Mapana Journal of Sciences
2020, Vol. 19, No. 4, 37-48
ISSN 0975-3303|https://doi.org/10.12723/mjs.55.4

Assessment of Neuropharmacological Profile of Ethanolic Extract of *Lawsonia Inermis* Flowers

Syeda Nishat Fathima[*†], G Hemalatha[*], T Smitha[*], M Rama[*]

Abstract

On preliminary basis, the neuropharmacological profile of ethanolic extract of flowers of *Lawsonia inermis* was carried out. For assessing the activity of flowers on central nervous system locomotor activity using actophotometer, muscle relaxant activity using rotarod apparatus, pentobarbital-induced hypnosis and anticonvulsant activity by maximal electroshock test was performed. Ethanolic extract of flowers of *Lawsonia inermis* decreased the motor activity by 48.63 % and showed 49.03% noteworthy muscle relaxation along with 127.73% potentiation of pentobarbital-induced sleeping time and 66.67% decreased the duration of tonic hind leg extension of seizures activity. The results conclude that the extract of flowers of *Lawsonia inermis* has significant central nervous system depressant activity. Further investigations are, however, necessary to explore mechanism(s) of action involved in these pharmacological activities.

Keywords: *Lawsonia inermis,* central nervous system depressant, neuropharmacological profile, actophotometer

[*] Department of Pharmacology, Jayamukhi College of Pharmacy, Narsampet, Telangana; India
[†] syeda.nishu.fathima@gmail.com (Corresponding author)

1. Introduction

Lawsonia inermis Linn., is a perennial herbal medicinal plant commonly called as Henna belonging to the family Lythraceae grown as an ornamental and dye plant throughout India having different vernacular names in India viz., Mehndi in Hindi, Mendika, Rakigarbha in Sanskrit, Mailanchi in Malayalam, Muruthani, Alvanam, Aivani in Tamil, Benjati in Oriya, Mayilanchi in Kannada, Mehedi in Bengali and Goranta, Kormmi in Telugu [1]. Flowers on steam distillation gave an essential oil (0.02 %) rich in ionones (90 %) in which β-ionones predominated as their main phytoconstituents [2]. Several studies are being carried on the plant towards its activities like cytotoxic [3], hypoglycaemic [4], antimicrobial [5], tuberculostatic [6], wound healing [7], hepatoprotective [8], anti-inflammatory [9], analgesic and antipyretic [9], antibacterial [10], antifungal [11], nootropic [12], anti-parasitic [13], antifertility [14], antioxidant [15], immunomodulatory [15] and protein glycation inhibitory [16] activities. Traditionally *Lawsonia inermis* flowers are known to possess sedative hypnotic activity. The present study has been carried out to explore the activity of flowers of *Lawsonia inermis* on the central nervous system.

2. Materials and Methods

2.1 Collection of Plant Material

The flowers of *Lawsonia inermis* were collected from the medicinal garden of Jayamukhi College of Pharmacy during the months of July - September 2018 and reserved in the herbarium at the Department of Pharmacognosy bearing number 2018JCPN/12. The identification and the authentication of flowers of *Lawsonia inermis* were done in the Department of Botany, Kakatiya University, Warangal, Telangana, India.

2.2 Drugs and Chemicals

Diazepam procured from Ranbaxy Research Laboratories, India; Phenobarbitone sodium obtained from Rhone-Poulenc India Limited, India; Phenytoin procured from Cadilla Healthcare Ltd,

India and Tween 80 were obtained from S.D Fine Chemicals, India. All other reagents used for the experiments were of analytical grade.

2.3 Preparation of Plant Extract

The flowers of *Lawsonia inermis* were shade dried. The dried flowers of *Lawsonia inermis* were pulverized in an electrical processor and then the powder was separated. 50 gram of dried powder material was extracted in a soxhlet apparatus with 200 ml. of ethanol for 8 hours at 75^{o}C. The ethanolic extract was then distilled, evaporated and dried in the vacuum. The obtained extract was saddle brown in color with sticky constituency and extractive value was found to be 33.8% The extract was kept in desiccator and stored in a refrigerator for pharmacological experiment.

2.4 Animals Used

Female Albino mice, weighing about 25-30 grams were used in the experiments. Mice were accommodated in polypropylene cages with not more than three animals per cage and kept under standard condition (12 hours light / dark cycle; relative humidity 48%; temperature 25± 3^{o}C) and had free access to standard mice pellet diet (Hindustan Lever Ltd., India) and water *ad libitum*. All the animals were acclimatized to laboratory condition for 7 days before commencement of experiments. Mice were divided into three groups containing 6 mice in each and treatments were given according to the assessment that was carried out. The experimental procedures were reviewed and approved by Institutional Animal Ethical Committee (PCOL-201106/JCP/IAEC/2018, dated 21st July 2018) and experiments conducted according to CPCSEA.

