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EVALUATION OF ANTI-PARKINSONIAN ACTIVITY OF SEED EXTRACT OF *NELUMBO NUCIFERA*

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ABSTRACT

Methanolic seed extract of *Nelumbo nucifera* was partitioned with chloroform and was investigated for its antioxidant and anticataleptic effects in the haloperidol-induced catalepsy rat model by measuring various behavioral and biochemical parameters. Catalepsy was induced by administration of haloperidol (1 mg/kg, *ip*) in male albino rats. A significant reduction in the cataleptic scores were observed in all the drug-treated groups as compared to the haloperidol-treated group; with maximum reduction observed in the *Nelumbo nucifera* (200 and 400 mg/kg body weight) administered group. The biochemical parameters like thiobarbituric acid reactive substances (TBARS); catalase; and superoxide dismutase (SOD), in the brain were assessed. Haloperidol administration resulted in the increased generation of TBARS and decrease in catalase & SOD levels. The study concluded that *Nelumbo nucifera* treatment restored the levels of TBARS, Catalase and SOD levels in haloperidol induced catalepsy in rats.

KEYWORDS: ***Nelumbo nucifera*, antioxidant, toxicological, haloperidol and catalepsy**

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INTRODUCTION

The brain is deficient in oxidative defense mechanisms and hence is at a greater risk of damage mediated by reactive oxygen species (ROS), resulting in molecular and cellular dysfunction.[1] ROS can damage virtually any biological molecule in its vicinity including; DNA, essential proteins, and membrane lipids.[2] Parkinson's disease is a neurodegenerative disease characterized by the selective loss of dopamine neurons of the *substantia nigra pars compacta*. The events which trigger and/or mediate the loss of nigral dopamine neurons, remains unclear. Neuroleptic-induced catalepsy has long been used as an animal model for screening drugs for parkinsonism.[3] Catalepsy is defined as the failure to correct an externally imposed posture. A condition characterized by inactivity, decreased responsiveness to stimuli, and a tendency to maintain an immobile posture. The limbs tend to remain in whatever position they are placed (waxy flexibility). Catalepsy may be associated with psychotic disorders (eg, schizophrenia, catatonia), nervous system drug toxicity, and other conditions. The catalepsy test is widely used to evaluate motor effects of drugs that act on the extrapyramidal system.[4] Evidence suggests that immense oxidative stress, free radical formation,[5] genetic susceptibility,[6] and programmed cell death[7] are the main causes for neurodegeneration associated with Parkinson's and other related diseases. The neuropathology of the disease is based on depigmentation and cell loss in the dopaminergic nigrostraital tract of the brain, with the corresponding decrease in the striatal dopamine (DA) concentration.[8] Besides, dopamine receptor blockade and catecholamine depletion, other neurochemical hypotheses have been proposed for the development of catalepsy such as striatonigral GABAergic (gamma-aminobutyric acid), cholinergic, glutamate, and serotonoergic.[9–11] Haloperidol is an antipsychotic drug which is used in the treatment of schizophrenia and other affective disorders. It blocks dopaminergic action in the nigrostraital pathway leading to a high frequency of extrapyramidal motor side effects.[12] In animal models, haloperidol induces a behavioral state known as catalepsy in which the animals are unable to correct externally imposed postures.[13] The use of haloperidol has been associated with an increased level of oxidative stress in the brain.[14] This evidence suggests a possible role for antioxidants in the treatment of haloperidol-induced catalepsy. The brain is made up of 70% lipid and any kind of stress is usually manifested by lipid peroxidative damage.[15]The extent of this damage can be used to evaluate the degree of cellular harm. Stress-induced lipid peroxidative damage in the brain can be quantified by either determining the amount of peroxidative products or the rates of enzyme-catalyzed reactions neutralizing free radical intermediates such as superoxide dismutase (SOD). SOD is a primary, natural, and free radical scavenging and antioxidant enzyme in the body. The estimation of the activity of such antioxidant enzymes such as SOD, catalase, or glutathione peroxidase, can be used to assess the therapeutic effects of different antioxidant agents.[16] *Nelumbo nucifera* Gaertn. (Nymphaeaceae), also known as sacred lotus, is a well-known medicinal plant. The extracts of rhizomes, seeds, flowers and leaves have been reported to have varied therapeutic potential. Several bioactive compounds have been derived from these plant parts belonging to different chemical groups, including alkaloids, flavonoids, glycosides, triterpenoid, vitamins etc., which all have their own therapeutic impact. Thus, the pharmacological effects and various active ingredients of different parts of *N. nucifera* are well understood[17]. Texts like Charaka Samhitha give a detailed description on the use of herbal medicine for a variety of ailments. Lotus seeds are commonly used in folk medicine in the treatment of inflammation, cancer, emesis and given to children as diuretic and refrigerant in skin diseases. The seeds have proven antioxidant and antifertility activity[17, 18, 19]. The plant has been reported to possess anticonvulsant[20] and CNS activity[21,22]. With this back ground, the present study is designed to explore the protective effect of *Nelumbo nucifera* seeds in haloperidol induced catalepsy.

MATERIALS AND METHODS

PLANT MATERIAL

The seeds of *Nelumbo nucifera* were obtained from the Peter & Margali distributors from Ernakulam, Kerala. The seeds were authenticated by Prof. B. Badraiah, Department of Botany, Osmania University, Hyderabad.

EXTRACTION

The dried seeds of ***Nelumbo nucifera,*** from both red and white types of flowers were collected, pulverized to a coarse powder, macerated using methanol. The methanolic extract was then partitioned between equal amount water (H_2O) and chloroform ($CHCl_3$). Using separating funnel, the chloroform and aqueous layer were separated. The aqueous layer was drained and the chloroform layer was filtered. The filtered extract was evaporated by heating up to the dryness and dried powder extract was collected and stored at room temperature. The extract was tested for various active chemical constituents.[22]

QUANTITATIVE PHYTOCHEMICAL SCREENING

TOTAL FLAVONOID CONTENT[23]

The plant extracts (50 mg each) were dissolved separately in 50 ml of methanol. These solutions were serially diluted with methanol to obtain lower dilutions. Phloroglucinol (50 mg) was dissolved in 50 ml of distilled water. It was serially diluted with water to obtain lower dilutions.0.2 ml of the extract was taken in a test tube and the final volume was made up to 2 ml with distilled water and to this 4 ml of vanillin reagent was added rapidly. Exactly after 15 min. absorbance was recorded at 500 nm against blank. The unknown was read from a standard curve prepared using different concentration of pholoroglucinol[23].Flavonoid content in NNSE was determined and their values were summarized in table 1.Result revealed that 8.01 mg/ml of extract is equivalent to 1mg/ml of Phloroglucinol for Total flavonoid content.

