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Short Communication

Anticonvulsant effect of *Sphaeranthus* flower extracts in mice

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ABSTRACT

Sphaeranthus indicus whole herb is included as a Rasayana drug in Ayurveda and is reported for the treatment of epilepsy. *S. indicus* flowers have anxiolytic, hypotensive, peripheral vasodilatory and cathartic activity. The objective of this study was to evaluate the anticonvulsant activity of the extract of flowers of *S. indicus* in various animal models of epilepsy. The anti-epileptic activity of Methanolic extract (ME) and Acetone extract (AE) of the flowers was evaluated using Maximal electro shock (MES) seizures, Pentyelenetetrazole (PTZ) induced convulsions and Picrotoxin induced convulsions. ME (50 mg/kg and 100 mg/kg) and AE (100 and 200 mg/kg respectively) protected animals against PTZ and Picrotoxin induced convulsion but did not have any effect against MES induced convulsion. In conclusion, the results of this study suggest that both the ME and AE possess promising anticonvulsant activity. It is further suggested that the flavonoids in the extract by the virtue of their effect on benzodiazepine site of GABA receptor, might be responsible for the effect, although no study is undertaken to prove this aspect. Nevertheless, the study provides pharmacological credibility to the anti-epileptic use of *S. indicus* suggested in Ayurveda.

1. Introduction

Ayurveda includes a class of drugs classified as Rasayana (rejuvenators). *Sphaeranthus indicus* is one of the plants in this class and has been used in the treatment of epilepsy and other mental disorders in *Ayurveda*. Epilepsy is a chronic neurological disorder that produces recurring convulsive or non-convulsive seizures affecting a variety of mental and physical functions. The increase in the number of patients with drug resistant seizure is reaching a sizeable population. Moreover, antiepileptic drugs show undesired CNS effects such as decreased cognitive abilities and psychiatric complications [1]. Thus, the traditional medicines with fewer side effects are gaining popularity.

S. indicus belongs to the Asteraceae family and is commonly known as Gorakhmundi in Hindi or *Shravani* in Sanskrit. Previous reports have suggested that the plant possesses anxiolytic and sedative effect [2], an effect commonly observed in many anticonvulsants. The plant is also reported to have neuroleptic effect [3] by inhibiting the nigrostriatal dopaminergic system via D1 and D2 mechanisms which is considered as the future of anti-epileptic treatment [4]. Considering mentioned findings, the anticonvulsant activity of the extracts of *S. indicus* was screened by using maximal electroshock (MES) induced convulsions, pentyelenetetrazole (PTZ) induced convulsions and Picrotoxin induced convulsions. Due to the difference in the mechanism of induction of convulsions, a battery of anti-epileptic models was used.

2. Material and methods

2.1. Drugs and chemicals

Methanol and acetone (S D fine chemical Ltd. Mumbai, India), Gallic acid (Loba Chemie Pvt. Ltd.,Mumbai, India), Quercetin and Stigmasterol (Sigma Aldrich, Mumbai, India), Caffeine (S D Fine chemical Ltd, Mumbai, India), Picrotoxin (Sigma Life Sciences, Mumbai, India), PTZ (Ozone international, Mumbai, India), Diazepam (Calmpose tablet), Phenytoin (Sisco Research Laboratories, Mumbai, India) Carboxy methyl cellulose-CMC (Ozone international, Mumbai, India). All other chemicals were from Fischer Scientific and S D Fine, Mumbai, India.

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2.2. Preparation of the extract

The dried flowers of *S. indicus* Linn. were purchased from local market in Mumbai, India. The flowers were authenticated at SPPSPTM, SVKM's, NMIMS as per the parameters laid down in the *Ayurvedic* pharmacopoeia [5]. A specimen voucher has been deposited at the same institute. Coarsely powdered plant material was extracted in a Soxhlet apparatus using methanol and acetone separately for 22 h. The extracts were cooled, filtered and evaporated to dryness under reduced pressure. Yield: 19.04% w/w of ME and 9.93% w/w of AE. Suspension of ME, AE, Diazepam and Phenytoin were prepared in distilled water by triturating with CMC (0.5% w/w). Dose of both extracts was optimised to obtain significant difference in all three models by conducting a pilot scale study.

2.3. Phytochemical analysis

Both the extracts were screened for the presence of phytoconstituents qualitatively [6], quantitatively for total phenolic content [7], total flavonoid [7], total alkaloids [8], total steroid content [9] and by HPTLC fingerprinting [10].

