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(Research Article)

Anti-convulsant Activity of Hydroalcoholic Extract of *Lawsonia Innermis* Leaves in Mice

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ABSTRACT

Epilepsy is a disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness. In the present study, anticonvulsant activity of Hydroalcoholic extract of *Lawsonia innermis* (HAELI) leaves was evaluated against Pentylentetrazol (PTZ) and Isoniazid (INH) induced convulsions in Swiss Albino Mice. The parameters such as onset of convulsion, Straub tail, Extension of hind limb (EOHL) and occurrence of death was determined. The extract showed protective effect in PTZ and INH induced convulsions. In conclusion, the results of HAEI leaves show anticonvulsant effect in both PTZ and INH induced convulsion models and hence suggesting their possible depressant action in the central nervous system.

Key Words: Anticonvulsant, *Lawsonia innermis*, PTZ, INH, Epilepsy, Seizures.

INTRODUCTION

Epilepsy is a collective term for a group of chronic seizure disorders having in common, sudden and transient episodes of loss or disturbance of consciousness, usually but not always with a characteristic body movement and sometimes with autonomic hyperactivity. Incidence of epilepsy in developing country is 100 per 100,000. India is home to about 10 million people with epilepsy (prevalence of about 1%). It has been observed that the presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients¹. The conventional antiepileptic agents like Phenytoin, Carbamazepine and Sodium Valporate carry with them several serious side effects notably neurotoxicity². As majority of antiepileptic drugs are consumed life long, concomitant administration of other drugs predisposes to the risk of drug interaction. However, newer antiepileptics like Gabapentin, Vigabatrin, Lamotrigine, etc are used supplemental to the conventional agents³. Thus, it is necessary to investigate for an antiepileptic agent that is highly efficacious as well as safe in terms of drug related toxicity. Ethno-pharmacological research on natural products can contribute to the discovery of new, safe active compounds with novel structure that may serve as leads to the development of new antiepileptic drugs⁴. Several plants of the families Euphorbiaceae, Leguminaceae, Labiatae, Liliaceae, Gentianaceae, Solanaceae, and Umbelliferae are used for the treatment of epilepsy in Indian traditional medicinal system⁵.

Lawsonia innermis Linn belonging to family Lythraceae commonly known as Henna/Mehandi in India. The plant is reported to contain carbohydrates, proteins, flavonoids,

tannins and phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthenes and fatty acids. This plant has been described in Charaka Samhita for the treatment of epilepsy and jaundice, and for dyeing grey hair. In Sushruta Samhita, it has been recommended as a remedy for malignant ulcers⁶. The Ayurvedic Pharmacopoeia of India indicated the use of leaves in dysuria, bleeding disorder, prurigo and other obstinate skin diseases⁷. The leaves have a bitter bad taste; vulnerary, diuretic, useful in headache, hemicranias, lumbago, bronchitis, boils, ophthalmia, syphilitis, sores, amenorrhoea, scabies, diseases of the spleen; favour the growth of the hair⁸.

There is no earlier study present in the literature that has deciphered the anticonvulsant activity of *Lawsonia innermis*. Hence, the present study was carried out to study the anticonvulsant activity of *Lawsonia innermis* in mice⁹.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The leaves of *Lawsonia innermis* was collected from Ambajogai area of Maharashtra in the month of October and was authenticated by the Agharkar Research Institute, Pune (Authentication No.: 09-101).

Extraction of Leaves

The 200 gm of coarsely powdered form of dried leaves of *Lawsonia innermis* was subjected to exhaustive extraction in percolater apparatus using 70% aqueous ethyl alcohol. Then obtained extract were evaporated at 45°C, the semisolid

mass obtained was 46gm (% yield = 23%). The extract was stored in air tight container in refrigerator for further use¹⁰. The extract was converted into a suspension and used for experimental purpose. Suspension was prepared using carboxymethyl cellulose powder in distilled water.

Phytochemical Screening of the Extract

The extract of *Lawsonia inermis* was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids¹¹.

Experimental Animals

Swiss albino mice 20-25 gm were used for the experiments. All the animals were obtained from Animal House of R.D's College of Pharmacy, Bhor. All the protocols of animal experiments were approved by the Institutional Animal Ethics Committee in accordance to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), ministry of Social Justice and Empowerment, Government of India, New Delhi¹².

Albino mice used for this work were obtained from the Yash farm and National Toxicological Centre, Pune. The animals were housed in Poly propylene cages and maintained at 24°C ± 2°C under 12 h light/ dark cycle and were fed ad libitum with standard pellet diet and had free access to water. The animals were given standard diet supplied by Pranav Agro Industries Ltd. Sangli. The composition of the diet are Energy 3615 (Kcal/Kg), Crude Protein 22.05%, Crude Oil 4.5%, Crude Fibre 4.10%, Ash 11.10%, Sand Silica 0.75%¹³.

