ANTICONVULSANT EFFECT OF ANNONA SQUAMOSA LINN. LEAVES IN MICE

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Summary
Anticonvulsant activity of Annona squamosa Linn. leaves extract and its combination with sub-effective and effective doses of diazepam and phenytoin was studied against maximum electroshock (MES), pentylenetetrazol (PTZ) and picrotoxin induced convulsions (PIC) in mice. The leaves extract of Annona squamosa Linn. at a dose of 250 and 500 mg/kg, p.o. demonstrated a significant dose dependent anticonvulsant effect against pentylenetetrazol and picrotoxin induced convulsion, while against MES no significant protection was observed. Further, subeffective dose of Annona squamosa Linn. extract potentiated the subeffective response of diazepam (1 mg/kg, i.p.). The present study clearly demonstrated that the leaves extract of Annona squamosa Linn. has anticonvulsant activity against pentylenetetrazol and picrotoxin induced convulsions. The study further concludes that it may be useful as an adjuvant therapy and can lower the potency and side effects of diazepam and phenytoin.

Keywords: Annona squamosa Linn., Neurotoxicity, Pentylenetetrazol, Maximal electroshock, Picrotoxin.
Introduction

Epilepsy is the condition of spontaneously recurrent seizures and is one of the major neurological disorders of the brain, affecting approximately 0.5-1.0% of the world population. Seizure is the characteristic feature in epilepsy and is associated with disordered and rhythmic high frequency discharge of impulses by a group of neurons in the brain. Abnormal cellular discharge may be associated with a variety of causative factors such as trauma, oxygen deprivation, tumors, infection and metabolic derangements. However, no specific factors are found in about half of patients suffering from epilepsy (1).

Annona squamosa Linn., Annonaceae, commonly known as sitaphal and custard-apple or sugar-apple, is a native of West Indies and is now cultivated throughout India, mainly for its edible fruit. The leaves contains several alkaloids (2) (annonaine, roemerine), flavanoids and acetogenins (3). Annona squamosa is reported to have numerous therapeutic uses viz: leaves extract has been used as antidiabetics, hypolipidemic, anticancer, expectorant and insecticidal agents.

The alcoholic extract of defatted seeds of Annona squamosa has been reported to possess some anticonvulsant activities in rats (4). However there is no study on the leaves. We therefore assessed the spectrum of anticonvulsant activities of ethanolic extract of leaves of Annona squamosa Linn. against pentylenetetrazol (PTZ), maximal electroshock (MES) and picrotoxin induced convulsions (PIC) in mice.

Materials and methods

Plant material and preparation of extract

The fresh leaves of Annona squamosa were collected and authenticated (RRI/BNG/SMP/Drug Authentication/ 2008-09/266) by Regional Research Institute (Ay.), Bangalore using standard description. The leaves were shade dried and grounded. The powdered material was then extracted twice using hydroalcoholic (30:70) solvent system in a soxhlet apparatus for 12 hrs. The extract was concentrated under reduced pressure using rotary evaporator.
and stored at 10°C (yield: 10%, w/w). The extract was reconstituted by dissolving it in 0.9% NaCl solution and then suspending the resultant solution in 0.5% tween 80 suspension freshly before use.

**Drugs and chemicals**

All standard chemicals used in this study were of analytical grade. Pentylenetetrazol and Picrotoxin were obtained from Sigma Chemical Company (USA), Phenytoin sodium from Cadila, Diazepam from Ranbaxy Laboratories, Tween 80 from Rankem labs, Ethanol from S.D.Fine Chemical Ltd.

**Animals**

Male swiss albino mice (*Mus musculus*), weighing 25-30 g procured from animal house, B.N.College of Pharmacy, Udaipur (Raj.). The animals were acclimatized for ten days under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C, relative humidity 65 ± 10% under 12 hours light/dark cycle. The animals were fed with commercial diet and water ad libitum. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC) with CPCSEA no. 870/ac/05/CPCSEA.

**Acute toxicity test of extract**

The acute toxicity test of extract in mice was estimated by p.o. routes (5). The extracts were administered at 10, 100, 1000 mg/kg in the first stage. When 100% mortality was induced at 10, 100, 1000 mg/kg, a second stage was tried in which doses were reduced. Conversely, when no mortality was induced at 10, 100, 1000 mg/kg, the dose was increased. Mice were kept under observation for the following 14 days and their weights registered, and at the end of the study a macroscopic evaluation was carried out.
Assessment of anticonvulsant activity (6)

**Pentylenetetrazol induced convulsions**

Pentylenetetrazol at a dose of 80 mg/kg, i.p. was given to eleven groups containing five animals in each pretreated 30 mins prior with varying i.p. doses and 60 mins prior with p.o. doses.

- Group I received 0.9% NaCl solution as control.
- Group II received diazepam (D1-0.5 mg/kg, i.p.)
- Group III received diazepam (D2-1.0 mg/kg, i.p.)
- Group IV received diazepam (D3-2.0 mg/kg, i.p.)
- Group V received diazepam (D4-4.0 mg/kg, i.p.)
- Group VI received AS 1 (A.squamosa)-125 mg/kg, p.o.
- Group VII received AS 2 (A.squamosa)-250 mg/kg, p.o.
- Group VIII received AS 3(A.squamosa)-500 mg/kg, p.o.
- Group IX received D1 + AS1
- Group X received D2 + AS2
- Group XI received D3 + AS3

Diazepam was taken as a reference standard drug. The latency to clonic convulsions was noted in all groups. All the extract treated groups were compared with control in order to determine the significant anticonvulsant activity.

