y, Maher P, Schubert D. Requirement for cGMP in nerve cell death caused by glutathione depletion. J Cell Biol, 139 (1997b) 1-8.

Lipton SA, Kater SB. Neurotransmitter regulation of neuronal outgrowth, plasticity and survival. Trends Neurosci, 1989; 12:265-270.

Lipton SA, Rosenberg PA. Excitatory amino acids as a final common pathway for neurologic disorders. New Eng J Med, 1994; 330:613-622.

Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr, 2003; 78:517S-520S.

Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev, 2010; 4:118-126.

Lorden JF, Caudle A. Behavioral and endocrinological effects of single injections of monosodium glutamate in the mouse. Neurobehav Toxicol Teratol, 1986; 8:509-519.

Lucas DR, Newhouse JP. The toxic effect of sodium L-glutamate on the inner layers f the retina. AMA Arch Ophthalmol, 1957; 58:193-201.

Maher P, Davis JB. The role of monoamine metabolism in oxidative glutamate toxicity. J Neurosis, 1996; 16:6394-6401.

Maheswari JK, Singh KK, Saha S. Ethnomedical uses of plants by the Tharus of Kheri district. UP Bulletin of Medic Ethnobot Res, 1980; 1:318.

Malterud KE, Rydland KM. Inhibitors of 15-lipoxygenase from orange peel. J Agric Food Chem, 2000; 48:5576-80

Manickam M, Ramanathan M, Jahromi MA, Chansouria JP, Ray AB. Antihyperglycemic activity of phenolics from *Pterocarpus marsupium*. J Nat Prod, 1997; 60:609-10.

Mankani KL, Krishna V, Manjunatha BK, Vidya SM, Singh SDJ, Manohara YN, Raheman AU, Avinash KR. Evaluation of hepatoprotective activity of stem bark of *Pterocarpus marsupium* Roxb. Indian J Pharmacol, 2005; 37(3):165-168.

Manlio T, Stefania G, Antonietta DC, Marinella R, Daniela P, Maria M. Pterostilbene and 3 hydroxypterostilbene are effective apoptosis inducing agents in MDR and BCR ABL expressing leukemia cells. Int J Biochem Cell Biol, 2005; 37:1709-1726.

Martinez JC, Cardenas F, Lamprea M, Morato S. The role of vision and proprioception in the aversion of rats to the open arms of an elevated plus-maze. Behav Processes, 2002; 60:15-26.

Matsumura T, Arai M, Yonemitsu Y, Maruoka D, Tanaka T, Suzuki T et al. The traditional Japanese medicine Rikkunshito increases the plasma level of ghrelin in humans and mice. J Gastroenterol, 2010; 45(3):300-307.

Mattson MP. Calcium and neuronal injury in Alzheimer disease. Contributions of ß-amyloid precursor protein mismetabolism, free radicals, and metabolic compromise. Ann NY Acad Sci, 1994; 747:50-76.

Matusuo M, Hamasaki Y, Fujiyama F, Miyazaki S. Eicosanoids are produced by microglia, not by astrocytes, in rat glial cell cultures. Brain Res, 1995; 685:201-204.

Maurya RS, Rajinder, Mundkinajeddu D, Handa SS, Prem PY, Mishra K, Pushpesh. Constituents of *Pterocarpus marsupium*: An ayurvedic crude drug. Phytochem, 2004; 65(7):915-20.

McCarthy HD, McKibbin PE, Holloway B, Mayers R, Williams G. Hypothalamic neuropeptide Y receptor characteristics and NPY-induced feeding responses in lean and obese Zucker rats. Life Sci, 1991; 49:1491-1497.

Michaelis EK. Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. Prog Neurobiol, 1998; 54:369-415.

Miller B, Sarantis M, Traynelis SF, Attwell D. Potentiation of NMDA receptor currents by arachidonic acids. Nature, 1992; 355:722-725.

Miller NJ, Rice-Evans CA. The relative contribution of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and black currant drink. Food Chem, 1997; 60:331-337.

Miller RJ. G-protein linked glutamate receptors. Seminars Neurosci, 1994; 6:105-115.

Mo´rotz GM, De Vos KJ, Vagnoni A, Ackerley S, Shaw CE, Miller CCJ. Amyotrophic lateral sclerosis-associated mutant VAPBP56S perturbs calcium homeostasis to disrupt axonal transport of mitochondria. Hum Mol Genet, 2012; 21:1979–1988.

