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Evaluation of aqueous extract of *Ocimum sanctum* in experimentally induced parkinsonism

[1]S. V. Joshi*, [2]S. B. Bothara, [3]S. J. Surana

[1]Department of Pharmacology, Maliba Pharmacy College, Gopal Vidhyanagar, Bardoli-Mahuva Road, Tarsadi, Gujarat, India
[2]ROFEL Shri GM Bilakhia College of Pharmacy, Namdha Campus, Vapi-Namdha Road, Vapi(W) Ta: Pardi, Dist. Valsad, Gujarat, India
[3]R.C.Patel College of Pharmacy, Shirpur, Dist: Dhule (M.S), India

ABSTRACT

Parkinson's disease (PD) is neurodegenerative disorder characterized by the progressive loss of the dopaminergic neurons in the substantia nigra pars compacta which innervates the dorsal striatum. Using haloperidol induced catalepsy in rat and muscle rigidity in mice effects of aq. extract of Ocimum sanctum (OSE_{aq}) were studied. Haloperidol was administered to induce either short term model (0.5mg/kg i.p) or long term model (4mg/kg s.c.) to observe changes in catalepsy and muscle rigidity as measured using rota-rod test and chimney test. Out of 100mg, 200mg, 300mg and 600mg/kg p.o, doses of O. sanctum, 300mg/kg dose showed maximum shortening of onset and duration of catalepsy as compared with other dose levels in short term and long term model. Pretreatment with OSE_{aq} (300mg and 450mg/kg p.o.) significantly reduces initial decrease in activity in rota rod and significantly improves performance of mice in chimney test as compared with control in short term and long term models. In conclusion, OSE_{aq} in the dose 300mg/kg i.p. shows anticataleptic action in rats as well as in the dose 300 and 450mg/kg i.p. improves the performance of mice in rota-rod and chimney test indicating reduction of muscle rigidity. Active constituent-linalool, modulation of central neurotransmission might be responsible for the antiparkinsonian activity exhibited by OSE_{aq}

Key words: - *Ocimum sanctum*, haloperidol, muscle rigidity, rota-rod, chimney test.

INTRODUCTION

Parkinson's disease (PD) is neurodegenerative disorder characterized by the progressive loss of the dopaminergic neurons in the substantia nigra pars compacta which innervates the dorsal striatum. Excitotoxicity by glutamate neurotransmitters, oxidative stress, apoptosis and certain

environmental triggers are believed to be responsible for dopaminergic neurodegenaration in the striatum. Along with dopamine involvement, other neurotransmitters such as glutamate, GABA, acetylcholine are also proposed in the progression of PD [1]. It has been shown that modulation of adenosine A_{2a} receptors [2] and adrenergic receptors [3] strongly influences the motor behavior in animals. In addition to several synthetic agents modulating various pathways, certain plant constituents also exhibited some activity against experimentally induced PD e.g. Linalool increase dopamine release from striatal slices in rats [4]. *Mucuna pruriens*, also known as cowhage or velvet bean, has been recommended for treatment of PD by ancient Ayurvedic texts, and the seeds of *M. pruriens* have been shown to contain levodopa. In three open label studies with *M. pruriens*, which involved 18 and 60 patients and used mean dosages of 45 g/day of mucuna seed powder extract (contains about 1500 mg L-dopa), reported significant improvements in parkinsonism for 12–20 weeks. One study suggested tolerability might be better with mucuna than with standard L-dopa preparations [5]. One another plant *Vicia faba* (broad or fava bean) has also been suggested to possess antiparkinsonism activity [6].

Ocimum sanctum (OS) is considered a sacred plant in the Hindu culture and known as "*Tulsi*" or "Tulasi" in Hindi or Holy Basil in English. Basils (Ocimum spp., Lamiaceae) contain a wide range of essential oils rich in phenolic compounds [7], linalool [8] and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins [7]. Basil leaves showed to be antidiabetic [9], wound healing [10], antibacterial [11], anti-tussive [12], anti-ulcer [13] and immunomodulatory [14]. Gupta et al. (2002) evaluated the actions of OS which was proposed in ancient literature of Aurveda such as catarrhal bronchitis, bronchial asthma, dysentery, dyspepsia, skin diseases, chronic fever, hemorrhage, helminthiasis [15] and topically for ring worms [16]. Leaves of OS possess hypoglycemic /antihyperglycemic actions in experimental animals [17]. This medicinal plant has many reported pharmacological effects viz. anticancer [18], antifertility [19,20] and radioprotective [21]. Lately, OS has been reported to possess anti-inflammatory activity which has been attributed to its cyclo-oxygenase inhibitory and antioxidant effects [22,23]. The same extract which was used in this study, was studied for cardioprotective effects and found to be antiapoptotic and antioxidant [24,25].

