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CNS Depressant and Antiepileptic Activities of the Methanol Extract of the Leaves of *Ipomoea Aquatica* Forsk

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Abstract: The central nervous system (CNS) depressant and antiepileptic activities of the methanol extract of the leaves of *Ipomoea aquatica Forsk* (IAF) were investigated on various animal models including pentobarbitone sleeping time and hole-board exploratory behavior for sedation tests and strychnine, picrotoxin and pentylenetetrazole-induced convulsions in mice. IAF (200 and 400 mg/kg, p.o.), like chlorpromazine HCl (1 mg/kg, i.m.), produced a dose-dependent prolongation of pentobarbitone sleeping time and suppression of exploratory behavior. IAF (200 and 400 mg/kg) produced dose-dependent and significant increases in onset to clonic and tonic convulsions and at 400 mg/kg, showed complete protection against seizures induced by strychnine and picrotoxin but not with pentylenetetrazole. Acute oral toxicity test, up to 14 days, did not produce any visible signs of toxicity. These results suggest that potentially antiepileptic compounds are present in leaf extract of IAF that deserve the study of their identity and mechanism of action.

Keywords: *Ipomoea aquatica* Forsk, Antiepileptic activity, Phenobarbital, Strychnine, Picrotoxin, Pentylenetetrazole.

Introduction

Epilepsy is an important health problem. Around 30,000 people develop epilepsy every year and the condition will affect about one person in 20 at some time during their lives. There are around 20 to 70 new cases of epilepsy per 100,000 people per year[1]. There are many classes of antiepileptics that are of clinical usefulness with good prognosis for controlling seizures in most patients[2]. Despite this, many patients, have seizures that are not adequately managed by the established antiepileptic drugs (AEDs)[3]. Moreover, the high incidence of detestable adverse effects from the use of AEDs is also a source of widespread concern in patients who use them chronically. These and treatment cost, have made traditional herbs

and herbalists very useful and indispensable in the struggle for seizure management and future AED development. There is therefore need for research into medicinal plants with possible antiepileptic effects; *Ipomoea aquatica Forsk* belongs to the family Convolvulaceae grows wild and is cultivated throughout Southeast Asia and is a widely consumed vegetable in the region. Many of the waters where IAF grows serve as recipients for domestic and other types of waste water. Water spinach is also supposed to possess an insulin-like activity according to indigenous medicine in Sri Lanka[4]. Only a very few scientific studies have been conducted on its medicinal aspects. These include the inhibition of effects on liver diseases[5], constipation[6]. IA is considered a tonic the species contains several vitamins, including A, B, C, E and "U" (S-methyl-methionine) and is used to treat gastric and intestinal disorders[7]. The species also contains aliphatic pyrrolidine amides, carotenoids, hentriacontane, β-sitosterol and its glycosides[8-11]. It is runner type plant with numerous small flowers[12]. The current study was undertaken to evaluate the CNS depressant and antiepileptic activity methanolic extract IAF by, till now no pharmacological evaluation has been done on IAF especially in leaf for its antiepileptic activity. This prompted us to pursue the activity and was examined for their efficacy and for determination of their possible mechanism of action.

Experimental

The fresh leaf's of IAF were collected from (Peranakkavur,Ramakrishna Mudaliar street, Changlepet, Tamilnadu, India) western Ghats of South India during June 2008. The plant was identified and authenticated by Dr.Sasikala Ethirajulu, Captain Sreenivasan research foundation, Chennai, Tamilnadu, India. The specimen voucher was deposited in the Department of Pharmacology and toxicology, C.L.Baid Metha College of Pharmacy, Chennai, Tamilnadu, India.

Preparation of the methanolic extract of IAF

The fresh leaf of IAF was collected and washed with running water. It was shade dried at room temperature and 1 kg of the dried leaf was made in to coarse powder. The powder was passed through a 60 No mesh sieve. The grounded power was extracted with methanol in water bath at room temperature. The solvent was then removed by filtration and fresh solvent was added to the plant material. The extract process was twice repeated. The combined filtrates were then evaporated under reduced pressure to give a dark green viscous mass. The extract was stored at 0-4 ˚C. The percentage yield was 19.5%w/w.