Table 1
Flavonoid content in Nelumbo nucifera seed extract (NNSE)

STANDARD (PHLOROGLUCINOL)		EXTRACT	
Concentration	Absorbance	Concentration	Absorbance
25	0.040	25	0.019
50	0.388	50	0.155
100	0.925	75	0.243
200	2.388	100	0.369
		200	0.498
		300	0.623
		500	0.914

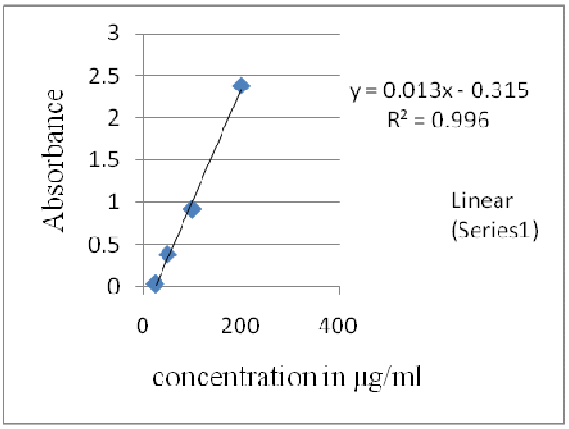


Figure 1
Standard calibration curve for Flavonoid estimation

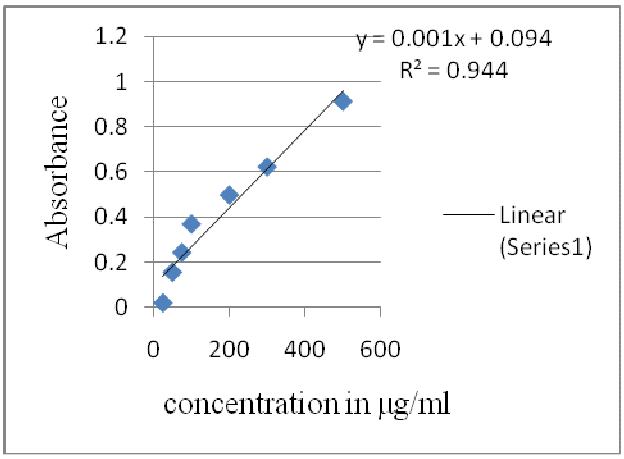


Figure 2
Test calibration curve for Flavonoid estimation

INVITRO ANTIOXIDANT ACTIVITY

i. SCAVENGING OF NITRIC OXIDE RADICAL[24]

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, pH 7.4, 1 ml) and extract in DMSO at various concentrations or standard was incubated at 25^{o} C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite ion was removed, 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 min for completion of diazotisation. Then, 1 ml of Naphthylethylene diaminedihydrochloride(NEDD) was added, mixed and allowed to stand for 30 min in diffused light. A pink coloured chromophore was formed. The absorbance of these solutions were measured at 540 nm[24]. Nitric oxide scavenging activity of *NNSE* was determined and their values were summarized in table 2.

ii. SCAVENGING OF HYDROGEN PEROXIDE[25]

Hydrogen peroxide is generated *in vivo* by several oxidase enzymes. There is increasing evidence that hydrogen peroxide, either directly or indirectly via its reduction product hydroxyl radical (OH^-) causes severe damage to biological system. In this method, when a scavenger is incubated with hydrogen peroxide, the decay or loss of hydrogen peroxide can be measured spectrophotometrically at 230 nm. A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1 ml of the extracts or standards in methanol were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min and the results are tabulated in table 2.

Table 2
Invitro Antioxidant Studies

Samples	IC_{50} values µg/ml by methods	
	NITRIC OXIDE	H_2O_2 Scavenging activity
TEST	349±1	156.67±5.77
	RUTIN	RUTIN
STANDARD	65.44±1.56	24.67±0.58

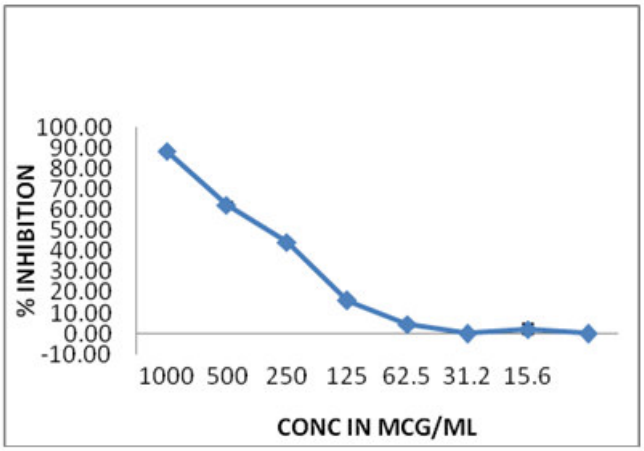


Figure 3
Nitric oxide Scavenging activity of NNSE

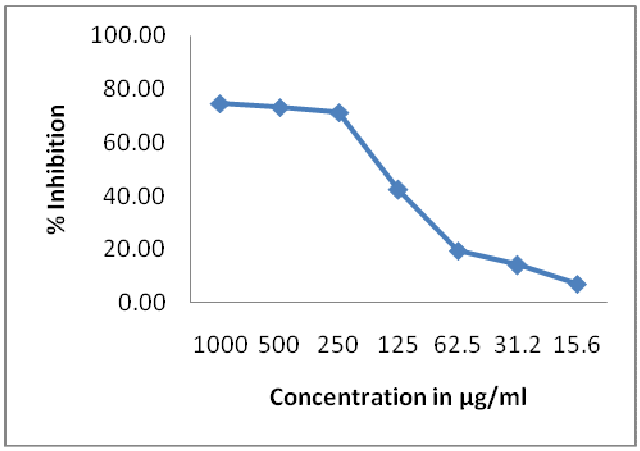


Figure 4
H_2O_2 Scavenging activity of NNSE

Experimental animals

Inbred adult Wistar rats of either sex, weighing 150–200 gm were obtained from Sainath agencies. The animals were maintained in a well-ventilated room with a 12-hour light/dark cycle in standard polypropylene cages under controlled temperature(26 ± 1°C) and humidity (30%–40%). They were fed with standard pellet diet. Water was supplied to the animals *ad libitum*.

