RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2015.125.130

Neuroprotective Activity of *Tephrosia purpurea* against Haloperidol Induced Parkinson's Disease Model

[1]Cuddapah Rajaram, [2]Kandula Ravindra Reddy and [3]Kothapalli Bonnth Chandra Sekhar
[1]Department of Pharmacology, P. Rami Reddy Memorial College of Pharmacy, Utukuru, Kadapa, 516003, Andhra Pradesh, India
[2]Department of Pharmaceutics, CES College of Pharmacy, Kurnool, 518 218, Andhra Pradesh, India
[3]Department of Chemistry, Jawaharlal Nehru Technological University Ananthapur, Ananthapuramu, 515 002, Andhra Pradesh, India

ABSTRACT

Background and Objectives: Parkinson's Disease (PD) is a chronic neurodegenerative disorder characterized by the progressive loss of the dopaminergic neurons in the substantia nigra pars compacta which innervates the dorsal striatum. Haloperidol was used to induce Parkinson's disease in experimental rats and the present study investigates the neuroprotective activity of *Tephrosia purpurea* on haloperidol induced Parkinson's disease model. **Methodology:** The present study was carried out on Sprague-Dawley rats, where Parkinson's disease was induced by haloperidol. In the current investigation, rats were randomly separated into five groups and then test animals received ethanol extract of *Tephrosia purpurea* (EET) at dose of 200 and 400 mg kg^{-1} for 21 days. Catalepsy and muscle rigidity were assessed. In addition, antioxidant levels and histopathological studies were carried out. **Results:** Ethanol extract of *Tephrosia purpurea* (EET) showed significant and dose dependent increase in behavioral activity and improved muscular coordination. Significant reduction of lipid peroxidation (LPO), increased antioxidant enzymes like Catalase (CAT) and non-enzymatic activity of reduced glutathione (GSH) in extract treated groups was observed as compared to control group. **Conclusion:** The EET possesses significant antioxidant activity and renders neuroprotection which was more pronounced at the dose of 400 mg kg^{-1} against haloperidol induced neurotoxicity.

Key words: Catalepsy, neurotoxicity, tremors, anti-parkinson effect, purple tephrosia

Pharmacologia 6 (4): 125-130, 2015

INTRODUCTION

Parkinson's Disease (PD) is a chronic, progressive neurodegenerative disease characterized by the classical signs of bradykinesia, rigidity and resting tremor with postural instability developing at a later stage. It involves primarily a degeneration of certain nerve cells in deep parts of the brain called the basal ganglia and in particular a loss of nerve cells (or) neurons in a part of the brainstem called the Substantia Nigra (SN). These cells make the neurochemical messenger dopamine which is partly responsible for starting a circuit of messages that coordinate normal movement[1]. The available treatments are levodopa, carbidopa, apomorphine, amantadine, orphenadrine, benzhexol, benztropine, selegiline, pergola and many more. This drug effectively reverses the symptoms of Parkinson and improves the level of dopamine. The greatest disadvantage in presently available potent synthetic drugs lies in their adverse effects like constipation, ulcer, respiratory depression and hypertension, toxicity and reappearance of symptoms after discontinuation[2]. Hence, search for new neuropharmacological agents that retain therapeutic efficacy and yet devoid of adverse effects are justifiable.

The drug that blocks the action of dopamine may result in parkinsonism[3]. The neuroleptic drug like haloperidol is one of the major causes for drug induced Parkinson's worldwide. The incidence of drug induced Parkinson's progresses with age[4]. It blocks dopamine D_2 receptors and produces a state of catalepsy in human or animals by reducing dopaminergic transmission in basal ganglion[5].

Corresponding Author: Cuddapah Rajaram, Department of Pharmacology, P. Rami Reddy Memorial College of Pharmacy, Kadapa, 516003, Andhra Pradesh, India Tel: +919642780790

Tephrosia purpurea is a polymorphic, much-branched suberect perennial herb, popularly known as "Sarapunkha" in Sanskrit, "Purple tephrosia" in English and "Vempali" in telugu. Experimental studies have demonstrated its analgesic[6], antihyperglycemic[7], antiulcer[8] and hepatoprotective effects. There were no scientific data on anti-Parkinson effect of *T. purpurea* in experimental parkinsonism. Hence, the present study was designed to evaluate neuroprotective effect of *T. purpurea* in haloperidol induced Parkinson's rats.

