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Effect of Acute and Chronic Administration of the Aqueous Extract of *Lawsonia Inermis* Leaves on Haloperidol Induced Catalepsy in Albino Mice

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disease characterized by selective loss of dopamine neurons of the *substantia nigra pars compacta*. The present study is carried out to investigate the effect of acute and chronic administration of aqueous extract of *Lawsonia inermis* Linn. leaves on haloperidol induced catalepsy in albino mice which is a useful animal model for screening drugs for PD. Catalepsy was induced by i.p. administration of haloperidol (1mg/kg). The aqueous extract at 100, 200 and 400 mg/kg b.w. were screened for its anticataleptic efficacy against haloperidol induced catalepsy in mice. The cataleptic score and superoxide dismutase (SOD) level were considered to correlate the levels of oxidative stress and degree of catalepsy. There is significant reduction in the cataleptic scores and increase in SOD activity was observed in *Lawsonia inermis* extract treated groups and maximum reduction was observed at a dose of 400mg/kg administered chronically. The study revealed significant anti-cataleptic activity of aqueous extract of *Lawsonia inermis* leaves. Hence the title plant can be used as an alternative agent in preventing and treating the extra pyramidal side effects of antipsychotic agents in clinical practice. However, further preclinical and clinical studies are required.

Key words: Catalepsy, haloperidol, scopolamine, *Lawsonia inermis* Linn.

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INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder. PD is observed in more than 1% of individuals over the age of 65 [1]. It affected approximately 5.2 million men and women worldwide, with 4-20 new cases reported per 1,00,000 people every year [2]. Parkinson's disease patients show age related decrease in the amount of brain neurotransmitter called dopamine. When the levels of dopamine continue to drop below 60 to 70 percent, the person will start to have Parkinson's disease symptoms. The production of toxic free radicals as by-products of cellular metabolism is one of the intrinsic factor which leads to neuronal degradation. There is currently no cure for PD and available therapies only treat the symptoms [1,3].

There is a widespread belief that the natural products are less toxic when compared to pure chemicals. Recent data suggests that 80% drug molecules are natural products or natural compound inspired [4]. The title plant *Lawsonia inermis* Linn. (Family: Lythraceae) commonly known as "Henna" have been used in traditional medicine in dysuria, jaundice, bleeding disorders, ulcers and other obstinate skin diseases. The plant is reported to contain carbohydrates, proteins, flavonoids, tannins, phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthones and fatty acids [5]. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products. The plant has been scientifically proved for antioxidant, antiulcer, hepatoprotective, wound healing, immunomodulatory, antibacterial, and anticancer properties [6-13].

Neuroleptic (Haloperidol) -induced catalepsy has long been used as an animal model for screening drugs for PD. In animal models, haloperidol induces a behavioural state known as catalepsy in which the animals are unable to correct externally imposed postures. The 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.

To best of our knowledge there is no scientific data regarding the anticataleptic activity of *Lawsonia inermis* Linn. is available. Hence, the present study was undertaken to evaluate the anticataleptic activity of *Lawsonia inermis* Linn. in mice.

MATERIALS AND METHODS

Chemicals

Haloperidol was procured from RPG Life Sciences Ltd., Mumbai, Scopolamine was procured from Cadila Healthcare Ltd, Goa. Sodium hydroxide, potassium dihydrogen phosphate, sodium carbonate and all other chemicals were of analytical grade were procured from High Media, Mumbai, India.

Plant material

The leaves of *Lawsonia inermis* Linn. were collected from the surroundings of Deralakatte, Mangalore, Karnataka, India, during May and was authenticated by Dr. Noeline J. Pinto, Head of Department of Botany, St. Agnes College, Mangalore. Dried and powdered leaves of *Lawsonia inermis* (100g) were extracted with 500ml of distilled water by the soxhlet apparatus [15]. The extract was concentrated using a rotary flash evaporator under reduced pressure to a syrupy consistency and then to dryness.

Preliminary phytochemicals screening

The preliminary phytochemicals studies were performed for testing different chemical constituents present in aqueous extract of *Lawsonia inermis* Linn. [16].

