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Evaluation of Anticonvulsant Activity of Leaf Extracts of *Ailanthus Glandulosa* (Linn) Planch in Experimental Animals

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A B S T R A C T

Ailanthus glandulosa (Linn). Planch has been used from long time in traditional medicine. The main objective of the work was to evaluate the anticonvulsant activity of *Ailanthus glandulosa*. The anticonvulsant activity of ethanolic extract of *Ailanthus glandulosa* leaves was evaluated using Pentylentetrazole [PTZ] as induced convulsions in mice and maximal electric shock (MES) induced convulsions and lithium-pilocarpine induced status epilepticus in mice. Preliminary phytochemical investigation of the ethanolic extract of *Ailanthus glandulosa* intrigifolia leaves are the presence of glycosides, saponins, quassinoids, steroid, amino acids, tannins, carbohydrates and flavanoids. The ethanolic extract (100 mg/kg and 300 mg/kg) delayed onset of [PTZ] as induced convulsions and also prolonged the onset of clonic convulsions in mice. The extract are protect the mice from (MES) induced convulsions. The extract also protected mice against seizures induced by lithium –pilocarpine. In lithium –pilocarpine models the ethanolic extract (100 mg/kg and 300 mg/kg) delayed the latency to rearing with forelimb clonus significantly. The results indicate that ethanol extract contained such phytochemical compounds which are active in case of *Pentylentetrazole* (PTZ) and Maximal electroshock (MES) and *lithium pilocarpine* induced status epilepticus, which support the ethno medicinal application of the plant as an anticonvulsant agent.

Keywords: *Ailanthus glandulosa*, Pentylentetrazole [PTZ], flavanoids, *lithium pilocarpine*

A R T I C L E I N F O

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1. Introduction

Epilepsy is a collective term for a group of chronic seizure disorder having in common, sudden and transient episodes (seizure) of loss or disturbance of consciousness, usually but not always with characteristic body movements (convulsions) and sometimes with autonomic hyperactivity. There are a number of synthetic anticonvulsant drugs currently available for use in the Management, control and treatment of individuals with epilepsy [1]. However, most of the synthetic drugs are not only inaccessible and unaffordable, but also possess many toxic adverse effects. Epilepsy is a common neurological disorder that demands immediate medical attention and, often, long-term therapy [2, 3].

In developed countries, annual new cases are between 40 and 70 per 100,000 people in the general population. This figure is often close to twice as high due to the higher risk of experiencing conditions that can lead to permanent brain damage. At the present day, six antiepileptic drugs, *gabapentin, lamotrigine, tiagabine, topiramate, vigabatrin* and *zonisamide*, have been used for the treatment of epilepsy. They have all been shown to be effective in short-term addon clinical trials in patients with uncontrolled epilepsy. Synthetic antiepileptic drugs are associated with side-effects, including teratogenicity, chronic toxicity and adverse effects, on cognition and behaviour [4, 5]. Therefore, there is a great need for the development of cheap, effective and safe anticonvulsant agents from plants and other sources.

In traditional systems of Ayurvedic medicine, *Ailanthus glandulosa is* a well known plant drug used for its Anti Epileptic property [6].*Ailanthus glandulosa* (Mill.) Swingle, (family: simaroubaceae) tree of heaven is used in traditional medicine for treatment of dysentery, gonorrhoea, haemorrhoids and a remedy for cough, gastric and intestinal upsets[7]. The leaves of *A. glandulosa* are prescribed to treat anomie, haemorrhage and spermatorrhea. It is also used as antispasmodic, antiasthma tic, cardiac depressant, astringent and for treatment of epilepsy [8]. Phytochemical studies on *A. glandulosa* have demonstrated the presence of flavanoids, glycosides, carbohydrates, amino acids, steroids, quassinoids, Saponins and tannins.

2. Materials and Methods

Plant material

The dried plants of *Ailanthus glandulosa* Linn were collected locally and received from Kurnool, Andhra Pradesh. Dr. C V S Bhaskars M.sc, PhD Govt. Venkatagiri Raja's College, authentified plant.

Preparation of Ailanthus glandulosa leaves extracts (AGLE)

Fresh leaves of Ailanthus glandulosa were collected and shade dried at room temperature. Dried leaves were powdered mechanically through mesh sieve. 100 g of freshly powdered leaves were evenly packed in soxhlet apparatus and the extraction was done 70% ethanol. The solvent was evaporated at low temperature under reduced pressure.