MATERIALS AND METHODS

Animals: Experimental animals of either sex weighing 150-200 g were obtained from Raghavendra enterprises, Bangalore. The animals were housed in polypropylene cages at a controlled room temperature of 24°C, under 12 h light and 12 h dark cycle and given standard laboratory feed (Pranav agro Ltd, Bangalore) and water *ad libitum*. After one week of acclimatization, the experiment animals were divided randomly into 5 groups (n = 6). The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee of PRRM College of Pharmacy (1423/PO/a/11/CPCSEA).

Chemicals: Haloperidol, glutathione and (±)-epinephrine were purchased from Sigma Aldrich, Bangalore. Carboxymethyl Cellulose (CMC), Thiobarbituric acids (TBA), Trichloroacetic acid (TCA), Hydrogen peroxide (H_2O_2) were obtained from SD fine chemicals Ltd. Mumbai. Sodium dihydrogen phosphate, potassium dihydrogen phosphate, tris buffer and all other reagents used were of analytical grade.

Plant material: The roots were collected from Kadapa, Andhra Pradesh. It was identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V University, Tirupati. The voucher specimen of the plant was kept in Department of Pharmacology, PRRM College of Pharmacy, Kadapa and Andhra Pradesh. The roots were cleaned then, after cutting them into small pieces, these were dried in shade and then subjected to coarse powdering and passed through a sieve No. 44 to get uniform powder size. The collected powder was extracted with 95% of alcohol by soxhlet apparatus. After the extraction, solvent was distilled, dried by lyophilization and stored in air tight container under refrigeration. The obtained ethanol extract of *Tephrosia purpurea* (EET) was used to assess anti-Parkinson's activity.

Experimental design: Animals were randomized and divided into five experimental groups (n = 6) as follows:

Groups I: Normal received vehicle (1% CMC, 10 mL kg^{-1} body weight, p.o.)
Group II: Control received haloperidol (1 mg kg^{-1}, p.o.)
Group III: Standard received L-Dopa and carbidopa (100+25 mg kg^{-1} p.o.) and haloperidol (1 mg kg^{-1}, p.o.)
Group IV: Low dose received low dose of EET (200 mg kg^{-1}, p.o.) and haloperidol (1 mg kg^{-1}, p.o.)
Group V: High dose received high dose of EET (400 mg kg^{-1}, p.o.) and haloperidol (1 mg kg^{-1}, p.o.)

The doses were given for 21 days. Animals were provided with food and water as usual before experiment. On 21st day, haloperidol (1 mg kg^{-1}, p.o.) was injected 60 min after oral administration for all groups except normal group. All the behavioral studies were performed at room temperature in a calm room without any external interference. After the 21 days, animals were sacrificed and their brains were harvested quickly to procure midbrain and striatum which was used to assay Lipid Peroxidation (LPO), reduced Glutathione (GSH) and Catalase (CAT).

Neurobehavioral studies

Catalepsy: Catatonia was assessed using a method reported earlier[9]. In brief, the animals were placed with their forepaws on a wooden box on height 9 cm and the time spent without deliberate move to step down was determined. An average of three trials was taken with each trail commencement up to maximum 15 sec.

Muscle rigidity: The major clinical symptom of PD is muscle rigidity. This was evaluated in an animal model by rotarod. This test was carried out similar to a reported method[10]. In a few words, the apparatus consist of a 70 cm long rod with diameter 3 cm placed at a height of 50 cm and divided into four sections. Five trials were taken before the main reading to all the groups by adjusting the rate of rotation at 30 rpm.

Biochemical estimation: Brain homogenate was used to measure oxidative degradation in parkinson rats. LPO, CAT and GSH were measured according to the procedure described earlier[11].

Histopathological studies: The brains from control and experimental groups were fixed with 10% formalin and embedded in paraffin wax and cut into longitudinal section of 5 μm thickness. The sections were stained with haematoxylin and eosin dye for histopathological observation.

Statistical analysis: All the data are expressed in Mean±SEM. The significance of difference in means between control and treated animals was determined by One-way Analysis Of Variance (ANOVA) followed by Turkey's multiple comparison test. Significance of difference between sham and control group were evaluated by un-paired student's t-test. $p<0.05$ was considered statistically significant.

RESULTS

Selection of dose: *Tephrosia purpurea* at a dose of 200 and 400 mg kg^{-1} were used for the present study according to the earlier studies of Rambabu *et al.*[12] reporting anti-inflammatory and analgesic activity of *T. purpurea*[12].