Experimental animals

Healthy Swiss albino mice weighing about 18 -22g between 2 and 3 months of age were used for the study. Animals were kept in the animal house of NGSM Institute of Pharmaceutical Sciences, Mangalore under controlled conditions of temperature (23±2ºC), humidity (50±5%) and 12 hrs light-dark cycle. Animals were fed pellet diet (Venkateshwara enterprises, Bangalore) and water *ad libitum*. All the animals were acclimatized for seven days before the study. The experimental protocol was approved by institutional animal ethical committee (approval number: KSHEMA/AEC/24/2011)

Acute toxicity test

Acute toxicity study for aqueous extract of *Lawsonia inermis* Linn. was carried out on mice according to OECD guidelines 425 [17].

Experimental design

For the evaluation of anticataleptic activity of *Lawsonia inermis* Linn. three dose levels 100 mg/kg, 200 mg/kg and 400 mg/kg were selected.

Haloperidol Induced Catalepsy: Catalepsy Bar Test: [18,19]

A. Acute study

Study comprises following groups of six animals each. (All observations made between 10:00 hrs and 16:00 hrs)

- Control group: received vehicle (1% gum acacia p.o.)
- Standard group: received scopolamine (1 mg/kg i.p. route)
- Aqueous extract of *Lawsonia inermis* Linn. (100 mg/kg p.o.)
- Aqueous extract of *Lawsonia inermis* Linn. (200 mg/kg p.o.)

- Aqueous extract of *Lawsonia inermis* Linn. (400 mg/kg p.o.)

Thirty minutes after administration of vehicle/drugs, haloperidol 1 mg/kg i.p. was administered to induce catalepsy. At 30, 60, 90 and 120 min after haloperidol administration, the degree of catalepsy was measured as the time the animal maintained an imposed position with both front limbs raised and resting on a four centimetre high wooden bar. The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. If the animal maintained the imposed posture for at least twenty seconds it was said to be cataleptic and given one point. For every, further twenty seconds that the animal continued to maintain the cataleptic posture one extra point was given. A cut-off cataleptic score of 60 was used during the recording of observations. After the recording of observations, the animals were returned to their individual cages and were maintained with a 12:12 h dark-light cycle for six more days. The same groups of animals were used for the chronic study.

B. Chronic Study

Similar treatments as in case of acute study for respective groups were continued for six more days. Catalepsy was again measured on the seventh day at 30, 60, 90 and 120 min post haloperidol administration. Soon after the behavioural study, the animals were sacrificed by cervical dislocation, and the superoxide dismutase (SOD) activity [20] of whole brain was estimated.

Statistical analysis

The data were expressed as mean ± standard error of the mean (S.E.M.) of 6 animals per group. Parametric one way analysis of variance (ANOVA) followed by Post Tukey test. Statistical analysis was performed using Graph pad prism 5.0. The minimal level of significance was identified at $P < 0.05$.

RESULTS

Phytochemical screening

The preliminary phytochemical screening of the aqueous extract of *Lawsonia inermis* Linn. revealed the presence of carbohydrates, flavanoids, saponins and tannins. (Table 1)

Acute toxicity study

The aqueous extract of *Lawsonia inermis* was found to be safe up to 2000mg/kg body weight by oral route. After 24 hrs animals were found well tolerated. There was no mortality and signs of toxicity after 24 hrs. Three dose levels i.e. 100mg/kg, 200mg/kg and 400mg/kg body weight were selected for the present study.

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Table 1: Qualitative analysis of aqueous extract of *Lawsonia inermis*.