The Ethanol extracts of *Ailanthus glandulosa* leaves were subjected to the following investigations,

1. Preliminary phytochemical screening.
2. Pharmacological activities

a. Determination of acute toxicity (LD50)

b. Anticonvulsant activity

Experimental Animals

Male Wistar albino mice weighing between 20–30 g each were used for this experiment. They were procured from Sri Venkateswara Enterprises, Bangalore, India. They were housed in polypropylene cages and maintained at 27 ± 20^0C relative humidity 65 ± 10% under 12 h light/dark cycles. The animals were given a standard diet supplied by Ratnam institute of pharmacy Andhra Pradesh, India. The study protocol was approved from the Institutional Animal Ethics Committee constituted in accordance with the rules and guidelines of the CPCSEA (Committee for the purpose of Control and Supervision of Experiments on Animals), India.

Determination of LD50 of Leaf Extract of Ailanthus glandulosa

The acute toxicity of leaf extracts of *Ailanthus glandulosa* was determined by using albino mice of either sex weight between (20-30 g) maintained under standard conditions. The animals were fasted for 3 hr prior to the experiments. Animals were administered with single dose of either Ethanolic leaf extract of *Ailanthus glandulosa* and observed for its mortality up to 48 hr study period (short term toxicity). Based on the short-term toxicity profile, the next dose was decided as per OECD guidelines No 425. Since no mortality was observed upto dose 2000mg/kg From the LD50 dose, 100 mg/kg and 300 mg/kg doses were selected and considered as low and high doses respectively.

Experimental Design

Assessment of anticonvulsant activity

Treatment schedule

Albino mice were used to evaluate anticonvulsant activity. Mice were used in the Maximal electroshock induced seizures and *Lithium pilocarpine* induced status-epilepticus and mice were used in *pentylentetrazole*-induced convulsions. Animals were divided in four groups. One group received vehicle and second group received the reference standard and the two groups received EEAG (100 & 300 mg/kg)

Pentylentetrazole (PTZ) induced convulsion

60 min after above mentioned drug treatment clonic seizures were induced in mice by Intraperitonially (i.p) of 80mg/kg *Pentylentetrazole*. The latency to the onset of clonic convulsions in non-protected mice and lethality during the following 24 hour was recorded and compared with those of vehicle treated control mice to assess the anticonvulsant activity [9, 10, 11]. One group received *Diazepam 4* mg/kg - i.p. as a reference standard 30 min before *PTZ*. The animals were observed for onset of convulsion up to 30 min after *PTZ*. Each animal was then placed into individual plastic cages and were observed initially for 30 min and later up to 24 hrs. The following parameters were recorded during test session of initial 30 min and up to 24 hrs respectively: Latency (onset of

clonus), Onset of tonic-clonic convulsions, and Status of animal after 1hr.

Maximal Electro Shock Induced seizures (MES)

One group received *phenytoin* (25 mg/kg- p.o.) as a as a reference standard. Tonic clonic convulsions were induced by giving maximal electroshock seizures (MES) (30 mA for 0.2sec) using an electroconvulsometer (INCO, Ambala, India) ear electrodes', 60 minutes after administration of either vehicle or test drug dosese and 90 minutes after *Phenytoin* (25mg/kg-p.o.). The number of animals protected from tonic hind limb extension seizure (i.e. abolition of tonic hind limb extension within 10 sec after delivery of the electroshock was considered as protected mice) and duration of observed tonic hind limb extension seizure (HLTE) was recorded in each dose group [9, 12]. For recording various parameters, mice were placed in clear rectangular plastic cages with an open top, permitting full view of the animal's motor responses to seizure. In the pilot study various phases of convulsions, viz., tonic flexion, extension, clonus, stupor and mortality due to convulsions were selected as the parameters.

***Lithium pilocarpine* induced status-epilepticus**

Status epilepticus was induced by administration of *pilocarpine* (30 mg/kg i.p) 24 h after *lithium carbonate* (3mEq/kg i.p). The effect EEAG (each 100 & 300 mg/kg, p.o) was studied on the rearing with forelimb clonus by administering both extracts 30min.Before injection of pilocarpine [13]. *Diazepam* was used as a reference standard in a dose of 1 mg/kg i.p.

3. Results and discussions

Preliminary phytochemical studies:

In the preliminary phyto chemical screening , the ethanolic extract of Ailanthus glandulosa leaves extract gave positive for tests for glycosides , saponins, steroid, amino acids, quassinoids, tannins, carbohydrates and flavanoids. The residual extract was dissolved in sterile water and used in the investigation [14]. So, the anticonvulsant activity of ethanolic extract of plant in different dose levels (100mg/kg and 300mg/kg) was studied.

Acute toxicity study:

The Ethanolic extract produced effect of toxicity.

Assessment of Anticonvulsant Activity of *Ailanthus glandulosa* Leaves.

