INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-4, Issue-1, Jan-Mar-2013 Coden : IJABPT Copyrights@2013

ISSN : 0976-4550

Received: 12th Jan-2013 *Revised: 19th Jan-2013* *Accepted: 20th Jan-2013*

Research article

MODULATION OF Na^+/K^+, Mg^{2+} and Ca^{2+} ATPase ACTIVITY IN DIFFERENT REGIONS OF RAT BRAIN DURING ROTENONE INDUCED PARKINSON'S DISEASE AND PROTECTIVE ROLE OF *BACOPA MONNIERI*

Gunduluru Swathi, Cherukupalle Bhuvaneswar and Wudayagiri Rajendra*

Department of Zoology, Division of Molecular Biology, Sri Venkateswara University, Tirupati-517502. A.P. INDIA.

ABSTRACT: *Bacopa monnieri* (BM; Family: Scrophulariaceae), also referred as Brahmi or Jalbrahmi has been used for centuries in Ayurvedic system of medicine as a brain tonic, memory enhancer, revitaliser of sensory organs, anti-anxiety, cardio-tonic, diuretic, antidepressant and anticonvulsant agent, and the pharmacological actions are mainly attributed to the saponin compounds present in the alcoholic extract of the plant. The present study was carried out with a specific aim to examine the neuroprotective effect of *Bacopa monnieri* during Rotenone (RT) induced Parkinson's disease (PD) with particular reference to Na^+/K^+, Mg^{2+} and Ca^{2+} -ATPase activities in different regions of rat brain. In the experiment conducted rats were divided into four groups of six in each group, group 1 received Saline water (1 ml/kg), group 2 received RT (2.5 mg/kg) through i.p. route administration for 60 days to induce PD. The third group received BM extract (180 mg/kg/day) for 20 days orally before induction of PD and group 4 received Levodopa (LD) (10 mg/kg/day) orally which is referred as drug control. The levels of Na^+/K^+, Mg^{2+} and Ca^{2+} -ATPase activities were measured. Na^+/K^+, Mg^{2+} and Ca^{2+} -ATPase activities were significantly depleted in different brain regions of rat during RT induced PD when compared to control rats. Treatment with BM and LD caused significant elevation in the activity levels of Na^+/K^+, Mg^{2+} and Ca^{2+} -ATPase in different brain regions of rats when compared to induced PD rats. Our results suggest the ability of BM extract to modulate Na^+/K^+, Mg^{2+} and Ca^{2+} - ATPase activities in different brain regions of RT induced rodent model of PD and thus offers effective management in the treatment of PD.

Keywords: Parkinson's disease (PD). *Bacopa monnieri* (BM). Rotenone (RT). Levodopa (LD). Na^+/K^+. Mg^{2+} and Ca^{2+} -ATPase.

INTRODUCTION

Parkinson's disease (PD) is a motor system disorder, resulting from the loss of dopamine-producing brain cells. PD exhibit cumulative symptoms as muscle rigidity, tremor, bradykinesia (Rascol et al. 2009), depression, memory loss, sleep disturbance, speech impairments and dysphagia (Harish et al. 2010). It is characterized by degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc).The prevalence of PD, worldwide, is 1% of the population over the age of 60 years (Samii et al. 2004). It has increased relative risk of mortality ranges from1.6 to 3.0 compared with matched control populations (Clarke et al. 2007). Among the toxic animal models of PD, chronic systemic exposure of rotenone represents one of the most recently used approaches (Betarbet et al. 2000). It is highly lipophilic and readily gains access to all organs (Talpade et al. 2000) and functions as Complex I mitochondrial inhibitor. The chronic exposure to rotenone causes highly selective nigrostriatal dopaminergic degeneration, the characteristic feature of PD (Betarbet et al. 2000; Alam and Schmidt 2002; Sherer et al. 2002). It develops slow onset of PD symptoms that makes suitable to study neuroprotective strategies (Sherer et al. 2003; Schmidt and Alam, 2006). Chronic use of current anti-parkinsonian medications including Levodopa therapy causes disabling abnormal involuntary movements known as drug-induced dyskinesias in the majority of PD patients (Deogaonkar and Subramanian 2005; Obeso et al. 2000). Hence, there is a need to discover newer pharmacologically active agents obtained from natural medicinal plant extracts which exhibit lesser or no side effects. *Bacopa monnieri* (called Brahmi in Sanskrit) an ayurvedic medicinal plant have been used as a brain tonic, which contains a mixture of triterpenoid saponins designated as bacosides A and B (Chatterjee et al. 1963, 1965). Several studies have reported its pharmacological roles as memory enhancer (Bhattacharya et al. 2000), cognition-enhancer (Das et al. 2002; Singh & Dhawan 1997; Sumathi et al. 2002), antidepressant and also antioxidant properties (Sairam et al. 2001).

