

NEUROPROTECTIVE EFFECTS OF S-ALLYL CYSTENE (SAC) ON HALOPERIDOL (HP) INDUCED NEUROTOXICITY IN RAT

K. R. REKHA¹, G. P. SELVAKUMAR¹, N. SRI KUMARAN^{2*} AND G. MARICHAMY²

¹Department of Biochemistry and Biotechnology, Annamalai University, Tamil Nadu, India, ²Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai 608502, Tamil Nadu, India. Email: s.kumaran08@gmail.com

Received: 08 Feb 2012, Revised and Accepted: 31 Mar 2012

ABSTRACT

This present study was conducted to evaluate the neuroprotective effects of S-allyl cysteine (SAC) on Haloperidol (HP) induced neurotoxicity in rat. In this study totally 4 groups of rats were used, each group was treated with saline (control), SAC, HP and HP+SAC separately. The behavioral abnormalities of the drug treated animals were noted by narrow beam test, rotarod and forelimb akinesia on 8th, 15th, 22th and 29th day. After 28th day the drug treated rats were dissected and the brains were used for analysis of biochemical changes. In the narrow beam walking, HP lesioned rats shows increase retention time compared to control rats and the HP+ SAC lesioned rats shows increase rate in retention time at 15th day test and reduced on 22 and 29th day test. In the rotarod analysis, the HP lesioned rats shows reduced retention time compared to control rats and the HP+SAC lesioned rats shows increase rate in retention time. The Akinesia score increased gradually in HP alone treated rats. In SAC pretreated rats were significantly reduces the score compared to HP treated rats. In biochemical test such as TBARS, GSH, SOD, CAT GPx shows great variation to compare each group. These results indicate that the S-allyl cysteine (SAC) is potential agent for treating neurochemical and behavioral abnormalities in rat.

Keywords: S-allyl cysteine, Haloperidol, Neuroprotective effects and neurochemical changes.

INTRODUCTION

The nervous system is the major communication network in the human body. Its normal functioning is strongly dependent on the maintenance of its structural integrity and many complex metabolic processes. Accordingly, the processes that disrupt normal structure or metabolism or both are capable of producing neurological disease. The degenerative diseases of the central nervous system (CNS) encompass a heterogeneous group of disorders characterized by spontaneous, progressive degeneration of neurons in specific regions of the brain, spinal cord, or both. The neurodegenerative disorders include Alzheimer's disease, Parkinson's disease, Huntington's disease and Amyotrophic Lateral Sclerosis (motor neuron disease). Alzheimer's disease is the most common cause of dementia in the elderly with cerebrovascular disease and several less common neurodegenerative disorders accounting for most of the remaining cases¹. The protection of neurons from damage and death is an important challenge in the development of treatment of brain ischemia and neurodegenerative diseases². Typical antipsychotics may also be used for the treatment of acute mania, agitation, and other conditions. Typical antipsychotics, sometimes referred to as first generation antipsychotics, conventional antipsychotics, classical neuroleptics, or major tranquilizers are a class of antipsychotic drugs used to treat psychosis (in particular, schizophrenia).

Haloperidol (HP) is commonly used in the treatment of schizophrenia, acute psychotic states and delirium causes neuronal damage and extrapyramidal symptoms after several years of treatment³. Haloperidol has been used clinically in psychiatry, obstetrics and anesthesiology, and its pharmacology has been extensively reported⁴. Chemically, it belongs to the butyrophenone series of neuroleptic compounds and it is a tertiary amine that tends to form inter phase between water/air or water/lipid at very low concentration of the order of 10⁻⁷ M. It has been shown that neuroleptic drugs tend to decrease the permeability of a variety of biological membranes for various organic and inorganic molecules, including water, and that they exert this effect in minute concentration⁵.

There are several reports on neuroprotective effect of SAC against various brain insults. S-allyl cysteine (SAC) has been reported to exert its neuroprotective effect by scavenging peroxynitrite and inhibiting the ERK signaling pathway activated during initial hypoxic/ischemic insults⁶. *In vitro* studies also suggested the neuroprotective effect of S-allyl cysteine against neurotoxicity in cultured hippocampal neurons^{7, 8}. Therefore based on the above

literatures, the present study was carried out to investigate the influence of the SAC on HP induced neuroprotective effect by evaluating behavioral changes and biochemical analysis.