Neuroprotective activity

Neurobehavioral studies: The results of this study revealed the potential neuroprotective activity of *T. purpurea* ethanol extract.

Effect of EET on catalepsy: The cataleptic score was significantly reduced after 60 min, with both the standard drug and the plant extracts. The reduction in cataleptic scores with EET was significant throughout the period of observations, till 240 min. But interestingly the high dose (400 mg kg^{-1}, p.o.) shows good significant decrease in immobility and muscular rigidity than low dose (Table 1).

Effect of EET on muscle rigidity: Haloperidol administration significantly decreased motor coordination and body balance when compared to normal control rats. Pretreatment with EET (200 and 400 mg kg^{-1}, p.o.) in Haloperidol induced rats significantly improved the motor coordination and body balance and showed increase in latency to balance on the beam significantly (Table 2).

Biochemical estimation: The brain homogenate showed significantly reduced activities of CAT and GSH and increase in LPO in control group as compared to normal group. The ethanol extract of *T. purpurea* showed a significant protection by reducing the elevated levels of LPO and increasing the CAT and GSH levels as compared to the control group Fig. 1(a-c).

Histopathological studies: The histopathological study confirmed the neuroprotective activity of EET as a significant recovery of neuronal damage and decreased necrosis was evident (Fig. 2).

Table 1: Effect of EET in haloperidol induced catalepsy on 21st day

	Immobility (sec)								
Groups	0 (min)	30 (min)	60 (min)	90 (min)	120 (min)	150 (min)	180 (min)	210 (min)	240 (min)
I	12.75±0.4	12.25±0.7	9.50±1.32	9.25±1.37	11.25±0.85	12.50±0.64	11.75±0.94	12.25±1.25	12.75±0.47
II	13.00±0.4	54.25±17.3	61.50±8.4$^{\#}$	78.25±2.73$^{\#\#\#}$	77.25±1.65$^{\#\#\#}$	96.25±2.56$^{\#\#\#}$	112.00±4.65$^{\#\#\#}$	130.80±4.51$^{\#\#\#}$	170.30±5.1$^{\#\#\#}$
III	12.00±1.2	21.75±1.1	40.50±1.6	68.25±2.13*	67.25±1.65***	48.75±2.39****	36.00±1.87****	39.00±2.91****	25.75±0.62****
IV	10.50±1.19	24.00±1.08	50.75±6.6	76.25±3.17	77.00±2.7	86.50±1.5*	92.50±2.5*	74.25±2**	54.25±3.51**
V	10.25±1.1	37.00±5.81	39.25±3.7	70.25±2.28	69.00±0.7**	82.50±2.21**	91.75±3.19**	59.75±3.96****	35.75±2.56****

All values were expressed as Mean±SEM (n = 6). $^{\#\#\#}p<0.001$, $^{\#\#}p<0.01$ vs. normal, ****$p<0.001$, **$p<0.01$, *$p<0.05$ vs. control

Table 2: Effect of EET on muscle rigidity on 21st day

	Fall of time (sec)								
Groups	0 (min)	30 (min)	60 (min)	90 (min)	120 (min)	150 (min)	180 (min)	210 (min)	240 (min)
I	25.08±2.33	26.38±2.08	28.68±1.03	22.90±1.79	30.55±2.65	33.15±2.64	33.37±2.4	26.30±2.71	21.90±1.64
II	26.38±1.3	13.01±1.45	11.30±1.7$^{\#\#}$	8.00±1.64$^{\#\#\#}$	10.14±2.63$^{\#\#\#}$	12.35±1.26$^{\#\#\#}$	11.06±0.5$^{\#\#\#}$	6.69±1.53$^{\#\#\#}$	7.26±0.64$^{\#\#\#}$
III	22.35±1.79	23.68±3.96	20.05±2.23	14.85±0.89	15.54±2.49	19.69±0.92*	19.64±2.35**	23.15±1.09****	27.39±1.12****
IV	24.73±2.63	14.03±1.69	13.11±3.71	9.16±1.16	11.98±4.68	13.97±1.13	18.13±0.26*	19.78±3.16**	15.74±0.71**
V	26.78±1.47	23.58±5.1	14.76±0.72	15.53±2.84	16.37±1.8*	19.44±0.7*	19.94±0.44**	23.34±1.95****	28.68±1.71****

All values were expressed as Mean±SEM (n = 6) $^{\#\#\#}p<0.001$, $^{\#\#}p<0.01$ vs. normal, ****$p<0.001$, **$p<0.01$, *$p<0.05$ vs. control