ATPases are membrane-bound enzymes and any perturbation in the activities of these enzymes affects membrane status by inflicting changes in electrophysiological energetics and normal homeostasis. Na^+/K^+-ATPase is responsible for the generation of the membrane potential through the active transport of sodium and potassium ions in the CNS necessary to maintain neuronal excitability. Na^+/K^+-ATPase is present at high concentrations in brain, consuming about 40-50% of the ATP generated in this organ (Erecinska and Silver, 1994). Na^+/K^+-ATPase is implicated in metabolic energy production as well as in the uptake, storage, and metabolism of catecholamines, serotonin, and glutamate (Carageorgiou et al. 2007). Ca^{2+}-ATPase activity is associated with neuronal excitability, cellular depolarization and fine tunning of Ca^{2+}-channel activity (Lees, 1991). Mg^{2+}-ATPase activity associated with mitochondrial membrane bound enzyme which is involved in turnover of ATP synthesis in conjugation with oxidative phosphorylation.

Keeping in view of the importance of ATPases in the neuronal metabolism, the present investigation is carried out with specific aim to evaluate the alterations in ATPases during induced PD and also to study the antiparkinsonian effect of Bacopa monnieri in different regions of rat brain.

MATERIALS AND METHODS

Collection of plant material:

Bacopa monnieri plant used in this work was collected in bulk from Tirumala Hills, Andhra Pradesh in India and authenticated by qualified botanist at Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh in India.

Extract Preparation:

The whole plant (including roots) of BM was dried in shade, and then powdered plant material was macerate with ethanol for 7 days. The plant material was percolated with circulating 95% ethanol (200 ml) for three rounds. The residue was extracted twice using the same procedure. The extract was filtrated and concentrated under reduced pressure in the Buchi rotavapour yielding a greenish-black sticky residue. Finally the extract was freeze- dried and was used for further studies.

Experimental design

The present work was conducted on male Wistar rats weighing 150±25g, they were maintained at a temperature of 25±2°C and relative humidity of 45-55% with 12:12 h dark: light cycle. The rats were maintained according to the ethical guidelines for animal protection and welfare bearing no.04a/a/CPCSEA/IAEC/08-09/SVU/zool/WR-GS/dt.1.9.2009. The rats were divided into 4 groups each consisted of 6 rats.
GROUP I: Served as normal control group, Oil (sunflower oil) was injected as vehicle to the control rats (1 ml/kg) of 1.0 ml/kg/day intraperitoneally (i.p.) for 60 days.

GROUP II: Rotenone (RT) emulsified in sunflower oil at 2.5 mg/ml was given i.p. once a day at 1 ml/kg for 60 days (Alam and Schmidt, 2002), which induces PD.

GROUP III: RT-induced PD rats were treated with BM extract with a dose of 180 mg/kg/day orally for 80 days, started before 20 days from induction of PD.

GROUP IV: RT -induced PD rats were treated with Levodopa (reference control) with a dose of 10 mg/kg/day orally started after 20 days from induction of PD (Alam and Schmidt, 2004).