Mohammadi M, Khole S, Devasagayam TPA, Ghaskadbi S. *Pterocarpus marsupium* extract reveals strong *in vitro* antioxidant activity. Drug Discov Ther, 2009; 3(4):151-161.

Mohire NC, Salunkhe VR, Bhise SB, Yadav AV. Cardiotonic activity of aqueous extract of heartwood of *Pterocarpus marsupium*. Indian J Exp Biol, 2007; 45:532-7.

Morel I, Cillard P, Cillard J. Flavonoid-metal interactions in biological systems. In: Rice-Evans CA, Packer L, editors. Flavonoids in health and disease. New York: Marcel Dekker Inc; 1998; pp.163-77

Muller-Delp JM, Lubahn DB, Nichol KE, Philips BJ, Price EM, Curran EM et al. Regulation of nitric oxide-dependent vasodilation in coronary arteries of estrogen receptor-alpha-deficient mice. Am J Physiol Heart Circ Physiol, 2003; 285(5):H2150-57.

Mura T, Dartigues JF, Berr C. How many dementia cases in France and Europe? Alternative projections and scenarios 2010-2050. Eur J Neurol, 2010; 17:252-259.

Murphy TH, Schnaar RL, Coyle JT. Immature cortical neurons are uniquely sensitive to glutamate toxicity by inhibition of cystine uptake. FASEB J, 1990; 4:1624-1633.

Nadkarni AK. In Nadkarni's Indian Materia. Vol. I. Popular Prakashan, Bombay, 1976; pp.662-663.

Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol, 2005; 29:41-47.

Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C et al. Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. J Clin Endocrinol Metab, 2004; 89(6):2832-36

Nemani VM, Lu W, Berge V, Nakamura K, Onoa B, Lee MK et al. Increased expression of alpha-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. *Neuron*, 2010; 65: 66–79.

Nicholis D, Attwell D. The release of excitatory amino acids. Trends Pharmacol Sci, 1990; 11:462-468.

Nishimura AL, Mitne-Neto M, Silva HCA, Richieri-Costa A, Middleton S, Cascio D et al. A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. Am J Hum Genet, 2004; 75:822–831.

Norris EH, Giasson BI, Ischiropoulos H, Lee VM. Effects of oxidative and nitrative challenges on alpha-synuclein fibrillogenesis involves distinct mechanisms of protein modifications. J Biol Chem, 2003; 278:27230-27240.

Novelli A, Reilly JA, Lysko PG, Henneberry RC. Glutamate becomes neurotoxic via the NMDA receptor when intracellular energy levels are reduced. Brain Res, 1988; 451:205-212.

O'Riordan JI, Thompson AJ, Kingsley DP et al. The prognostic value of brain MRI in clinically isolated syndromes of the CNS. A 10-year follow-up. Brain, 1998; 121:495–503.

O'Hare E, Shaw DL, Tierney KJ, Eam K, Levine AS, Shephard RA. Behavioral and neurochemical mechanisms of the action of mild stress in the enhancement of feeding. Behav Neurosci, 2004; 118:173–177.

Oka A, Belliveau NJ, Rosenberg PA, Volpe JJ. Vulnerability of oligodendroglia to glutamate: pharmacology, mechanisms and prevention. J Neurosis, 1993; 13:1441-1453.

Olesen J, Gustavsson A, Svensson M, Wittchen HU, Jonsson B. The economic cost of brain disorders in Europe. *Eur J Neurol*, 2012; 19:155-162.

Olney JW, Ho OL, Rhee V. Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system. Exp Brain Res, 1971; 14:61-76.

Olney JW. Excitotoxic amino acids and neuropsychiatric disorders. Annu Rev Pharmacol Toxicol, 1990; 30:47-71.

Olney JW. Neurotoxicity of excitatory amino acids. In: Kainic acid as a tool in neurobiology. McGeer EG, Olney JW, McGeer PL, eds., Raven Press, New York, USA (1978) pp.95-121.

Olney JW. The toxic effects of glutamate and related compounds in the retina and the brain. Retina, 1982; 2:341-359.

Pandey MC, Sharma PV. Hypoglycaemic effect of bark of *Pterocarpus marsupium* Roxb. (Bijaka) on alloxan induced diabetes. Med. Surg, 1976; 16:9-11.

