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Neuroprotective Effect of *Orthosiphon thymiflorus*roth on Haloperidol Induced Parkinson Disease in Animal Models

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Abstract
Objective: The aim of the present work was to investigate anti-Parkinson's activity of *Orthosiphon thymiflorus* Roth ethanolic extract (OTEE) in haloperidol induced experimental animal models. **Material and Methods:** The experiment was designed by giving haloperidol to induce catalepsy and to induce Parkinson's disease-like symptoms. The effects of *Orthosiphon thymiflorus* (200, and 400 mg/kg, p.o.) were studied using in vivo behavioral parameters like catalepsy, muscle rigidity, locomotor activity and antioxidant activities in rats. **Results:** The increased cataleptic scores were significantly ($P<0.05$) found to be reduced with the OTEE at a dose of 200 and 400 mg/kg. OTEE administration showed significant increase in lipid peroxidation level and depleted catalase, and reduced glutathione level. Daily administration of OTEE (400 mg/kg) also significantly($P<0.05$) improved motor performance and increase locomotor activity. **Conclusion:** The present study was confirmed that prepared extract was significantly attenuated the motor defects and also protected the brain from oxidative stress.

Keywords
Orthosiphon thymiflorus, Parkinson's, Haloperidol, Catalepsy, Antioxidant.

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INTRODUCTION
Parkinson's disease (PD) is a neurodegenerative disease that occurs due to progressive damage to the dopaminergic neurons in the nigrostriatal tract of the brain. Oxidative stress, low glutathione levels, DNA damage and iron deposition are the main causes for degradation of dopaminergic neurons in PD. Oxidative stress not only responsible for degradation of the dopaminergic neurons, but it also compromises mitochondrial oxidative phosphorylation, leading to decreased energy output and eventually to secondary cell death. Loss of dopaminergic neurons in nigrostrictem tract results in the depletion of dopamine which is involved in coordinating movement [1,2]

The neuroleptic drug like haloperidol is one of the major cause for drug induced Parkinson's worldwide. The incidence of drug induced Parkinson's progresses with age [3]. It blocks dopamine D_2 receptors and produces a state of catalepsy in human or animals by reducing dopaminergic transmission in basal ganglion [4]. WHO currently encourages, recommends and promotes traditional as well as natural remedies in national health care programmes, as they are easily available at low cost, comparatively safe, and are culturally acceptable. The usage of herbs in Parkinson's disease as they are safe and alternative medicine.

Orthosiphon thymiflorus Roth belonging to family Lamiaceae is used for treating the ailments of the

kidney, since it has a mild diuretic effect. It is also claimed to have anti-allergenic, anti-hypertensive and anti-inflammatory properties, and is commonly used for kidney stones and nephritis. Orthosiphon is sometimes used to treat gout, diabetes, hypertension and rheumatism. It is reportedly effective for anti-fungal and anti-bacterial purposes. It has been claimed to be useful in PD. But no systemic pharmacological studies were reported in the literature. Hence in the present study the whole plant of *Orthosiphon thymiflorus* Roth was evaluated for its antiparkinson's potency.

MATERIALS AND METHODS

Collection and Preparation of Plant extract

The whole plant of *Orthosiphon thymiflorus* Roth was collected from Tirumala Hills, Tirupati, India. This plant was identified and authenticated by Dr. Madhava Chetty, Professor of Botany, Sri Venkateshwara University, Tirupati and voucher specimen of the plant were preserved at institute herbarium library for future reference. The dried plant was subjected to size reduction to a coarse powder by using dry grinder and passed through sieve (40 mesh). About 200gm of dried powder was defatted by treating with pet-ether and then extracted with 70% ethanol by using soxhlet apparatus till solvent was colorless. The extract was filtered and then solvent was evaporated under reduced pressure to a solvent free concentrated mass, which was then stored in air-tight container in a cool and dry condition.

Preliminary phytochemical screening

Preliminary qualitative phytochemical screening was done for the presence of different group of chemicals, that is, alkaloids, flavonoids, saponins, tannins, sterols, carbohydrates, and glycosides [5].

