A STUDY OF ANTICONVULSANT ACTIVITY OF ALCOHOLIC EXTRACT OF LEAVES OF PASSIFLORA INCARNATA ON MICE

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ABSTRACT
Leaves of *P. incarnata* were powdered and subjected to successive extraction with solvents like ethanol, methanol and water using soxhlet apparatus. All the extracts were administered as suspension in water for injection in all the experiments. Preliminary phytochemical investigation of the AELPI (alcoholic extract of leaves of *Passiflora incarnata*) revealed the presence of alkaloids, glycoside and flavonoids. It was found to be non-toxic even up to the dose level of 2000mg/kg [LD50]. In PTZ induced convulsion models medium and high doses 200 and 400mg/kg. Anticonvulsant effect of high dose 400mg/kg was found to be better than the medium dose 200mg/kg. In MES induced convulsion model medium and high doses 200 and 400mg/kg but not the low dose 100mg/kg of AELPI had exhibited significant anticonvulsant effect by decreasing the duration of tonic-extensor phase and increasing the latency of clonus convulsion. Thus the result recorded with above experimental models confirms the anticonvulsant activity of AELPI. The present investigation revealed that, the AELPI possessed anticonvulsant activity.

Keywords: *P. incarnata leaf*, alcoholic extract, diazepam, PTZ, anticonvulsant activity.

1. INTRODUCTION

Epilepsy is a chronic noncommunicable disorder of the brain, occurs all over in all areas of the world and not less than three out of every thousand people – and in several places over 40 per thousand (4%) – are affected. As per the WHO, epilepsy is the commonest serious disorder of the brain, and epileptic fit or seizure is caused by brief, excessive and abnormal discharge of nerve cells in the brain, like a small "electrical storm" or "short circuiting" in the brain.

A majority of those with a seizure disorder (66 percent) were given a prescription for phenobarbital or phenytoin, and most (65 percent) of those with a psychiatric diagnosis who were given a prescription for an anticonvulsant received carbamazepine.

In day today life of stress and strain there is a dire need for agents having neuroprotective and neuropharmacological activity enhancing learning and memory caliber of the brain. Stress involves complex biochemical, neurological and immunological mechanisms and plays a crucial role in the genesis/progression of a variety of disease states ranging from psychiatric disorders like depression and anxiety, immunosuppression, endocrine disorders including diabetes mellitus, impotency and cognitive dysfunctions.

Anxiety related disorders such as generalized anxiety, panic, obsessive-compulsion, phobias or post traumatic stress disorders are common and major cause of disability and 1/8th of the total population worldwide affected with anxiety and became a very important area of research interest in psychopharmacology. Anxiety is also an obvious component of many psychiatric and medical conditions.

Traditionally pharmacological research in the area of anxiety and stress treatment is very much influenced by the availability of anxiolytic drugs. Throughout history recorded, ethanol was and is the standard drug for treatment of feelings of discomfort, tension, anxiety and stress.

Benzodiazepines (bdz) as anxiolytic agents have brought tremendous progress in understanding the physiological, biochemical and pathological status of the disease. However the use of tranquillizer and psychotropic drugs leads to variety of autonomic, neurologic and hematopoietic disorder, but these agents primarily relieve the symptoms and offer a palliative relief of a temporary nature.

In recent years use of alternative medicine in particular, derived from plant have been increased in a number of patient with condition that affect the mind.

In traditional system of Indian medicine (Ayurveda) *Passiflora incarnata* (fig 1) is widely used for its antipyretic, antispasmodic, nerve tonic and also for various ailments like, convulsions, paralysis and similar nervous complaints.

![Figure 1: Herb of *P. incarnata.*](image1)

**Figure 1:** Herb of *P. incarnata.*
2. OBJECTIVES OF THE STUDY

The main objective of the proposed work is to evaluate the anticonvulsant activity of a various extracts of *Passiflora incarnata*. The whole study is divided into two phases

**Phase I:**
- Preparation of various extracts with leaves of *P. incarnata* using soxhlet apparatus.
- Determination of LD$_{50}$ and dose selection for the study (i.e., selection of three doses 1/20, 1/10 and 1/5 from the LD$_{50}$ value) those considered as low, medium and high doses.

**Phase II:**
To evaluate anticonvulsant activity of the extracts in various experimental animal models like
- PTZ (Pentylenetetrazole) Induced convulsion model
- Maximal Electro Shock (MES) Induced convulsion model

It is also planned to evaluate the following parameters:
1. Number of seizures and tonic clonic convulsions in PTZ model.
2. Study of various phases of convulsions in MES model.