Acute toxicity studies

Rats selected by random sampling technique were used for the study. Acute oral toxicity were performed as per Organization for Economic Co-operation and Development (OECD)-423 guidelines[26]. In toxicity study three groups of male wistar strain rats were administered with *Nelumbo nucifera* seed extract in graded doses of 50 mg/kg, 300 mg/kg and 2000 mg/kg *p.o.,* respectively. The drug was administered orally to rats, which were fasted overnight with water *ad libitum* before the administration of the drug. For 14 days, animals were kept under observation for change in behavior or deaths which are summarized in table 3.It was observed that the test extract was not lethal to the rats even at the 2000 mg/kg doses. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study.

Table 3
Acute toxicity studies

S. No	Treatment	Dose	Weight of the animal (Day 1)	Weight of the animal (Day 14)	Signs of toxicity	Onset of toxicity	Reversible or Irreversible	Duration
1.	*N. nucifera*	50mg/kg	150 gm	158 gm	NIL	NIL	NIL	14 Days
2.	*N. nucifera*	50mg/kg	150 gm	152 gm	NIL	NIL	NIL	14 Days
3.	*N. nucifera*	50mg/kg	150 gm	154 gm	NIL	NIL	NIL	14 Days
4.	*N. nucifera*	300mg/kg	150 gm	153 gm	NIL	NIL	NIL	14 Days
5.	*N. nucifera*	300mg/kg	150 gm	151 gm	NIL	NIL	NIL	14 Days
6.	*N. nucifera*	300mg/kg	150 gm	155 gm	NIL	NIL	NIL	14 Days
7.	*N. nucifera*	2 g/kg	140 gm	143 gm	NIL	NIL	NIL	14 Days
8.	*N. nucifera*	2 g/kg	140 gm	146 gm	NIL	NIL	NIL	14 Days
9.	*N. nucifera*	2 g/kg	140 gm	141 gm	NIL	NIL	NIL	14 Days

EXPERIMENTAL DESIGN

Adult male Wistar rats (180-250g)were divided into five groups, each containing six animals.

Group I: Treated with the vehicle 1% Tween 80solution and served as control.

Group II: Treated withhaloperidol (1mg/kg B.W) alone and served as negative control/cataleptic control.

Group III: Treated with combination of L-dopa and Carbidopa (100mg + 25mg/kg by i.p) and served as positive control.

Group IV and **V:** Treated with haloperidol and *Nelumbo nucifera* seed extract at low and high doses (200 & 400 mg/kg) respectively.

After 30min of drug administration, haloperidol (1 mg/kg) was administered i.p for 12 days. Behavioral studies were performed at room temperature in a calm room. Animals were sacrificed by cervical dislocation after 12 days and the whole brain was dissected immediately and washed with ice cold saline to remove the blood traces. 10% of brain homogenate was prepared in 0.025M Tris-Hcl buffer at pH 7.5, which is used to measure the activities of TBARS. 10% brain homogenate was prepared in 0.2M phosphate buffer at pH 8.0 to assay the enzyme activity.

INVESTIGATION OF ANTIPARKINSONIAN ACTIVITY

I. MEASUREMENT OF CATALEPSY BY BLOCK METHOD

This scoring method is followed in 3 steps.

Step 1: The mice was taken out of the home cage and placed on a table. If the rat failed to move when touched or pushed gently on the back a score of 0.5 was assigned.

Step II: The front paws of the rats were placed alternatively on a 3cm high block. If the mice failed to correct the posture within 15 seconds, a score of 0.5 is assigned for each paw and was added to the score of step 1.

Step III: The front paws of the mice were placed alternatively on a 9cm high block, if the mice failed to correct the posture within 15 seconds a score of 1 for each paw was added to the scores of step I and II. Thus the highest score for any animal was 3.5 (cut off score) and that reflects total catalepsy[27].

II. BEHAVIORAL ASSESSMENT BY METAL BAR TEST

Behavioral assessment in haloperidol- induced cataleptic mice was studied by the method as prescribed by S.K. Kulkarni. Cataleptic behavior was measured with a high bar test method. Catalepsy score was measured for 4 hours at one- hour intervals after haloperidol

administration by gently placing the fore paws of the mice over a metal bar(diameter 2-5mm suspended 6cm above the table top). The intensity of catalepsy is assessed by counting the time in seconds until the mice bring both the forepaws down to the table top, with a maximum cut off time of 3 minutes. Finally, scores at different time points (0, 60, 120, 180 and 240 minutes after haloperidol injection) were added and expressed as a cumulative catalepsy score for comparison purposes[28].

BIOCHEMICAL INVESTIGATION

LIPID PEROXIDATION

Malondialdehyde and other Thiobarbituric acid reactive substances (TBARS) are quantified by their reactivity with Thiobarbituric acid (TBA) in acidic conditions. The reaction resulted in the generation of pink colour, which was observed observed in a colorimeter at 535nm. 500mg of rat brain was homogenated using 5ml of normal saline. The 10% homogenate was centrifuged at 4000rpm for 10minutes. The supernatant was used for estimation of lipid peroxidation. 1ml of supernatant was added to 2ml of reaction mixture (mixture of equal parts of the reagent 1, 2 and 3). The reaction solutions were kept in water bath for 15minutes at 80^{o}C, cool it and centrifuged at 1500rpm for 10minutes. The resultant pink colour was measured at 535nm against a reagent blank. 1,1,3,3 tetra methoxy propane was used as external standard. The amount of TBARS was expressed as nmoles/mL for plasma or nmoles/mg for haemolysate[29].

ESTIMATION OF ANTIOXIDANT ENZYMES

The enzymatic antioxidants analyzed were superoxide dismutase and catalase.

- ***ASSAY OF SUPEROXIDE DISMUTASE (SOD)***

Superoxide dismutase uses the photochemical reduction of riboflavin as oxygen generating system and catalyses the inhibition of Nitro blue tetrazolium (NBT) reduction, the extent of which can be assayed spectrophotometrically at 600nm.0.5g of rat brain was homogenized in 5ml of potassium phosphate buffer. The homogenates were centrifuged at 2000 rpm for 10 minutes and the supernatants were used for the assay. The incubation medium contained, in a final volume of 3.0 ml, 50 mM potassium phosphate buffer (pH 7.8), 45 µM methionine, 5.3 mM riboflavin, 84 µM NBT and 20 µM potassium cyanide. The amount of homogenate added to this medium was kept below one unit of enzyme to ensure sufficient accuracy. The tubes were placed in an aluminium foil-lined box maintained at 25°C and equipped with 15W fluorescent lamps. After exposure to light for 10 minutes, the reduced NBT was measured spectrophoto-metrically at 600nm. The maximum reduction was observed in the absence of the enzyme. One unit of enzyme activity was defined as the amount of enzyme giving a 50% inhibition of the reduction of NBT. The values were calculated as units/mg protein[30].