S. No	Tests	Inference
1	Alkaloids a) Dragendorff's test b) Hager's test c) Wagner's test d) Mayer's test	 - ve - ve - ve - ve
2	Carbohydrates a) Anthrone test b) Benedict's test c) Fehling's test d) Molisch's test	 + ve + ve + ve + ve
3	Flavanoids a) Shinoda's test	 + ve
4	Glycosides a) Molisch's test	 - ve
5	Triterpenoids a) Liebermann – Burchard test	- ve
6	Resins	- ve
7	Saponins	+ ve
8	Steroids a)Liebermann -Burchard's test b)Salkowski reaction	 - ve - ve
9	Tannins	+ ve

Haloperidol Induced Catalepsy: Catalepsy Bar Test

A. Effect of aqueous leaf extracts of *Lawsonia inermis* on haloperidol induced catalepsy– Acute study

The cataleptic score was significantly reduced after 30 min. with both the standard drug scopolamine (1mg/kg, $P<0.001$) and the aqueous extract of *Lawsonia inermis* at the doses 200 and 400 mg/kg ($P<0.05$ and $P<0.01$ respectively). The group treated with the dose of 100 mg/ kg, showed the significant reduction ($P<0.05$) in cataleptic score at 90 min of observation. The reduction in cataleptic scores with 200 and 400 mg/kg was significant throughout the period of observations till 120 min. Maximum reduction in cataleptic activity was seen in the 400mg/kg extract treated group. (Table 2 and Figure 1)

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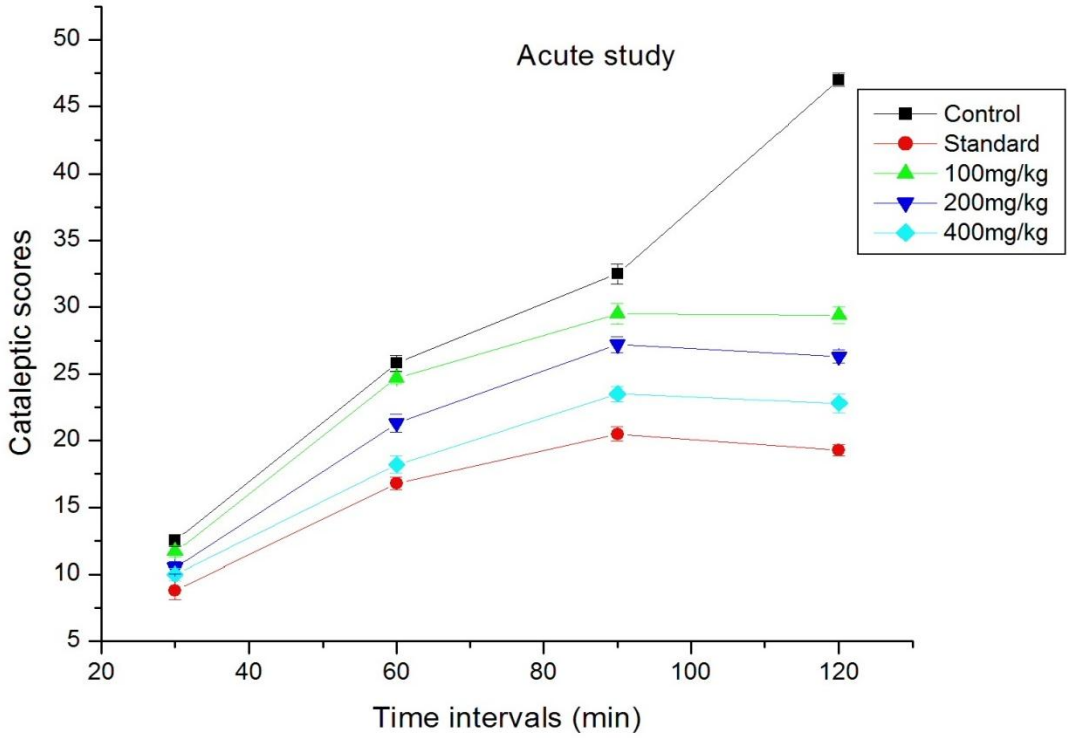

Figure 1: Effect of *Lawsonia inermis* on haloperidol induced catalepsy– Acute study

Table 2: Effect of *Lawsonia inermis* on haloperidol induced catalepsy– Acute study