2.5 Acute Toxicity Studies

A preliminary pharmacological study was carried out to evaluate the gross behavioral effects and safety effects of the flowers of *Lawsonia inermis* extract. The acute toxicity study was carried on mice weighing about 20-25gm as per ICH Topic S7A guidelines. Overnight fasted mice received the flower extract at a dose of 100mg/kg orally and mortality was observed for 14 days. If no death was observed for any mice, then the procedure was repeated

again with doses of 300 and 2000 mg/kg orally as per Section 4 of Organization for Economic Cooperation and Development (OECD) 423 guidelines [17].

The animals were observed continuously for 2 hours for behavioral, neurological and autonomic profiles along with the percentage of mortality observations that were tabularized according to Irwin's table [18]. For this the following checklist was utilized:

Stimulation: Hyperactivity, Piloerection, Twitching, Rigidity, Irritability, Jumping, Clonic convulsions, Tonic convulsions

Depression: Ptosis, Sedation, Loss of righting reflex (sleep), Loss of traction, Loss of Pinnal reflex, Catatonia, Ataxia, Loss of muscle rigidity, Analgesia.

Autonomic reflexes: Straub's tail, Laboured respiration, Cyanosis, Reddening, Abnormal secretions, balancing.

3. Methods Employed In Screening of CNS Activity

3.2 Assessment of Locomotor activity using actophotometer:

This activity was measured using an actophotometer which works on photoelectric cells, which were joined in circuit with a counter. When the beam of light falling on the photoelectric cell is interrupted by the mice, a count was recorded. This test can demonstrate a CNS depressant or stimulant activity profile of the drug. Animals were divided into three groups containing six mice each. The equipment was turned on and animals were placed individually inside the activity cage of the actophotometer for 10 min and basal activity score was noted. Group 1 was treated as the control and administered with 1ml of 1% Tween 80 by oral route; Group 2 was treated with ethanolic extract of flowers of *Lawsonia inermis* (500 mg/kg, oral route) and Group 3 was treated with standard (diazepam 4 mg/kg, intraperitoneal route) [19]. After 30 minutes mice were placed again in the Actophotometer for 10 min and the activity was checked. The difference in activity before and after treatment was noted and the percentage change in the activity was calculated. [20]

3.3 Assessment of muscle relaxant activity using rotarod Apparatus

The loss of muscle grip is a sign of skeletal muscle relaxation. The difference in the fall off time from the rotating rod between the control and treated animal was taken as an index of muscle relaxation. Before performing this experiment, the animals were trained to remain on rotarod apparatus (with the rod rotating at a speed of 25 rpm) for 3 minutes. Animals remaining on rotarod for 2 minutes or more in low successive trials were selected for testing; after training, the mice were divided into three groups of six mice each. Group 1 was treated as the control and was administered with 1ml of 1% Tween 80 by oral route; Group 2 was treated with ethanolic extract of flowers of *Lawsonia inermis* (500 mg/kg, oral route) and Group 3 was treated with standard (diazepam, 4 mg/kg intraperitoneal route). 30 minutes after treatment the same test was repeated once again. The fall off time from the rotating rod was then noted. The difference in the fall off time from the rotating rod between the control and the treated mice (standard-diazepam/extract) was taken as an index of muscle relaxation [21].

3.4 Assessment of pentobarbital-induced hypnosis

In this method, mice of either sex were randomly taken and divided into three groups containing six mice in each. Group 1 was treated as control and administered with 1ml of 1% Tween 80 by oral route; Group 2 was treated with ethanolic extract of flowers of *Lawsonia inermis* (500 mg/kg, oral route) and Group 3 was treated with standard (diazepam, 4 mg/kg intraperitoneal route). After 30 min mice received an intraperitoneal injection of pentobarbital sodium (40 mg/kg) [22]. The time between the loss and recovery of righting reflex was taken as that of sleeping time. The percentage effect of pentobarbital-induced hypnosis was calculated considering the right reflex in control as 100% [23].