2.4. Animals

Male albino mice weighing between 20 and 25g, were placed in cages and kept in standard environmental conditions [23°C ± 5, 60% ± 5 RH, and 12:12 h dark and light cycle], fed with standard diet and allowed free access to drinking water during the period of acclimatization. All animal experiments were carried out in accordance with guidelines of Committee for the Purpose and Supervision of Experiments on Animals (CPCSEA) and the study was approved by the Institutional Animal Ethics committee (IAEC).

2.5. Pentyelenetetrazole (PTZ) induced convulsions [11]

Animals were divided into six groups of six animals each. Group 1 received vehicle (p.o.) and served as the control group, Group 2 received the standard drug Diazepam (5 mg/kg, p.o). Group 3 and 4 received ME extracts (50 mg/kg and 100 mg/kg, p.o., respectively), Group 5 and 6 received AE extracts (100 mg/kg and 200 mg/kg, p.o., respectively). PTZ (80 mg/kg) was injected sub cutaneously to mice 45 min after vehicle, extracts and the standard drug. Immediately after PTZ administration mice were observed for latency of clonic convulsions (elapsed time from PTZ injection until convulsion occurred) and mortality for the duration of 30 min [11].

2.6. Picrotoxin induced convulsions [11]

Animals were divided into six groups as stated in 2.5. One hour after administration of vehicle, extracts and diazepam all mice were treated with 3.5 mg/kg Picrotoxin by subcutaneous route. Immediately after picrotoxin injection, mice were observed during the next 1 h for: latency of clonic convulsions (elapsed time from Picrotoxin injection until convulsion occurred), episodes of clonic convulsions (Repeated attacks) and mortality [11].

2.7. MES seizures induced by Electro-convulsometer [11]

Animals were divided into six groups as stated in 2.5. Exactly 45 min after vehicle, extracts and Phenytoin administration, maximal electroshock seizures were elicited by the application of electric shock (60 Hz AC, 150 mA) for 0.2 s (s) using electrodes at the ear pinna. After electric stimuli, duration of various phases of epileptic attacks like jerking, grooming, tail straub, extension of hind limb, recovery, total duration of convulsion and mortality were recorded [11].

2.8. Statistical analysis

The results for all groups were statistically compared with control group. Data presented as Mean ± SEM. Data for mortality was analysed by Fischer's exact test. All the other data was analysed using one way ANOVA followed by Dunnett's multiple comparison test.

3. Results

It is reported for the first time that the ME and AE of *S. indicus* flowers showed anti-convulsant activity in experimentally induced seizures in mice. Single subcutaneous dose of PTZ (80 mg/kg) caused clonic convulsions and mortality in mice. Mice pre-treated with ME and Diazepam showed a significant increase ($p < 0.0001$) in the latency whereas AE failed to show any increase in latency at both the doses (Table 2). Recovery time was significantly reduced ($p < 0.0001$) at both the doses for ME, AE and Diazepam (Table 2). There was no mortality observed at both the

Table 1
Phytochemical analysis of ME and AE.

Phytoconstituent	ME (%w/w)	AE (%w/w)
Steroids (g Stigmasterol)	9.012	10.437
Flavonoids (g Quercetin)	10.135	6.055
Phenolics (g gallic acid)	4.120	4.302
Alkaloids (g Caffeine)	2.570	4.420

Table 2
Effect of ME and AE on convulsions.

Groups	Parameters for PTZ induced convulsions			Parameters for Picrotoxin induced convulsions			Parameter for MES induced convulsions
	Duration of latency (min)	Duration of recovery (min)	% mortality	Duration of latency (min)	Number of episodic attacks	% mortality	Duration of convulsions (min)
Control	0.402 ± 0.055	1.17 ± 0.039	(5/6) 83.33	13.170 ± 1.249	2 ± 0.00	(6/6) 100	14.66 ± 0.666
Diazepam 5 mg/kg	29.455 ± 1.409[a]	0.0383 ± 0.073[a]	(0/6) 0[*]	33.5 ± 0.833[a]	1 ± 0.00[b]	(0/6) 0[**]	–
Phenytoin 25 mg/kg	–	–	–	–	–	–	6 ± 0.957[a]
ME 50 mg/kg	6.122 ± 0.270[a]	0.147 ± 0.006[a]	(0/6) 0[*]	14.83 ± 1.478	2 ± 0.00	(6/6) 100	16.167 ± 0.258
ME 100 mg/kg	8.772 ± 0.310[a]	0.17 ± 0.012[a]	(0/6) 0[*]	13.67 ± 0.703	1 ± 0.00[b]	(0/6) 0[**]	14.5 ± 0.401
AE 100 mg/kg	1.243 ± 0.045	0.272 ± 0.013[a]	(3/6) 50	9.167 ± 0.918	1.833 ± 0.3073	(2/6) 33.33	18.167 ± 0.562
AE 200 mg/kg	1.683 ± 0.205	0.197 ± 0.111	(0/6) 0[*]	17.83 ± 1.108[c]	1.33 ± 0.2108[c]	(0/6) 0[**]	21.5 ± 0.542