Acute Oral Toxicity Study (AOT)

Healthy adult Swiss mice (20-30 gm) were subjected to acute oral toxicity studies as per Organization for Economic Co-operation and Development (OECD) guidelines 2001 (AOT-423). Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. The changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor activity and behaviour pattern were noted (OECD guidelines, 2001)¹⁴.

Preparation of Drug Solutions

- 1) PTZ - (Dose: 60mg/kg i.p.): Prepared a stock solution containing 8mg/ml of the drug and injects 1ml/100gm of body wt. of mice.
- 2) Diazepam - (Dose: 4mg/kg i.p.): Prepared a suspension of Diazepam in 1% (w/v) gum acacia containing 0.4mg/ml of the drug and inject 1ml/100 gm of body wt. of mice.
- 3) INH- (Dose: 300mg/kg i.p.): Prepared sufficient quantity of stock solution of INH in gum acacia and inject 300mg/kg according to body wt. of animal¹⁵.

Experimental Design

PTZ induced Convulsions

Animals were randomly divided into four groups of six animal each (n=6). Group I- Control receives PTZ (60mg/kg i.p.) only, Group II- Standard receives Diazepam (4mg/g i.p.), Group III- HAELI 200mg/kg, and Group IV- HAELI 400mg/kg. The parameters such as onset of convulsion,

Straub tail, extension of hind limb and percent protection was determined¹⁶.

Isoniazid (INH) Induced Convulsions

Animals were randomly divided into four groups of six animal each (n=6). Group I - Control receives INH (300mg/kg i.p.) only, Group II- Standard receives Diazepam (4mg/g i.p.), Group III- HAELI 200mg/kg, and Group IV- HAELI 400mg/kg. The parameters such as onset of convulsion, Straub tail, extension of hind limb and percent protection was determined¹⁷.

STATISTICAL ANALYSIS

The values were expressed as mean ± SEM (n=6). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnet's test and P<0.05, P<0.01, and P<0.001 were considered to be statistically significant¹⁸.

RESULTS

Phytochemical Screening of the Extract

Phytochemical study of HAELI showed the presence of steroids, Triterpenoids, glycosides, flavonoids, proteins and carbohydrates¹⁹.

Acute Oral Toxicity (AOT) of AEAM

According to OECD guidelines for acute oral toxicity at the dose of 2000mg/kg, animals in the group treated with HAELI did not showed any symptoms of toxicity at this dose level and no mortality was observed during the 14 days of observational period. Hence, according to the guideline, the different doses of HAELI selected present study for per oral administration were 200 mg/kg (Middle dose) and 400 mg/kg (Upper dose)²⁰.

Effect of Hydroalcoholic Extract of *Lawsonia inermis* on PTZ induced convulsions

Animals treated with HAELI 200mg/kg showed significant effects P<0.05 on onset of convulsion and straub tail. Whereas animals treated with HAELI 400mg/kg showed significant effects on onset of convulsion (P<0.01), straub tail (P<0.05) and extension of hind limb (P<0.01). There is no onset of convulsion; straub tail and extension of hind limbs are seen in animals treated with Diazepam. Animals treated with HAELI 200mg/kg and HAELI 400mg/kg showed 66.66% and 83.33% protection respectively whereas Diazepam showed 100% protection in PTZ induced convulsions²¹(Table-1 and 3).

Effect of Hydroalcoholic Extract of *Lawsonia inermis* on INH induced Convulsions

Animals treated with HAELI 200mg/kg showed significant effects P<0.05 on straub tail and extension of hind limbs. Whereas animals treated with HAELI 400mg/kg showed significant effects on onset of convulsion (P<0.01), straub tail (P<0.05) and extension of hind limb (P<0.05). There is no onset of convulsion; straub tail and extension of hind limbs are seen in animals treated with Diazepam. Animals treated with HAELI 200mg/kg and HAELI 400mg/kg showed 50% and 66.66% protection respectively whereas Diazepam showed 100% protection in INH induced convulsions²²(Table-2 and 4).

DISCUSSION

A seizure reflects an imbalance between excitatory and inhibitory activity in the brain, with an increment of excitation over inhibition. Gamma amino butyric acid is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion, respectively²³.

