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RESEARCH ARTICLE

Evaluation of anticonvulsant activity of *Saraca asoca* flower (Roxb.) Wilde in Swiss albino mice

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ABSTRACT

Background: Epilepsy is a neurological disorder distressing a large scale of the inhabitants, which accounts for about 1% of the world's burden of diseases. A large number of groups called antiepileptic drugs are accessible to treat several types of seizures with the objective to decrease seizure incidence and ruthlessness within an outline of an acceptable level of side effects, search for an alternative antiepileptic from natural source, i.e., herbal remedies, which were used traditionally, and safe on human health, is gaining global attention. **Aims and Objectives:** The objective of the study is intended to evaluate the antiepileptic activity of *Saraca asoca* flower (Roxb.) Wilde extract of *S. asoca* flower (ESAF) in Swiss albino mice. **Materials and Methods:** Ethanolic ESAF was prepared by a continuous method using Soxhlet apparatus with respected temperature. EASF in the doses 50, 100 mg/kg bodyweight along with valproate was administrated to albino mice by oral route followed by antiepileptic activity was evaluated by maximal electroshock (MES) and pentylenetetrazole (PTZ)-induced seizure models. Abolition of tonic hind limb extension (THLE) phase and increase in seizure latency period, when compared to control group, were taken as a measure of protection in MES- and PTZ-induced convulsion model, respectively. The results are expressed as mean ± standard error of the mean. Statistical analysis was done by one-way analysis of variance test followed by *post hoc* Dunnett's multiple comparison tests. $P < 0.05$ was considered statistically significant. **Results:** ESAF in the dose of 50 and 100 mg/kg bodyweight showed significant antiepileptic property in both MES and PTZ seizure models. There was significant abolition of THLE phase in MES model. There was also significant increase in seizure latency in PTZ-induced seizure model. **Conclusion:** ESAF possesses significant antiepileptic activity. Further, investigations are required to determine its active constituents and also its mechanism of action.

KEY WORDS: *Saraca asoca* Flower; Maximal Electroshock; Pentylenetetrazole; Anticonvulsant; Immobility

INTRODUCTION

Epilepsy, which has a high prevalence among people of all ages, is a serious and diverse set of chronic neurologic disorders. It is characterized by paroxysmal cerebral dysrhythmia, manifesting as a brief episode (seizures) of loss or disturbance of consciousness, with or without characteristics body movements (convulsion) with sensory or psychiatric phenomenon.[1] Epilepsy is most frequently diagnosed in infantile and in peoples over 60 years of age, but it can affect anyone.[2] It has been estimated that 50 million people worldwide live with epilepsy and that >85% of this disease occurs in low- and middle-income countries.[3,4]

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Seizures can be defined as brief episodes of signs and symptoms due to abnormal, excessive synchronous neuronal activity in the brain. Epilepsy can be idiopathic or secondary to infection, neoplasm, or head injury. In some case, it may be hereditary.[5]

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Epilepsy is a major public health issue in many nations. Despite the massive scale of the problem and much research undertaken therapy of epilepsy remains poorly understood.[6]

Most therapeutics currently used in the treatment of epilepsy is either directed toward blocking voltage-gated sodium and calcium channels or potentiating gamma-aminobutyric acid (GABA)-mediated neurotransmission, with little focus on voltage-gated potassium ion channels, despite these channels having a major role in the control of all aspects of neuronal excitability. It is reported that functional impairment of potassium ion channels, either by mutation or inhibition result in epilepsy.[7]

A group of synthetic antiepileptic drugs (AEDs) are available in practice; however, their effectiveness does not hold true with the entire range of population suffering from this disorder. The conventional antiepileptic agents such as phenytoin and sodium valproate carry with them several adverse effects notably neurotoxicity, gum hypertrophy (20% incidence), more in younger, and hirsutism.[7,8]

However, seizures are controlled successfully with currently available AEDs in most patients with epilepsy, >30% of patients still have medically refractory epilepsy.[3] Furthermore, there are still about 30–40% of epileptic patients affected by numerous side effects and seizures resistance to current AEDs.[4]

Ancient remedy includes the use of herbal medicine, plant sources, animal sources, and minerals. However, herbal medicines are the most commonly used of the above three. Herbal medicines contain an active ingredient, aerial, or underground parts of plants as their petal or seeds, bark, roots, flower materials, or combinations thereof, whether in the crude state or as plant preparations. Furthermore, about 80% of the world population is dependent on plant-based drugs (WHO, 1996).[9]