The development of Parkinson's disease was detected after 20 days from induction with rotenone, by occurrence of tremors and exhibiting specific symptoms such as bradykinesia and rigidity in rats. The treatment with BM extract was started 20 days before induction of PD and LD was started after 20 days from induction of PD and continued for 60 days. After stipulated duration, the animals were sacrificed by cervical dislocation and the brain regions [Cerebral cortex (CC), Cerebellum (CB), Mid brain(MB) and Pons-Medulla (PM)] were immediately isolated, frozen in liquid nitrogen and were stored at -40°C until further analysis.

Biochemical analysis:

The activities of Na^+/K^+, Mg^{2+} and Ca^{2+} ATPases in all the brain regions were estimated by the method of Desaiah and Ho (1979). Protein concentrations of the tissue homogenates were determined by the standard method of Lowry et al. (1951) using bovine serum albumin as the standard.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Statistical analyses:

Results are presented as mean ± SEM. One-way analysis of variance (ANOVA) followed by Student–Newman–Keuls (SNK) test was used to compare differences between means in more than two groups. All statistical analysis were analysed using the SPSS statistical software package.

RESULTS

To examine efficacy of BM on ATPase activity in the PD rat brain regions as CC, CB, MB and PM, activities of Na /K^+, Mg^{2+} and $Ca2^+$ ATPases were measured.

Na^+ /K^+ ATPase activity

The PD induced rat brain regions (CC, CB, MB and PM) showed significantly reduced ATPase activities of Na /K^+ ($P < 0.05$) compared to controls (Table 1). BM pretreated and LD treated PD induced rat brain regions showed significantly improved activities of Na /K^+ ($P < 0.05$) compared to untreated PD rat brain regions.

Table 1: Changes in the Sodium, Potassium – ATPase (Na^+/K^+ -ATPase) in different brain regions of rats during RT-induced PD and pretreatment with ethanolic extract of BM.

Na^+/K^+ - ATPase	SC	RT	BM+RT	LD+RT
CC	3.37±0.06	1.05±0.13	2.69±0.18	2.38±0.10
CB	3.74±0.04	1.76±0.09	2.80±0.07	2.48±0.12
MB	2.81±0.24	1.10±0.08	2.43±0.10	2.40±0.27
PM	3.00±0.03	1.30±0.09	2.26±0.14	2.31±0.13

Values are expressed in μ moles of inorganic phosphate formed/mg protein/hr

Mg^{2+} ATPase activity

The PD induced rat brain regions (CC, CB, MB and PM) showed significantly reduced ATPase activities of Mg^{2+} ($P < 0.05$) compared to controls (Table 2). BM pretreated and LD treated PD induced rat brain regions showed significantly improved activities of Mg^{2+} ($P < 0.05$) compared to untreated PD rat brain regions.

Table 2: Changes in the Magnesium – ATPase (Mg^{2+} -ATPase) in different brain regions of rats during RT-induced PD and pretreatment with ethanolic extract of BM.

Mg^{2+} - ATPase	SC	RT	BM+RT	LD+RT
CC	3.27±0.08	1.17±0.05	2.47±0.16	2.45±0.14
CB	3.54±0.11	1.16±0.08	2.63±0.11	2.24±0.10
MB	2.69±0.26	1.18±0.06	2.43±0.10	2.15±0.07
PM	2.33±0.19	1.17±0.08	2.09±0.20	2.18±0.20

Values are expressed in μ moles of inorganic phosphate formed/mg protein/hr

Ca^{2+} ATPase activity

The PD induced rat brain regions (CC, CB, MB and PM) showed significantly reduced ATPase activities of Ca^{2+} ($P < 0.05$) compared to controls (Table 3). BM pretreated and LD treated PD induced rat brain regions showed significantly improved activities of Ca^{2+} ($P < 0.05$) compared to untreated PD rat brain regions.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Table 3: Changes in the Calcium – ATPase (Ca^{2+} -ATPase) in different brain regions of rats during RT-induced PD and pretreatment with ethanolic extract of BM.