***PTZ* - Induced seizures**

Ailanthus glandulosa leaves were screened for anticonvulsant activity using *PTZ* induced convulsion model in mice. Study was conducted using low and high doses of EEAG (100 & 300 mg/kg respectively). The above mentioned doses were administered as mentioned earlier. It was observed that both low and high doses of EEAG exhibited a significant anticonvulsant exhibited a significant anticonvulsant effect. The ethanolic extract was found to be more effective. The standard drug *Diazepam* (4 mg/kg-i.p.) exhibited a significant anticonvulsant activity and offered 100% protection. The observations are given in Table 1.

MES Induced seizures

Ailanthus glandulosa leaves were screened for anticonvulsant activity using MES induced convulsion model in mice. Study was conducted using low and high doses of EEAG (100 & 300mg/kg) respectively. The above mentioned doses were administered as mentioned earlier. It was observed that both the low dose and high doses of EEAG exhibited a significant anticonvulsant effect as compared to control by reducing the duration of tonic extensor phase and tonic-clonic seizures. The standard drug *phenytoin* (25 mg/kg- p.o.) exhibited a significant anticonvulsant activity and offered 100% protection. The Observations are given in Table 2.

***Lithium pilocarpine* induced status-epilepticus**

Ailanthus glandulosa leaves were screened for anticonvulsant activity using *Lithium pilocarpine* induced status epilepticus model in mice. In vehicle treated group latency to forelimb clonus was observed at 20.36±0.69 min after *pilocarpine*. Study was conducted using low and high doses of EEAG (100 & 300 mg/kg-p.o. respectively). The above mentioned doses were administered as mentioned earlier. It was observed that both low and high doses of EEAG exhibited a significant anticonvulsant effect by showing significant delay in latency to rearing with forelimb clonus when compared to control group. The Ethanolic extract was found to be active extract. The standard drug *Clonazipam* (1mg/kg-i.p.) exhibited a significant anticonvulsant activity. The animals were normal in behaviour after 180 min. The observations are given in Table 3.

Discussion

Epilepsy is characterized by recurrent episodes of seizures. A seizure is due to abnormal discharges of some neurons in the brain. Anti epileptic drugs may have a stabilizing influence on neuronal membrane: prevent detonation of normal brain cells by the focal discharge, these drugs act only on those neurons with are firing repeatedly. Some drugs reduce low threshold Ca++ current and abolish absence seizures whereas some drugs increase GABA activity in the synapse causing neuronal inhibition hence antiseizure effect. GABA is the primary inhibitory neurotransmitter in the central nervous system (CNS).

Diminution of brain GABA level has been reported after PTZ. Diminution of brain GABA level has been reported after subconvulsive dose of PTZ [15]. Many plants having anticonvulsant activity are known to inhibit GABA transaminase activity thereby increasing brain contents of GABA. The MES test against generalized tonic clonic and cortical focal seizures and the PTZ test against absence seizure, while Lithium-pilocarpine was found useful in status epilepticus [14]. Pretreatment of *lithium* initiates limbic seizures after administration of subconvulsant doses of *pilocarpine* and other cholinergic agonist; still *lithium* does not have proconvulsant activities [16]. If *lithium* and *pilocarpine* administered concurrently it results in an accumulation of inositol monophosphate and reduction in cortical inositol that are about 10 times greater than the effects obtained with either drugs alone[16,17]. *Lithium-pilocarpine* induced convulsions have used to study the effect of *fluoxetine* on post- status epilepticus induced depression in mice. The study has shown that depression in epilepsy may have specific mechanisms and not only

altering serotonergic pathways. Serotonergic or cholinergic mechanisms may be responsible for inhibition of *lithiumpilocarpine*- induced convulsion [18].

Phenobarbitone, sodium valproate, diazepam and *trimethadione* prevent the limbic seizures induced in rats by *pilocarpine;* however, *phenytoin* and *carbamazepine* are ineffective [19]. *Lithium-pilocarpine*induced seizures were inhibited by blocking of serotonergic transmission and inhibition of post-synaptic 5-HT receptors [20]. On observation and reference to reported data from Phytochemical test Ethanolic of *Ailanthus glandulosa* leaves showed the presence of flavonoids, Glycosides, steroids, Carbohydrates, Amino acids and Tannins been implicated in various pharmacological actions on central nervous system including anticonvulsant and anxiolytic activity[21,22]. Flavonoids and sterols have been involved in central inhibitory and neuromodulatory effects [23]. The anticonvulsant activity may be due to the presence of flavonoids and sterols in the extracts. From t he above data it is concluded that *Ailanthus glandulosa* leaves possesses significant anticonvulsant activity against *pentylentetrazole*, *Lithiumpilocarpine* and against MES induced convulsions