Since certain plants shows release of dopamine and also extract of *O. sanctum* can reduce oxidative stress, one of the causative factors for PD and contains linalool which can increase Dopamine (DA) concentration we found it appropriate for verifying its effects in experimentally induced PD like condition. Further litearure review suggests that this extract/plant is not yet studied for its antiparkinson's like activity.

Out of various animal models viz. 6-hydroxydopamine (6-OHDA) induced lesions in rat [26,27], haloperidol induced catalepsy [28,29], reserpine induced muscle rigidity [1,30], tacrine induced tremulous jaw movement [31], MPTP induced neurotoxicity [32,33] were used for studying antiparkinsonism activity. 6-OHDA model is well accepted as gold standard model and haloperidol induced muscle rigidity model is the second most model which is used largely by the researchers as per literature survey. However 6-OHDA model requires stereotaxic instrument for injecting the 6-OHDA in brain while haloperidol model being comparatively simpler is used extensively for the antiparkinson effect study. Lebsanft et al., (2005) used rota-rod test and chimney test along with catalepsy test for evaluation of muscle rigidity [29].

EXPERIMENTAL SECTION

Animals: -

Wistar rats (180-250g) and Albino Mice (15-30g) of either sex were used for the study. Animals were obtained from in house facility and housed in the room on an artificial light/dark cycle

(12/12 hr, light on from 7 a.m. to 7 p.m.), under standard conditions with free access to food and water. The study was performed in accordance with the guidelines issued by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) an authority regulating animal experiments and was approved by the Institutional Animal Ethics Committee formed as per CPCSEA guidelines.

Drugs: -

Haloperidol (Serenace, LPG Life Sciences) solution was diluted in saline. Aqueous extract of *Ocimum sanctum* (OSE_{aq}) (Hydro-alcoholic lyophilized extract of *Ocimum sanctum* was gifted to us by Mr. Mukesh Nandave Research Scholar working under Dr. D. S. Arya Professor of Pharmacology, All India Institute of Medical Science, New Delhi, India; which was procured from Dabur Research Foundation by them) and was dissolved in DW and administered. All the solutions were freshly prepared prior to each experiment.

Models: -

Haloperidol is widely used to induce Parkinsonism like conditions in the dose 0.5mg/kg and 1mg/kg *i.p.* in rats [34] and 0.5-2mg/kg *i.p.* in mice [35]. The study was performed using short term and long term models as given below-

Short term Model – Haloperidol (0.5 mg/kg *i.p.)* was administered for short term model [28]. OSE_{aq} was administered orally 60 min before the haloperidol treatment. The study was started in the morning and readings were noted for each 10min interval after haloperidol treatment. The short term model showed duration of catalepsy for approximate 4 hours accordingly the observations were noted for 4 hours. Following groups were randomly made to administer the drugs as shown-

Animals were divided in five groups each containing 6 male and 6 female rats. The treatment scheme followed for each group was as follows,

Group 1:- Control. Received equivalent volume of vehicle p.o.
Group 2:- OSE_{aq} 100- Treated with OSE_{aq} 100mg/kg p.o. Short term
Group 3:- OSE_{aq} 200- Treated with OSE_{aq} 200mg/kg p.o. Short term
Group 4:- OSE_{aq} 300- Treated with OSE_{aq} 300mg/kg p.o. Short term
Group 5:- OSE_{aq} 600- Treated with OSE_{aq} 600mg/kg p.o. Short term

Haloperidol solution in the dose 0.5 mg/kg i.p. was injected to animals in each group after 60min of OSE treatment.