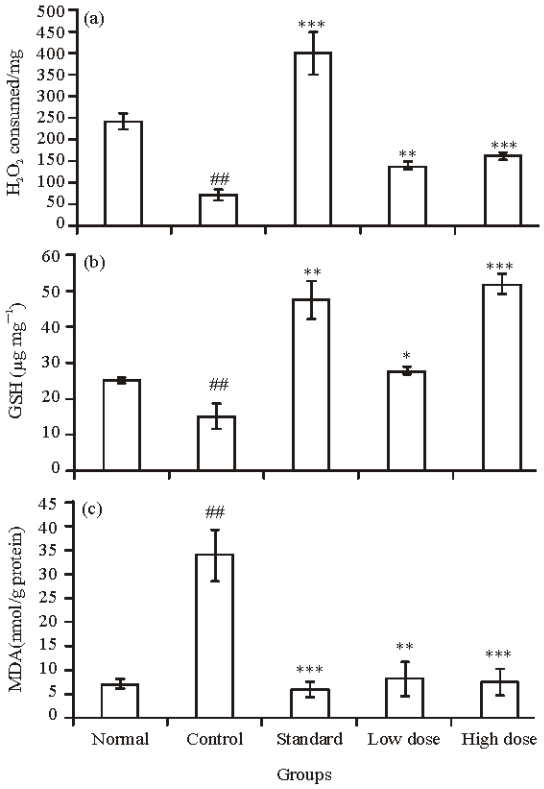

Fig. 1(a-c): Effect of EET on (a) Catalase, (b) Reduced glutathione and (c) Lipid peroxidation. $^{\#\#}p<0.01$ vs. normal, $^{**}p<0.01$ and $^{***}p<0.001$ vs. control

DISCUSSION

Oxidative stress to dopaminergic neurons of SNpc is believed to be one of the leading causes of neurodegeneration in PD. Antioxidants may play an important role in the prevention of PD and combat against oxidative stress induced progressive neurodegeneration by reactive oxygen species. However, medicinal plants like *Gingko biloba* and *Stereospermum suaveolens* have shown neuroprotective activity due to their antioxidant property[13,14]. Extract of *T. purpurea* has proven free radical scavenging activity[15].

Animal models of Parkinson's disease are widely used to investigate its pathophysiological mechanisms and for exploring potential treatments. Neuroleptics such as haloperidol can produce a sustained but reversible akinesia, due to blockade of dopamine D_2 receptors and this neuroleptic-induced Parkinsonism is a major side effect of their use in treatment of schizophrenia. The D_2 antagonists may act directly to reduce the ability of cortical and basal ganglia motor pathways to generate descending commands. Thus, neuroleptics have been used as an acute model of Parkinson[16]. In haloperidol induced catalepsy, EET at the dose of 200 and 400 mg kg^{-1} along with haloperidol showed a significant and dose dependent decrease in immobility and muscle impairment. Amazingly, the high dose of *T. purpurea* showed comparable results with standard L-Dopa and carbidopa.

The muscular rigidity tested using a rota-rod, is an established method used for the assessment of neurological deficits in rodents[17]. Significantly enhanced muscular coordination was seen in ethanol extract of *T. purpurea* treated groups as compared to control group. Haloperidol blocks dopamine receptors and may contribute to haloperidol neurotoxicity due to the generation of free radicals and the increase in lipoperoxidation products. In the present study, a marked increase in LPO and depletion of GSH and CAT in control group was observed which is similar as shown in the earlier reports[18]. The ethanolic extract of *T. purpurea* significantly reversed these toxic effects of haloperidol, revealing the antioxidant activity of the