Each values represent Mean ± S.E.M. for the number of animals utilized during experiment.
[*]$p < 0.05$, [**]$p < 0.01$, when compared to control using Fischer's exact test., [a]$p < 0.0001$, [b]$p < 0.001$, [c] $p < 0.05$, when compared to control using one way ANOVA followed by Dunnett's multiple comparison test.

doses for ME and Diazepam, whereas it was reduced to 50% and 0% at the dose of 100 mg/kg and 200 mg/kg for AE respectively as shown in Table 2.

Single subcutaneous administration of Picrotoxin (3.5 mg/kg) induced clonic convulsions and mortality in mice. Animals pre-treated with ME and AE did not show any significant difference in the latency of convulsions (Table 2). There was no significant difference observed in number of episodic attacks in ME (50 mg/kg) and AE (100 mg/kg and 200 mg/kg). However, the number of attacks were significantly reduced ($p < 0.0001$) ME (100 mg/kg) pre-treated animals (Table 2). The mortality was significantly reduced ($p < 0.001$) in animals pre-treated with all the extracts and Diazepam except ME (50 mg/kg) (Table 2).

Application of electric shock using electrodes at the ear pinna induced tonic clonic convulsions in mice. Pre-treatment with both the extracts did not show any significant difference in the occurrence or duration of tonic hind limb extension as well as duration of convulsion, however Phenytoin pre-treatment significantly reduced ($p < 0.0001$) this duration (Table 2). There was no mortality observed in any of the groups.

4. Discussion

PTZ enhances the glutamatergic neurotransmission by activating the NMDA receptors [12,13]. This results into neuronal toxicity, thus accelerating the onset of convulsions, prolonging the convulsive phase and finally causing mortality. The extracts helped in reversing these symptoms suggestive of probable modulation of glutamatergic neurotransmission.

Picrotoxin is a $GABA_A$ antagonist modifying the function of Cl^- ion channel which prevents the conductance of Cl^- ions into the brain thus hindering GABA mediated inhibition and causes excitation [14]. This excitation of the neurons further causes convulsions and death of animals. Though the extracts didn't alter the onset of convulsions, they helped in reducing mortality and number of episodic attacks, which is suggestive of GABA mediated inhibition of the neurons. The effectiveness of extracts in above two models is suggestive that they may either be modulating the GABAergic or glutamatergic activity and further studies are warranted to establish this modulatory effect.

MES induced convulsion model causes the activation of $Ca^{2+}$and Na^+ channels and drugs inhibiting this influx can prevent MES induced tonic hind limb extension. Since the extracts do not show efficacy in this model, it is suggestive that the extracts do not act on the voltage gated Na channels and hence are not able to inhibit the seizure spread.

This study hypothesizes to designate the anti-convulsant effect to one of the phytoconstituent-namely the flavonoids. This statement is based on the following evidences:

a) Both the extracts show the presence of steroids, alkaloids, phenolic compounds and flavonoids and the standard -7-hydroxyfrullanolide (Rf-0.69) as shown in Table 1 and Suppl. Fig. 1, but ME has higher flavonoid content than AE and accordingly shows higher anti-convulsant efficacy.
b) Few reports stated that flavonoids act on $GABA_A$-Cl^--channel complex due to structural similarity to benzodiazepines, potentiating its effects [15].

5. Limitations

The data shows preliminary evidence of ME and AE showing anti-convulsant potential but fails in establishing the mechanism of action of the active phytoconstituent.

6. Conclusion

The study reveals that both ME and AE show dose dependant anti-convulsant activity, Moreover, it provides a pharmacological evidence for the use of *S. indicus* in traditional system of medicine for the treatment of epilepsy.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jaim.2018.06.008.

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