Phytochemical screening

The freshly prepared leaf extract of IAF was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Mayer's, Hager's and Dragendorffs reagent; Flavonoids with the use of sodium acetate, ferric chloride, amyl alcohol; Phenolic compounds and tannins with lead acetate and gelatin; carbohydrate with Molish's, Fehling's and Benedict's reagent; proteins and amino acids with Millon's, Biuret and xanthoprotein test. Saponins was tested using hemolysis method; Gum was tested using Molishs reagent and Ruthenium red; Coumarin by 10% sodium hydroxide and Quinones by Concentrated Sulphuric acid. These were identified by characteristic color changes using standard procedures[13].

These were identified by characteristic color changes using standard procedures.

The screening results were as follows: Alkaloids + ve; Carbohydrates + ve; Proteins and amino acids +ve; Steroids - ve; Sterols + ve; Phenols + ve; Flavonoids + ve; Gums and mucilage + ve; Glycosides + ve; Saponins – ve; Terpenes + ve and Tannins + ve Where + ve and – ve indicates the presence and absence of compounds.

Animals

Young adult Swiss albino ma;mice of either sex, weighing (18-20 g) were obtained from animal house of C.L.Baid Metha College of pharmacy, Chennai, Tamilnadu, India. Animals were kept in raised mesh bottom cages to prevent coprophagy. The animals were maintained in colony cages at 25±2 ^{o}C, relative humidity 50-55% maintained under 12:12 h light and dark cycle. The animals were fed with Standard animal feed (Hindustan Lever Ltd. Bangalore, India) and water ad libitum, animals were acclimatized to the laboratory conditions prior to experimentation. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).the study was approved by the Institutional Animal Ethical Committee**.** C.L.Baid Metha College of Pharmacy, Chennai, Tamilnadu, India.

Acute toxicity studies

Acute toxicity study was performed for the extracts to ascertain safe dose by acute oral toxic class method of Organization of Economic Co-operation and Development, as per 423 guidelines (OECD)[14].

Pentobarbitone induced sleeping time

Mice of either sex were randomly allocated to the different control and test groups (6 animals per group). They were treated with 200 and 400 mg/kg, methanolic leaf extract of *Ipomoea aquatica* Forsk (MEIA) pentobarbitone sodium (40 mg/kg, i.p.) was administered 30 min later. The control group received 10 mL/kg normal saline, i.p.15 min before 40 mg/kg, i.p. pentobarbitone. For positive control group; pentobarbitone (40 mg/kg, i.p.) was administered 15 min after chlorpromazine hydrochloride (1 mg/kg, i.m.)[15]. Onset of sleep was taken as the time when mice accepted the decubitodorsal position for three consecutive trials. Conversely, the duration was considered completed when mice did not accept the decubitodorsal position for three consecutive trials[16].

Exploratory activity

This study was carried out by the hole-board method using a white painted wooden board (40 cm×40 cm) with four equidistant holes (1 cm diameter×2 cm depth). Each mouse was placed at one corner of the board and the animal moved about and dipped its head into the holes indicating exploratory behavior. The number of dips in 7.5 min was recorded[17]. The test was carried out 30 min after oral treatment with MEIA at doses of 200 and 400 mg/kg or chlorpromazine (1 mg/kg, i.m.) to different groups of mice.

Strychnine induced seizures

Mice of either sex were randomly allotted to the different control and test groups. The control mice were administered with strychnine (4 mg/kg, i.m.) 30 min after normal saline (10 mL/kg,p.o.). The positive control group of mice received 4 mg/kg, i.m. strychnine, 15 min after phenobarbitone sodium (40 mg/kg, i.p.).Test group of mice received MEIA 200 and 400 mg/kg, p.o. 30 min before 4 mg/kg, i.m. strychnine. Onset to forelimb cloni and tonic seizures was recorded. Mice that did not convulse 30 min after strychnine administration were considered protected[18].

Picrotoxin-induced seizures

In this test, picrotoxin (6 mg/kg, i.p.) was used to induce seizure. Animals were treated with MEIA at dose of 200 and 400 mg/kg p.o., respectively, 30 min before picrotoxin. Phenobarbitone (10 mg/kg) was used as a standard drug. Seizure stage and seizure latency were the two parameters used to evaluate antiepileptic activity of the drugs[19.]