Long term model – The dose i.e. 4mg/kg *s.c.* was finalized after trying series of doses of haoperidol to evaluate the effect of test compound on continuous and long term Parkinsonism like conditions. OSE_{aq} was administered 60min before the haloperidol treatment. The study was of one day which started in the morning with dosing of OSE_{aq} and readings were noted for each 10min interval after haloperidol treatment. The long term model showed duration of catalepsy for approximate 8-9 hours.

Animals were divided in five groups each containing 6 male and 6 female rats. The treatment scheme followed for each subgroup was as follows,

Group 1:- Control. Received equivalent volume of vehicle p.o.
Group 2:- OSE_{aq} 100- Treated with OSE_{aq} 100mg/kg p.o. Long term.
Group 3:- OSE_{aq} 200- Treated with OSE_{aq} 200mg/kg p.o. Long term.
Group 4:- OSE_{aq} 300- Treated with OSE_{aq} 300mg/kg p.o. Long term.
Group 5:- OSE_{aq} 600- Treated with OSE_{aq} 600mg/kg p.o. Long term.

Haloperidol solution in the dose 4mg/kg s.c. was injected to the animals in each group after 60min of OSE treatment.

Parameters-

Scoring for catalepsy [**36, 37]:-**

The following scoring system was used:

0 – rat moved normally when placed on the table;

0.5 – rat moved only when touched or pushed;

0.5 - rat placed on the table with front paws set alternately on a 3 cm high wooden block, failed to correct the posture. Time (in sec.) taken to correct the posture (2 min. was considered as cut off time) was multiplied by the score for each paw;

1 .O - rat failed to correct posture when front paws are placed on a 9 cm high wooden block, Time (in sec.) taken to correct the posture (2 min. was considered as cut off time) was multiplied by the score for each paw

Some modifications were made in the above reported scoring method. In previous method scoring is done at 30 min interval, but we scored the catalepsy at the interval of 10 minutes so as to explore the exact time at which catalepsy was developed and to observe the slight change in degree of induction of catalepsy with respect to time. The scoring pattern was slightly modified and multiplied with time (in sec.) taken to correct the posture by animal for each score. This was done to differentiate the intensity of catalepsy of animals showing same score (below the highest score).

Formula to calculate catalepsy score: -

Total Score =0.5 + (0.5 x Time in sec. of front right paw on 3 cm high wooden block) + (0.5 x Time in sec. of front left paw on 3 cm high wooden block) + (1 x Time in sec. of front right paw on 9 cm high wooden block) + (1 x Time in sec. of front left paw on 9 cm high wooden block)

For example, rat move only when touched or pushed then score 0.5. Then rat placed on the table with front right paw set on a 3 cm high wooden block, fails to correct the posture in specific time (in sec.) say 100 sec. (2 min. was considered as cut off time), the score 0.5 multiplied by the time taken to correct the posture, the score become 0.5 x 100 = 50. Similarly with left paw, taking time 90sec to correct the posture, score become 0.5 x 90=45. In case of right paw placed on 9 cm high wooden box, taking time of 60 sec to correct the posture, the score will be 1 x 60 = 60. Similarly with left paw, taking time 80 sec to correct posture, score will be 1 x 80 = 80.

Total score in the above example will be 0.5+50+45+60+80=235.5

Thus, according to the formula, for a single rat, the maximum possible cumulative score of catalepsy was 360.5. The catalepsy score at the given time interval was plotted against time and following observations were noted.

1. **Onset of overall catalepsy** was the time at which animals started showing the catalepsy.
2. **Duration of overall catalepsy** was total duration of catalepsy produced.
3. **Onset of maximum catalepsy** was the time at which animals started to show maximum score of catalepsy i.e. above 335
4. **Duration of maximum catalepsy** was the duration of catalepsy at which the score was nearer to the maximum score i.e. above 335

Tests for Muscle Rigidity using Rota rod test and Chimney Test: -

Albino mice (Swiss) were randomly divided in 4 sets of experiments having 4 groups (n=6). Out of 4 sets, 2 sets were for short term model of haloperidol induced muscle rigidity (0.5mg/kg) and 2 for long term model of haloperidol induced muscle rigidity(4mg/kg). The grouping for short term model are-

Group 1: - Normal- Received equivalent volume of saline i.p. and DW p.o /kg body wt. as in control except haloperidol and OSE_{aq}
Group 2:-. Control-haloperidol i.p
Group 3:- OSE_{aq} 300- Treated with OSE_{aq} 300mg/kg p.o.
Group 4:- OSE_{aq} 450- Treated with OSE_{aq} 450mg/kg p.o.