MATERIALS AND METHODS

Animals

Male Albino Wistar rats (140 to 150 g) purchased from the Institute Central Animal House (RMMC). The animals were kept under 12-hrs light / dark cycles, at 22°C and 60% humidity with food and water and *libitum*. The experimental protocols met with the National Guidelines on the proper care and use of Animals in Laboratory Research (Indian National Science Academy, New Delhi, (2000) (Approval No: 760/16.12.2010)

Chemicals

S-allyl cysteine (SAC) Haloperidol (HP), Thiobarbituric acid (TBA), Reduced Glutathione and 3, 5-Dithio-bis-nitrobenzoic acid (DTNB) were purchased from Sigma chemical Co. USA. All other chemicals were of analytical grade.

Experimental induction of neurotoxicity

Haloperidol (1.0 mg/kg of body weight) was administered chronically to rats for a period of 28 days⁹. All the behavioral assessments were carried out on 8th, 15th, 22nd and 29th days of treatment period¹⁰.

Experimental design

The mice were randomized and divided into four groups of six animals each group.

Group I - Rats with intraperitoneal (*i.p*) injection of saline served as control rat.

Group II - Rats treated with SAC (120 mg /kg body weight, *i.p*) for 28 days¹¹.

Group III - Rats treated with HP (1 mg/kg body wt, *i.p*) daily for 28 days.

Group IV - Rats received *i.p* injection of HP (1 mg/kg body wt, *i.p*) after 1hrs SAC (120mg/kg body wt, *i.p*) were administered¹¹.

24 hours after the 7th, 14th, 21th and 28th daily injection of HP or vehicle solution all the animals were analysed for behavioural studies¹⁰ such as narrow beam walking¹² Rotarod¹³ and Akinesia

were performed. Animals were tested 30 min after *i.p.* injection at all time points in three consecutive trials.

Narrow beam walking test

The narrow beam test was performed according to the descriptions of Hicks and D'Amato,¹⁴.

Rotarod Test

Rota rod was performed according to the descriptions of Dunham and Miya,¹⁶. After 10 sec the speed was accelerated linearly up to 20 rpm within 240 sec and the time spent on the rotarod was recorded automatically.

Forelimb akinesia (Step test)

The stepping test^{17,18} was performed at three time points during the experiment. At each time point the animals were tested twice daily on 3 consecutive days. Briefly, the rat was held by the experimenter fixing its hindlimbs with one hand and the forelimb not to be monitored with the other, while the unrestrained forepaw was touching the table. The number of adjusting steps was counted while the rat was moved sideways along the table surface (90 cm in 5 sec), in the forehead direction, (*i.e.*, the animal is pulled to left when right paw is unrestrained), for both forelimbs.

Biochemical Analysis

On 28th day, after behavioral assessment, animals were sacrificed by decapitation. The brain regions were removed and striatum dissected out. A 10% tissue homogenate was prepared in 0.1M phosphate buffer (pH 7.4). Homogenate were centrifuged for 20min at 10,000rpm and supernatant was used for the biochemical analysis.

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS in the striatum was determined by the method of Utely *et al.*¹⁸.

Estimation of Reduced glutathione (GSH)

Reduced glutathione in the striatum was determined by the method of Jollow *et al.*¹⁹.

Estimation of Catalase

Catalase activity was assayed by the method of Claiborne²⁰.

Assay of Superoxide dismutase

Superoxide dismutase activity was measured by the method of Beauchamp *et al.*²¹.

Assay of Glutathione Peroxidase

Determination of glutathione peroxidase (GPx) activity measured according to the procedure described by Mohandas *et al.*²².

Data analysis

All data were expressed as Mean ± SD of number of experiments (n=6). Statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 11.5 software and individual comparisons were obtained using Duncan's Multiple Range Test (DMRT). Values were considered statistically significant.