Pentylenetetrazole (PTZ)-induced seizures

Mice of either sex were randomly allotted to the different control and test groups. The control mice were administered with pentylenetetrazole (75 mg/kg, i.p.) 30 min after normal saline 10 mL/kg, p.o.). The positive control group of mice received pentylenetetrazole (75 mg/kg, i.p.), 15 min after phenobarbitone sodium (40 mg/kg, i.p.) Test group of mice received MEIA 200 and 400 mg/kg, p.o. 30 min before 75 mg/kg, i.p. pentylenetetrazole. Onset to forelimb cloni, as well as hindlimb extension (tonic convulsion) was recorded. The onset and number of death after showing tonic hindlimb extension were also recorded. Mice that did not convulse 30 min after pentylenetetrazole administration were considered protected[20].

Statistical analysis

All values are expressed as mean ±SEM. Data were analyzed by non-parametric ANOVA followed by Dunnett's multiple comparison tests, and other data was evaluated using Graph Pad PRISM software. A p-value <0.05 was considered significantly different.

Results and Discussion

MEIA produced significant (P < 0.05) and dose-dependent reduction in the onset and prolongation of sleep duration induced by pentobarbitone, MEIA at the dose of 200 and 400 mg/kg showed a significant prolongation of sleep duration, where as MEIA at high dose 400 mg/kg is more significant when compare of 200 mg/kg and the result is comparable to that produced by chlorpromazine (Table 1). Moreover, MEIA treated mice showed decreased exploratory activity showed decrease in the number of head dipping, in a dose dependent manner, MEIA at the dose of 400 mg/kg showed significant reduction in head dipping response when compare to 200 mg/kg , effect comparable to chlorpromazine (4 mg/kg, i.p.) (Table 2). Prolongation of pentobarbitone sleeping time, as well as suppression of exploratory behaviour indicates a central nervous system depressant activity of the extract.

Table 1. Effect of MEIA on pentobarbitone induced sleeping time in mice

Treatment, mg/kg	Onset to sleep±S.E.M., min	Sleeping time±S.E.M, min
Control: NS, 10 mL/kg, p.o.	8.1 ± 0.5	42.0 ± 0.9
MEIA, 200 mg/kg, p.o.	4.9 ± 0.2*	50.8 ± 0.6*
MEIA, 400 mg/kg, p.o.	3.2 ± 0.9*	55.6 ± 0.8*
Chlorpromazine, 1 mg/kg, i.m.	2.8 ± 0.5*	68.4 ± 0.3*

*NS: normal saline; MEIA: Methanol extract of Ipomoea aquatica Forsk. *Significant P < 0.05 compared to control, ANOVA; n =6*

Table 2. Effect of MEIA on exploratory behavior in mice

Treatment, mg/kg	Number of head-dips in 7.5 min.	
	Pre treatment mean±S.E.M	Post treatment mean±S.E.M.
Control: NS, 10 mL/kg, p.o.	12.2 ± 0.7	9.0 ± 0.1
MEIA, 200 mg/kg, p.o.	8.8 ± 0.2	5.0 ± 0.9*
MEIA, 400 mg/kg, p.o.	8.6 ± 0.1	2.9 ± 0.9*
Chlorpromazine, 1 mg/kg, i.m.	8.5 ± 0.4	1.0 ± 0.5*

*NS: normal saline; MEIA: Methanol extract of Ipomoea aquatica Forsk. * Significant P < 0.05 compared to control, ANOVA; n =6*

At 200 and 400 mg/kg, MEIA produced significant ($P < 0.05$) prolongation of both clonic and tonic seizure latencies, and MEIA at high dose 400 mg/kg, showed complete protection against strychnine (Table 3) and picrotoxin (Table 4) induced seizures. In the PTZ model, however MEIA at both doses 200 and 400 mg/kg did not increase onset to either clonic or tonic seizure, (Table 5). Prolongation of both clonic and tonic seizure latencies induced by strychnine and picrotoxin indicates MEIA extract posses promising antiepileptic activity.