All the groups except the first were treated with haloperidol solution in the dose 0.5 mg/kg *i.p.* after 60 min of OSE treatment for short term model.

The grouping for long term model are-
Group 1: - Normal- Received equivalent volume of saline s.c. and DW p.o /kg body wt. as in control except haloperidol and OSE_{aq}
Group 2: -. Control-haloperidol s.c..
Group 3:- OSE_{aq} 300- Treated with OSE_{aq} 300mg/kg p.o. Long term.
Group 4:- OSE_{aq} 450- Treated with OSE_{aq} 450mg/kg p.o. Long term.

It is one day study in which all the groups except the first were treated with haloperidol solution in the dose 4 mg/kg *s.c.* after 60 min of OSE treatment once in the morning for long term model.

The OSE dose regimen was modified, as in catalepsy model no significant effects were observed in dose of 100, 200 and 600 mg/kg. This minimized use of animals and gave chance to explore activity at 450 mg/kg.

Out of two sets of each short term and long term model, one set of groups was subjected to following tests.

Rota rod test [**38] : -**

The speed of the rotating rod was set at 20 rpm. Animals were placed on the rod and the fall off time before haloperidol and for each 10 min after haloperidol treatment was determined. Percent decrease in time compared to fall off time before haloperidol was calculated.

Chimney Test [**39, 40]: -**

Each mouse was introduced into the transparent plastic tube having 3cm inner diameter and 25cm length, with the head forward. When the mouse reached to the other end of tube (gentle push was given when necessary with a rod), the tube was moved to a vertical position (head of mouse downwards). The mouse tried to climb backwards. The time required for the mouse to climb backwards out of cylinder was noted. Cut off time was of 240 sec.

The time (sec.) required to climb back was plotted against time (min) and following observations were noted. Delayed in climbing back time indicate muscle rigidity in mouse.

1. **Onset of overall muscle rigidity** was the time at which animals showing 50 sec to climb back in chimney.
2. **Duration of overall muscle rigidity** was total duration of muscle rigidity produced.
3. **Onset of maximum muscle rigidity** was the time at which animals show maximum time to climb back i.e. above 225.

4. **Duration of maximum muscle rigidity** was the duration of muscle rigidity in which the time required by mice to climb back was nearer to the maximum score i.e. above 225.

Statistical Analysis: -

All results were expressed as Mean ± SEM. Statistical analysis was carried out using One-way ANOVA with Dunnette's multiple comparison test.

RESULTS

Catalepsy test:-

Table 1 and 2 shows effect of different doses of OSE on induction of catalepsy as observed in change in catalepsy score with respect to time in haloperidol induced catalepsy in short term and long term model. The results were summarized to depict the various parameters like onset and duration of overall and maximum catalepsy in short term and long term models respectively. The lowest dose of OSE i.e 100 mg/kg did not show any change in all these parameters in short term as well as long term model. Although there were some positive changes i.e delayed onset and shortened duration in OSE_{aq}200 and OSE_{aq}600 groups of short term model, the effects were not significant except delayed overall catalepsy at dose of 200 and 600 mg ($P< 0.05$). However at 300 mg/kg the OSE_{aq} exhibited highly significant ($P< 0.01$) changes in all the parameters of catalepsy. Similar results were observed in long term model too. (Table 2)

Table 1: - Effect of OSE on onset and duration of overall catalepsy and onset and duration of maximum catalepsy, in haloperidol induced catalepsy (0.5mg/kg i.p.) – short term model in rats