Animals

Wistar albino rats of either sex weighing were used in the present study. All the animals were maintained under controlled conditions of temperature (23 ± 2 C), humidity (50 ± 5%) and 12 h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile husk as bedding. They had free assessed to standard pellets as basal diet and water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Malla Reddy Institute of Pharmaceutical sciences (Reg. No: 1662/PO/Re/S/12/CPCSEA).

Acute toxicity study

LD50 of the extract was determined following the Organizations for Economic Cooperation and Development (OECD) guidelines. Acute toxicity class method (OECD guideline no. 423) and revised up-and-down method (OECD guideline no. 425) were followed for the testing of chemicals. Animals were observed for 24 hours for toxic symptoms such as behavioral changes, convulsions, locomotion, and mortality. If one animal showed mortality, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, then the same procedure was repeated for further higher doses [6].

EXPERIMENTAL PROCEDURE

Haloperidol Induced Catalepsy

Haloperidol causes dysfunction of various neurotransmitters such as acetylcholine, GABA, and serotonin. Pathology of haloperidol induced catalepsy underlying increased oxidative stress. Haloperidol, an antipsychotic drug, blocks central dopamine receptor in striatum. It also produces a behavioral state in animals like mice and rats in which they fail to correct externally imposed postures (called catalepsy); thus, keeping the above fact in mind, the haloperidol induced catalepsy model was selected. The method described by Elliott and Close in 1990 was followed for the anti-cataleptic activity. The animals were divided into five groups (n=6). Group I served as vehicle control, Group II served as standard, L-Dopa & Carbidopa (100mg+25mg/kg p.o) and Groups III–V served as test group treated with OTEE (200, and 400 mg/kg, p.o.), respectively. Standard bar test was used to measure the catalepsy. Catalepsy was induced by haloperidol (1 mg/kg, i.p.) and examined at every 30 min interval for 210 min. The duration for which the rat retains the forepaws extended and resting on the elevated bar was considered as cataleptic score [7].

Pharmacological Evaluation

In this research, Wistar albino rats were randomly divided into four groups consisting of six animals in each group. The first group is normal which received vehicle (1% CMC, p.o) weight p.o., the second group is negative control which received haloperidol 1mg/kg body weight i.p., and the third and fourth groups were test groups which received 200 and 400 mg/ kg body weight of OTEE. All the groups were treated for 21 days. The tabulated treatment schedule is given in Table 1.

Table 1: Treatment schedule - Evaluation of antiparkinson's activity of OTEE

S.No	Group	No.of Animals	Treatment	Treatment period (Days)
1	Normal	6	Vehicle (1% CMC, p.o.)	21
2	Negative Control	6	Haloperidol (1mg/kg, i.p.,)	21
3	Standard	6	L-Dopa & Carbidopa (100mg+25mg/kg p.o) + Haloperidol (1mg/kg, p.o.)	21
4	Test-1	6	OTEE (200 mg/kg, p.o) + Haloperidol (1mg/kg, p.o.)	21
5	Test-2	6	OTEE (400 mg/kg, p.o) + Haloperidol (1mg/kg, p.o.)	21

ESTIMATION OF BEHAVIOURAL PARAMETERS

Locomotor Activity

The spontaneous locomotor activity was monitored using digital actophotometer (Hicon instrument, India) equipped with infrared sensitive photocells. The apparatus was placed in a darkened, light and sound attenuated, and ventilated testing room. Each interruption of a beam on the axis generated an electric impulse, which was presented on a digital counter. A time interval of 30 min was maintained in between administration of haloperidol and test/standard drug. Standard and test drugs were administered for a period of 21 days [8].

Rota rod Activity

All animals were evaluated for grip strength by using the rotarod. The rotarod test is widely used in rodents to assess their "minimal neurological deficit" such as motor function and coordination. Each rat was given a prior training session before initialization of therapy to acclimatize them on a rotarod apparatus (EIE instrument, India). Animal was placed on the rotating rod with a diameter of 7 cm (speed 25 rpm). A time interval of 30 min was maintained in between administration of haloperidol and test and standard drugs. The average results were recorded as fall of time [9].