Groups	Cataleptic scores at different time intervals			
	30 min	60 min	90 min	120 min
Control group (vehicle) + Haloperidol	12.5 ±0.43	25.8 ±0.60	32.5 ±0.76	47.2 ±0.48
Standard group (scopolamine 1 mg/kg) + Haloperidol	8.83±0.70***	16.80±0.48***	20.5 ±0.56***	19.3±0.42***
Aqueous extract of *Lawsonia inermis* 100 mg/kg + Haloperidol	11.7 ±0.42	24.7 ±0.43	29.5 ±0.76*	29.4 ±0.60**
Aqueous extract of *Lawsonia inermis* 200 mg/kg + Haloperidol	10.5 ±0.43*	21.3 ±0.67***	27.2 ±0.60***	26.3±0.49***
Aqueous extract of *Lawsonia inermis* 400 mg/kg + Haloperidol	10.0 ±0.45**	18.2 ±0.65***	23.50±0.56***	22.8±0.70***

The values are expressed as Mean ± SEM, n=6 mice in each group. Significance *P<0.05, **p < 0.01, ***P<0.001 compared to haloperidol treated group.

B. Effect of aqueous leaf extracts of *Lawsonia inermis* on haloperidol induced catalepsy– Chronic study

The standard drug scopolamine (1mg/kg) and aqueous extract of *Lawsonia inermis* at the higher doses (200 and 400 mg/kg) significantly (P<0.001) reduced the cataleptic score right

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from the start of the study. The group treated with the dose of 100mg/kg, P<0.001, showed significant reduction in cataleptic score only at 120 min of observation. In the chronic study, reduction in the cataleptic score was more compared to acute study. (Table 3 and Figure 2)

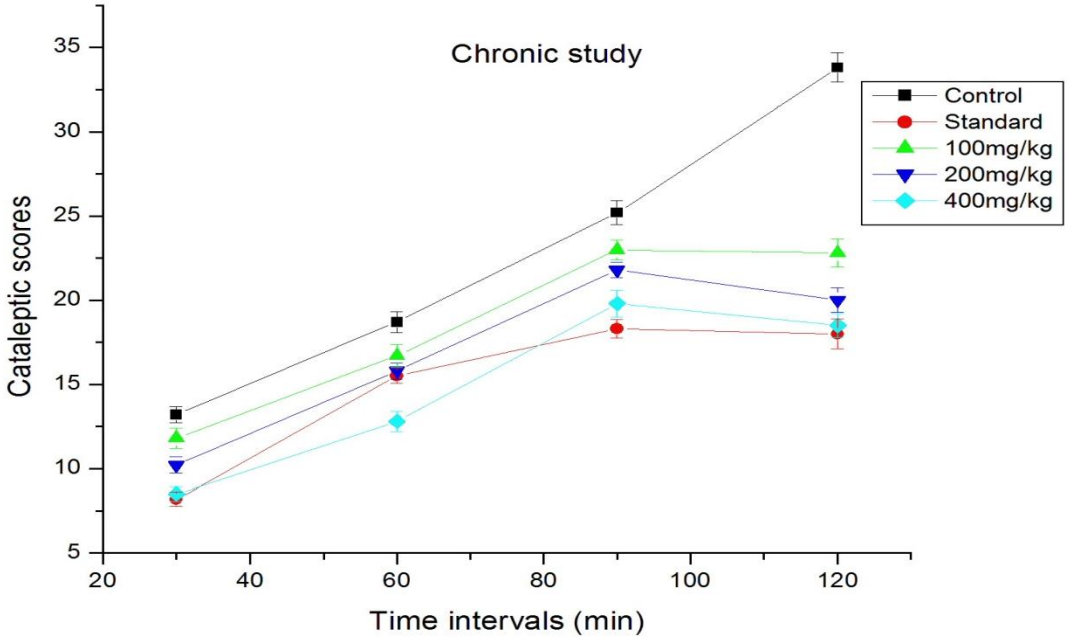

Figure 2: Effect of *Lawsonia inermis* on haloperidol induced catalepsy– Chronic study

Table 3: Effect of *Lawsonia inermis* on haloperidol induced catalepsy– Chronic study