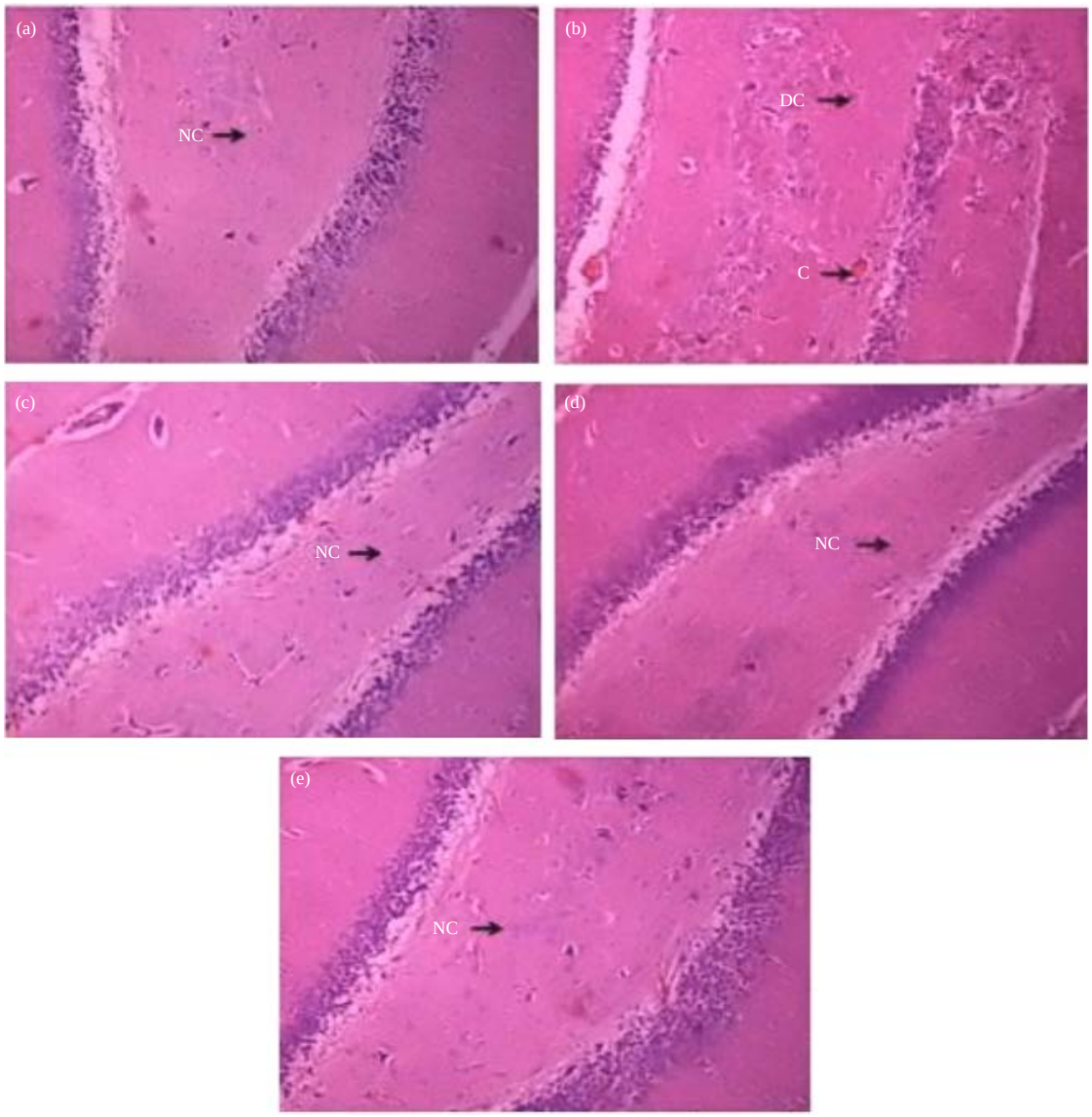

Fig. 2(a-e): Effect of EET on neurodegeneration in substantia nigra, (a) Group I, (b) Group II, (c) Group III, (d) Group IV and (e) Group V

extract which is supported by previous reports of *in vitro* antioxidant activity[15]. Decreased LPO and increased levels of CAT and GSH in both doses of EET has offered protection against oxidative stress. Inhibition of oxidative stress may be one of the possible mechanisms for the anti-Parkinson effects of *T. purpurea*.

Histopathological findings showed haloperidol treated group showed the congestion of degenerative changes due to decrease the number of neural cells in SNpc in brain tissue. Pre-treatment with EET (200 and 400 mg kg^{-1}, p.o.) rats showed regenerative changes in SNpc in brain. Surprisingly, high dose treated rats showed almost normal architecture of SNpc in brain tissue, further substantiates the neuroprotective activity against haloperidol induced Parkinson's disease model.

CONCLUSION

In conclusion, the present study suggests a potential role of ethanolic extract of *T. purpurea*. against haloperidol induced Parkinson's disease model. Further, studies are required for understanding the basic mechanism and characterization of active constituents responsible for neuroprotective effect.

ACKNOWLEDGMENT

The authors are thankful to the authorities of PRRM College of pharmacy, Kadapa (Andhra Pradesh) for providing support to the study and other necessary facility like internet surfing, library and other technical support to write a research.