Biochemical analysis

Animals were euthanized 24 hours after haloperidol-EEOT treatment by decapitation under ether an aesthesia, brain was excised, washed with ice-cold saline solution (0.9 % NaCl), weighed and stored for the biochemical analyses. The brain was homogenized with 0.1 M phosphate buffer saline at pH 7.4, to give a final concentration of 10 % w/v for the bio chemical assays.

Statistical Analysis

Statistical Analysis. All the values were expressed as mean SEM. Statistical evaluation of the data was done by one-way ANOVA (between control and drug treatments) followed by Dunnett's *t*-test for multiple comparisons and two-way ANOVA followed by Bonferroni's multiple comparison test, with the level of significance chosen at $P< 0.05$ using Graph-Pad Prism 5 (San Diego, CA) software.

RESULTS

Preliminary Phytochemical Screening

Phytochemical screening showed the presence of glycosides, flavonoids, alkaloids, proteins, carbohydrates and phenolic compounds.

Acute toxicity studies

The whole plant of *Orthosiphon thymiflorus* Roth was found to be safe since no animal died at the maximum single dose of 2000 mg/kg when administered orally and the animals did not show any gross behavioral changes. Hence1/10th and 1/5th of the maximum tolerated of this dose i.e. 200mg/kg and 400mg/kg was fixed to the experimental animals.

Effect of OTEE on Cataleptic Activity

An increase in the degree of catalepsy was noticed in the haloperidol group after 60 and 90minutes of administration. The score was significantly reduced after 60 minutes with the test drug OTEE at 200 and 400 mg/kg doses. During the period of observation till 210 minutes, there has been a significant reduction. The 400 mg/kg treated group exhibited maximum reduction in the catalepsy (table-2).

Rotarod Test

Animals treated with haloperidol (1mg/kg, s.c.,) alone for 21 days showed a non-significant decrease on 21st day in the latency of fall when compared to normal group. Animals treated with low dose and high doses of OTEE (200mg/kg and 400mg/kg)) along with haloperidol (1mg/kg, s.c.,) for 21days showed a significant($P<0.05$) increase on 21st day in the latency of fall when compared to negative control group (table-3).

Effect of EEOT on Behavioral Parameters-locomotor Activity

Animals treated with haloperidol (1 mg/kg, s.c.,) alone for 21days showed a significant ($P < 0.05$) decrease on 21st day in the locomotor activity when compared to normal group. Animals treated with low

dose of OTEE (200 mg/kg and 400mg/kg) along with haloperidol (1 mg/kg, i.p.,) for 21 days showed a significant ($P < 0.05$) increase on 21st day in the locomotor activity when compared to negative control group (table-4).

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. Treatment with OTEE showed significant protection by reducing the elevated levels of LPO and increasing the CAT and GSH levels as compared to the control group (Table-5).

Table 2: Effect of OTEE on catalepsy by metal bar test

Treatment	Immobility (Fall of time in min) (Mean ± S.E.M) on 21st day						
	30	60	90	120	150	180	210
1% CMC (p.o.)	12.25±0.7	9.5 ± 1.32	9.25 ± 1.37	11.25 ± 0.85	12.50 ± 0.64	11.75 ± 0.94	12.25 ± 1.25
Haloperidol (1mg/kg, p.o.)	54.25±17.3[a]	61.5±8.4[a]	78.25±2.73[a]	77.25±1.65[a]	96.25±2.56[a]	112±4.65[a]	130.8±4.51[a]
L-Dopa & carbidopa (100+25 mg/kg,p.o.)+Haloperidol (1mg/kg, p.o.)	21.75 ± 1.1[b]	40.5±1.6[b]	68.25 ± 2.13[b]	67.25 ±1.65[b]	48.75±2.39[b]	36 ±1.87[b]	39 ±2.91[b]
OTEE (200mg/kg, p.o.) + Haloperidol (1mg/kg, p.o.)	24 ±1.08[c]	50.75 ±6.6[c]	76.25±3.17[c]	77±2.7[c]	86.50±1.5[c]	92.5±2.5[c]	74.25±2.0[c]
OTEE (400mg/kg, p.o.) + Haloperidol (1mg/kg, p.o.)	37±5.81[c]	39.25 ±3.7[c]	70.25 ±2.28[c]	69 ± 0.7[c]	82.50 ±2.21[c]	91.75±3.19[c]	59.75±3.96[c]