Sr. No.	Groups	Overall catalepsy (min)		Maximum catalepsy (min)	
		Onset	Duration	Onset	Duration
1	Control- haloperidol (0.5mg /kg, *i.p*)	15±2.24	211.66±6.00	30±3.65	158.33±14.47
2	OSE_{aq} (100mg/kg, *p.o.*) + haloperidol (0.5mg /kg, *i.p*)	15±2.24	215±7.19	30±3.65	156.66±8.03
3	OSE_{aq} (200mg/kg) + haloperidol(0.5mg/kg)	25±2.24*	175±6.71**	36.66±3.33	123.33±6.66*
4	OSE_{aq} (300mg/kg) + haloperidol(0.5mg/kg)	43.33±2.11**	155±3.42**	56.67±3.33**	106.67±4.22**
5	OSE_{aq} (600mg/kg) + haloperidol(0.5mg/kg)	25±2.24*	195±4.28	38.33±1.67	146.67±5.58

**- P<0.05, **- P<0.01 - Individual readings were compared with readings of control. (n = 6)*

Rota-rod Test: -

Fig. 1 and Fig. 2 depicts effect of different doses of OSE on time spent on Rota-rod for studying reduction in movement with respect to time in haloperidol induced Muscle rigidity in short term and long term model respectively.

Haloperidol administration significantly ($P<0.01$) reduced movement of mice in both models as compared to normal animals. In short term model, pretreatment with *OSE aq* (both doses i.e. 300 mg and 450 mg/kg) significantly ($P< 0.01$) delayed and shortened the reduction in movement as compared with control. There were no significant differences in effects exerted by different doses.

Table 2: - Effect of OSE on onset and duration of overall catalepsy and onset and duration of maximum catalepsy, in haloperidol induced catalepsy (4mg/kg i.p.) – long term model in rats.

Sr. No.	Groups	Overall catalepsy (min)		Maximum catalepsy (min)	
		Onset	Duration	Onset	Duration
1	Control- haloperidol (0.5mg /kg, *i.p*)	10	543.33±4.94	13.33±4.94	496.67
2	OSE_{aq} (100mg/kg, *p.o.*) + haloperidol (0.5mg /kg, *i.p*)	10	543.33±4.94	16.67±2.11	491.67±4.01
3	OSE_{aq} (200mg/kg) + haloperidol(0.5mg/kg)	20**	530±4.47	28.33±1.67**	481.67±5.43
4	OSE_{aq} (300mg/kg) + haloperidol(0.5mg/kg)	33.33±3.33**	501.67±7.03**	45±2.24**	448.33±7.03*
5	OSE_{aq} (600mg/kg) + haloperidol(0.5mg/kg)	20**	530±4.47	25±2.24*	483.33±4.94