PTZ induced convulsion is one of the most commonly used model for assessment of anticonvulsant effect of medicinal plants. PTZ is believed to be an antagonist of GABA-A receptor and produces convulsion by inhibiting the GABA pathway in the CNS responsible for hyper excitation of neurons leads to convulsion. Animals treated with HAELI showed significant effect on PTZ induced convulsion and it may be due to the correction of GABA partway in the CNS and hence control the hyper excitation of. Neurons occurred due to the induction of PTZ²⁴.

INH is an anti-tubercular drug which induces convulsion at higher doses by interfering with the synthesis of GABA through the inhibition of pyridoxal-5-phosphate, a cofactor for the Glutamic acid Decarboxylase (GAD), an enzyme those catalyses the synthesis of GABA from glutamic acid²⁵. Animals treated with HAELI showed significant effect on INH induced convulsion and it may correlate with improvement in GABAergic pathway in the CNS. Diazepam is commonly used anticonvulsant drug acts on beta subunit of GABA-A receptor and facilitate opening of GABA gated chloride channel which leads to avoid the hyper excitation of neurons and convulsions²⁶.

CONCLUSION

On the basis of the results obtained in this study we conclude that HAELI shows anticonvulsant activity in both PTZ and INH induced convulsion in mice.

Phytochemical tests carried out in the present study show that the HAELI contains saponins, tannins and flavonoids. The plants containing saponins or flavonoids exhibit anticonvulsant activity^{27, 28}. We may hypothesize that saponins and flavonoids present in HAELI might contribute to its anticonvulsant activity. Further research is warranted in which the pure isolated components of HAELI may be individually evaluated for their anticonvulsant activity to discover the exact mechanism accounting for its described activities.

Table 1: Effect of Hydroalcoholic extract of *Lawsonia innermis* on PTZ induced convulsions

| Groups | Treatment | Dose | Convulsions | | |
|--------|-----------|----------|-----------------|---------------|---------------|
| | | | Onset (Sec) | Straub Tail | EOHL |
| I | PTZ | 60mg/kg | 62.50 ± 7.81 | 40.17 ± 8.34 | 24.67 ± 5.20 |
| II | Diazepam | 4mg/kg | 0 | 0 | 0 |
| III | HAELI | 200mg/kg | 106.2 ± 13.13* | 17.83 ± 4.37* | 16.17 ± 3.82 |
| IV | HAELI | 400mg/kg | 121.8 ± 14.15** | 16.67 ± 5.35* | 8.00 ± 3.06** |

All values are reported as means ±SEM and were analyzed for statistical significance by one-way analysis of variance followed by Dunnett's test, The minimum level of significance considered was *p<0.05, **P<0.01.

Table 2: Effect of Hydroalcoholic extract of *Lawsonia innermis* on INH induced convulsions

| Groups | Treatment | Dose | Convulsions | | |
|--------|-----------|----------|-----------------|---------------|--------------|
| | | | Onset (Sec) | Straub Tail | EOHL |
| I | INH | 300mg/kg | 54.50 ± 7.62 | 28.50 ± 8.23 | 19.50 ± 4.75 |
| II | Diazepam | 4mg/kg | 0 | 0 | 0 |
| III | HAELI | 200mg/kg | 96.33 ± 17.81 | 10.33 ± 2.12* | 8.83 ± 1.13* |
| IV | HAELI | 400mg/kg | 131.5 ± 16.66** | 9.50 ± 3.23* | 6.66 ± 2.72* |

All values are reported as means ±SEM and were analyzed for statistical significance by one-way analysis of variance followed by Dunnett's test, The minimum level of significance considered was *p<0.05, **P<0.01.

Table 3: Percent protection of Hydroalcoholic extract of *Lawsonia innermis* on PTZ induced convulsions

| Groups | Treatment | Status of animals | | | |
|--------|------------------|-------------------|---------------|---------------|--------------------|
| | | Animals used | Animals alive | Animals death | Percent Protection |
| I | PTZ | 6 | 0 | 6 | 0 |
| II | Diazepam | 6 | 6 | 0 | 100% |
| III | HAELI (200mg/kg) | 6 | 4 | 2 | 66.66% |
| IV | HAELI (400mg/kg) | 6 | 5 | 1 | 83.33% |

Table 4: Percent protection of Hydroalcoholic extract of *Lawsonia innermis* on INH induced convulsions

| Groups | Treatment | Status of animals | | | |
|--------|------------------|-------------------|---------------|---------------|--------------------|
| | | Animals used | Animals alive | Animals death | Percent Protection |
| I | PTZ | 6 | 0 | 6 | 0 |
| II | Diazepam | 6 | 6 | 0 | 100% |
| III | HAELI (200mg/kg) | 6 | 3 | 3 | 50% |
| IV | HAELI (400mg/kg) | 6 | 4 | 2 | 66.66% |

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