Ca^{2+} - ATPase	SC	RT	BM+RT	LD+RT
CC	8.54± 0.15	5.34± 0.10	7.53± 0.15	7.78±0.17
CB	8.69±0.13	5.28±0.11	7.48±0.15	7.54±0.15
MB	7.93±0.08	5.44±0.08	7.68±0.07	7.37±0.14
PM	6.68±0.28	5.29±0.12	7.26±0.18	7.70±0.09

Values are expressed in μ moles of inorganic phosphate formed/mg protein/hr

DISCUSSION

In the central nervous system, mitochondrial dysfunction and consequent ATP depletion are the major causes of oxidative stress and alterations in calcium homeostasis (Cassarino and Bennett 1999; Fighera et al.2006), which produce loss of cellular integrity and cell death. Indeed, various CNS disorders, such as seizures, Parkinson's disease, Huntington, and Alzheimer diseases (Poon et al.2005) have been associated with mitochondrial dysfunction, excitotoxicity, and generation of reactive oxygen species (Fighera et al.2003, 2006). In the present investigation the activity levels of Na^+/K^+, Mg^{2+} and Ca^{2+} ATPases were decreased in all the areas of brain during RT induced PD. The decreased levels of ATPases (Na^+/K^+, Ca^{2+} and Mg^{2+}) in this study, during the rotenone induction suggest that impairment of energy metabolism resulting in abnormal neuronal function. Further, as sodium and potassium ions are also important as calcium ions for the development and conduction of action potentials, the decrease in activities of Na^+/K^+, Mg^{2+} and Ca^{2+} -ATPases, may alter the rate of influx and efflux of the cations, namely Na^+ and K^+ respectively. The decrement in the activities of Na^+/K^+, Mg^{2+} and Ca^{2+} ATPases, may also reflect the decreased turnover of ATP, presumably due to inhibition of oxidoreductase system and uncoupling of the same from the electron transport system. The ability to maintain membrane polarity depends on functional ion pumps, in particular, the Na^+/K^+ ATPase (Glynn and Karlish 1975). The Na^+/K^+ ATPase, in turn, depends on an adequate supply of ATP, more than 90% of which is derived from mitochondrial oxidative metabolism (Erecinska and Dagani 1990). Thus, the inhibition in the activity of Na^+/K^+ -ATPase in specific regions of rat brain upon rotenone induction may be correlated with the altered membrane permeability properties. Impaired mitochondrial function depletes ATP, disrupts Na^+/K^+ ATPase activity and causes depolarization (Erecinska and Dagani 1990). Decreased Na^+/K^+ ATPase, may cause innocuous concentrations of glutamate to become excitotoxic, as the relationship between excitotoxicity and bioenergetics may play a role in the pathophysiology of a variety of neurodegenerative diseases (Beal, 1992) and may be particularly relevant to PD. Excitotoxicity triggers free radicals which causes oxidative stress and also depletion of ATPase activity. Protective and/or rescuing treatments have also been proposed trying to suppress the possible causes of dopaminergic neurons apoptosis such as: oxidative stress, age dependent mitochondrial dysfunction, neurotoxins, decrease of neurotrophic factors, excitotoxicity, disturbances of calcium homeostasis, immunologic and infectious mechanisms (Naoi and Maruyama 2001). Among these, oxidative stress has been suggested as playing a major role in the pathophysiology of PD. May be due to the impairment of ATPase activity in RT induced PD rat brain regions (Table 1, 2 and 3), may have lead to inadequate supply of energy which leads to production of reactive oxygen species (ROS) inducing oxidative stress. RT, also a Complex I inhibiter, leading to ATP depletion and generation of toxic oxygen free radicals.The substantia nigra is exposed to a high degree of oxidative stress as a consequence of formation of cytotoxic oxygen radicals. It is apparent that there are several potential sources of oxidative stress to dopaminergic neurons. Post-mortem studies also support the notion that oxidative damage is central to the neurodegeneration of PD (Blandini et al.1996).

Several micronutrients and antioxidants of natural origin of plants have been experimentally proved as effective protective agents against the oxidative stress and also improving ATPase activities as it had been shown that ischemia significantly reduced Na^+, K^+ -ATPase activity by about 40% and increased malondialdehyde content and *Ginkgo biloba* extract pretreatment abolished these effects (Pierre et al.1999).