Table 3. Effect of MEIA on strychnine-induced seizure in mice

Treatment, mg/kg	Seizure onset±S.E.M, min	
	Clonic.	Tonic.
Control: NS, 10 mL/kg, p.o.	6.6 ± 0.2	4.8 ± 0.5
MEIA, 200 mg/kg, p.o.	8.0 ± 0.2*	4.5 ± 0.5*
MEIA, 400 mg/kg, p.o.	NC	NC
Phenobarbitone, 40 mg/kg, i.p.	NC	NC

*NS: normal saline; MEIA: Methanol extract of Ipomoea aquatica Forsk. * Significant $P < 0.05$ compared to control, ANOVA; n =6*

Table 4. Effect of MEIA on picrotoxin-induced seizure in mice

Treatment, mg/kg	Seizure onset±S.E.M., min	
	Clonic.	Tonic.
Control: NS, 10 mL/kg, p.o.	7.8 ± 0.4	11.4 ± 0.9
MEIA, 200 mg/kg, p.o.	11.2 ± 0.9*	16.1 ± 1.1*
MEIA, 400 mg/kg, p.o.	NC	NC
Phenobarbitone, 40 mg/kg, i.p)	NC	NC

*NS: normal saline; MEIA: Methanol extract of Ipomoea aquatica Forsk. * Significant $P < 0.05$ compared to control, ANOVA; n =6*

Table 5. Effect of MEIA on pentylenetetrazole-induced seizure in mice

Treatment, mg/kg	Seizure onset±S.E.M, min	
	Clonic.	Tonic.
Control: NS, 10 mL/kg, p.o.	3.8 ± 0.2	7.6 ± 0.7
MEIA, 200 mg/kg, p.o.	3.9 ± 0.8*	7.9 ± 0.1*
MEIA, 400 mg/kg, p.o.	4.2 ± 0.5*	8.2 ± 0.6*
Phenobarbitone, 40 mg/kg, i.p.	NC	NC

*NS: normal saline; MEIA: Methanol extract of Ipomoea aquatica Forsk. *Significant $P < 0.05$ compared to control, ANOVA; n =6*

We studied the CNS depressant and antiepileptic activity of *Ipomoea aquatica Forsk* using Strychnine, Picrotoxin and PTZ induced seizure models and pentobarbitone induced sleeping behavior, in which both clonic and tonic seizure latencies and prolongation of sleep duration, exploratory activity measured at 200 and 400 mg/kg, MEIA. An important mechanism of Strychnine has been demonstrated to have a well defined mechanism of convulsant action reported to be by directly antagonizing the inhibitory spinal cord and brainstem reflexes of glycine[21] and thus increasing spinal reflexes. Picrotoxin, on the other hand, is a selective non-competitive antagonist of gamma amino butyric acid (GABA) at GABAA receptor, which has been widely implicated in epilepsy[22].

GABA is the major inhibiting neurotransmitter in the brain and its inhibition is thought to be an underlying factor in epilepsy[23]. Furthermore picrotoxin acts by blocking the action of GABA, but they differ, being competitive and non-competitive antagonists of GABAA receptor, respectively[24]. Ability of MEIA to inhibit clonic seizure in the PTZ test suggests that it may not have the ability to raise seizure threshold[25]. Essentially the effectiveness of a drug against PTZ seizure indicates its probable effectiveness against absence seizures[26]. PTZ has been reported to inhibit chloride conductance by binding to picrotoxin sites of GABAA receptor complex[27]. Based on this partial effectiveness, it is difficult to report MEIA as having antiepileptic effect against PTZ seizure; however, usefulness of a drug against neurotoxicity which normally causes death following extensive seizures by chemicals. Based on these results, it is therefore probable that the methanol leaf extract of *Ipomoea aquatica Forsk* has considerable antiepileptic action that might involve both GABAergic and glycinergic inhibitory mechanism. Preliminary phytochemical investigations of MEIA revealed the presence of carbohydrates, flavonoids, sterols, saponins, phenols and terpenes. The sedative effects of MEIA might therefore be due to any one or combination of these phytochemicals. Since MEIA exhibited anti-seizure activity, it might be clinically useful in the control of human epilepsies. Thus, we conclude that successive studies are mandatory to establish the precise nature of active constituents as well as their mechanism of action.

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