- ***ESTIMATION OF CATALASE (CAT)***

The UV light absorption of hydrogen peroxide can be easily measured between 230 – 250 nm. On decomposition of hydrogen peroxide by catalase, the absorption decreases with time. The enzyme activity can be estimated by this decrease in absorption.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 1.0ml 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[31].

- ***ESTIMATION OF DOPAMINE***

Method Parameters

Analyte: Dopamine

Internal standard: Adrenaline

Sample homogenization Procedure

The straitum was homogenized with the Heidolph Sensitive homogenizer with 360 rpm in clean glass homogenizer test tube with the addition of Ringer's solution under cold conditions (approximately, -40^{o}C).

Sample Extraction

Sample (20µL) was acidified with 50 *µ*L of formic acid (98-100%, J.T Baker, USA), the samples were extracted from the supernatant by solid-phase extraction using mixed-mode strong cation-exchange and reversed-phase cartridges (Oasis MCX 150 mg, 6 cc, Waters, MA). The catridge was maintained with proper conditioning with 3ml of methanol and 3 ml of 0.1% aqueous formic acid followed by addition of 5ml of supernatant. Then the catridge was washed with 4ml of 2% aqueous formic acid and 4ml of methanol and the compounds were eluted with 10 ml methanol containing 2% ammonia. 2ml of methanol and 750µl of formic acid were added to neutralize ammonia. Methanol was evaporated using a rotary evaporator followed by fractionation of dopamine.

STATISTICAL ANALYSIS

Data was expressed as the mean ± SEM. The results of the study were subjected to analysis of variance (ANOVA) using graph pad prism followed by Dunnett's t-test.

RESULTS

The dried seeds of *N. nucifera* were extracted with methanol by maceration method and were partitioned with equal quantities of water and chloroform. The % yield of the seed extract was found to be 3.36%.The active constituents viz; Alkaloid, Glycoside, Flavonoids, Tannins, Phenolics, Carbohydrates, Proteins and Amino acids were estimated from the extract, and the results of the active constituents present in seed extract of *Nelumbo nucifera* were depicted in table 4.

Table 4

Qualitative phytochemical investigation of Nelumbo nucifera seed extract (NNSE)

S. No.	TEST	RESULT
1.	Alkaloid	+
2.	Glycoside	+
3.	Flavonoids	+
4.	Tannins	+
5.	Phenolic compounds	+
6.	Proteins &aminoacids	+
7.	Carbohydrates	+

"+" represents Present.

ASH VALUES

Table 5

Ash values of dried powder of N. nucifera seeds.

S. No.	Method	Yield
1.	Total Ash value	3.5%
2.	Water insoluble ash value	2.6%
3.	Acid insoluble ash value	0.4%

INVESTIGATION OF ANTIPARKINSONIAN ACTIVITY

Antiparkinson activity of Seed extract of *Nelumbo nucifera* was evaluated using block and metal bar model in rats.

I. BLOCK METHOD

Haloperidol induced catalepsy in animals which was significantly minimized by *Nelumbo nucifera* dose dependently as shown in Table 6.

Table 6
Effect of administration of N. nucifera seed extract on behavioral assessment by Block method.

Group	30min	60min	90min	120min
I	0.00	0.00	0.00	0.00
II	2.5±0.13	3.0±0.18	3.5±0.15	3.5±0.06
III	0.83±0.10**	1.17±0.10**	1.33±0.10**	1.08±0.08**
IV	2.0±0.13*	2.5±0.12	2.5±0.12**	2.08±0.15**
V	1.33±0.10**	1.83±0.16**	1.83±0.10**	1.75±0.21**

The values are expressed as Mean ± SEM, where n=6. All the data were analysed by using One way ANOVA followed by dunnet's test. **P<0.01,*P<0.05 as compared to group II toxicant control.

II. METAL BAR TEST

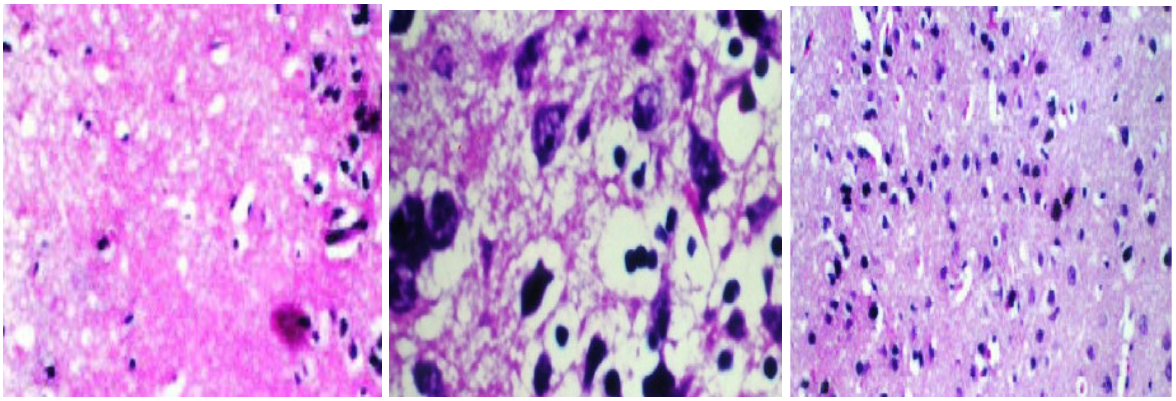
Haloperidol produced extrapyramidal disorder in rats due to which they produce immobility in metal bar test. Immobility induced by haloperidol was significantly minimized towards normal level by seed extract of *Nelumbo nucifera* dose dependently as shown in Table 7.

Table 7
Effect of administration of N. nucifera seed extract on behavioral assessment by Metal bar test.