Values are expressed as mean±SEM, one-way ANOVA followed by Dunnett's multiple comparisons test. [a]p<0.05 as compared with control group. [b,c]p<0.05 as compared with haloperidol induced rats. OTEE: *Orthosiphon thymiflorus* ethanolic extract

Table 3: Effect of OTEE on muscle rigidity by using rota rod

Treatment	Fall of time (min) (Mean ± S.E.M) on 21st day						
	30	60	90	120	150	180	210
1% CMC (p.o.)	26.38±2.08	28.68±1.03	22.9 ± 1.79	30.55 ± 2.65	33.15 ± 2.64	33.37 ± 2.4	26.30 ± 2.71
Haloperidol (1mg/kg, i.p)	13.01±1.45[a]	11.3±1.7[a]	8 ± 1.64[a]	10.14±2.63[a]	12.35±1.26[a]	11.06±0.5[a]	6.69±1.53[a]
L-Dopa & carbidopa (100+25 mg/kg, p.o.) +Haloperidol (1mg/kg,i.p)	23.68±3.96[b]	20.05±2.23[b]	14.85±0.89[b]	15.54±2.49[b]	19.69±0.92[b]	19.64±2.35[b]	23.15±1.09[b]
OTEE (200mg/kg, p.o.) +Haloperidol (1mg/kg,p.o)	14.03±1.69[c]	13.11±3.71[c]	9.16±1.16[c]	11.98±4.68[c]	13.97±1.13[c]	18.13±0.26[c]	19.78±3.16[c]
OTEE (400mg/kg, p.o.) +Haloperidol (1mg/kg, p.o.)	23.58±5.1[c]	14.76±0.72[c]	15.53±2.84[c]	16.37 ± 1.8[c]	19.44 ± 0.7[c]	19.94±0.44[c]	23.34±1.95[c]

Values are expressed as mean±SEM, one-way ANOVA followed by Dunnett's multiple comparisons test. [a]p<0.05 as compared with control group. [b,c]p<0.05 as compared with haloperidol induced rats. OTEE: *Orthosiphon thymiflorus* ethanolic extract

Table 4: Effect of OTEE on Locomotor activity

Treatment	On 21st day (Mean ± S.E.M)			
	Peripheral movements /5min	Central movements/5min)	Rearings (5 min)	Groomings (5 min)
1% CMC (p.o.)	469.8 ± 9.95	443 ± 14.91	23 ± 0.91	14.25 ± 1.79
Haloperidol (1mg/kg, p.o.)	213.3 ± 12.34[a]	200.5 ± 2.21[a]	10 ± 0.91[a]	4.25 ± 0.47[a]
L-Dopa & carbidopa (100+25 mg/kg, p.o) + Haloperidol (1mg/kg, p.o.)	404.8 ± 4.6[b]	348 ± 3.3[b]	19 ± 0.4[b]	12.5 ± 0.64[b]
OTEE (200mg/kg, p.o.) +Haloperidol (1mg/kg, p.o.)	266 ± 7.16[c]	251 ± 1.73[c]	14.5 ± 0.28[c]	10 ± 0.40[c]
OTEE (400mg/kg, p.o.) +Haloperidol (1mg/kg, p.o.)	423 ± 9.29[c]	309 ± 6.8[c]	17.5 ± 0.64[c]	12.75 ± 0.25[c]

Values are expressed as mean±SEM, one-way ANOVA followed by Dunnett's multiple comparisons test. [a]p<0.05 as compared with control group. [b,c]p<0.05 as compared with haloperidol induced rats. OTEE: *Orthosiphon thymiflorus*ethanolic extract