3.5 Assessment of Anticonvulsant activity by Maximal electroshock test

Maximal electroshock test is the most widely used animal model in the antiepileptic drug discovery. Maximal electroshock produces

convulsions mainly by opening the voltage dependent sodium ion channels thereby triggering the repetitive firing of action potential. In brief, tonic convulsions of the hind extremities of the animals were induced by passing 50 Hz alternating electrical current of 150 milliamps for 0.2 seconds through corneal electrodes. Animals were divided into three groups containing six mice in each. Group 1 was treated as control and administered with 1ml of 1% Tween 80 by oral route; Group 2 was treated with ethanolic extract of flowers of *Lawsonia inermis* (500 mg/kg, oral route) and Group 3 was treated with standard (phenytoin, 25 mg/kg intraperitoneal route) for 15 days prior to the induction of seizures. The percentage protection from hind limb tonic extension seizure and the duration of seizure were recorded [24].

3.6 Statistical Analysis

Values were expressed as mean ± standard error of the mean. The Significance of differences among each group was assessed using one-way analysis of variance (ANOVA). The test followed by Dunnett's multiple comparisons test of significance. p values less than 0.05 were considered as statistically significant [25].

4. Results and Discussion

This study has been carried out to establish the central nervous system properties of ethanolic extract of flowers of *Lawsonia inermis*. For the screening of neuropharmacological profile of extract assessment of locomotor activity using actophotometer, muscle relaxant activity using rotarod apparatus, pentobarbital-induced hypnosis and anticonvulsant activity by maximal electroshock test was done.

Gamma aminobutyric acid (GABA) activity in the brain can be augmented by three approaches i.e., by GABA agonists; barbiturates and benzodiazepines which directly increase inhibitory chloride conductance and/or upregulate the effect of GABA release at synapse on the GABAA receptor respectively. Different drugs that are used in various psychological and neurological disorders possibly will modify the GABAergic system at the level of the synthesis of GABA, thereby inducing anxiolysis

or hypnosis in animals by potentiating the GABA mediated postsynaptic inhibition through an allosteric modification of GABA receptors, and thereby directly augmenting chloride conductance or indirectly by potentiating GABA-induced chloride conductance with simultaneous decrease of voltage activated calcium currents like barbiturates. As the result of calcium channel blockade, calcium entry into presynaptic nerve terminals is blocked, leading to inhibition of release of excitatory neurotransmitters such as glutamate. This results in net reduction of excitatory synaptic transmission thereby producing CNS depression. [26]

One of the important pharmacological actions of antianxiety agents of benzodiazepine class of drugs is central nervous system depressant effect along with muscle relaxing property. The skeletal muscle relaxant together with taming or calming effects, these agents decrease anxiety and tension. Locomotor activity indicates an index of wakefulness or alertness whereas loss of muscle grip on Rota rod shows muscle relaxation [27]. Ethanolic extract of flowers of *Lawsonia inermis* reduced the spontaneous locomotor activity from 48.63% when compared with control 4.27% (Table 1) and showed significant (49.03%) muscle relaxation (Table 2) which is an indication of CNS depressant property as like diazepam which acts by potentiating GABAergic inhibition triggering chloride channel opening with resulting membrane hyperpolarization.

Table 1: Effect of *Lawsonia inermis* extract on Locomotor activity in mice

Treatment	Dose	Locomotor activity observed for 10 min		Percentage change in activity
		Before treatment	30 minutes after treatment	
Control	1ml of 1% Tween	412 ± 5.74	366 ± 4.35	4.27
Extract	500 mg/kg	408±7.33	198 ± 7.34*	48.63*
Diazepam	4 mg/kg	426± 6.87	108 ± 6.24**	78.83**

Values are mean ± S.E.M. (n=6) One-way ANOVA followed by Dunnet's test. *P < 0.05 and **P < 0.01 when compared to control.

Table 2: Effect of *Lawsonia inermis* extract on muscle relaxant activity in mice

Treatment	Dose	Fall of time (sec)		Percentage change in activity
		Before treatment	After treatment	
Control	1ml of 1% Tween	86±3.72	89±3.61	3.98
Extract	500 mg/kg	78±6.21	30±6.33*	49.03*
Diazepam	4 mg/kg	83±5.63	14±4.33**	83.88**

Values are mean ± S.E.M. (n=6) One-way ANOVA followed by Dunnet's test. *P < 0.05 and **P < 0.01 when compared to control

Pentobarbital sleeping time test was also used to confirm the possible depressive-like effects. Decrease in sleep latency and increase in sleeping time are classically related to central nervous system depressant drugs. Further, the present results suggest that ethanolic extract of *Lawsonia inermis* flowers possesses CNS-depressant action. Extract showed marked potentiation of pentobarbital-induced sleeping time with significant decrease in the onset and prolongation of sleep duration induced by pentobarbitone which is indicated by the loss of righting reflex (Table 3). When compared with that of the control 27.73% effect was observed in the mice treated with extract [28].