Group	60min	120min	180min	240min
I	2.60±1.25	4.29±2.15	3.80±2.25	2.88±2.0
II	62.5±5.5***	90.81±5.93***	135.3±4.1***	175.1±3.2***
III	30.8±4.49***$^{\#\#\#}$	27.65±3.84**$^{\#\#\#}$	19.8±7.1$^{\#\#\#}$	8.21±1.29$^{\#\#\#}$
IV	52.27±1.78***	54.65±2.63***$^{\#\#\#}$	48.70±3.25***$^{\#\#\#}$	44.62±9.6***$^{\#\#\#}$
V	38.3±5.81***$^{\#\#}$	34.16±6.12***$^{\#\#\#}$	27.43±4.9$^{\#\#\#}$	16.19±7.40$^{\#\#\#}$

The values are expressed as Mean ± SEM, where n=6. All the data were analysed by using One way ANOVA followed by dunnet's test. ***P<0.001,**P<0.01 as compared to group I control; $^{\#\#\#}$P<0.001, $^{\#\#}$P<0.01 as compared to group II toxicant control.

HISTOPATHOLOGICAL STUDIES OF RAT BRAIN



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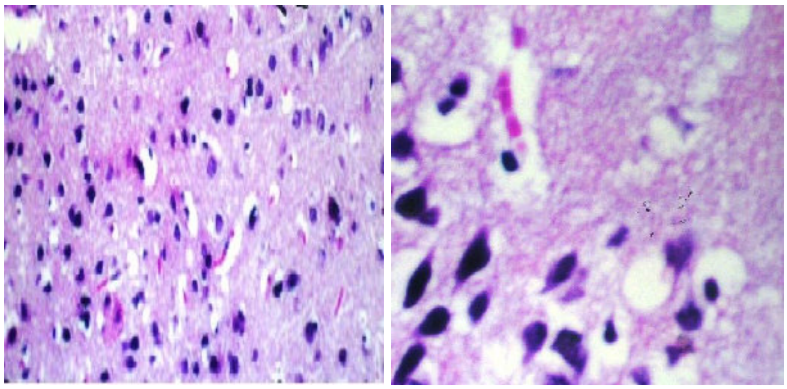

Figure 5
Histopathological studies of midbrain

A: Rats treated with Normal saline for 12 days showing a typical histopathological architecture of rat brain; **B**: Rats treated with Haloperidol alone for 12 days showing Pericellular oedema, revealed alterations in brain histology in the form of typical necrosis, nucleus shrinkage or disappearance and cellular edema; **C & D**: Rats treated with 200 & 400 mg/kg body weight of *NNSE* respectively for 12 days showing mild diffuse gliosis in cerebral cortex; **E**: Rats treated with Haloperidol followed by combination of L-dopa and Carbidopa (100mg + 25mg/kg by i.p) for 12 days showing mild pericellular oedema.

LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES

Table 8
Effect of administration of N. nucifera seed extract on lipid peroxidation and antioxidant enzymes.

Group	TBARS (nmoles MDA/mg protein)	SOD (Units/mg protein)	CAT (nmolH2O2/mg protein)
I	29.27 ± 0.08	36.5 ± 0.17	39.28 ± 1.47
II	56.88 ± 0.13***	23.2 ± 0.14***	20.7 ± 1.59***
III	35.01 ± 0.15$^{***###}$	32.1 ± 0.14$^{***###}$	32.42 ± 1.32$^{*###}$
IV	44.14 ± 0.26$^{***###}$	28.98 ± 0.11$^{***###}$	27.42 ± 1.44$^{***#}$
V	39.94 ± 0.20$^{***###}$	30.92 ± 0.11$^{***###}$	28.8 ± 1.38$^{***##}$

The values are expressed as Mean ± SEM, where n=6. All the data were analysed by using One way ANOVA followed by dunnet's test. ***P<0.001,*P<0.05 as compared to group I control; ###P<0.001, ##P<0.01, #P<0.05 as compared to group II toxicant control.

- ***TBARS Activity***

The TBARS levels were found to be significantly increased in the brain tissue of the haloperidol (1 mg/kg p.o.) treated animals, as shown in table 9. Extract minimized the level of TBARS activity towards normal level dose dependently.

- ***SOD Activity***

In the animals pretreated with *N. nucifera* seed extract, the levels of SOD were significantly increased as compared to those in the haloperidol 1 mg/kg p.o. treated rats. Table 9 showed the effect of the significant and dose dependent recovery on the haloperidol induced reduction of the SOD levels in animals.

- ***CATALASE Activity***

N. nucifera seed extract significantly increased the level of catalase towards normal level and the results were comparable to standard as shown in table 9.

ESTIMATION OF DOPAMINE CONCENTRATION

Dopamine concentration in rat brain was determined in various groups and the results were shown in Table 11. Present investigation revealed that haloperidol reduced the dopamine concentration while NNSE restored the dopamine level towards normal level in a dose dependent manner.

Table 9
Concentration of Dopamine (in nM).

S.No	Sample Name	Concentration (nM)
1	Control (Blank Matrix)	0.00
2	Normal	7.20±0.09
3	Toxicant treated (Haloperidol)	1.95±0.02***
4	Standard	5.30±0.10***###
5	Drug Treated (Low dose)	3.80±0.08***###
6	Drug Treated (High Dose)	4.95±0.06***###

*The values are expressed as Mean ± SEM, where n=3. All the data were analysed by using One way ANOVA followed by dunnet's test. ***P<0.001 as compared to group I control; ###P<0.001 as compared to group II toxicant control.*

Graphical Representation

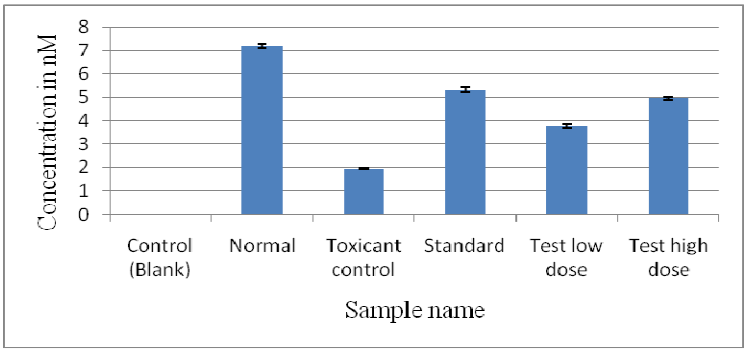

Figure 6
Concentration of Dopamine (nM)

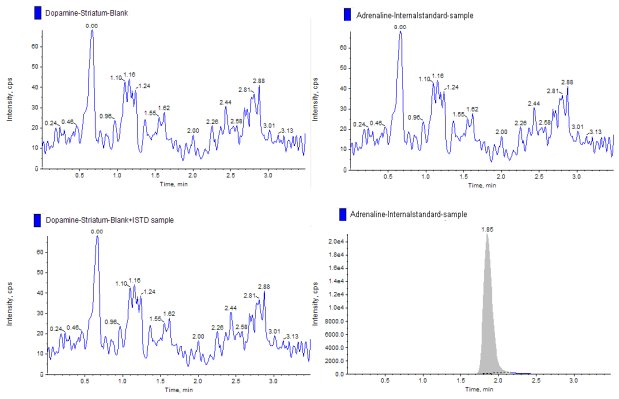

Figure 7
Chromatogram of Dopamine sample (Blank)