On the other hand, herbal medicines are widely used across the globe due to their wide applicability and therapeutic efficacy coupled with least side effects, which in turn has accelerated the scientific research regarding the antiepileptic activity. Natural products have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of AEDs with novel structures and better safety and efficacy profiles. Thus, it is necessary to investigate for an antiepileptic agent that is highly efficacious as well as safe in items of drug-related toxicity.[10]

Many indigenous medical plants which are safe and well tolerated are used since ages for treating neurological disease including epilepsy. *Saraca asoca* of "Caesalpiniaceae" family is known as a small to medium-sized handsome evergreen tree. In Hinduism term, "Ashoka" means "one of that relieves pain and grief of women" is considered as a sacred tree. It has been widely used by folk medicine as an anticancer, antioxidant, antibacterial, anti-inflammatory, antifertility, antiarthritic, cardioprotective, larvicidal, antimutagenic/genoprotective, antidepressant, etc., and also has been extensively used in Ayurveda, Unani, and Homeopathy practices.[11] Our previous study demonstrated significant antiepileptic activity of *Mimosa pudica* root plant because of its active phytoconstituents "mimosin."[12] Hence, the present study aimed to the evaluation of antiepileptic activity of *S. asoca* flower (Roxb.) Wilde in animal models. These element object researcher to develop novel antiepileptic constituents from herbal medicine.[13,14]

MATERIALS AND METHODS

Chemicals

Ethanol, pentylenetetrazole (PTZ) (Sigma Laboratories Pvt. Limited), Propylene glycol, and Valproic acid (Sun Pharmaceuticals).

Plant Materials and Extract Preparation

The fresh plant flower was collected during the months of April (2015–2016) from West Bengal. It was taxonomically identified and was authenticated by Dr. Mruthunjaya, Department of Pharmacognosy, JSS Pharmacy College, Mysore, and herbarium of the plant is preserved for future references (Specimen Voucher No. SAF-10601/Pharma). The collected flowers were washed and shade dried at room temperature for 7 days. Dried flowers were coarsely powdered and fine powder was separated. The coarse powder of flower (800 g) was subjected to extraction with ethanol by Soxhlet apparatus and extracts were concentrated to dryness by vacuum. The extract was then weighed to calculate the percentage of yield in terms of air-dried crude material. The resultants ethanolic extract of *S. asoca* flower (ESAF) was kept in refrigerator for further use. Before administration, the extract was freshly prepared with normal saline and three doses (50 mg/kg and 100 mg/kg) were selected based on the results of previous studies.

Animals

Adult Swiss albino mice of either sex weighing between 25 and 30 g were randomly selected from Central Animal Facility of JSS Medical College, Mysore. Animals were housed in five groups of six each, at an ambient temperature of 25°C ± 1°C with *ad libitum* access to food and water. The animals were fasted overnight just before the experiment but allowed free access to drinking water and food.

Grouping

Animals were randomly divided into six groups of six each.

Group 1: Control group received 0.25 ml propylene glycol p. o.
Group 2: Standard group received 200 mg/kg valproate p. o.[13]
Group 3: Test Group 1 received 50 mg/kg of ESAF p. o.

Group 4: Test Group 2 received 100 mg/kg of ESAF p. o.
Group 6: Test Group 3 received 50 mg/kg of ESAF + 70 mg/kg valproate p. o.

All the drugs were administered orally for 5 days. The experimental seizures were induced on the 5th day of the experiment. The institutional ethical committee approval was obtained before the experimentation.

Phytochemical Screening

The extract obtained from the powdered flower of *S. asoca* was subjected to phytochemical tests to determine the presence of active metabolites using standard procedure.[14]

Acute Toxicity Study

The animals were treated with increasing doses of the ethanolic extract (ESAF): 0.5, 1, and 2 mg/kg p. o. The toxicity studies were conducted according to the Organization for Economic Cooperation and Development 423 guidelines.[15] All the treated animals were observed for any abnormal or toxic manifestations and mortality.