**- P<0.05, **- P<0.01 - Individual readings were compared with readings of control. (n = 6)*

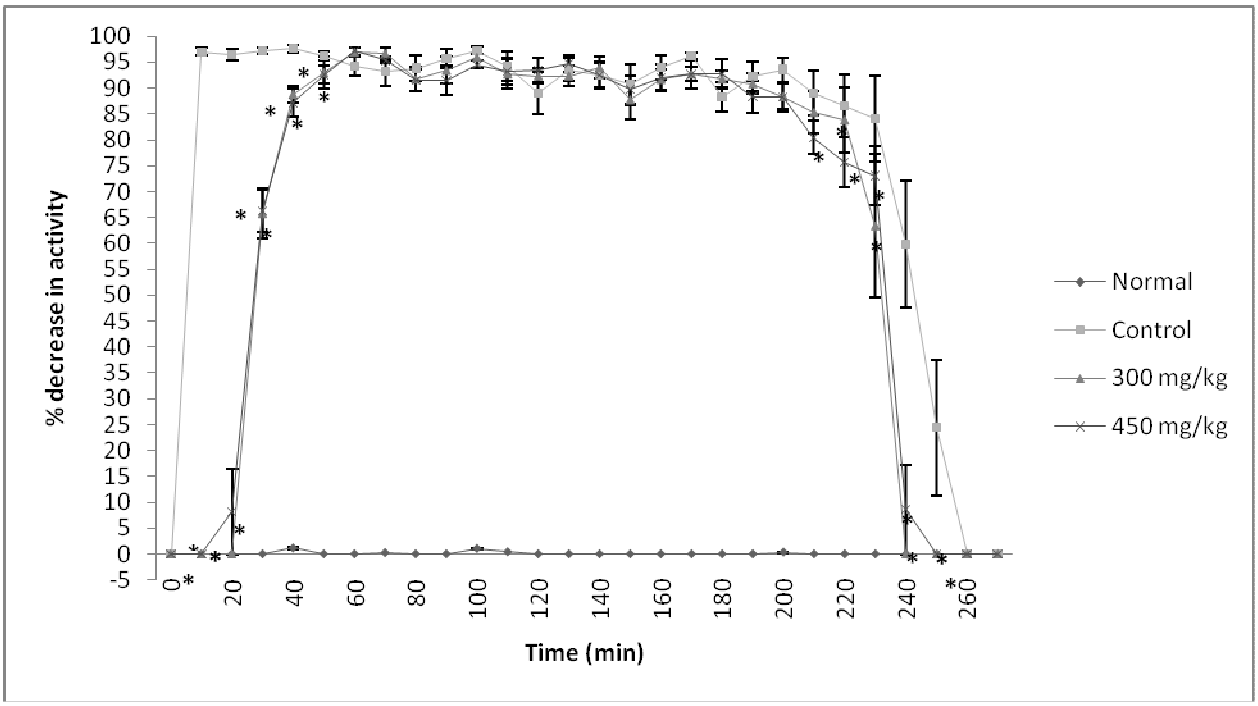

Figure 1: - Effect of different doses of OSE on time spent on Rota-rod for studying reduction in movement with respect to time in haloperidol induced Muscle rigidity in short term model in rats.

**- P<0.05 - Test was compared with control. (n = 6)*

Chimney test: -

In chimney test, in both the models, pretreatment with OSE significantly improves performance of mice as compared with the control. In initial phase, mice pretreated with OSE take significantly less time for climbing back (P<0.01) in chimney apparatus.

The results were summarized in table 3 and 4 to depict the various parameters like onset and duration of overall and maximum muscle rigidity in short term and long term models respectively. 300 mg/kg and 450 mg/kg dose of the OSE_{aq} exhibited highly significant (P< 0.01) changes in all the parameters of muscle rigidity. Similar results were observed in long term model too. (Table 4)