**Picrotoxin induced convulsions**

Picrotoxin (8 mg/kg, s.c.) was injected into eleven groups (mentioned above) containing 5 animals in each, pretreated 30 min prior with varying i.p. doses and 60 min prior with p.o. doses. Onset of clonic convulsions was noted in all groups. All the extract treated groups were compared with control in order to determine the significant anticonvulsant activity.
Maximal electroshock induced convulsions

Maximal electroshock convulsion model was used to evaluate the anticonvulsant activity of the extract. Convulsion was induced in mice by transauricular electroshock of 50 mA for 0.2 s by means of convulsiometer (Inco, Ambala, India), through a pair of crocodile ear clips. Eight groups of mice (n=5) each pretreated i.p., with phenytoin (reference standard) were treated after 30 min and varying p.o. doses of extract treated after 60 min for MES seizure response. Duration of tonic hind limb extension was noted in all groups.

- Group I received 0.9% NaCl solution as control.
- Group II received Phenytoin (PH, 25 mg/kg, i.p.)
- Group III received AS 1 (A.squamosa)-125 mg/kg, p.o.
- Group IV received AS 2 (A.squamosa)-250 mg/kg, p.o.
- Group V received AS 3 (A.squamosa)-500 mg/kg, p.o.
- Group VI received PH1(10 mg/kg, i.p.) + AS1
- Group VII received PH2(15 mg/kg, i.p.) + AS2
- Group VIII received PH3(20 mg/kg, i.p.) + AS3

Statistical analysis

Results were expressed as mean ± sem. Comparison among groups were done by using ANOVA followed by Dunnett’s t-test. A statistical difference was determined for values p < 0.05.

Results

Toxicity assessment

The LD50 value of ethanolic extract of Annona squamosa was found to be 2.5 g/kg
Assessment of anticonvulsant activity

Pentylenetetrazol induced convulsions

In animals treated with vehicle, myoclonic jerks were observed at 41.512 ± 2.403 secs after PTZ administration and convulsions appeared at 74.646 ± 1.248 secs. All the animals died after seizures. The extract at a dose of 500 mg/kg, p.o. delayed onset of clonic seizures after PTZ administration (Fig. 1). The combination of subprotective doses of diazepam (1 mg/kg, i.p.) and hydroalcoholic extract of leaves of *Annona squamosa* L. (250 mg/kg, p.o.) showed significant delayed in the onset of clonic seizures.(Fig. 1).

![Graph showing effect of different doses of Annona squamosa L. and diazepam on onset of clonic seizures against PTZ induced convulsions.](image.png)

Fig. 1- Effect of different doses of *Annona squamosa* L. and diazepam on onset of clonic seizures against PTZ induced convulsions. (data are analysed by one-way ANOVA followed by Dunnett test, *p < 0.05).

Picrotoxin induced convulsions

The hydroalcoholic extract of leaves of *Annona squamosa* L. caused a dose (500 mg/kg, p.o.) dependent delay to onset of clonic convulsions in picrotoxin induced convulsion model. Here, the combination of subprotective doses of diazepam (1 mg/kg, i.p.) and hydroalcoholic extract of leaves of *Annona squamosa* L. (250
mg/kg, p.o.) showed significant delayed onset of clonic seizures. (Fig. 2).

Fig. 2- Effect of different doses of *Annona squamosa* L. and diazepam on onset of clonic seizures against PIC induced convulsions. (data are analysed by one-way ANOVA followed by Dunnett test, *p < 0.05).

**Maximal electroshock induced convulsions**

The leaves extract of *Annona squamosa* L. having dose of 500 mg/kg and the combination of subprotective dose of phenytoin (20 mg/kg) showed significant decrease in duration of tonic hind limb extension (Fig. 3)
Fig. 3- Effect of different doses of *Annona squamosa* L. and phenytoin on hind limb extension in MES induced convulsions. (data are analysed by one-way ANOVA followed by Dunnett test, *p < 0.05*).

**Discussion**

The search for novel pharmacotherapy from medicinal plants for neurological and psychiatric diseases has progressed significantly owing to their less side effects and better tolerability (7). The observation emanated in the present study provided the evidence in the favour of anticonvulsant activity of hydroalcoholic extract of leaves of *Annona squamosa* L. in PTZ induced convulsions in mice. The dose of 500 mg/kg, p.o. delayed onset of seizures and significantly suppresses the clonic convulsions induced by PTZ. Moreover, the combination of subprotective doses of diazepam (1 mg/kg, i.p.) and hydroalcoholic extract of leaves of *Annona squamosa* L. (250 mg/kg, p.o.) showed significant delayed the time to the onset of seizures which provides the information regarding potentiation of anticonvulsant activity of diazepam, which also acts on GABA binding site.

There was a significant (*P < 0.05*) decrease in the duration of tonic hind limb extension only at a dose of extract (500 mg/kg, p.o.) in MES model with maximum protection observed at 250 and 500 mg/kg, p.o., as compared to control group. The combination of subprotective doses of phenytoin (20 mg/kg, i.p.) and
hydroalcoholic extract of leaves of *Annona squamosa* L. (250 mg/kg, p.o.) showed significant decrease in the duration of tonic hind limb extension.

The hydroalcoholic extract of leaves of *Annona squamosa* L. caused a dose dependent delayed to the onset of clonic convulsions in picrotoxin induced convulsion model. Picrotoxin (PIC), is a potent, selective GABA \(_A\) receptor antagonist, produces seizures by blocking the effect of GABA at central GABA \(_A\) receptors, which have been associated with epilepsy (8).

Thus *Annona squamosa* Linn. can be very useful in treating seizures. Moreover, the potentiation of phenytoin and diazepam by the hydroalcoholic extract of leaves of *Annona squamosa* Linn. may be useful as an adjuvant therapy and can lower the potency and side effects of diazepam and phenytoin.

**References**