Antiepileptic Models

Maximal electroshock-induced seizures (MES) in mice

The tonic-clonic convulsions were induced by MES using an electroconvulsiometer (INCO, Ambala, India) by passing an alternating electric current of 50 Hz and 150 mA for 0.2 s through ear clip electrodes. The animals were preliminarily screened and the mice which showed the extension of hind limb on electric shock were included in the study. All the drugs and propylene glycol were administered 1 hour before induction of convulsion. The duration of tonic hind limb flexion (THLF), tonic hind limb extension (THLE), clonus, and stupor was noted. The vehicle-treated mice showed the characteristic extensor tonus. The abolition of extensor (tonic) phase in drug-treated groups was taken as criteria for their anticonvulsant activity.

PTZ-induced seizures in mice

The albino mice were selected 2 weeks before the experiment by injecting the PTZ in a dose of 30 mg/kg intraperitoneally. Only those mice which showed clonic convulsions within 30 min during the preliminary examination were chosen for the study. After 1 hour of drug treatment, PTZ (70 mg/kg) was injected intraperitoneally and animals were observed for clonic convulsion episode. The clonic convulsions onset time, duration of clonic convulsions, and postictal depression (PID) were observed for 30 min.

Statistical Analysis

The results were computed using GRAPH PRISM PAD Version 7, one-way analysis of variance (ANOVA) test followed by Dunnett's multiple comparison tests was applied using the software. The differences between means were considered to be statistically significant at $P < 0.05$, and the results were tabulated as below.

RESULTS

Phytochemical Screening

Phytochemical screening of ESAF showed that the crude extract contained tannins, carbohydrates, saponins, flavonoids, sterols, glycosides, and proteins.

Acute Toxicity Study

There was no mortality among the mice treated with the graded dose of ESAF up to a dose of 2000 mg/kg for a duration of 72 h. ESAF dose-dependently protected the mice against the MES- and PTZ-induced seizures. At the dose of 500 mg/kg and 4000 mg/kg p. o, the ESAF provided, respectively, 23% and 100% protection against seizures in PTZ-induced seizures model. Based on the preliminary toxicity data and logarithmic dose-response curve, the ESAF dose of our further study was determined between 1000 and 2000 mg/kg.

MES-induced Seizure Model

The average duration of THLF, THLE, clonus, and stupor along with the percentages of inhibition of convulsions is presented in Table 1. Albino mice pretreated with ESAF at the doses of 50 and 100 mg/kg exhibited a significant delay in the onset time and also a significant decrease in duration of THLF, THLE, clonus, and stupor phases when compared to the control group mice. The albino mice pretreated with ESAF at doses of 50 and 100 mg/kg also exhibited significant protection from convulsion induced by electroshock method in a dose-dependent manner. The animal group treatment with a combination of both 50 mg/kg of ESAF and low dose (70 mg/kg) of valproate exhibited significant antiepileptic activity comparable to the standard valproate (100 mg/kg)-treated group.

PTZ-induced Seizure Model

The average seizure latency time, duration of myoclonic jerks, generalized clonic seizures, and PID along with the percentages of protection against convulsions are presented in Table 2. Albino mice pretreated with ESAF at the doses of 50 and 100 mg/kg and the combination group exhibited a significant delay in the onset time of clonic seizures when compared to the control group mice. ESAF (50 and 100 mg/kg)-treated mice also showed a significant decrease in number and duration of myoclonic jerks, clonic seizures, and duration of PID when compared to the control group mice. ESAF provided significant protection from convulsion

Table 1: Effect of ethanolic ESAF on MES-induced seizures in mice

Treatment	Duration of THLF	Duration of THLE	Clonus	Stupor	PID
Control	8.2±0.67	9±0.7	18±2.1	284±53	296±8.4
Valproic (200 mg/kg)	3.4±0.33****	1.9±0.28****	8.2±0.48***	106±4.6***	237±7.7****
ESAF 50 mg/kg	5.8±0.34*	1.8±0.35****	15±1.7 ns	127±16**	273±11 ns
ESAF 100 mg/kg	5.1±0.77**	3±011****	13±0.52**	98±6.7***	239±6.8*
ESAF 50 mg/kg+valproic acid 70 mg/kg)	3.6±0.31***	4±0.29****	12±0.72**	87±4.1****	240±24*

