IJPSR (2016), Vol. 7, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 February, 2016; received in revised form, 05 April, 2016; accepted, 04 May, 2016; published 01 July, 2016

ANTICONVULSANT ACTIVITY OF THE METHANOLIC EXTRACT OF *LAWSONIA INERMIS* LEAVES IN ALBINO RATS

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Keywords:

Anticonvulsant Maximum electroshock Pentylenetetrazole *Lawsonia inermis*

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ABSTRACT: The aim of the study was to determine the anticonvulsant activity of methanolic extract of *Lawsonia inermis* leaves in albino rats. The anticonvulsant activity of methanolic extract of leaves of *Lawsonia inermis* (200 mg/kg and 400 mg/kg) was assessed in rats using maximum electroshock seizure (MES) test and pentylenetetrazole (PTZ) induced seizure test. The methanolic extract of *Lawsonia inermis* leaves significantly ($p<0.01$) reduced the hind limb tonic extension in the MES test in a dose dependent manner. In the PTZ model also, the extract signicantly ($p<0.01$) reduced the duration of clonic convulsions as well as delay the onset of seizures in a dose dependent manner. The study demonstrates that *Lawsonia inermis* has significant anticonvulsant activity possibly through a GABA-ergic interaction.

INTRODUCTION: Epilepsy is the second most common neurological disorder after stroke, affecting approximately 1% of the world's population. It is a heterogeneous symptom complex- a chronic disorder characterized by recurrent seizures.[1] Seizure is defined as a paroxysmal event which occurs due to abnormal, excessive hyper-synchronous discharges from aggregates of central neurons.[2] The incidence of epilepsy ranges from 40-70 per 100,000 in most developed countries and from 100-190 per 100,000 in developed countries. [3] Thus close to 80% of epilepsy cases worldwide are found in developing countries alone. India is home to about 10 million people with epilepsy with an overall prevalence of 1%. [4]

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(7).3068-72

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (7).3068-72

The introduction of anticonvulsant therapy has significantly contributed to the management of epilepsy. 60-70% of patients with epilepsy achieve control of their seizures with the use of conventional anti-epileptic drugs (AED). However seizure control is not achieved in nearly one-third of the epileptic patients, even with the continued use of AED. [5] Moreover the use of AEDs are associated with a vast array of side effects, dose-related and chronic toxicity as well as teratogenic effects. [6] As such, there is an increased need to discover drugs which are effective in refractory epilepsy and have lesser adverse effects. The use of medicinal plants, which are popular in developing countries, offer important sources of new chemical substances with potential therapeutic benefits.

Lawsonia inermis, commonly known as Henna/Mehndi, is a small branched, deciduous shrub or tree belonging to the family Lythraceae. It is found native in tropical and subtropical zones of North Africa and Southern Asia including India. The plant has been traditionally used for dyeing of

hair and in the treatment of epilepsy, jaundice, rheumatoid arthritis, leprosy and a multitude of other disorders.[7] The Charaka Samhita has described the use of extract of this plant in the treatment of epilepsy. The plant has been scientifically proven have antidiabetic, immunomodulatory, hepatoprotective, antioxidant, antibacterial, antifungal, molluscicidal, nootropic, analgesic and anti-inflammatory and cytotoxic activity. [8-15]

However the anticonvulsant activity has not been thoroughly investigated scientifically. Hence our present study has been undertaken to evaluate the anticonvulsant activity of *Lawsonia innermis* leaves.

MATERIALS AND METHODS:

Collection and preparation of plant extract:

The leaves of *Lawsonia inermis* were collected from Silchar distict, Assam, India. The plant material was authenticated by Mrs. Purnima Dutta Choudhury, Associate Professor, Department of Botany, Cachar College, Silchar, Assam. The leaves of *Lawsonia inermis* were washed with tap water and air dried at room temperature for 14 days. The dried leaves were then powdered mechanically with the help of a commercial grinder. The powder was then subjected to extensive extraction with the help of a Soxhlet apparatus using methanol (boiling temperature of 45ºC) as a solvent for 72 hours.[16] The extract was filtered through a Buchner funnel with Whatman number 1 filter paper and then evaporated to dryness with a rotary evaporator at a temperature of 50ºC. The extract obtained was then stored at 4ºC until further used as suspension in 2% gum acacia.

Drugs:

Phenytoin was obtained from Zydus Cadila Healthcare Limited, Diazepam obtained from Ranbaxy Laboratories, New Delhi and Pentylenetetrazol was obtained from Sigma Aldrich India, Bangalore.