Groups	Cataleptic scores at different time intervals			
	30 min	60 min	90 min	120 min
Control group (vehicle) + Haloperidol	13.2 ±0.48	18.7 ±0.62	25.2 ±0.70	33.8 ±0.87
Standard group (scopolamine 1 mg/kg) + Haloperidol	8.2 ±0.42	15.5 ±0.43***	18.3 ±0.56***	18.0±0.88***
Aqueous extract of *Lawsonia inermis* 100 mg/kg + Haloperidol	11.7 ±0.60	16.7 ±0.67	23.0 ±0.58	22.8 ±0.83***
Aqueous extract of *Lawsonia inermis* 200 mg/kg + Haloperidol	10.2±0.48***	15.8 ±0.48**	21.8 ±0.48***	20.0±0.73***
Aqueous extract of *Lawsonia inermis* 400 mg/kg + Haloperidol	8.50±0.43***	12.8±0.60***	19.80±0.79***	18.5±0.70***

The values are expressed as Mean ± SEM, n=6 mice in each group. Significance *P<0.05, **p < 0.01, ***P<0.001 compared to haloperidol treated group.

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C. Effect of aqueous leaf extracts of LI on SOD activity

The SOD activity in the brain tissue was found to be significantly decreased (P<0.001) in the haloperidol treated group as compared to normal group. The treatment of aqueous extract of *Lawsonia inermis* groups [100 mg/kg (P<0.05), 200 mg/kg and 400 mg/kg (P<0.001)] increases SOD activity significantly towards the normal levels. The maximum increase in SOD activity was seen in the group treated with the dose of 400 mg/kg which is comparable with standard scopalamine. (Table 4)

Table 4: Effect of *Lawsonia inermis* on SOD activity

Groups	SOD activity (units/mg protein)
Normal group	5.590±0.05
Control group (vehicle) + Haloperidol	2.560±0.08###
Standard group (scopolamine 1 mg/kg) + Haloperidol	4.872±0.06***
Aqueous extract of *Lawsonia inermis* 100 mg/kg + Haloperidol	2.854±0.05*
Aqueous extract of *Lawsonia inermis* 200 mg/kg + Haloperidol	4.140±0.05***
Aqueous extract of *Lawsonia inermis* 400 mg/kg + Haloperidol	4.510±0.04***

The values are expressed as Mean ± SEM, n=6 mice in each group. Significance *P<0.05, **p < 0.01, ***P<0.001 compared to haloperidol treated group. ###P<0.001 compared to normal group

DISCUSSION

Haloperidol induced catalepsy in rodents has been used as an animal model for screening drugs for Parkinsonism and it is a robust behavioural method for studying nigrostriatal function. In the present haloperidol induced catalepsy model, there is a blockade of postsynaptic striatal dopamine D1 and D2 receptors. Furthermore, cataleptic symptoms produced in rodents have been compared to the Parkinson's-like extra pyramidal side effects seen clinically with administration of antipsychotic drugs. In addition to various neurotransmitters, many preclinical and clinical studies have also proposed reactive oxygen species (ROS) as causes of haloperidol induced toxicity [18, 19, 21].

Under diseased conditions there is decreased activity of protective antioxidant enzymes like SOD, glutathione peroxidase (GSH- PX) and catalase in the brain leads to accumulation of oxidative free radicals which may resulting in neurodegenerative effects. Therefore an increase in these enzyme levels would represent increased antioxidant activity and a protective mechanism in neuronal tissue, thus constituting the first line of defence against oxidative stress induced damage in our body [22, 23].

The present study was designed to evaluate the anticataleptic activity of aqueous extract of leaves of *Lawsonia inermis* Linn. The preliminary qualitative phytochemical analysis of aqueous leaf extract showed the presence of flavonoids, saponins carbohydrates and tannins. The treatment of *Lawsonia inermis* Linn. shows significant decrease in the cataleptic score as well as increase in SOD activity in both acute and chronic study but in the chronic study, the reduction in the cataleptic score was more compared to those of acute study. The activities at higher dose of 200 mg/kg and 400 mg/kg are comparable with that of standard scopolamine.