Table 1: Effect of ethanolic extract of *Ailanthus glandulosa* Leaves on PTZ (80mg/kg-i.p) Induced convulsions in mice

Groups	Treatment[mg/kg]	Onset of first clonus[mg/kg]	No. of animals survived/used	Percentage mortality[%]
I	Vehicle control	221.13±8.42	0/6	100%
II	Diazepam 4 i.p	Absent	6/6	0%
III	EEAG 100 p.o	232.72±6.59	2/6	66.6%
IV	EEAG 300 p.o	272.16±5.26	1/6	83.33%

Values are mean ± SEM; n=6; One way analysis of variance (ANOVA) followed by Dunnet's test.
Where, ***P<0.001; EEAG: Ethanolic extract of *Ailanthus glandulosa*

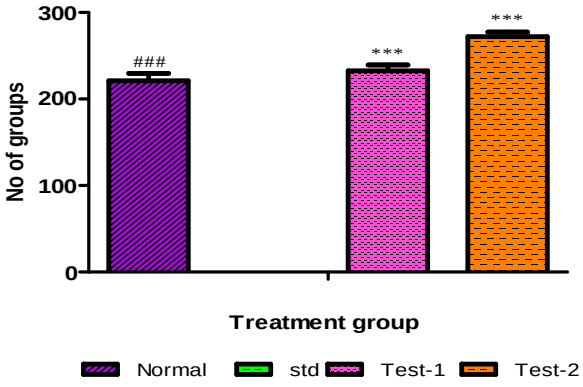


Table 2: Effect of ethanolic extract of *Ailanthus glandulosa* Leaves on MES induced convulsions in mice

Groups	Treatment[mg/kg]	Duration of hind limb extension [second]	Mice convulsed/ Mice used
I	Vehicle control	30.13±1.92	6/6
II	Phenytoin 25 I.P	4.3±0.31	0/6
III	EEAG 100 P.O	12.42±2.09	0/6
IV	EEAG 300 P.O	6.35±1.73	0/6

Values are mean ± SEM; n=6, NS non significant, One way analysis of variance (ANOVA) followed by Dunnet's test.
Where ***P<0.001; EEAG: Ethanolic extract of *Ailanthus glandulosa*

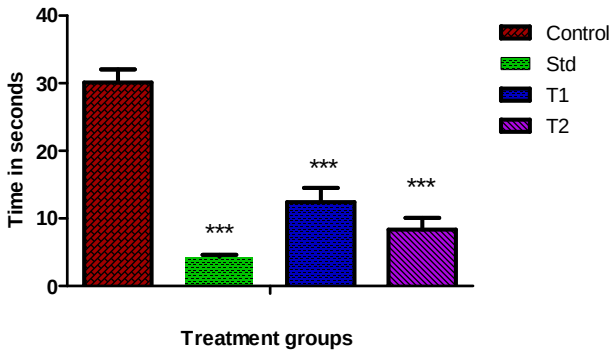
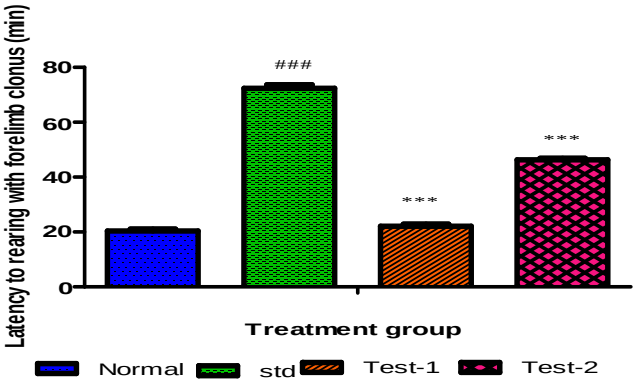


Table 3: Effect of Ethanolic extracts of *Ailanthus glandulosa* Leaves on *Lithium pilocarpine* Induced statusepilepticus

Groups	Treatment (mg/kg)	Latency to rearing with forelimb clonus (min)
I	Vehicle control	20.36±0.69
II	Diazepam 1 i.p	72.35±1.29
III	EEFG 100 p.o	22.13±0.68
IV	EEAG 300 p.o	46.29±0.53

Values are mean ± SEM; n=6, NS non significant, One way analysis of variance (ANOVA) followed by Dunnet's test, Where***P<0.001; EEAG: Ethanolic extract of *Ailanthus glandulosa*



4. Conclusion

The Ethanolic extract of *Ailanthus glandulosa* leaves is more potent for showing the anticonvulsant activity. The extract showed dose dependent effect .Further studies are required to find and isolate active principles and determine the mechanism of their anticonvulsant action, also our study suggests the application of *Ailanthus glandulosa* leaves in the treatment of convulsive disorders as a need of modern health science.

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