Table 5: Effect of OTEE on tissue antioxidant levels

Treatment	On 21st day (Mean ± S.E.M)		
	CAT (µM of H_2O_2 decomposed/mg protein/min)	GSH (µg of GSH/mg)	LPO (µM /mg protein)
1% CMC (p.o.)	64.68 ± 1.9	34.98 ± 1.48	10.69 ±0.63
Haloperidol (1mg/kg, p.o.)	30.63 ± 2.12[a]	23.93 ± 1.4[a]	25.87 ± 0.72[a]
L-Dopa+ Carbidopa (100+25mg/kg, p.o.) + Haloperidol (1mg/kg, p.o.)	58.85 ± 1.22[b]	44.82 ± 0.09[b]	14.12 ± 0.67[b]
OTEE (200mg/kg, p.o.) + Haloperidol (1mg/kg, p.o.)	40.75 ± 1.37[c]	30.86 ± 0.51[c]	21.87 ± 0.3[c]
OTEE (400mg/kg, p.o.) + Haloperidol (1mg/kg, p.o.)	56.67 ± 1.76[c]	46.49 ± 0.83[c]	12.69 ± 0.84[c]

Values are expressed as mean±SEM, one-way ANOVA followed by Dunnett's multiple comparisons test. [b]p<0.05 as compared with control group. [b,c]p<0.05 as compared with haloperidol induced rats. OTEE: *Orthosiphon thymiflorus* ethanolic extract

DISCUSSION

Parkinsonism disease is a neurodegenerative disorder characterized by the selective loss of dopamine neurons of the substantia nigra pars compacta (SNpc). The events which trigger and/or mediate the loss of nigral dopaminergic neurons, however, remain unclear. Current treatment of PD is based on dopamine replacement therapy, but this leads to long term complications, including dyskinesia. Herbal medicines are currently used as a safer alternative to PD. The World Health Organization has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines.

In the present study, the animals which were treated for 21 days with haloperidol showed severe cataleptic responses along with decreased locomotor and motor coordination. Further, the animals (haloperidol treated) showed decreased levels of glutathione and catalase and increased levels of LPO products and superoxide dismutase as compared to the control animals. The exact mechanism by which haloperidol increases free radical production was not clear. The enzymatic degradation by MAOs was associated with the production of hydrogen peroxide, which was readily converted to the hydroxyl radical in the presence of iron [10] Thus, it could initiate a destructive LPO cascade, but an increased dopamine (DA) turnover, leading to hydrogen peroxide production which might not be exclusively involved in the degeneration of oxidative stress [11].The auto-oxidation of DA which resulted in the production of superoxide radicals might have contributed to the unbalanced production of the free radicals. However, other mechanisms may also be involved. Haloperidol was reported to suppress the activity of certain detoxifying enzymes, thus leaving the cell unprotected, especially if the basal enzyme activity was low or if the free radical scavenging mechanisms were less effective (Hyun, 2010). Haloperidol (HP) is converted to potentially toxic (HHP+) metabolites which may play a role in the extrapyramidal side effects which are observed in the patients who are treated with haloperidol. Another possible mechanism could be the effect of neuroleptics on the

mitochondrial respiration. The metabolites of haloperidol inhibit complex-I of the electron transport chain. The capability of the anti-psychotic drugs to clinically induce the extrapyramidal syndrome seems to correlate well with their inhibitory effect on the complex-I inhibition.[11] Whatever could have been be the mechanism of the unbalanced production of the reactive oxygen species and the oxidative stress by haloperidol, HABO was found to be effective in decreasing the oxidative stress in the haloperidol-treated animals. The anti-oxidative properties of EEOT reduced the duration of the catalepsy and increased the locomotor activity along with motor coordination. The group treated with EEOT (400 mg/kg) showed normal locomotor activity and motor coordination without any cataleptic behavior when compared with the haloperidol treated group. The group treated with HABO 250mg/kg showed some cataleptic behavior when compared to EEOT (400 mg/kg) treated group. Treatment with EEOT (200 and 400 mg/kg) decreased the elevated levels of LPO in the haloperidol treated animals and elevated the cellular defense mechanisms such as glutathione, further suggesting the role of free radicals in the pathophysiology of the haloperidol-induced extrapyramidal syndrome.

CONCLUSION

The results of the present study conclusively showed that *Orthosiphon thymiflorus* has beneficial effects in metal bar test, locomotor activity, muscle rigidity by using rota rod test. In this regard, future studies on this topic may provide an elaborate view to use *Orthosiphon thymiflorus* in clinical medicine for treatment of Parkinson's disease and its neurological sequel.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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