RESULTS

In the narrow beam walking (Fig. 1) the HP lessoned rats shows increase retention time compared to control (group I) rats. The SAC lessoned rats not show that much variation compare to control rat group. The HP+ SAC lessoned rats shows increase rate in retention time at 15th day test and reduced on 22 and 29th day test. In the rotarod analysis (Fig. 2) the HP lessoned rats shows reduced retention time on the rotarod compared to control rats (group I). The HP+ SAC lessoned rats shows increase rate in retention time in rotarod analysis. The SAC pretreated HP lessoned rats (group IV) were withstand on the rotarod and narrow beam walking for appreciable time compared to HP intoxicated (group III) rats. Akinesia score increased gradually in HP alone treated rats. In SAC pretreated rats were significantly reduces the score compared to HP treated rats. There was no significant difference in SAC and control treated rats (Fig. 3).

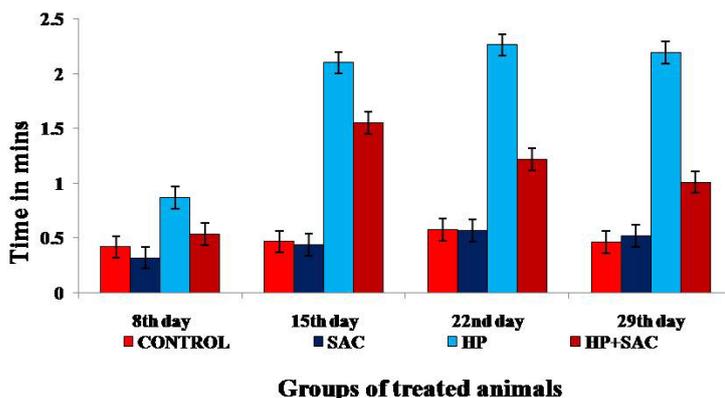


Fig. 1: Effects of narrow beam walking test

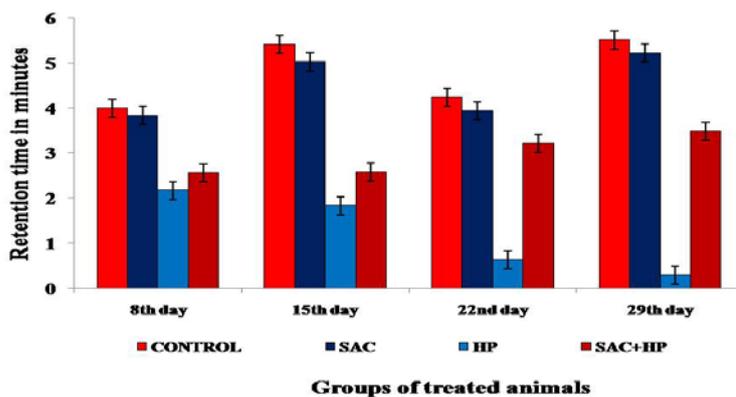


Fig. 2: Effects of Rotarod test

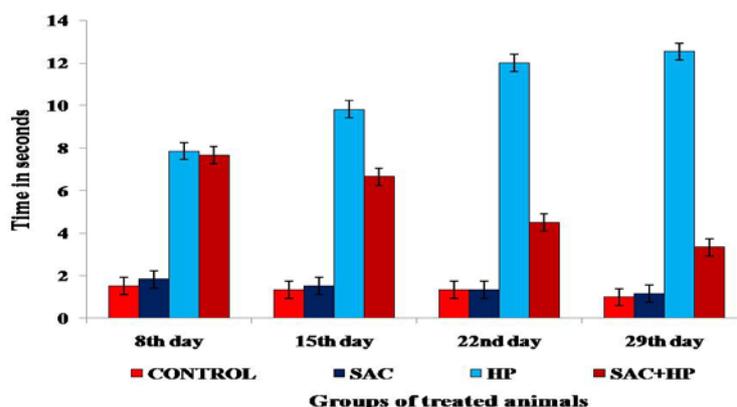


Fig. 3: Effects of forelimb akinesia (step test)