Wang et al. (2003) have shown that the polysaccharides isolated from *Rheum tanguticum* reduced malondialdehyde (MDA), increased total SOD activity and Na^+, K^+ -ATPase activity in the traumatic brain injury. Earlier studies have shown that the beans of *Mucuna pruriens* (Damodaran and Ramaswamy 1937) and *Vicia faba* (Lattanzio et al.1982) have been successfully used as PD treatment. In the present study BM had been used in treatment of PD. It is widely accepted that neuronal damage can be significantly minimized by free radical scavengers. *Bacopa monnieri* showed significant antioxidant effect per se and in stressed animals (Sairam et al.2001). BM prophylaxis significantly reduces the endogenous levels of oxidative markers and also RT induced oxidative dysfunctions in brain regions. Neuroprotective efficacy of BM was clearly demonstrated by its propensity to significantly attenuate PQ-induced oxidative stress and neurotoxicity (Ravi kumar and Muralidhara 2010).

In the present study, pretreatment with BM significantly increased the ATPases (Na^+/K^+, Ca^{2+}, and Mg^{2+}) activity in the brain of induced PD rats (Table 1, 2 and 3).The BM extract group showed that it had significance difference compared to RT induced PD which indicates its protective effect on PD induced rats. It also showed that there was no significance difference compared to controls, which clearly indicates the recovering or stopping the progression of PD. The BM treated group results are similar to the results of LD treated group which shows that it can act as antiparkinsonian agent. In agreement with our results, earlier studies have shown Bacosides isolated from *Bacopa monnieri* caused significant inhibition in lipid peroxidation, improved the activities of Na^+, K^+ -ATPase, Ca^{2+} -ATPase and Mg^{2+} -ATPase and maintained the ionic equilibrium. The results of BM group were similar to the LD treated group. Preclinical and clinical studies have shown that *Bacopa monnieri* improves memory and mental function (Roodenrys et al.2002). The chronic effects of an extract of *Bacopa monnieri* on cognitive function in human subject have been reported (Stough et al. 2001) and also its pharmacological roles as memory enhancer (Bhattacharya et al.2000), antidepressant and also antioxidant properties (Sairam et al.2001). Human consumption of BM is on the increase owing to its multiple beneficial effects (Anbarasi et al. 2005).

In conclusion, our findings demonstrated the ability of BM extract, which suggests the bioactive factors present in the plant extract has a significant effect to modulate ATPases activity in different brain regions of RT induced rodent model of PD. Further, the BM ethonalic extract, effectively regulated ATPase activity by decreasing oxidative stress / recovering the energy loss that has occurred due to PD and thus can be used as “antiparkinsonian agent”.

ACKNOWLEDGEMENTS

One of the authors (G. Swathi) is grateful to CSIR-UGC (NET) (India) for providing Junior Research Fellowship.

REFERENCES

A. Das, G. Shanker, C. Nath, R. Pal, S. Singh, H.K. Singh; (2002). A comparative study in rodents of standardized extracts of *Bacopa monniera* and *Ginkgo biloba* anticholinesterase and cognitive enhancing activities. Pharmacol Biochem Behav 73:893-900

A. Samii, J.G. Nutt, B.R. Ransom; (2004). Parkinson’s disease. Lancet 363:1783–1793

C. Stough, J. Lloyd, J. Clarke; (2001).The chronic effects of an extract of *Bacopa monniera* (Brahmi) on cognitive function in healthy human subjects. Psychopharmacol 156: 481-488

C.E. Clarke, A.P. Moore; (2007). Parkinson’s disease. Am Fam Physician 75:1045–1048

Desaiah D, Ho IK (1979).Effect of acute and continuous morphine administration on catecholamine-sensitive adenosine triphosphatase in mouse brain. J Pharmacol Exp Ther 208: 80-85

DJ Talpade, JG Greene, DS Higgins, J.T. Greenamyre; (2000). *Invivo* labeling of mitochondrial complex I (NADH:ubiquinone oxidoreductase) in rat brain using [(3)H]dihydrorotenone. J Neurochem 75:2611-2621

DS Cassarino, JP Bennett; (1999).An evaluation of the role of mitochondrial in neurodegenerative diseases: mitochondrial mutations and oxidative pathology, protective nuclear responses and cell death in neurodegeneration. Brain Res Rev 29: 1-25.