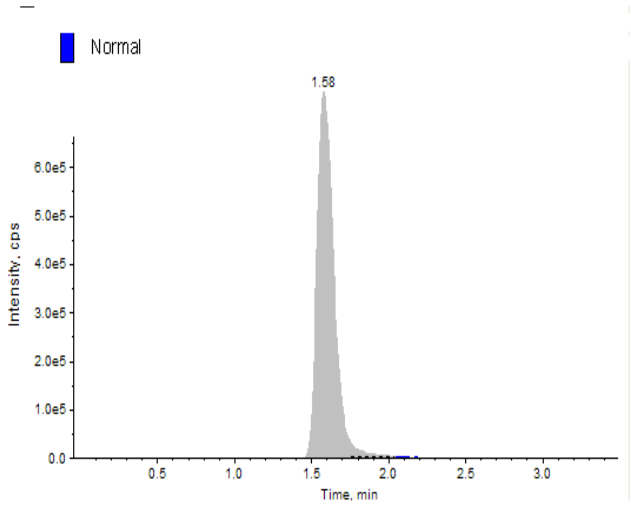

Figure 8
Chromatograms of Dopamine Samples of Normal

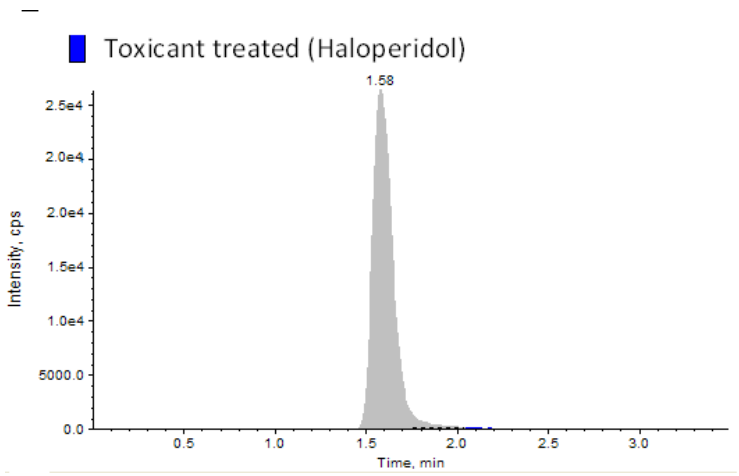

Figure 9
Chromatograms of Dopamine Samples of toxicant

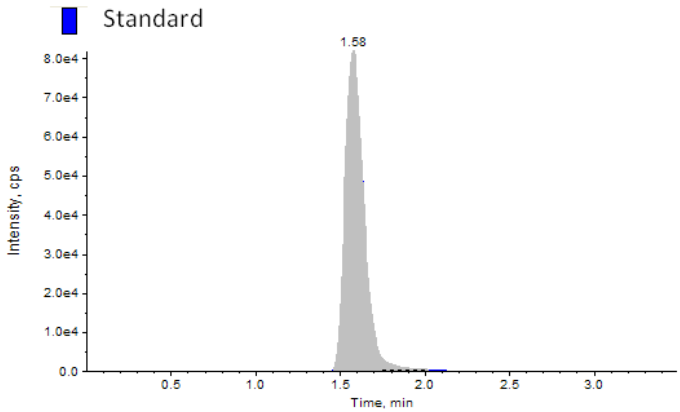

Figure 10
Chromatograms of Dopamine Samples of standard

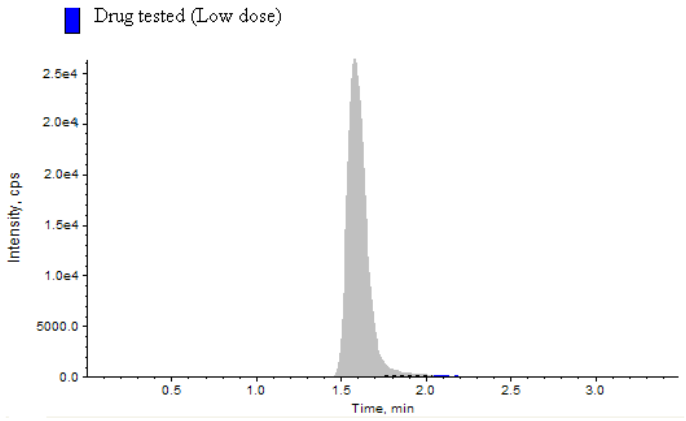

Figure 11
Chromatograms of Dopamine Samples of low dose

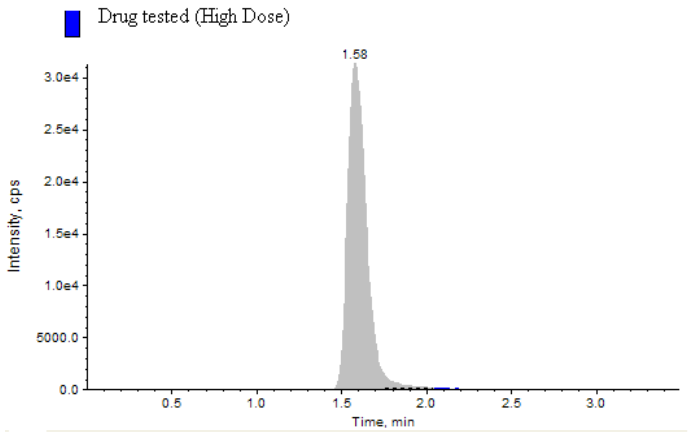

Figure 12
Chromatograms of Dopamine Samples of high dose

DISCUSSION

The central nervous system is especially vulnerable to free radical damage because of the brain's high oxygen consumption, its abundant lipid content, and the relative paucity of antioxidant enzymes as compared with other tissues[32]. Evidence also indicates that Reactive Oxygen Species (ROS) may stimulate extracellular release of excitatory amino acids[33]. Glutamate is the major excitatory amino acid in the brain which acts through various types of ionotropic receptors, the most significant being *N*-methyl *D*-aspartate (NMDA) receptors. There seems to be a bidirectional relationship between the ROS production and the release of excitatory amino acids.[34] Free radicals generated in the brain are also reported to influence gene expression, subsequently effecting apoptosis and neuronal death[35]. In the brain, an array of cellular defence systems exists to counterbalance the ROS. These include enzymatic and non enzymatic antioxidants that lower the concentration of free radical species and repair oxidative cellular damage. The brain is known to synthesize molecules like glutathione and NADPH. Glutathione functions as a major antioxidant in tissue defence against free radicals in the brain. However, the concentration of glutathione is, relatively, in lesser quantities in the brain as compared to the other organs of the body[32]. The natural antioxidant system present in brain can be in the form of enzymes like catalase,