Table 1 exhibit the biochemical changes of the drug treated rats brains. The table .1 summarized the levels of TBARS, GSH activities, SOD, CAT, and GPX in striatum of the control (group I) and experimental animals. Lipid peroxidation levels in striatum were significantly elevated in HP treated animals (group III) relative to the control rats (group I). Prior treatment of SAC administered rats (group IV) significantly reduced the levels of lipid peroxidation. The levels of reduced glutathione decreased in striatum regions in HP treated animals (group III) compared to the control rats (group I). Prior treatment of SAC administered rats (group IV) significantly increased the reduced glutathione level. No significant changes in

the levels of TBARS and reduced glutathione were found in rats treated with SAC alone (group II) compared to saline treated control rats (group I).

The activities of SOD, CAT and GPX in various brain regions were significantly decreased in HP treated animals (group III) relative to the control rats (group I). Prior *ip* administration of SAC to HP injected rats (group IV) tends to increase the activities of SOD, CAT and GPX as compared to the HP treated rats (group III). There is no significant changes in the activities of enzymatic antioxidants were found in SAC alone treated rats (group II) compared to control treated rats.

Table 1: Depicts the levels of TBARS, GSH, SOD, CAT and GPx in Striatum

Groups	TBARS (nmoles/g)	GSH ($\mu\text{g}/\text{gram tissue}$)	SOD ($\text{U}^{\text{A}}/\text{mg protein}$)	CAT ($\text{U}^{\text{B}}/\text{mg protein}$)	GPx ($\text{U}^{\text{C}}/\text{mg protein}$)
Control (Group 1)	0.240 \pm 0.39 ^a	1.045 \pm 0.01 ^a	2.767 \pm 0.02 ^a	1.48 \pm 0.14 ^a	0.0967 \pm 0.08 ^a
SAC (Group 2)	0.201 \pm 0.30 ^a	0.936 \pm 0.01 ^a	2.683 \pm 0.02 ^a	1.394 \pm 0.1 ^a	0.0891 \pm 0.08 ^a
HP (Group 3)	1.370 \pm 1.5 ^b	0.17 \pm 0.07 ^b	0.217 \pm 0.55 ^b	0.25 \pm 0.02 ^b	0.020 \pm 0.001 ^b
SAC+HP (Group 4)	0.666 \pm 0.68 ^c	0.711 \pm 0.06 ^c	1.400 \pm 1.07 ^c	0.85 \pm 0.72 ^c	0.062 \pm 0.05 ^c

^a= amount of enzyme required to inhibit 50% of NBT reduction

^b= μmoles of H_2O_2 consumed /min/mg protein

^c= amount of glutathione utilized/minute

Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

TBARS, GSH, SOD, CAT GPx

DISCUSSION

Neuroleptics act by blocking DA receptors²³. Such blockade results in increased DA turnover, which in turn leads to increased production of hydrogen peroxide resulting in oxidative stress²⁴. Existing evidence indicates that excessive production of free radicals is associated with chronic neuroleptic use, and might contribute to the onset of TD and other movement disorders, such as dystonias and Parkinsonism²⁵. In the present study, HP treatment significantly induced neurotoxicity in rat brain and develops the extra pyramidal symptoms such as TD, facial jerking, vacuous chewing movements and tongue perfusion as compared to vehicle-treated control animals. SAC treatment significantly reversed HP induced VCMs and tongue perfusions. SAC alone treated rats did not show any significant behavioral changes as compared with the vehicle treated control group. Motor functions in this study on experimental TD are normally measured by performing behavioral test namely rotarod performance²⁶, hang test, open field test and narrow beam walking²⁷. These methods are reported to be sensitive in detecting functional impairments in experimental PD models and to quantify the potential efficacy of treatments designed to prevent dopamine loss, to reduce oxidative stress and to maintain the muscular coordination. The narrow beam walking, used to test the balance,