So, decrease in cataleptic scores and increase in SOD activity in *Lawsonia inermis* Linn. treated groups indicates the ability of the extract to combat oxidative stress in the brain tissue and reduce the severity of haloperidol induced catalepsy in experimental animals.

CONCLUSION

Our findings confirm the anticataleptic activity of aqueous extract of *Lawsonia inermis* Linn. The study suggests that the test drug can be used as an alternative agent in preventing and treating the extrapyramidal side effects of antipsychotic agents in clinical practice.

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REFERENCES

[1] Standaert DG, Young AB. Treatment of central nervous system degenerative disorders. In: Brunton LL, Lazo JS, Parker KL, editors. Goodman and Gilman's the pharmacological basis of therapeutics. 11th ed, Mc Graw Hill, New York (NY), 2006. pp. 527.
[2] Parkinson's disease. Available from: URL:http://www.lundbeck.com/global/braindisorders/diseaseareas/parkinsonsdisease.
[3] Discovery could provide new treatment options for parkinson's disease. Available from: URL:http://www.elements4health.com/discovery-couldprovide-new-treatment-options-for-parkinsons-disease.html.
[4] Bhutani KK, Gohil VM. Ind J Exp Biol 2010; 48: 199-207.
[5] Borade AS, Kale BN, Shete RV. Int J Pharm Life Sci 2011; 2(1): 536-41.
[6] Kamal M, Jawaid T. Int J Biomed Res 2010; 1(2): 37-43.
[7] Hosein MHK, Zinab D. World J Dairy Food Sci 2007; 2(1): 38-41.
[8] Sravanthi P, Kumar KM, Begum DT, Kumar SN, Ravindrareddy K, Khanhare RS. J Pharm Res 2011; 4(6): 1872-4.
[9] Sanni S, Thilza IB, Ahmed MT, Sanni FS, Talle M, Okwor GO. Academia Arena 2010; 2(6): 87-9.
[10] Sakarkar DM, Sakarkar UM, Shrikhande VN, Vyas JV, Mandavgade S, Jaiswal SB, et al. Nat Product Rad 2004; 3(6): 406-12.

[11] Mikhaeil BR, Badria FA, Maatooq GT, Amer MA. Naturforsch 2004; 59: 468-76.
[12] Arun P, Purushotham KG, Jayarani JJ, Kumara V. Int J PharmTech Res 2010; 2(2): 1178-81.
[13] Zumrutdal ME, Ozaslan M, Tuzcu M, Kalender ME, Daglıoglu K, Akova A. Afr J Biotechnol 2008; 7(16): 2781-6.
[14] Rasheed AS, Venkataraman S, Jayaveera KN, Fazil AM, Yasodha KJ, Aleem MA, et al. Int J General Med 2010; 3: 127-36.
[15] Berenji F, Rakshsandheh H, Ebrahimipour H. Jundishapur J Microbiol 2010; 3(3): 125-8.
[16] Kokate CK, Purohit PA, Gokhale SB. Pharmacognosy Analytical pharmacognosy: a method of drug evaluation. 9th ed, Nirali Prakashan, Pune, 1998, pp. 92-3.
[17] OECD/OCDE. 2001; 425: 1-26. Available from: URL:http://www.oecd.org/dataoecd/17/51/1948378.pdf
[18] Gopalakrishna HN, Sudhakar P, Dorababu P, Pai MRSM, Colaco N, Vineetha V. J Clin Diagnos Res 2010; (4): 2134-8.
[19] Nair V, Arjuman A, Dorababu P, Gopalakrishna HN, Rao UC, Mohan L. Ind J Med Res 2007; 126: 480-4.
[20] Kono YA. Archives Biochem Biophy 1978; 186(1): 189-95.
[21] Aswar MK, Joshi RH. Int J Pharm Res Develop 2010; 2(6): 1-7.
[22] Arjuman A, Nair V, Gopalakrishna HN, Nandini M. Ind J Pharmacol 2007; 39(3): 151-4.
[23] Ahemad RS, Venkataraman S, Mohammed, Fajayveera KN. Int J Pharm Pharmaceut Sci 2012; 4(3): 323-7.