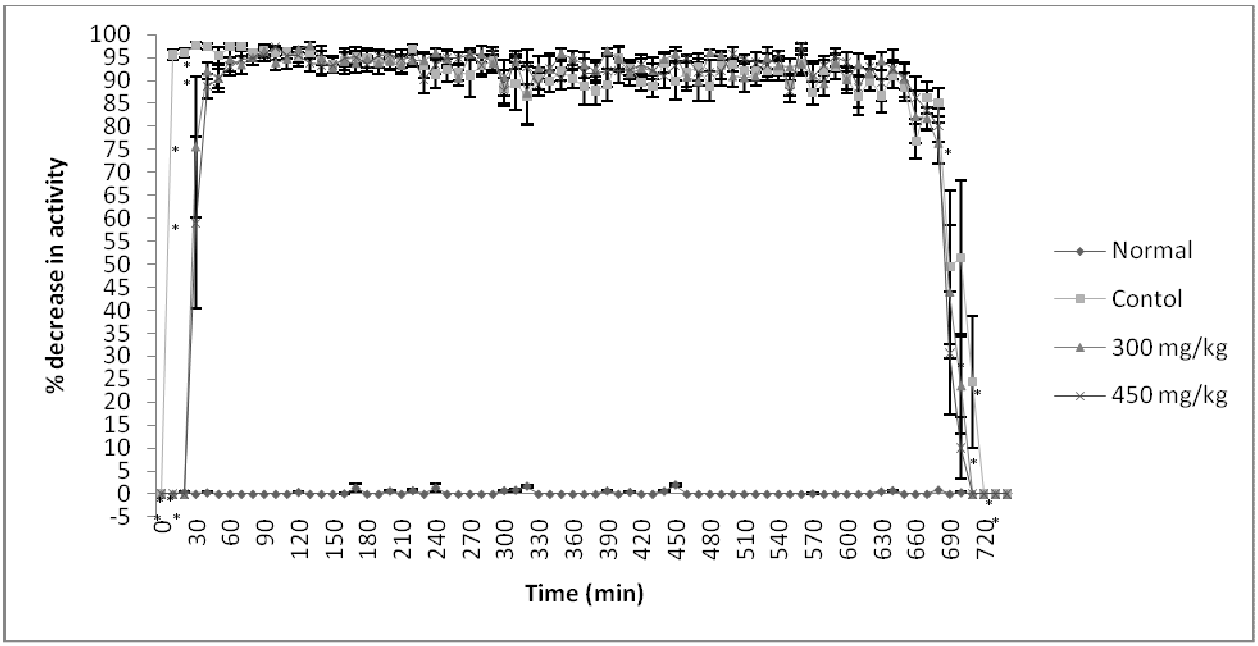

Figure 2: - Effect of different doses of OSE on time spent on Rota-rod for studying reduction in movement with respect to time in haloperidol induced Muscle rigidity in long term model in rats.

**- P<0.05 - Test was compared with control. (n = 6)*

Table 3: - Effect of ose on onset and duration of maximum response in chimney test (0.5mg/kg i.p.) – short term model in mice

Sr. No.	Groups	Overall muscle rigidity (min)		Maximum muscle rigidity (min)	
		Onset	Duration	Onset	Duration
1	Normal	0	0	0	0
2	Control	23.33±2.11$^{\#}$	183.33±3.33$^{\#}$	30$^{\#}$	161.66±1.66$^{\#}$
3	300mg/kg	41.66±1.66**	165±2.23**	51.66±1.66**	131.66±3.07**
4	450mg/kg	38.33±3.07**	166.66±3.33**	51.66±1.66**	141.66±1.66**

*# -P<0.01 - Control compared with Normal, *- P<0.05, **- P<0.01 - Individual readings were compared with readings of normal. (n = 6)*

Table 4: - Effect of ose on onset and duration of maximum responce in chimney test (4mg/kg i.p.) –long term model in mice.

Sr. No.	Groups	Overall muscle rigidity (min)		Maximum muscle rigidity (min)	
		Onset	Duration	Onset	Duration
1	Normal	0	0	0	0
2	Control	10$^{\#}$	618.33±4.01$^{\#}$	15±2.23$^{\#}$	590±5.16$^{\#}$
3	300mg/kg	28.33±3.07**	605±2.23**	38.33±1.66**	573.33±4.94*
4	450mg/kg	25±2.23**	611.66±3.07**	41.66±1.66**	565±4.28**

*# -P<0.01 - Control compared with Normal, *- P<0.05, **- P<0.01 - Individual readings were compared with readings of normal. (n = 6)*

DISCUSSION

Above results indicated that haloperidol induced muscle rigidity, which is prime characteristic of PD, in rat and mice. Further results also indicated significantly delayed onset and shortened duration with OSE_{aq} 300 in short term and long term model of catalepsy and both doses in short term and long term models of muscle rigidity. Such effects may be due to variety of reasons.

Angers et al. (1996) study showed that four Ocimum species, *O. basilicum*, *O. canum*, *O. gratissimum*, and *O. sanctum* contains 71-82% of linalool, a monoterpine found in most of the

aromatic species as major component of essential oils[4]. Linalool treatment in rat increased the secretion of adrenaline, noradrenaline and dopamine[41]. It was also found that 2.5 µg linalool admministration in rat led to increase in dopamine release from striatal slices by 3-fold as compared to basal levels[4].

Moreover OSE may shows similar activity with some plants like *Mucuna pruriens*[5] and *Vicia faba*[6] which contained precursors of dopamine and therefore exhibited anti-PD activity.

However our results, particularly reduction in activity at 600 mg/kg indicating dose independent activity, either rule out above hypothesis of dopamine release/ dopaminergic activity or indicate possibility of additional mechanisms responsible for antiparkinsonian activity by OSE_{aq}. Being aqueous extracts linalool may less but some other similar water soluble compound may be present causing dose independent activity. The active dose may stimulate other mechanism (other than modification in dopaminergic system), modulating the anti parkinsonian effects.

In conclusion, the present study for the first time showed that OSEaq significantly delayed the onset and reduced duration of haloperidol induced catalepsy in rat along with muscle rigidity as shown by changes in rota-rod movement and chimney test. However the effect was dose independent giving rise to speculation and warranting further studies on plausible mechanism.

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