F. Bladini, R.H. Porter, T.Greenamyre; (1996).Glutamate and Parkinson's disease. Mol Neurobiol 12:73-93

G. Harish, C. Venkateshappa, R.B. Mythri, S.K. Dubey, K. Mishra, N. Singh, S. Vali, M.M. Bharath; (2010).Bioconjugates of curcumin display improved protection against glutathione depletion mediated oxidative stress in a dopaminergic neuronal cell line: implications for Parkinson's disease. Bioorganic and Medicinal Chemistry 18:2631-2638

G.J. Lees; (1991). Inhibition of sodium-Potassium ATPase: a potentially ubiquitous mechanism contributing to central nervous system neuropathology. Brain Res Rev 16:283-300

H. Carageorgiou , C. Pantos , A .Zarros; (2007). Changes in acetylcholinesterase, Na^+, K^+-ATPase and $Mg2^+$-ATPase activities in the frontal cortex and the hippocampus of hyper and hypothyroid adult rats. Metabolism 56:1104-1110

H. Ravikumar, Muralidhara; (2010).Prophylactic treatment of *Bacopa monnieri* leaf powder mitigates paraquat-induced oxidative perturbations and lethality in Drosophila melanogaster. Ind J Biochem Biophy 47:75-82

H.F. Poon, M. Frasier, N. Shreve, V. Calabrese, B. Wolozin, D.A. (2005). Butterfield; Mitochondrial associated metabolic proteins are selectively oxidized in A30P alpha-synuclein transgenic mice-A model of familial Parkinson's disease. Neurobiol Dis 18 (3):492–498

H.K. Singh, B.N. Dhawan; (1997). Neuropsychopharmacoloical effects of the Ayurvedic nootropic *Bacopa monnieri* Linn (Brahmi). Indian J Pharmacol 29:S359-S365

J.A. Obeso, C.W. Olanow, J.G. Nutt; (2000).Levodopa motor complications in Parkinson's disease. Trends Neurosci 23(10 Suppl): S2-7

K. Anbarasi, G. Vani, K. Balakrishna, C.S. Shyamaladevi; (2005). Effect of bacoside A on membrane-bound ATPases in the brain of rats exposed to cigarette smoke. J Biochem and Mol Toxicol 19:9-65

K. Sairam, C.V. Rao, M.D. Babu, R.K. Goel; (2001).Prophylactic and curative effects of *Bacopa monniera* in gastric ulcer models. Phytomedicine 8:423-430

M. Alam, W.J. Schmidt; (2004). L-DOPA reverses the hypokinetic behaviour and rigidity in rotenone-treated rats. Behav Brain Res 153(2):439-446

M. Alam, W.J. Schmidt; (2002). Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. Behav Brain Res 136(1):317-324

M. Damodaran, R. Ramaswamy; (1937).Isolation of L-DOPA from the seeds of *Mucuna pruriens*. Biochemistry 31: 2149-2151

M. Deogaonkar, T. Subramanian; (2005). Pathophysiological basis of drug-induced dyskinesias in Parkinson's disease. Brain Res Rev 50:156-68

M. Erecinska, F. Dagani; (1990).Relationships between the neuronal sodium/potassium pump and energy metabolism. Effects of K^+, Na^+ and adenosine triphosphate in isolated brain synaptosomes. J Gen Physiol 95: 591-616