Parent JM. Adult neurogenesis in the intact and epileptic dentate gyrus. Prog Brain Res, 2007; 163:529–540.

Pasinelli P, Brown R.H. Molecular biology of amyotrophic lateral sclerosis: insights from genetics. Nat Rev Neurosci, 2006; 7:710–723.

Patil GG, Mali PY, Bhadane VV. Folk remedies used against respiratory disorders in Jalgaon District, Maharashtra, Nat Prod Radiance, 2008; 7(4):354-358.

Patil UH, Gaikwad DK. Phytochemical screening and microbicidal activity of stem barks of *Pterocarpus marsupium*. Int J Pharma Sci Res, 2011; 2(1):36-40.

Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods, 1985; 14:149-167.

Pennetta G, Hiesinger PR, Fabian-Fine R, Meinertzhagen IA, Bellen HJ. Drosophila, VAP-33A directs bouton formation at neuromuscular junctions in a dosage dependent manner. Neuron, 2002; 35:291–306.

Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol, 1978; 47:379-391.

Pradhan SN, Lynch LF Jr. Behavioral changes in adult rats treated with monosodium glutamate in the neonatal stage. Arch Int Pharmacodyn, 1972; 197:301-304.

Pueyo IU, Calvo MI. Phytochemical Study and Evaluation of Antioxidant, Neuroprotective and Acetylcholinesterase Inhibitor Activities of *Galeopsis ladanum L*. extracts. Pharmacog Mag, 2009; 5:287-290.

Qi HY, Wang R, Liu Y, Shi YP. Studies on the chemical constituents of *Codonopsis pilosula*. Zhong Yao Cai, 2011; 34(4):546-48

Rada P, Avena NM, Hoebel BG. Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. Neuroscience, 2005; 134:737-744.

Raff M. Cell suicide for beginners. Nature, 1998; 396:119-122.

Ramanathan M, Sivakumar S, Anandvijaykumar PR, Saravanababu C, Pandian PR. Neuroprotective evaluation of standardized extract of Centella asciatica in monosodium glutamate treated rats. Ind J Exp Biol, 2007; 45:425-431.

Ramanathan M, Sivakumar S, Anandvijayakumar PR, Saravanababu C, Pandian PR. Neuroprotective evaluation of standardized extract of *Centella asciatica* in monosodium glutamate treated rats. *Indian J Exp Biol*, 2007; 45:425-431.

Randall RD, Thayer SA. Glutamate-induced calcium transient triggers delayed calcium overload and neurotoxicity in rat hippocampal neurons. J Neurosis, 12 (1992) 1882-1895.

Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology, 5/e. Elsevier India Pvt Ltd., New Delhi, India, (2006) pp.490-501.

Rasmusson AM, Hauger RL, Morgan CA, Bremner JD, Charney DS & Southwick SM. Low baseline and yohimbine-stimulated plasma neuropeptide Y (NPY) levels in combat-related PTSD. *Biol Psychiatry*, 2000; 47: 526–539.

Ratnaparkhi A, Lawless GM, Schweizer FE, Golshani P, Jackson GR. A Drosophila model of ALS: human ALS-associated mutation in VAP33A suggests a dominant negative mechanism. PLoS One, 2008; 3:2334.

Ratty AK, Das NP. Effects of flavonoids on nonenzymatic lipid peroxidation: structure-activity relationship. *Biochem Med Metab Biol*, 1988; 39(1): 69-79

Redding TW, Schally AV, Arimura A, Wakabayashi I. Effect of monosodium glutamate on some endocrine functions. Neuroendocrinol, 1971; 8:245-255.

Reetz K, Lencer R, Hagenah JM, Gaser C, Tadic V, Walter U et al. Structural changes associated with progression of motor deficits in spinocerebellar ataxia. Cerebellum, 2010; 17:210–217.

Remsberg CM, Yanez JA, Ohgami Y, Vega Villa KR, Rimando AM, Davies NM. Pharmacometrics of pterostilbene: Preclinical pharmacokinetics and metabolism, anticancer, antiinflammatory, antioxidant and analgesic activity. Phytother Res, 2008; 22:169-179.

Rice AC, Khaldi A, Harvey HB, Salman NJ, White F, Fillmore H et al. Proliferation and neuronal differentiation of mitotically active cells following traumatic brain injury. Exp Neurol, 2003; 183:406-417.