Values are expressed as mean±SEM. Comparison between control v/s all the other groups. Statistical test done by one-way ANOVA followed by Dunnett's multiple comparison tests *P<0.05, **P<0.01; ***P<0.001; ****P<0.0001. *S. asoca*: *Saraca asoca*, ESAF: Extract of S. asoca flower, MES: Maximal electroshock, THLF: Tonic hind limb flexion, THLE: Tonic hind limb extension, PID: Postictal depression, SEM: Standard error of the mean, ANOVA: Analysis of variance

Table 2: Effect of ethanolic ESAF on PTZ-induced seizures in mice

Treatment	Seizure latency period (s)	Duration of myoclonic jerks (s)	Duration of clonic seizures (s)	PID
Control	294±28	5.2±1.8	8.7±0.07	305±3.6
Valproic (200 mg/kg)	453±16***	1.5±0.13****	6.6±0.27****	253±3.6***
ESAF 50 mg/kg	275±24 ns	3.4±0.49*	8.2±0.45 ns	290±4.6 ns
ESAF 100 mg/kg	380±12*	3.3±0.26*	6.9±0.38*	274±3*
ESAF 50 mg/kg+valprioc acid 70 mg/kg)	448±21***	2.4±0.24***	6.8±0.37*	264±9.8**

Values are expressed as mean±SEM. PID: Postictal depression; comparison between control versus all the other groups. Statistical test is done by one-way ANOVA followed by Dunnett's multiple comparison tests. *P<0.05, **P<0.01; ***P<0.001; ****P<0.0001. *S. asoca*: *Saraka asoca*, ESAF: Extract of *S. asoca* flower, PTZ: Pentylenetetrazole, ANOVA: Analysis of variance

induced by PTZ in a dose-dependent manner. The animal group treatment with a combination of both 50 mg/kg of ESAF and low dose (70 mg/kg) of valproate exhibited a significant antiepileptic activity even better than the standard valproate (100 mg/kg)-treated group.

DISCUSSION

In the present study, MES and PTZ were used to evaluate the anticonvulsant activity of ethanolic ESAF in albino mice.

MES test in mice primarily indicates the compounds which are effective in grand mal epilepsy. The tonic extension of the hind limb evoked by electrical stimuli is suppressed by antiepileptics. AEDs that block MES-induced seizure are known to act by blocking the seizure spread.[16] The drugs which antagonize the PTZ-induced convulsions are known to be effective in petit mal epilepsy. PTZ is known to possess GABA antagonistic activity.[16] The AEDs diazepam and phenobarbitone are proved to produce their antiepileptic effects by enhancing GABA-mediated inhibition in the brain. Valproate is known to act by multiple mechanisms: Prolongation of Na^+ channel inactivation-like phenytoin, attenuation of T-type Ca^{++}currents like ethosuximide, and by augmenting GABA transmission. It is known to inhibit both PTZ- and MES-induced convulsions.[17]

The present study revealed that ESAF possesses a dose-dependent protection against tonic extensor phase in MES-induced seizure model and seizure latency in PTZ-induced seizure model. ESAF showed a better anticonvulsant activity in the PTZ model than MES antiepileptic model. The ESAF combined with the low dose of valproate (70 mg/kg) showed a similar anticonvulsant activity as the standard dose of valproate (200 mg/kg). This combination of low doses of ESAF and valproate is beneficial as it reduces the incidences and severity of undue side effects of the drugs.

The phytochemical screening of the ESAF extract revealed the presence contained tannins, carbohydrates, saponins, flavonoids, sterols, glycosides, and proteins. Based on the present data of the chemical constituents, it is not possible to attribute with certainty the detected active principle/s for its anticonvulsant activity. However, several flavonoids could act as benzodiazepine-like molecules in the central nervous system and modulate GABA-mediated chloride channels in animal models of anxiety, sedation, and convulsion. Certain triterpenic steroids are reported to possess anticonvulsant activity in MES and PTZ experimental seizure models.[18] Further studies are required for isolation of bioactive principles responsible for these activities. These findings justify the traditional use of this plant in the control and treatment of convulsions and epilepsy.

CONCLUSION

The ethanolic flower extract of *S. asoca* has demonstrated potential antiepileptic properties and safe in the experimental animals at the doses used. However, further studies still needed to be evaluated the precise mechanism/s, bioactive

principles, and safety profile of the plant as a medicinal remedy for convulsive disorders.

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