M. Erecinska, I.A. Silver; (1994).Ions and energy in mammalian brain. Prog Neurobiol 43:37–71

M. Glynn, S.J.D. Karlish; (1975). The sodium pump. Annu Rev Physiol 37:13-55

M. Naoi, W. Maruyama; (2001).Future of neuroprotection in Parkinson's disease. Parkinsonism Relat Disord 8:139-145

M.F. Beal (1992).Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? Ann Neurol 31:119 -130

M.R. Fighera, L.F. Royes, A.F. Furian, M.S. Oliveira, N.G. Fiorenza, R. Frussa-Filho, J.C. Petry, R.C. Coelho, C.F. Mello; (2006).GM1 ganglioside prevents seizures, Na^+, K^+-ATPase activity inhibition and oxidative stress induced by glutaric acid and pentylenetetrazole. Neurobiol Dis 2: 611–623

N. Chatterjee, R.P. Rastogi, M.L. Dhar; (1963).Chemical examination of *Bacopa monniera* Wettst: Part I. Isolation of chemical constituents. Indian J Chem 1:212-5

N. Chatterjee, R.P. Rastogi, M.L. Dhar; (1965).Chemical examination of *Bacopa monniera* Wettst: Part II. The constitution of bacoside A. Indian J Chem 3:24–9

O. Rascol; (2009).Disease-modification trials in Parkinsondisease: targetpopulations, endpoints and study design. Neurology 72:S51–S58

O.H. Lowry, N. Rosenbrough, A.L. Farr, R.J. Randall; (1951).Protein measurement with folin phenol reagent. J Biol Chem 193:265-275.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

R. Betarbet, T.B. Sherer, G. MacKenzie, M.Garcia-Osuna, A.V.Panov, J.T. Greenamyre; (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci 3:1301–1306

S. Pierre, I. Jamme, M.T. Droy-Le faix, A. Noubelot, J.M. Maixent; (1999).*Ginkgo biloba* extract (EGB 761) protects Na^+, K^+- ATPase activity during cerebral ischemia in mice. Neuroreport 10:47-51

S. Roodenrys, D. Booth, S. Bulzoni, A. Phipps, C. Micallef , J. Smoker; (2002).Chronic effects of Brahmi (*Bacopa monnieri*) on human memory. Neuropsycho pharmacol 27:279-281

S.K. Bhattacharya, A. Bhattacharya, A. Kumar, S. Ghosal; (2000).Antioxidant activity of *Bacopa monniera* in rat frontal cortex, striatum and hippocampus. Phytother Res 14:174-179

S.W. Brusilow, A.L. Horwich; (1995).Urea cycle enzymes: The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, pp 1187-1232

T. Sumathi, M. Nayeem, K. Balakrisshna, G. Veluchamy, N.S. Devarraj; (2002).Alcoholic extract of *Bacopa monniera* reduces the *invitro* effects of morphine withdrawal in guinea-pig ileum. J Ethnopharmacol 82:75-81

T.B. Sherer, J.H. Kim, R. Betarbet, (2003).Subcutaneous rotenone exposure causes highlyselective dopaminergic degeneration and alpha-synuclein aggregation. Exp Neurol 179:9-16

T.B. Sherer, R. Betarbet, A.K. Stout, S. Lund, M. Baptista, A.V. Panov, M.R. Cookson, J.T. Greenamyre; (2002).An *invitro* model of Parkinson's disease: linking mitochondrial impairment to altered-synuclein metabolism and oxidative damage. J Neurosci 22:7006-7015

V. Lattanzio, V.V. Bianco, D. Lafiandra; (1982).High-performance reversed-phase liquid chromatography (HPLC) of favism-inducing factors in Vicia faba L. Experientia 38: 789-790

W.J. Schmidt, M. Alam; (2006). Controversies on new animal models of Parkinson's disease pro and con: the rotenone model of Parkinson's disease (PD). J Neural Transm Suppl 70:273-276

Z.P. Wang, L. Liu, Q.B. Mei, R. Zang, T.W. Gu, X. Zhang, D.K. Gao; (2003). Protective effect of Rheum tanguticum polysaccharides (RTP) on traumatic brain injury in rats. Zhongguo Zhong Yao Za Zhi 28:974-976