Preliminary phytochemical screening:

The phytochemical screening of the methanolic extract of *Lawsonia inermis* leaves was performed by standard methods.[17]

Experimental animals:

Forty-eight albino rats (Rattus norvegicus) weighing 150-250 mg each of either sex were used in the experiments. The animals were housed in standard cages and maintained under standard conditions (12 hours light/dark cycle; 25 ± 3ºC). The animals were given standard diet of Bengal gram, maize, wheat and fasted overnight before the day of experiment. Water was given ad libitum. The animals were well acclimatized to laboratory conditions before commencement of experiments. The experiment protocol was duly approved by the Institutional Animal Ethics Committee.

Acute oral toxicity studies:

Acute oral toxicity study was done as per OECD guideline 423. A group of three Wistar rats of either sex selected randomly and were used for acute toxicity study. The extracts were administered orally at the dose level of 5 mg/kg body weight to the animals and observed for 14 days. Since no mortality was observed, the procedure was repeated for further higher doses of 50, 300 and 2000 mg/ kg body weight. The extract showed no mortality at doses upto 2000mg/kg. Hence $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected as the dose levels for the study.

Evaluation of antiepileptic activity:

Maximum electro shock (MES) induced seizure model:

Twenty-four albino rats were taken and divided into four groups containing 6 animals each. Group I served as control and received 2% gum acacia solution(10 ml/kg, p.o). Rats in groups II and III received methanolic extract of *Lawsonia inermis* (MELI) orally at the doses of 200 mg/kg and 400 mg/kg body weight respectively. Group IV received the standard drug phenytoin at a dose of 25mg/kg intraperitoneally. All drugs were administered 1 hour prior to induction of seizures by MES. After 1 hour, electric current of 150 mA for 0.2 seconds was administered through ear electrodes to induce convulsions in all the experimental animals with the help of a convulsiometer. The different phases of convulsions were noted down along with the duration of each phase. Abolition or reduction in the duration of hind limb tonic extensor (HLTE)

phase was taken as a measure of protection against MES induced seizures.[18]

Pentylenetetrazole (PTZ) induced seizure model:

Twenty-four albino rats were taken and divided into four groups containing 6 animals each. Group I served as control and received 2% gum acacia solution(10 ml/kg, p.o). Rats in groups II and III received methanolic extract of *Lawsonia inermis* (MELI) orally at the doses of 200 mg/kg and 400 mg/kg body weight respectively. Group IV received the standard drug diazepam at a dose of 4 mg/kg intraperitoneally. All drugs were administered 1 hour prior to induction of seizures by PTZ. After 1 hour, all the animals received convulsive doses of pentylenetetrazole (80 mg/kg) intraperitoneally. The animals were observed for 30 minutes after the administration of PTZ. The different parameters noted were the onset and duration of clonic convulsions. The anticonvulsant property was assessed by the ability to reduce the duration of clonic convulsions and increase the latency of seizures.[18]

Statistical analysis:

All the results were expressed as Mean ± SD. The statistical significance was analysed by performing one-way ANOVA followed by post hoc Dunnett's test. The difference was taken to be statistically significant at p value < 0.05.

RESULTS:

Phytochemical screening:

The preliminary phytochemical screening of the methanolic extract of *Lawsonia inermis* revealed the presence of carbohydrates, phytosterols, glycosides, alkaloids, saponins, tannins and flavonoids.

Effect of MELI on MES induced seizures:

The MELI at doses 200mg/kg and 400mg/kg did not completely abolish the hind limb tonic extensor (HLTE) phase as seen with phenytoin, however there was a significant (p<0.01) reduction in the duration of HLTE phase in a dose dependent manner. Phenytoin treated animals showed 100% protection against MES induced seizures whereas MELI 200 mg/kg and 400 mg/kg showed 48% and 70% protection respectively. (**Table 1**)

TABLE 1: EFFECT OF METHANOLIC EXTRACT OF *LAWSONIA INERMIS* LEAVES ON MES INDUCED SEIZURES.

Groups	Treatment	Flexion (sec)	Extensor (sec)	Clonus (sec)	% protection
Group I	2%gum acacia 10 ml/kg p.o	6.41 ± 0.58	12.16 ± 1.47	15.23 ± 0.50	0
Group II	MELI 200 mg/kg p.o	5 ± 0.70*	6.33 ± 0.75*	10.61 ± 0.46*	48
Group III	MELI 400 mg/kg p.o	3.71± 0.25*	3.58 ± 0.58*	9.38 ± 0.76*	70
Group IV	Phenytoin 25 mg/kg i.p	3.25 ± 0.52*	0 ± 0*	9.25 ± 1.08*	100

n = 6. Values are expressed as Mean ± SD. Statistical analysis done by one-way ANOVA followed by Dunnett's test. * p value < 0.01 when compared to control.