REFERENCES

1. Roger, C.D., I.G. Lawrence, H.M. Margery, I.S. Jacob and S.W. Arthur, 1996. Parkinson's Disease Handbook. American Parkinson Disease Association, USA., pp: 30-40.
2. Karch, A.M., 2009. Focus on Nursing Pharmacology. Lippincott Williams and Wilkins, USA., pp: 850-874.
3. Dauer, W. and S. Przedborski, 2003. Parkinson's disease: Mechanisms and models. Neuron, 39: 889-909.
4. Joy, A.E., M. Shyamjith and K. ShankarBhat, 2012. Acute effect of ethanolic Extract of *Moringaoleifera* on haloperidol induced catalepsy in mice models. Drug Invention Today, 4: 543-545.
5. Nair, V., A. Arjuman, P. Dorababu, H.N. Gopalakrishna, U.C. Rao and L. Mohan, 2007. Effect of NR-ANX-C (a polyherbal formulation) on haloperidol induced catalepsy in albino mice. Indian J. Med. Res., 126: 480-484.
6. Gopalakrishnan, S., E. Vadivel and K. Dhanalakshmi, 2010. Anti-inflammatory and analgesic activities of *Tephrosia purpurea* Linn. aerial and root extracts. J. Pharm. Res., 3: 1103-1106.
7. Pavana, P., S. Sethupathy and S. Manoharan, 2007. Antihyperglycemic and antilipidperoxidative effects of *Tephrosia purpurea* seed extract in streptozotocin induced diabetic rats. Indian J. Clin. Biochem., 22: 77-83.
8. Despande, S.S., G.B. Shah and N.S. Parmar, 2003. Antiulcer activity of *Tephrosia purpurea* in rats Inidan. Indian J. Pharmacol., 35: 168-172.
9. Zazpe, A., I. Artaiz, A. Innerarity, E. del Olmo and E. Castro *et al*., 2006. *In vitro* and *in vivo* characterization of F-97013-GD, a partial 5-HT 1A agonist with antipsychotic-and antiparkinsonian-like properties. Neuropharmacology, 51: 129-140.
10. Abdel-Aal, R.A., A.A.A. Assi and B.B. Kostandy, 2011. Rivastigmine reverses aluminum-induced behavioral changes in rats. Eur. J. Pharmacol., 659: 169-176.
11. Kraus, R.L., R. Pasieczny, K. Lariosa-Willingham, M.S. Turner, A. Jiang and J.W. Trauger, 2005. Antioxidant properties of minocycline: neuroprotection in an oxidative stress assay and direct radical-scavenging activity. J. Neurochemistry, 94: 819-827.
12. Rambabu, B., S. Kokkula, T. Kiran, D. Rajakumar, T. Ramchander and D. Kemisetti, 2012. Studies on the anti-inflammatory and analgesic activity of the ethanolic fraction of the root extract of *Tephrosiapurpurea* (Linn). Int. J. Pharm. Pharm. Sci., 4: 275-278.
13. Kim, M.S., J.I. Lee, W.Y. Lee, S.E. Kim, 2004. Neuroprotective effect of *Ginkgo biloba* L. extract in a rat model of Parkinson's disease. Phytother. Res., 18: 663-666.
14. Shalavadi, M.H., V.M. Chandrashekhar, S.P. Avinash, C. Sowmya and A. Ramkishan, 2012. Neuroprotective activity of *Stereospermum suaveolens* DC against 6-OHDA induced Parkinson's disease model. Indian J. Pharmacol., 44: 737-743.
15. Patel, A., A. Patel, A. Patel and N.M. Patel, 2010. Determination of polyphenols and free radical scavenging activity of *Tephrosia purpurea* linn leaves (Leguminosae). Pharmacognosy Res., 2: 152-158.
16. Chandra, S., X. Chen, J. Rizo, R. Jahn and T.C. Sudhof, 2003. A broken α-helix in folded α-synuclein. J. Biol. Chem., 278: 15313-15318.
17. Rogers, D.C., J.O. Peters, J.E. Martin, S. Ball and S.J. Nicholson *et al*., 2001. SHIRPA, a protocol for behavioral assessment: Validation for longitudinal study of neurological dysfunction in mice. Neurosci. Lett., 306: 89-92.
18. Kabra, M.P., S.S. Bhandari, A. Sharma and R.B. Gupta, 2014. Evaluation of anti-parkinson's activity of gentisic acid in different animal models. J. Acute Dis., 3: 141-144.