Table 3: Effect of *Lawsonia inermis* extract on pentobarbital-induced hypnosis in mice

Treatment	Dose	Onset of action (min)	Duration of action (min)	% Effect
Control	1ml of 1% Tween	9.21±1.01	27.28±3.17	100
Extract	500 mg/kg	6.37±0.66*	39.33±4.63*	127.73
Diazepam	4 mg/kg	2.84±0.73**	54.61±3.23**	163.33

Values are mean ± S.E.M. (n=6) One-way ANOVA followed by Dunnet's test. *P < 0.05 and **P < 0.01 when compared to control

The MES test determines the electroconvulsive threshold and seizure magnitude in mice and mainly used for screening of drugs such as valproic acid and phenytoin which acts by voltage dependent blockade of sodium ion channels responsible for generation of action potential [29]. MES produced hind limb tonic extension seizures in all the animals used. The Ethanolic extract of

Lawsonia inermis at the dose of 500 mg/kg protected 66.67 % of mice and decreased the duration of tonic hind leg extension and altered the frequency of seizures elicited by Maximal Electroshock to a significant extent when compared with the control 33.33% (Table 4). The present result showed that *Lawsonia inermis* shows the anticonvulsant effect by blocking the frequency of voltage dependent sodium channel conductance there by preventing the repetitive firing of action potential.

Table 4: Effect of Lawsonia *inermis* extract on Maximal Electroshock Induced Seizures in mice

Treatments	Dose	Duration of HLTE (sec)	Quantal protection	% protection
Control	1ml of 1% Tween 80	19.53±0.29	4/6	33.33
Extract	500 mg/kg	13.36 ± 0.38*	5/6	66.67
Phenytoin	25 mg/kg	7.71±0.31**	6/6	100

Values are mean ± S.E.M. (n=6) One-way ANOVA followed by Dunnet's test. *P < 0.05 and **P < 0.01 when compared to control

5. Conclusion

From the present series of experiments, it can be concluded that the ethanolic extract of *Lawsonia inermis* flowers possessed positive locomotor depressant, skeletal muscle relaxant, sedative potentiating and anticonvulsant effects in the experimental rodent models indicating its significant depressant action on the central nervous system, as shown by these important neuropharmacological properties in Swiss albino mice. Purification of the plant extract and further definitive studies may reveal the exact mechanisms and constituents behind the observed neuropharmacological activities of *Lawsonia inermis* flowers.

References

[1] M. Singh, M. Kaur, C. B. S. Dangi and H. Singh, "Phytochemical & TLC Profile of Lawsonia Inermis (Heena)," International Journal for Pharmaceutical Research Scholars. 3(1):624-634 (2014).

[2] S. B. Amit, N. K. Babasaheb and V. S. Rajkumar, "A phytopharmacological review on Lawsonia inermis (Linn.)," International Journal of Pharmacy & Life Sciences. 2(1): 536-541 (2011).
[3] M. Ali and M. R, Grever, "A cytotoxic naphthoquinone from Lawsonia inermis," Fitoterapia. 69(2):181-183 (1998).
[4] Yamsudin and H. Winarno, "The effects of Inai (Lawsonia inermis) leave extract on blood sugar level: An Experimental Study" Research Journal of. Pharmacology. 2(2):20-23 (2008).
[5] F. Malekzadeh, "Antimicrobial activity of Lawsonia inermis", L. Appl. Microbiol. 16:663-664 (1968).
[6] V.K. Sharma, "Tuberculostatic activity of henna Lawsonia inermis Linn." Tubercle. 71(4):293-296 (1990).
[7] B.S. Nayak, G. Isitor, E. M. Davis and G. K. Pillai, "The evidence based wound healing activity of Lawsonia inermis Linn," Phytotherapy Research. 21(9):827-831 (2007).
[8] K. Hemalatha, H. N. Natraj and A.S. Kiran, "Hepatoprotective activity of leaves of Lawsonia alba", Indian Journal of Natural Product 20(4): 14-17. (2004)
[9] B. H. Ali, A. K. Bashir and M. O. M. Tanira, "Anti Inflammatory, antipyretic and analgesic effects of Lawsonia inermis L. (henna) in rats," International Journal of Experimental and Clinical Pharmacology. 51(6):356-363 (1995).
[10] P. Arun, K.G. Purushotham, J. Jayarani and V. Kumari, "In vitro Antibacterial activity and Flavonoid contents of Lawsonia inermis (Henna)," International Journal of PharmTech Research. 2(2):1178-1181 (2010).
[11] M. R. Natarajan and D. K. Lalitha, "Leaf extracts of Lawsonia inermis as antifungal agent," Current Science. 56(19):1021-1022 (1987).
[12] M. R. Iyer, S. C. Pal, V. S. Kasture and S, B. Kasture, "Effect of Lawsonia inermis on memory and behaviour mediated via Monoamine neurotransmitters," Indian Journal of Pharmacology. 30(3):181-185 (1998).
[13] T. Okpekon, S. Yolou, C. Gleye, et al., "Antiparasitic activities of medicinal plants used in Ivory Coast," Journal of Ethnopharmacology. 90(1):91-97 (2004).
[14] S. R. Munshi, T. A. Shetye and R.K. Nair, "Antifertility activity of three indigenous plant preparations," Planta Medica. 31(1):73-75 (1977).
[15] B. R. Mikhaeil, F. A. Badria, G. T. Maatooq and M. M. Amer, "Antioxidant and immunomodulatory constituents of henna leaves," Zeitschrift für Naturforschung C. 59:468-476 (2004).