Effect of MELI on PTZ induced seizure:

The MELI at both the doses significantly (p< 0.01) increased the latency of seizures as well as reduced the duration of seizures in a dose dependent manner. With diazepam, a complete abolition of seizures was observed. The MELI 200 mg/kg and 400 mg/kg exhibited 42% and 74% protection respectively against PTZ induced seizures whereas 100% protection was observed with diazepam. (**Table 2**).

TABLE 2: EFFECT OF METHANOLIC EXTRACT OF *LAWSONIA INERMIS* ON PTZ INDUCED SEIZURE.

Groups	Treatment	Onset of clonic convulsions (sec)	Duration of clonic convulsions (sec)	% protection
Group I	2% gum acacia 10 ml/kg p.o	69.17 ± 5.84	279 ± 15.83	0
Group II	MELI 200 mg/kg p.o	124.33 ± 8.52*	161.67 ± 7.74*	42
Group III	MELI 400 mg/kg p.o	177.67 ± 10.83*	72.83 ± 18.12*	74
Group IV	Diazepam 4 mg/kg i.p	0 ± 0*	0 ± 0*	100

n = 6. Values are expressed as Mean ± SD. Statistical analysis done by one-way ANOVA followed by Dunnett's test. * p value < 0.01 when compared to control.

DISCUSSION: MES and PTZ are the most popular and widely used amongst seizures models. The MES test is the most common initial screening model for identification of anticonvulsant activity of drugs. The MES test corresponds to the generalized tonic clonic seizures or "grand mal" epilepsy in humans.[19] PTZ induced seizure test is an experimental model for generalized absence seizures and corresponds to human generalized seizures of myoclonic and petit mal type.[20]

In our study, it was found that treatment with MELI in rats significantly reduced the hind limb tonic extensor (HLTE) phase in MES induced seizure model. Current available antiepileptic drugs such as phenytoin, carbamazepine, phenobarbitone, valproate and lamotrigine, which are clinically effective in the management of generalized tonic clonic and partial seizures, all suppress the hind limb tonic extension in the MES model.[19, 20] Protection against HLTE indicates the ability of a test substance to inhibit or abolish the spread of seizure discharges within the brain. In our present study, the ability of the methanolic extract of *Lawsonia inermis* leaves to inhibit the HLTE in the MES model as compared to phenytoin (100% protection) suggests the presence of anticonvulsant compounds in the extract.

Similarly, it was found that treatment with MELI significantly reduced the duration of convulsions as well as increased the onset of convulsions in the PTZ seizure model. Although the exact mechanism of PTZ induced seizure is unknown, recent studies suggest that PTZ may cause seizure by inhibiting the chloride ion channel associated with gamma amino butyric acid type A ($GABA_A$ receptors).[18] PTZ has been shown to interact with GABA neurotransmission and drugs such as benzodiazepines and phenobarbitone, which enhance the $GABA_A$ receptor mediated inhibitory neurotransmission, can prevent PTZ induced seizures.[18,20] Hence, the ability of methanolic extract of *Lawsonia inermis* leaves to antagonize the PTZ induced seizure suggests a possible interaction with GABA-ergic neurotransmission.

Preliminary phytochemical analysis of *Lawsonia inermis* showed that the methanolic leaf extract contains flavonoids, saponins and phytosterols. Several studies have indicated that plants containing flavonoids and saponins have significant anticonvulsant activity. Many flavonoids and phytosteroids have been found to be ligands for the $GABA_A$ receptors and hence can act like benzodiazepine like molecules.[21, 22] Therefore, these phytoconstituents may be responsible for the anticonvulsant activity of *Lawsonia inermis*. However, further research work is needed to establish the active constituent(s) of the extract and the exact mechanism of action.

Thus, the present investigation establishes the anticonvulsant activity of *Lawsonia inermis* leaves and also suggests the possibility of a GABA-ergic interaction to be responsible for the observed effect.

ACKNOWLEDGEMENTS: We are thankful to Dr. P. Chakravarty, Associate Professor and Mr. B. Dewan, Technical Associate (PvPI) of the department of Pharmacology, Silchar Medical College and Hospital for their guidance in our study. We also thank Mrs. Purnima D. Choudhury, Associate Professor, Botany department, Cachar College, Silchar, for her help in taxonomical identification.