vestibular integrity and muscular co-ordination²⁸ was significantly altered by HP treatment. In narrow beam walking, the TD animals (HP) took more time to cross the beam and also exhibits frequent foot slip errors compared to the normal rats. The animals which had SAC pretreatment showed a reduced time consume to cross the beam and also had a few foot slip errors. The animal to balance and walk on a rotating rod is widely used to measure the coordinated motor skills²⁹. The HP treated animals were shown a much reduced retention time on rotarod compared to normal rat group. The SAC pretreated animals shown appreciable retention on rotarod compared to HP treated animals. Administration of HP may also result in accumulation of HP metabolites in the brain and thus lead to the death of dopaminergic neurons. Formation of quinone species is also responsible for the decrease in DA levels. Increase in DA receptor density after chronic HP administration is well reported³⁰. The results of the study are well corroborated to those previous studies, where co-administration of SAC prevented the decrease in striatal DA levels in HP rat model of TD³¹. The antioxidative action of SAC might play a role in the prevention of the formation of quinone species as well as death of the dopaminergic neurons. It is clear that SAC may prevent the increase in DA receptor super sensitivity as suggested by behavioural experiments of measurement of catalepsy and facial dyskinesia; swim test and total locomotor activity (rearing, grooming and ambulatory activity).

Existing evidence indicates that an unbalanced production of free radicals is associated with chronic neuroleptic use and might contribute to the onset of TD and other movement disorders, such as dystonias and Parkinsonism³². DA is primarily metabolized through oxidation by monoamine oxidase to 3, 4-dihydroxyphenyl-acetic acid. This reaction produces hydrogen peroxide. DA is also metabolized by auto-oxidation yielding superoxide radical. Hydrogen peroxide can further react with iron or copper ions to produce the hydroxyl radical, which is the most toxic of free radicals. Increased DA turnover by neuroleptics could lead to excessive production of these potentially damaging free radicals²⁴. Oxygen free radicals are also reported to diminish the DA transporter function further increasing the extracellular DA levels³³. However, this does not seem to be the only mechanism responsible for the reduced glutathione/ATP depletion observed during HP treatment³⁴. Using rat primary cortical neurons and the mouse hippocampal cell line HT-22, Sagara³⁵ showed that HP causes a sequence of cellular alterations that leads to cell death, and that the production of reactive oxygen species (from mitochondria but not from the metabolism of catecholamines) is an integral part of this cascade. Thus, the possibility exists of a direct interaction with specific membrane components. It is conceivable that the changes in membrane properties may be related to free radical production. In addition, chlorpromazine causes an increase in the level of brain manganese, which in turn may potentiate the damage caused by free radicals³⁶. Another possibility is that neuroleptics suppress the activity of certain detoxifying enzymes, leaving cells unprotected especially if basal enzyme activity is low or the free radical-scavenging mechanisms are less effective. Free radicals are highly reactive with specific cellular components and have cytotoxic properties³⁷, and neuronal loss in the striatum has been reported in animals treated chronically with neuroleptics³⁸.

There are several reports on neuroprotective effect of SAC against various brain insults. Moreover, SAC has been reported to exert its neuroprotective effect by scavenging peroxynitrite and inhibiting the ERK signaling pathway activated during initial hypoxic/ischemic insults. *In vitro* studies also suggested the neuroprotective effect of SAC against neurotoxicity in cultured hippocampal neurons⁸ and SAC protects against the neurotoxic effects of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridinium (MPTP)¹¹ induced PD in rat brain.

Findings of this study demonstrate that SAC exerts neuroprotective effects in experimental rat model. The protective actions of SAC reported in models of HP neurotoxicity might be attribute to its potent antioxidant nature, it is also likely that all the properties of this agent may be acting in a concerted manner to produce neuroprotective responses in this paradigm, thus supporting the therapeutic potential of this and other cysteine containing compounds and garlic preparations. Nonetheless, further and detailed studies are required to the anti-inflammatory and therapeutic potential of SAC in this and other toxic model.

ACKNOWLEDGEMENTS

The authors are thanks to Annamalai University for providing necessary facilities to carry out this work.