3. MATERIALS AND METHODS

### Table 1: Materials and equipments used during experiment

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Materials and equipments</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Diazepam</td>
</tr>
<tr>
<td>2</td>
<td>Phenytoin</td>
</tr>
<tr>
<td>3</td>
<td>Pentylenetetrazole</td>
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<tr>
<td>4</td>
<td>Distilled water</td>
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<tr>
<td>5</td>
<td>Electro convulsive meter</td>
</tr>
</tbody>
</table>

#### 3.2. Animals

Swiss albino mice of either sex weighing between 20-30g were procured for experimental purpose. All mice were maintained in an institutional animal ethical committee (IAEC) accredited facility under guidelines set forth by the Committee for the purpose of control and supervision of experiments on animals (CPCSEA), India. The protocol was approved and permitted by IAEC. All the animals were acclimatized for seven days under standard husbandry conditions i.e.; room temperature of 24$^\circ$ ± 1$^\circ$ C; relative humidity 45-55% and a 12:12h light/ dark cycle. The animals had free access to standard diet with water provided *ad libitum* under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress.

The alcoholic extract of *P. incarnata* was subjected to the following investigations:

1. Preliminary phytochemical screening.
2. Pharmacological activities
   - Determination of acute toxicity (LD$_{50}$)
   - Anti-convulsant activity
   - Anxiolytic activity

#### 3.3. Preparation of leaves extracts of *Passiflora incarnata*

Leaves powder of *P. incarnata* will be successively extracted with ethanol, methanol and water. Each time before extracting with the next solvent, marc will be dried in Hot air oven below 50$^\circ$C. Finally the marc will be macerated with chloroform water (i.e.; chloroform acts as a preservative) for 24 hrs to obtain the aqueous extract. Each extract will be concentrated by distilling off the solvent and then evaporating to dryness on the water bath.

#### 3.4. Preliminary phytochemical Screening

The preliminary phytochemical investigations will be carried out with the leaves extracts of *P. incarnata* for qualitative identification of phytoconstituents.

#### 3.5. Pharmacological Activities

3.5.1. Determination of LD$_{50}$ of seed extract of *P. incarnata*

The acute toxicity of *P. incarnata* will be determined by using albino mice of either sex (20-25 g), maintained under standard conditions. The animals will be fasted for 3 hr prior to the experiment. Animals will be administered with single dose of leaves extract of *P. incarnata* and observed for its mortality up to 48 hr study period (short term toxicity). Based on the short-term toxicity profile, the next dose will be determined as per OECD guidelines No 425. From the LD$_{50}$ dose 1/20, 1/10 and 1/5 $^{0.6}$ doses are to be selected and considered as low, medium and high dose respectively.

3.5.2. Determination of anticonvulsant activity

3.5.2.1. PTZ (Pentylenetetrazole) induced convulsions

Albino mice of either sex between 22-25g each group consisting of six animals will be divided into five groups.

- Group A - Normal control (PTZ 60 mg/kg, s.c)
- Group B - Standard (Diazepam 5mg/kg p.o)
- Group C - AELPI (100mg/kg p.o)
- Group D - AELPI (200 mg/kg p.o)
- Group E - AELPI (400 mg/kg p.o)
Experimental procedure

Albino mice of either sex with a body weight 22-25g will be divided into five groups of 6 animals in each. Group A will be served as control treated with PTZ 80 mg/kg, intraperitonially, Group B with diazepam (5mg/kg i.p). Groups C, D and E with three different doses of leaves extracts of *P. incarnata* (low, medium and high) for seven consecutive days. On the eighth day one hr after oral administration of the std/extracts in respective groups, PTZ 80 mg/kg is administered intraperitonially. Each animal was placed into individual plastic cage and were observed initially for 30min and later up to 24 hrs. The following parameters were recorded during test session of initial 30min and up to 24 hrs:

- Latency (onset of clonus)
- Onset of tonic-clonic convulsions
- Status of animal after 30 minutes
- Status of animal after 24 hrs
- Percent protection

The values were expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Dunnett’s- t-test test to calculate the significance difference if any among the groups. P<0.05 was considered significant.

3.5.2.2. Maximal electro shock (MES) induced convulsions

Albino mice of either sex weighing between 22-25g, each group consisting of six animals will be divided into five groups.

- Group A - Normal control (water for injection p.o)
- Group B - Standard (Phenytoin 25mg/kg p.o)
- Group C - AELPI (100mg/kg p.o)
- Group D - AELPI (200 mg/kg p.o)
- Group E - AELPI (400 mg/kg p.o)

Experimental procedure

Albino mice of either sex with a body weight 22-25g will be divided into five groups of 6 animals in each. Group A will be served as control treated with water for injection, Group B with diazepam (5mg/kg i.p). Group C, D and E with three different doses of leaves extracts of *P. incarnate* (100,200 and 400mg/kg) for seven consecutive days. On the eighth day one hr after oral administration of the std/extracts in respective groups, MES seizures will be induced by electroconvulsometer. A 60mA current will be delivered transvascularly for 0.2sec in mice via small alligator clips attached to each pinna. This current intensity elicited complete tonic extension of the hind limbs in control mice. For recording various parameters, mice will be placed in a clear rectangular plastic cage 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 will be selected as the parameters. Here phenytoin will be used as standard instead of diazepam.