Rodgers RJ, Dalvi A. Anxiety, defence and the elevated plus-maze. Neurosci Biobehav Rev, 1997; 21:801-810.

Rosen DR, Siddique T, Patterson D et al. Mutations in Cu/Zn-superoxide dismutase gene are associated with familial ALS. Nature, 1993; 362:59-62.

Rosmond R. Stress induced disturbances of the HPA axis: a pathway to Type 2 diabetes. Med Sci Monit, 2003; 9:35–39.

Rothman SM, Thurston JH, Hauhari RE. Delayed neurotoxicity of excitatory amino acids in vitro. Neurosci, 1987; 22:471-480.

Saija A, Scalese M, Lanza M, Marzullo D, Bonina F, Castelli F. Flavonoids as antioxidant agents: importance of their interactions with biomembranes. Free Radic Biol Med, 1995; 19(4):481-86.

Sailer M, O'Riordan JI, Thompson AJ et al. Quantitative MRI in patients with clinically isolated syndromes suggestive of demyelination. Neurology, 1999; 52:599–606.

Saldanha CJ. Flora of Karnataka Vol I. Oxford IBH Publishing; 1984, pp.21.

Salunkhe VR, Yadav AV, Shete AS, Kane SR, Kulkarni AS. Anti-inflammatory acitivity of hydrogels of extracts of *Pterocarpus marsupium* and *Coccinia indica*. Indian Drugs, 2005; 42:319-321.

Samii A, Nutt JG, Ransom BR. Parkinson's disease. Lancet, 2004; 363:1783-1793.

Sanacora G, Kershaw M, Finkelstein JA, White JD. Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. Endocrinology, 1990; 127:730-737.

Sapha B. Clinical observation of antidiabetic properties of *Pterocarpus marsupium*, *Eugenia jambolona*. J Indian Med Assoc, 1956; 27:388-390.

Saxena HO & Brahmam M. The Flora of Orissa, 1994; Vol. I, pp.574.

Schlotz W, Hellhammer J, Schulz P & Stone AA. Perceived work overload and chronic worrying predict weekend–weekday differences in the cortisol awakening response. Psychosom Med, 2004; 66:207–214.

Selvam AB. Inventory of vegetable crude drug samples housed in Botanical Survey of India, Howrah. Pharmacognosy Rev, 2008; 2:61-94.

Seress L, Lazar G, Kosaras B, Robertson RT. Regional effect of monosodium-L-glutamate on the superficial layers of superior colliculus in rat. Cell Tissue Res, 1984; 235:453-457.

Shah CS, Qadry JS. Volatile oils. In: A Text Book of Pharmacognosy. Shah BS, eds., Prakashan Publications, India, 1988; pp.216-220.

Shah DS. A preliminary study of indigenous hypoglycemic action of heart wood of *Pterocarpus marsupium* Roxb. Indian J Med Res, 1967; 55:166-168.

Shah RS, Lee HG, Xiongwei Z, Perry G, Smith MA & Castellani RJ. Current approaches in the treatment of Alzheimer's disease. Biomed Pharmacother, 2008; 62:199-207.

Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal Plants Used in Ayurveda, 2005; Vol.3, pp.32-33.

Sharma R. Medicinal plants of India-An encyclopedia, 2003; pp.206.

Sharp AH et al., Widespread expression of Hungtinton’s disease gene (IT15) protein product. Eruon, 1995; 14:1065-1074.

Sherer TB, Kim JH, B.etarbet R & Greenamyre JT. Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and alpha-synuclein aggregation. Exp Neurol, 2003; 179:9-16.

Shigenaga MK, Hagen TM & Ames, BN. Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci USA, 1994; 91:10771-10778.

Shiraishi I, Honma K, Honma S, Hiroshige T. Ethosecretogram: relation of behavior to plasma corticosterone in freely moving rats. Am J Physiol, 1984; 247:40–45.

Siesjö BK. Calcium-mediated processes in neuronal degeneration. Ann NY Acad Sci, 1994; 747:140-161.

Siesjö BK. Cerebral circulation and metabolism. J Neurosurg, 1984;60:883-908.

Singh RP, Sharad S & Kapur S. Free radicals and oxidative stress in neurodegenerative diseases: Relevance of dietary antioxidants. JIACM, 2004;5:218-225.