REFERENCE

- Vinaykumar, Burns DK. The nervous system, Robbins Basic Pathology, 7th edition, Saunders, Philadelphia; 2003.
- Akaike A, Takatori TY, Kume T, Izumi Y. Mechanisms of Neuroprotective Effects of Nicotine and Acetylcholinesterase Inhibitors: Role of $\alpha 4$ and $\alpha 7$ Receptors in Neuroprotection, J. Mol. Neurosci. 2010; 40:211-216.
- Gloria BK, Leonardo OL, Graciela JR, Gerardo R. Haloperidol causes cytoskeletal collapse in N1E-115 cells through tau hyperphosphorylation induced by oxidative stress: Implications for neurodevelopment. Europe. Jour. Of. Pharmaco. 2010; 644: 24-31.
- Eyles DW, Mcgrath JJ, Pond SM. Formation of pyridinium species of haloperidol in human liver and brain. Psychopharmacol. 1996; 125: 214-219.
- Lau BHS, Yamasaki T, Gridley DS. Garlic compounds modulate macrophage and T-lymphocyte functions. Mol. Biother. 1991; 3: 103-107.
- Kim JM, Lee JC, Chang N, Chun HS, Kim WK. S-Allyl-L-cysteine attenuates cerebral ischemic injury by scavenging peroxynitrite and inhibiting the activity of extracellular signal-regulated kinase. Free. Radic. Res. 2006; 40: 827-835.
- Kosuge Y, Koen Y, Ishige K, Minami K, Urasawa H, Saito H, et al. S-allyl-L-cysteine selectively protects cultured rat hippocampal neurons from amyloid beta-protein- and tunicamycin induced neuronal death. Neurosci. 2003; 122: 885-895.
- Kosuge Y, Sakikubo T, Ishige K, Ito Y. Comparative study of endoplasmic reticulum stress induced neuronal death in rat cultured hippocampal and cerebellar granule neurons. Neurochem. Int. 2006; 49: 285-293.
- Kulkarni SK, Naidu PS. Animal models of tardive dyskinesia-a review. Indian. J. Physiol. Pharmacol. 2001; 45: 148-160.
- Texeira B, Muller LGC, Reckziegel PB, Boufler NC, Pase CSC. Beneficial effects of an innovative exercise model on motor and oxidative disorders induced by haloperidol in rats. Neuropharmacology. 2011; 60: 432-438.
- Garcia E, Limon D, De La Cruz PV, Giordano M, Munoz DM, Maldonado PD, et al. Lipid peroxidation, mitochondrial dysfunction and neurochemical and behavioral deficits in different neurotoxic models: Protective role of S-allyl cysteine. Free. Radic. Res. 2008; 42: 892-902.
- Fleming L, Mann JB, Bean J, Briggles T. Parkinson's disease and brain levels of organo-chlorine pesticides. Ann. Neurol. Sanchez-Ramos. JR. 2003; 36:100-103.
- Katrin M, Kirschbaum A, Henken S, Hiemke C, Schmitt U. Pharmacodynamic consequences of P-glycoprotein-dependent pharmacokinetics of risperidone and haloperidol in mice. Behav. Brain. Res. 2008; 188: 298-303.
- Hicks S, D'Amato CJ. Motor-sensory cortex-corticospinal system and developing locomotion and placing in rats. Am. J. Anat. 1975; 143: 1-42.
- Dunham NW, Miya T S. A note on a simple apparatus for detecting neurological deficit in mice and rats, J. Am. Pharm. Assoc. 1957; 46: 208.
- Schallert T, Norton D, Jones TA. A clinically relevant unilateral rat model of Parkinsonian akinesia. J. Neural. Transpl. Plast. 1992; 3: 332-333.
- Olsson M, Nikkiah G, Bentlage C, Bjorklund A. Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. J. Neurosci. 1995; 15: 3863-3875.
- Uteley HC, Bernheim F, Hochslein P. Effect of sulfhydryl reagent on peroxidation in microsome. Arch. Biochem. Biophys. 1967; 118: 29-32.
- Jollow DJ, Mitchell JR, Zampagloine N, Gillete JR. Bromobenzene-induced liver necrosis: protective role of glutathione and evidence for 3, 4 bromobenzeneoxide as the hepatotoxic intermediate. Pharmacology. 1974; 11: 151-169.
- Claiborne A. Catalase activity. In Green Wald RA ed. CRC Handbook of methods for oxygen radical research. Boca Raton, FL: CRC Press; 1985.
- Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Ann. of. Biochem. 1971; 44: 276-87.
- Mohandas J, Marshall JJ, Duggin GG. Differential distribution of glutathione and glutathione related enzymes in rabbit kidneys: Possible implication in analgesic neuropathy. Cancer. Res. 1984; 44: 5086-5091.
- Creese G, Burt D, Synder SIL. Dopamine receptor binding and pharmacological potencies and antischizophrenic drugs. Science. 1976; 92: 481-483.
- Elkashaf AM, Wyatt RJ. Tardive dyskinesia: possible involvement of free radical and treatment with vitamin E. Schizophr. Bull. 1999; 25: 731-740.
- Andreassen OA, Jorgensen HA. Neurotoxicity associated with neuroleptic induced oral dyskinesias in rats implications for tardive dyskinesia? Prog. Neurobiol. 2000; 61:525-541.