peroxidase, superoxide dismutase or low molecular weight antioxidants (ascorbic and lipoic acids, carotenoids or indirectly acting chelating agents)[35]. Free radical scavengers or antioxidants works as biological bodyguards for essential molecules. They do either neutralize reactive species before they mutilate a molecule or repairs the damage that has been inflicted. Typical neuroleptic agents such as chlorpromazine, haloperidol and reserpine induce a cataleptic state in rodents which is widely used as a model to test the extrapyramidal side effects of antipsychotic agents. Neuroleptic-induced catalepsy has been linked to a blockade of postsynaptic striatal dopamine D1 and D2 receptors[36].Despite this evidence, several other neurotransmitters such as acetylcholine, serotonin, angiotensin, adenosine or opioids have also been implicated in the catalepsy induced by neuroleptic agents[37].In addition to various neurotransmitters, many preclinical and clinical studies have also proposed reactive oxygen species as causes of haloperidol-induced toxicity[38]. Evidence indicates that drugs which potentiate or attenuate neuroleptic catalepsy in rodents might also aggravate or reduce the extrapyramidal signs respectively in human beings[39]. The mechanisms responsible for the degeneration of dopamine neurons are not known, but hypotheses include effects such as oxidative stress and excitotoxicity.

In the present study, *Nelumbo nucifera* seed extract protected rats from catalepsy induced by haloperidol. This study demonstrates the antioxidant effects of *Nelumbo nucifera* seed extract in haloperidol-induced, cataleptic oxidative stress in rats. The induction of free radicals in mammals by haloperidol is well established. Previous studies have shown that dopamine receptors in the striatum are involved in neuroleptic-induced catalepsy. It has been demonstrated that the cataleptic effects of haloperidol are apparently mediated by dopamine receptors localized postsynaptically on striatal neurons[40]. It is also well established that the administration of haloperidol leads to an increase in the oxidative stress in the brain tissue[41]. The increase in SOD observed in the present study supports this concept. Superoxide formation is a major factor in oxygen toxicity and the superoxide dismutase enzyme constitutes an essential defense against it. Under normal conditions, decreased activity of antioxidant enzymes, such as SOD, glutathione peroxidase and catalase, in the brain leads to the accumulation of oxidative free radicals resulting in degenerative effects[42]. An increase in these enzymes under normal conditions would represent increased antioxidant activity and a protective mechanism in neuronal tissue, thus, constituting the first line of defense against oxidative stress in our body. However, in the presence of a free radical-quenching agent, the induction of the antioxidant enzymes is minimized. So, any overall decrease in cataleptic scores and increased SOD activity in the drug treated groups indicates the ability of the drug extract to combat oxidative stress in brain tissue and reduce the severity of haloperidol-induced catalepsy. The altered balance of the antioxidant enzymes caused by the decrease in CAT, SOD activities may be responsible for the inadequacy of the antioxidant defenses in combating ROS mediated damage. The decreased activities of CAT and SOD may be a response to increased production of H_2O_2 and O_2 by the auto oxidation[43]. It has been suggested that these enzymes play an important role in maintaining physiological levels of oxygen and hydrogen peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides. Treatment with *Nelumbo nucifera* seed extract increased the activity of these enzymes by quenching the free radicals. Previously *Nelumbo nucifera* has been reported to be a well known antioxidant and the methanol extract of *N. nucifera* is reported to possess potent antioxidant activity that scavenges free radicals generated after the induction of catalepsy. Lower levels of lipid peroxides in the brains of the drug-treated group and increased activities of enzymatic and non-enzymatic antioxidants in the brain suggest that the extract reduces oxidative stress. The methanolic extract of *Nelumbo nucifera* showed no signs of lethality up to 2000 mg/kg. So initially two doses i.e. 200 and 400 mg/kg were selected for the

evaluation of anticataleptic activity using the Haloperidol induced catalepsy model. At a dose of 400 mg/kg the methanolic extract showed a significant reduction (P<0.01) of the cataleptic score. To conclude, the brain exhibits numerous morphological and functional alterations during oxidative stress, a factor implicated in the pathogenesis of many CNS disorders. Treatment of such neuronal disorders with *Nelumbonucifera* seed extract significantly decreases lipid peroxidation and significantly increases the antioxidants in the brain. The findings of this study suggest the possible antioxidant role of *Nelumbonucifera* seed extract in overcoming behavioral and neurochemical changes during oxidative stress. Since the catalepsy test has predictive value regarding extrapyramidal effects, the possibility of pharmacological interactions between haloperidol and *Nelumbo nucifera* seed extract can be further extrapolated to clinical studies for better therapy in humans.

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REFERENCES

1. Gupta YK, Madhur Gupta, Kohli K, Neuroprotective role of melatonin in oxidative stress vulnerable brain, *Indian J Physiol Pharmacol;*47(4):373–386, 2003.
2. Wolff SP, Garner A, Dean RT, Free radicals lipids and protein degradation, *Trends Biol Sci*;11:27–31, 1986.
3. Anil kumar, Kulkarni SK, Effect of BR-16A(Mentat), a polyherbal formulation on drug induced catalepsy in mice, *Indian J Exper Biol*;44:45–48, 2006.
4. Sanberg PR, Bunsey MD, Giordano M, The catalepsy test: it's up and downs, *Behav Neuro sci*;102:748–759, 1988.
5. Jenner P, Oxidative stress and the pathogenesis of Parkinson's disease, *Neurology*; 47:161–20, 1996.
6. Bandmann O, Marsden DC, Wood NW, Genetic aspects of Parkinson's disease, *Mov Disord*;13:203–211,1998.
7. Ziv I, Melamed E, Nardi N, Role of apoptosis in the pathogenesis of Parkinson's disease: a novel therapeutic opportunity, *Mov Disord*;13: 865–870, 1998.
8. Von Bohlen, Halbach O, Schober A, Krieglstein K, Genes, proteins, and neurotoxins involved in Parkinson's disease, *Prog Neuro biol*;73:151–27, 2004.
9. Bazian AS, Divergent and convergent mechanism of integrative activity of mammalian brain, *Zh. Vyssh. Nerv. DeiatIm. I P Pavlova*;51: 514–528, 2001.
10. Okuyama S, Atypical antipsychotic profile of sigma receptor ligands, *Nippon Yakurigaku Zasshi*;114:13–23, 1999.
11. Neal Belveau BS, Joyce JN, Lucki I, Serotonergic involvement in haloperidol-induced catalepsy, *J Pharmacol Exp. Ther.*;265:207, 1993.
12. Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G, Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine:Relation to extra-pyramidal side effects, *Arch Gen Psychiatry*; 49:538–544, 1992.
13. Sanberg PR, Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors, *Nature;*284:472–473, 1980.
14. Sagara Y, Induction of reactive oxygen species in neurons by haloperidol, *Jour Neuro chem;*71:1002–1012, 1998.
15. Kedar NP, Can we prevent Parkinson's and Alzheimer's disease? *J Postgrad Med*;49:236–245, 2003.