Singh U, Wadkwani AM, Johri BM. Dictionary of economic plants in India. Indian council of agricultural Research, New Delhi, 1965; 1:176-184.

Singhal AK, Gupta M, Edwin S, Soni R. Evaluation of wound healing potential of *Pterocarpus marsupium* heart wood extract in normal and diabetic rats. Chron Young Scient, 2012; 3(1):42-47.

Small SA. Measuring correlates of brain metabolism with high resolution MRI: a promising approach for diagnosing. Alzheimer disease and mapping its course. Alzheimer Dis Assoc Disord, 2003; 17:154–161.

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature, 1997; 388:839-840.

Squibb RE, Tilson HA, Meyer OA, Lamartiniere CA. Neonatal exposure to monosodium glutamate alters the neurobehavioral performance of adult rats. Neurotoxicol, 1981; 2:471–484.

Standaert DG, Young AB. Treatment of central nervous degenerative disorders. In: Goodman & Gilman's The Pharmacological basis of therapeutics, 11/e. Brunton LL, Lazo P, Parker KL, eds., McGraw-Hill, New Delhi, India (2006) pp.527-546.

Stone WT, Addae JI. The pharmacological manipulation of glutamate receptors and neuroprotection. Eur J Pharmacol, 2002; 447:285-296.

Stricker-Krongrad A, Max JP, Musse N, Nicolas JP, Burlet C, Beck B. Increased threshold concentrations of Neuropeptide Y for a stimulatory effect on food intake in obese Zucker rats: changes in the microstructure of the feeding behavior. Brain Res, 1994; 660:162-166.

Stricker-Krongrad A, Beck B, Burlet C. Enhanced feeding response to neuropeptide Y in hypothalamic neuropeptide Y-depleted rats. Eur J Pharmacol, 1996; 295:27-34.

Suralkar AA, Vaidya GS, Borate AR, Jadhav AS, Gaikwad KK. Antiallergic, Antianaphylactic and Mast Cell Stabilizing Activity of *Pterocarpus marsupium* Roxb. Int J Pharm Biomed Sci, 2012; 3(4):1691-1697.

Suzuki YS, Forman HJ, Sevanian A. Oxidants as stimulators of signal transduction. Free Radic Biol Med, 1997; 22:269-285.

Swain B.K, Das S.K. Visual guide to wild medicinal plants of Orissa, 2007; pp.37.

Takasaki Y, Sekine S, Matsuzawa Y, Iwata S, Sasoka M. Effects of parenteral and oral administration of monosodium-l-glutamate (MSG) on somatic growth in rats. Toxicol Lett, 1979; 4:327-343.

Takeda H, Sadakane C, Hattori T, Katsurada T, Ohkawara T, Nagai K et al. Rikkunshito, an herbal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT2 receptor antagonism. Gastroenterol, 2008; 134(7):2004-13

Tan S, Wood M, Maher P. Oxidative stress induces a form of programmed cell death with characteristics of both apoptosis and necrosis in neuronal cells. J Neurochem, 71 (1998) 95-105

Thomas DW, Scharrer E, Mayer J. Effects of alloxan induced diabetes on the feeding patterns of rats. Physiol Behav, 1976; 17:345-349.

Tiwari M, Khare HN. Chemical constituents and medicinal uses of *Pterocarpus marsupium* Roxb. Flora Fauna, 2015; 21(1): 55- 59

Tolosa E, Poewe W. Premotor Parkinson disease. Neurology, 2009; 72:1.

Trojanowski JQ, Lee VM. Fatal attractions of proteins, A comprehensive hypothetical mechanism underlying Alzheimer's disease and other neurodegenerative disorders. Ann N Y Acad Sci, 2000; 924:62–67.

Trout JJ, Koeniq H, Goldstone AD, Iqbal Z, Lu CY, Siddiqui F. NMDA receptor excitotoxicity involves activation of polyamine synthesis: protection by alpha-difluoromethylornithine. J Neurochem, 1993; 60:352-355.

Tsuda H, Han SM, Yang Y, Tong C, Lin YQ, Mohan K et al. The amyotrophic lateral sclerosis 8 protein VAPB is cleaved, secreted, and acts as a ligand for Eph receptors. Cell, 2008; 133:963–977.

Vats V, Yadav SP, Biswas NR, Grover JK. Anticataract activity of *Pterocarpus marsupium* bark and *Trigonella foenumgraecum* seed extracts in alloxan-induced diabetic rats. J Ethnopharmacol, 2004; 93(2-3):289-294.