26. Rozas G, Martin LE, Guerra MJ and Garcia JL. The overall rod performance test in the MPTP treated mouse model of Parkinsonism. *J. Neurosci. Meth.* 1998; 83:165-175.
27. Rajasankar S, Manivasagam T, Surendran S. Ashwagandha leaf extract: A potential agent in treating oxidative damage and physiological abnormalities seen in a mouse model of Parkinson's disease. *Neurons. Lett.* 2009; 454: 11-15.
28. Fleming SM, Salcedo J, Fernagut PO. Early and progressive sensory motor anomalies in mice overexpressing wild-type human α -synuclein. *J. Neurosci.* 2004; 24: 9434-9440.
29. Mohanasundari M, Srinivasan MS, Sethupathy S, Sabesan M. Enhanced neuroprotective effect by combination of bromocriptine and Hypericum perforatum extract against MPTP-induced neurotoxicity in mice. *J. Neurol. Sci.* 2006; 249:140-144.
30. Bishnoi M, Chopra, K, Kulkarni SK. Involvement of adenosinergic receptor system in an animal model of tardive dyskinesia and associated behavioural, biochemical and neurochemical changes. *Eur. J. Pharmacol.* 2006; 552: 55-66.
31. Zhang Z, Cao X, Xiong N, Wang H, Huang J, Sun S, et al. Morin exerts neuroprotective actions in Parkinson disease models in vitro and in vivo. *Acta. Pharmacologica. Sinica.* 2010; 31: 900-906.
32. Cadet JL, Lohr JB, Jeste DV. Free radicals and tardive dyskinesia. *Trends. Neurosci.* 1986; 9: 107-108.
33. Fleckenstein AE, Metzger RR, Beyeler ML, Gibb JW, Hanson GR. Oxygen radicals diminish dopamine transporter function in rat striatum. *Eur. J. Pharmacol.* 1997; 334: 111-114.
34. Vairetti M, Feletti F, Battaglia A, Pamparana F, Canonico PL, Richelmi P et al. Haloperidol induced changes in glutathione and energy metabolism effect of nicergolin. *Eur. J. Pharmacol.* 1999; 367: 67-72.
35. Sagara Y. Induction of reactive oxygen species in neurons by haloperidol. *J. Neurochem.* 1998; 71: 1002-1012.
36. Weiner WJ, Nausieda PA, Klawans HL. Regional brain anganese levels in animal model of tardive dyskinesia. In *Tardive Dyskinesia Research and Treatment*. New York: SP Medical and Sciences Books; 1980.
37. Ravindranath V, Reed DJO. Glutathione depletion and formation of glutathione protein mixed disulfide following exposure of brain mitochondria to oxidative stress. *Biochem. Biophys. Res. Commun.* 1990; 169: 150-158.
38. Nielsen EB, Lyon M. Evidence for cell loss in corpus striatum after long-term treatment with a neuroleptic drug (hupenthixol) in rats. *Psychopharm.* 1978; 59: 85-89.