16. Albina Arjuman, Vinod Nair, Gopalkrishna HN, Nandini M, Evaluation of the antioxidant potential of NR-ANX-C (a polyherbal formulation) and its individual constituents in reversing haloperidol induced catalepsy in mice, *Indian J Pharmacol*;39(3):151–154, 2007.
17. Khare CK. Indian Medicinal Plants: an Illustrated Dictionary. Berlin: *Springer*; 10–49, 2007.
18. Mukherjee PK, Mukherjee D, Maji AK, Rai S, Heinrich M, The sacred lotus (*Nelumbo nucifera*) - phytochemical and therapeutic profile, J Pharm Pharmacol; 61(4):407-22, 2009.
19. Mukherjee PK, Saha K, Balasubramanian R, Pal M and Saha BP, Studies on the psycho pharmacological effects of *Nelumbo nucifera* Gaertn. Rhizome extract, *J. Ethno pharmacol*;54: 63-67, 1996.
20. Rai, Antimicrobial Activity of Essential Oil from *Nelumbo nucifera* Gaertn, Pollen, *J. Ethno pharmacol*;104**:**322-327, 2006.
21. Maharshi K Joshi, Harsh B Joshi, kruti T Joshi, Ujjawal T Joshi, Hemangini B. Joshi, Anticonvulsant activity of *Nelumbo nucifera*, *IJPRD*; 3 (1): 84-90, 2011.
22. Sugimoto Y, Furutani S, Itoh A, Tanahashi T, Nakajima H, Oshiro H, Sun S, Yamada J, Effects of extracts and neferine from the embryo of *Nelumbo nucifera* seeds on the central nervous system, Phytomedicine;15(12): 112-24, 2008.
23. Swain T, Hills WE, The phenolic constituents of Purmus domestica I. The quantitative analysis of phenolic constituents, *J. Sci. Food. Agric*; 10: 63-68, 1959.
24. Garrat DC, *The Quantitative Analysis of Drugs*, Chapman and Hall Ltd; Japan, 3rdEd 456-58, 1964.
25. Jayaprakash GK, Lingamallu JR and Kunnumpurath KS, Antioxidant activities of flavidin in different *in-vitro* model systems, *Organic Medicinal Chemistry*; *12:* 5141-5146, 2004.
26. Ecobichnon DJ, *The Basis of Toxicity Testing*, 2nd Ed, New York, NY: CRC Press; 1997.
27. Deepa PK, Usha PTA, Nair AMC and Prasanna kumari KT, Assessment of CNS activity of *Nelumbo nucifera* (Red and White type) using Rota rod**,** *Indian J. Anim. Res*;42(4): 273-275**,** 2008.
28. Chopde CT, Khisti RT, Mandane SN, Haloperidol- induced catalepsy: A model for screening antidepressants effective in treatment of depression with Parkinson's diseases, *Ind J Exp Biol;*35:1297-1301, 1997.
29. Niehans (Jr.) WG, Samuelson B, Formation of Malondialdehyde from phospholipid sarachidonate during microsomal lipid peroxidation, *Eur. J Biochem*; 6: 126, 1968.
30. Misra, H.P, Fridovich I. *Biochemistry*; 15: 681- 687, 1967.
31. Aebi H. Methods of enzymatic analysis, ed., New York, Academic Press. 1974; 2: 674.
32. Skaper SD, Floreani M, Ceccon M, Facci L, Giusti P, Excitotoxicity, oxidative stress, and the neuroprotective potential of melatonin, *Ann NY Acad Sci*;890: 107–118, 1999.
33. Gilman SC, Bonner MJ, Pellmar TC, Effect of oxidative stress on excitatory aminoacid release by cerebral cortical synaptosomes, *Free Rad Med Biol*;15: 671–675, 1993.
34. Coyle JT, Puttfarcken P, Oxidative stress, glutamate, and neuro degenerative disorders, *Science*; 262: 689–695, 1993.
35. Gilgun-Sherki Y, Rosenbaum Z, Melamed E, Offen D, Antioxidant therapy in acute central nervous system injury: the current state, *Pharmacol Rev*;54: 271–284, 2002.
36. Samberg PR. Haloperidol induced catalepsy is mediated by postsynaptic dopamine receptors, *Nature* ;284:472-3, 1980.
37. Ossowska K, Neuronal basis of neuroleptic-induced extrapyramidal side effects, *Polish J Pharmacol*; 54: 299-312, 2002.
38. Polydoro M, Schroder N, Lima MN, Caldana F, Laranja DC, Bromberg E, Haloperidol and clozapine induced oxidative stress in the rat brain, *Pharmacol Biochem Behav*;78: 751-66, 2004.
39. Marti-Masso JF, Poza JJ, Lopez DMA, Drugs inducing or aggravating

Parkinsonism: A review, *Therapy*;51: 568-77, 1996.

40. Sanberg PR, Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors, *Nature*;284: 472–473, 1980.
41. Sagara Y, Induction of reactive oxygen species in neurons by haloperidol, *Jour Neuro chem*;71: 1002–1012, 1998.
42. Aragno M, Brignardello E, Tamagno O, Boccuzzi G, Dehydroeppiandrosterone administration prevents the oxidative damage induced by acute hyperglycemia in rats, *J Endocrinol*;155: 233–240, 1997.
43. Tripathi YB, Ekta T, Anil U, Antilipid-peroxidative property of *Nardostachys Jatamansi*, *Ind J Exp Biol*,34:1150–1151, 1996.