CONFLICT OF INTERESTS: Nil

REFERENCES:

1. Porter RJ, Meldrum BS. Antiseizure Drugs. In: Katzung BG, Masters SB, Trevor AJ(eds). Basic and Clinical Pharmacology, 12th edition.USA: McGraw Hill; 2012. p 403-426
2. Lowenstein DH. Diseases of the Central Nervous System: Seizures and Epilepsy. In: Longo, Fauci, Kasper, Hauser, Jameson and Loscalzo (eds). Harrison's Principles of Internal Medicine. 18th edition.USA: McGraw-Hill 2012; p. 3251-65.
3. Sander JW. The epidemiology of epilepsy revisited. Curr Opin Neurol 2003;16: 165-170.
4. Santhosh NS, Sinha S, Satishchandra P. Epilepsy:Indian perspective. Ann Indian Acad Neurol. 2014; 17(Suppl 1): s3-s11.
5. Brodie MR. Antiepileptic drug therapy the story so far. Seizure 2010;19(10): 650-655.
6. Swann AC. Major system toxicities and side effects of anticonvulsants. J Clin Psychiatry 2001;62(Suppl 14): s16-s21.
7. Dev S. A selection of prime Ayurvedic Plant Drugs, Ancient- modern concordance. Anamaya Publishers.New Delhi 2006: 276-279.
8. Chikaraddy A, Yasmeen M and Mannapur B. Hypoglycaemic activity of ethanol extract of Lawsonia

inermis Linn.(Henna) in alloxan-induced diabetic albino rats. Int J Pharm Bio Sci 2012;2(4): 287-292
9. Wiem A, Smail A, Wissem M, Faleiro M and Miguel M. Antioxidant, anti-inflammatory and anti-acetylcholinesterase activity of leaf, flower and seed aqueous extract of Lawsonia inermis from Tunisia. Int J Pharm Pharm Sci 2014;6(5): 445-452.
10. Selvanayaki R and Ananthi T. Hepatoprotective activity of aqueous extract of Lawsonia inermis against paracetamol induced rats. Asian J Pharm Res 2012;2(2): 75-77.
11. Habbal O, Hasson U, El-Hag AH, Al-Mashrooqi Z, Al-Hashmi N, Al- Bimani Z et al. Antibacterial activity of Lawsonia inermis Linn (Henna) against Pseudomonas aeruginosa. Asian Pac J Trop Biomed 2011;1(3): 173-176.
12. Rahmoun N, Boucherit- Otmani Z, Boucherit K, Benabdallah M and Choukchou-Braham M. Antifungal avtivity of Algerian Lawsonia inermis(Henna). Pharm Biol 2013;51(1): 131-135.
13. Singh A and Singh DK: Molluscicidal activity of Lawsonia inermis and its binary and tertiary combinations with other plant derived molluscicides. Indian J Exp Biol 2001; 39(3): 263-268.
14. Nesa L, Munira S, Mollika S, Islam M, Choin H, Choudhuri AU et al. Evaluation of analgesic, antiiflammatory and CNS depressant activities of methanolic extract of Lawsonia inermis barks in mice. Avicenna J Phytomed 2014;4(4): 287-296
15. Priya R, Ilavenil S, Kaleeswaran B, Srigopalram S and Ravikumar S. Effect of Lawsonia inermis on tumor expression induced by Dalton's lymphoma ascites in Swiss albino mice. Saudi J Biol Sci 2011;18(4): 353-357.
16. Bhat JU, Nizam Q, Aslam M, Parray SA, Asiaf A, Khanam R *et al*. Anticonvulsant activity of methanolic extract of Melissa parviflora in experimentally induced swiss albino mice. Int J Pharm Pharm Sci 2012; 4(2): 428-430
17. Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H. Phytochemical screening and extraction: a review. Internationale Pharmaceutica Sciencia 2011; 1(1): 98-106.
18. Kulkarni SK. Experiments on Intact Preparations. In: Hand book of Experimental Pharmacology. 4th edition reprint. New Delhi: Vallabh Prakashan 2013. p 142-146.
19. Castel-Branco MM, Alves GL, Fiqueredo IV, Falcao AC and Caramona MM.The maximum electroshock (MES) model in the preclinical assessment of potential new antiepileptic drugs. Methods Find Exp Clin Pharmacol 2009;31(2): 101-106.
20. Loscher W. Critical review of current animal models of seizures and epilepsy used in discovery and development of new antiepileptic drugs. Seizure 2011;20(5): 359-368.
21. Jager AK and Saaby L. Flavonoids and the CNS. Molecules 2011;16:1471-85
22. Singh D, Singh B,Goel RK. Role of saponins for the anticonvulsant effect of adventitious roots of Ficus religiosa. Pharm Biol 2012; 50(7): 816-22

How to cite this article:
Saikia A, Purkayastha A, Tigga R and Roy D: Anticonvulsant Activity of the Methanolic Extract of *Lawsonia Inermis* Leaves in Albino Rats. Int J Pharm Sci Res 2016; 7(7): 3068-72.doi: 10.13040/IJPSR.0975-8232.7(7).3068-72.