Volles MJ, Lansbury PT. Jr. Zeroing in on the pathogenic form of alpha-synuclein and its mechanism of neurotoxicity in Parkinson's disease. Biochem, 2003; 42:7871-7878.

Volterra A, Trotti D, Cassuti P, et al., High sensitivity of glutamate uptake to extracellular free arachidonic acid levels in rat cortical synaptosomes & astrocytes. J Neurochem, 1992; 59:600-606.

Vornov JJ, Coyle JT. Glutamate neurotoxicity and the inhibition of protein synthesis in the hippocampal slice. J Neurochem, 1991; 56:996-1006.

Walsh DM, Selkoe DJ. A beta oligomers – a decade of discovery. J Neurochem, 2007; 101: 1172–1184.

Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS et al. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*. Nature, 2002; 416:535–539.

Wang BS, Huang GJ, Tai HM, Huang MH. Antioxidant and anti-inflammatory activities of aqueous extracts of *Schizonepeta tenuifolia* Briq. Food Chem Toxicol, 2012; 50(3-4):526-31

Weinshenker BG, Bass B, Rice GPA, et al., The natural history of multiple sclerosis: a geographically based study I. Clinical course and disability. Brain, 1989; 112:133–146.

Wellman PJ, Clifford PS, Rodriguez JA. Ghrelin and ghrelin receptor modulation of psychostimulant action. Front Neurosci, 2013; 7:171

WHO. Second Report of the WHO Expert Committee on Diabetes Mellitus. World Health Organization, Geneva, 1980.

Wilfred CO, Christ'l MD. Detection of the Addition of Citrus reticulata and Hybrids to Citrus sinensis by flavonoids. J Agric Food Chem, 1997; 45(5):1633-37

Williams G, Gill JS, Lee YC, Cardoso HM, Okpere BE, Bloom SR. Increased neuropeptide Y concentrations in specific hypothalamic regions of streptozocin-induced diabetic rats. Diabetes, 1989; 38:321-327.

Wisden W, Seeberg PH. Mammalian ionotropic glutamate receptors. Curr opin Neurobiol, 1993; 3:291-298.

Yakabi K, Kurosawa S, Tamai M, Yuzurihara M, Nahata M, Ohno S et al. Rikkunshito and 5-HT2C receptor antagonist improve cisplatin-induced anorexia via hypothalamic ghrelin interaction. Regul Pept, 2010; 161(1-3):97-105

Yehuda R, Brand S, Yang RK. Plasma neuropeptide Y concentrations in combat exposed veterans: relationship to trauma exposure, recovery from PTSD and coping. Biol Psychiatry, 2006; 59:660–663.

Yogesh B, Yadav AS, Ajit K et al. *Pterocarpus marsupium* Roxb. biological activities and medicinal properties. Int J Adv Pharm Sci, 2010; 1:350-357.

Youdim KA, Qaiser MZ, Begley DJ. Flavonoid permeability across an in situ model of the blood-brain barrier. Free Radic Biol Med, 2004; 36:592-604.

Youdim KA, Spencer JP, Schroeter H, Rice-Evans C. Dietary flavonoids as potential neuroprotectants. Biol Chem, 2002; 383:503-519.

Zaid AM, Sharma KK, Rizvi SI. Effect of (-)-epicatechin in modulating calcium-ATPase activity in normal and diabetic human erythrocytes. Indian J Clin Biochem, 2002; 17(2):27-32.

Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I et al. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol, 2004; 55:164-173.

Zdenek Hlinak, Gandalovicova D, Krejci I. Behavioral deficits in adult rats treated neonatally with glutamate. Neurotoxicol Teratol, 2005; 27:465-473.

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Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

Institutional Animal Ethics Committee (IAEC)

REG: NO: 887/PO/Re/S/2005/CPSCEA

CERTIFICATE

Title of the Project	:	Evaluation of neuroprotective role of ethanol extract of Pterocarpus marsupium Roxb. bark against monosodium glutamate-induced excitotoxicity model in SD rats .
Department	:	Pharmacology.
Proposal Number	:	JKKNCP/MP/OCT/05/2015-16
Approval date	:	18.01.2016
Animals	:	Sprague Dawley Rats (Female)
No of Animals Sancioned	:	30

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Chairman IAEC