[16] N. Sultana, M. I. Choudhary and A. J. Khan, "Protein glycation inhibitory activities of Lawsonia inermis and its active principles," Journal of Enzyme Inhibition and Medicinal Chemistry. 24(1):257-261 (2009).
[17] N. F. Syeda, S. Amreen, S. Anusha, and F. Somaiya, "Study of Antiasthmatic Activity of Ethanolic Extract of *Alternanthera Sessilis*," International Journal of Pharma Research and Health Sciences. 4 (6): 1284-1290 (2016).
[18] S. Irwin, "Comprehensive behavioral assessment: 1a A systematic quantitative procedure for assessing the behavioral and physiologic state of the mouse," Psychopharmacologia. 13(3):222-57 (1968).
[19] M. C. Prabhakar, "Experimental Pharmacology. Orient Longman Pvt Ltd.; Some Pharmacology Techniques," 51-61(2007).
[20] H. Zhang, J. Lu, Y. Zhang, Y. Zhao, J. Wei and L. Zhou, "Anticonvulsant and sedative effect of Fufang Changniu pills and probable mechanism of action in mice," Trop J Pharm Res. 5(6):1251-1257 (2016).
[21] N. W. Dunham and T. S. Miya, "A note on a simple apparatus for detecting neurological deficit in rats and mice," J Am Pharmaceut Assoc. 46:208–210 (1957).
[22] H. G. Vogel, "Drug Discovery and Evaluation-Pharmacological Assays. 2nd Edition. New York: Springer-Verlag Berlin Heidelberg. Chapter E Hypnotic activity," 495-496 (2002)
A. Ghorbani, H. Rakhshandeh and H. R. Sadeghnia, "Potentiating Effects of Lactuca sativa on Pentobarbital-Induced Sleep," Iran J Pharm Res. 12(2):401-406 (2013).
[23] E. A. Swinyard, "Electrically induced convulsions. In: Experimental Models of Epilepsy. A manual for the Laboratory Worker, In Purpura, D.P., J.K., Tower, D.B., Woodbury, D.M. and Walter, R.D. Eds. New York: Raven Press," 433 – 458 (1982).
[24] G. N. Dakhale, S.K. Hiware, A.T. Shinde and M. S. Mahatme, "Basic biostatistics for postgraduate students," Indian J Pharmacol. 44(4):435-42 (2012).
[25] U. Bhosale, R. Yegnanarayan, P.Prachi, M. Zambare and R.S. Somani, "Study of CNS depressant and behavioral activity of an ethanol extract of Achyranthes Aspera (Chirchita) in mouse model," Ann Neurosci. 18(2):44-7 (2011).
[26] H. Fujimori, "Potentiatiom of barbital hypnosis as an evaluation method for central nervous system depressants," Psychopharmacologia. 7:374-378 (1965).
[27] Y. Shi, J. W. Dong, J. H. Zhao, L.N. Tang and J.J. Zhang, "Herbal Insomnia Medications that Target GABAergic Systems: A Review of

the Psychopharmacological Evidence," Current Neuropharmacology. 12(3):289-302 (2014).

[28] S. N. Fathima, M. Rama and P. Ushasree, "Evaluation of Anticonvulsant Potential of Methanolic Extract of Stem Bark of Bombax Ceiba in Albino Mice," Asian Journal of Pharmaceutical Research and Development. 3(6): 1-7 (2015).