The following parameters were recorded during 30min test session.

- Tonic flexion
- Tonic extension
- Clonus convulsions
- Percent protection

4. RESULTS

The values were expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using ANOVA followed by Dunnett’s-t-test test to calculate the significance difference if any among the groups. P<0.05 was considered significant. The results were shown in figure 2-5.

![Figure 2](image_url): Anti-convulsant activity of AEPI with PTZ induced convulsion in mice.

![Figure 3](image_url): Anti-convulsant activity of AELPI with PTZ induced convulsion in mice.

![Figure 4](image_url): Anti-convulsant activity with AELPI on MES induced convulsion in mice.
Anticonvulsant Activity

There are a number of synthetic anticonvulsant drugs currently available for use in the management, control and/or treatment of individuals with epilepsy. However, most of the synthetic drugs are not only inaccessible and unaffordable, but also possess many toxic adverse effects. There is, therefore, a dire need for the development of cheap, effective and safe anticonvulsant agents from plants and other sources.

In folk core medicine Passiflora incarnata leaves oil is used in the treatment of convulsions and paralysis. Based on the above data the alcoholic extract was prepared with leaves and studied for its anticonvulsant effect in different experimental animal models.

In most common forms of epileptic seizures, effective drugs appear to work either by promoting the inactivated state of voltage activated Na+ channels or enhance GABA mediated synaptic inhibition19.

Prevention of PTZ induced seizures in laboratory animals is the most commonly used preliminary screening test for characterizing potential anti-convulsant drugs20. The test is assumed to identify anticonvulsant drugs effective against generalized clonic seizures21, as PTZ produces clonic and tonic convulsions. It has been demonstrated that, a neural pathway subserving clonic PTZ convulsions is located in the forebrain while the brain stem is involved in the network of tonic PTZ induced convulsions22. The antiepileptic drug should abolish or increase the threshold for clonic and tonic convulsions. The mechanism by which PTZ exert its convulsant action is by acting as an antagonist at the GABAA receptor complex23.

Drugs offer protections against tonic–clonic seizures induced by PTZ are considered to be useful to control myoclonic and absence seizures in humans24. Various factors like age, sex, species, diet, water, day/night cycle, temperature, preparation dose and route of administration are known to affect the response of the animal to PTZ induced seizures25.

AEPI at medium and high doses (200 and 400mg/kg) but not lower dose had significantly increased the threshold for clonic and tonic convulsions and the Percentage protection against convulsions were 62.5%, 75% and 0% respectively as compared to control group. Standard drug diazepam (5mg/kg) had abolished the clonic and tonic seizures with intraperitonital injection of PTZ and offered 100% protection.

Diazepam acts through the activation of GABAA receptors and facilitate the GABA mediated opening of chloride channels. A dose depended activity has seen i.e., increase in the latency (onset) of convulsion as well as decrease in duration with tonic-clonic seizure threshold.

PTZ induced seizures suggest that, the extract of AEPI might have affecting GABA-ergic neurotransmission as PTZ has been shown to interact with the GABA Neurotransmitter26.

MES is also one of the commonly used models for preliminary testing of anticonvulsant drugs that produces generalized tonic-clonic seizures. i.e. hind limb tonic extensor (HLTE), tonic flexion and clonic convulsions.

In untreated animals a single MES produced an immediate tonic hind limb extension for 5-10 sec duration followed by clonic seizures27. Previous studies have reported that immediate to MES transmitters in vivo increase were over the 20-30 min post-ictal period28.

It has often been stated that antiepileptic drugs that block MES induced tonic extension act by blocking seizure spread, moreover MES induced tonic extension can be prevented either by drugs that inhibit voltage dependant Na+ channels (phenytoin, valproate)29,30 or by drugs that block glutaminergic excitation mediated by the N-methyl-D-aspartate (NMDA) receptor (felbamate)32,33.

AEPI at medium and high doses (200 and 400mg/kg) but not lower dose (400mg/kg) had significantly increased the duration of tonic extensor phase and onset of clonus as compared to control and thus exhibited anticonvulsant effect and the percent protection was 62.5%, 75% and 25% respectively. Standard drug (phenytoin 25mg/kg) had abolished the tonic extensor phase and showed 100% anticonvulsant effect by preventing seizure spread24. The percentage protection (Anticonvulsant effect) was found to be increased dose dependent.
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