EVALUATION OF ANTI-CONVULSANT ACTIVITY OF HYDROALCOHOLIC EXTRACT OF SAUROPUS ANDROGYNUS LEAVES

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Abstract- The anticonvulsant activity of hydroalcoholic extract of sauropus androgynus leaves was evaluated in the validated experimental animal models. Anticonvulsant activity of 70% ethanolic extract was investigated against PTZ and Picrotoxin induced convulsions in mice. Two doses: 200 mg/kg and 400 mg/kg b.w p.o of the extract were subjected for the evaluation of Anticonvulsant potential against PTZ (80mg/kg) and Picrotoxin (3.5mg/kg) in mice and Diazepam (4 mg/kg, i.p), used as standard. The parameters such as onset of convulsion, duration of convulsion and mortality were studied. It was observed that Both SA-200 and SA-400 showed dose dependent significant reduction in mortality rate and increased recovery in the rats treated with the extract, when compared with the vehicle control. Both extract showed decrease in the duration of convulsion and increased time for the onset of convulsion. Maximum protection was observed at 400mg/kg. The present study concluded that hydroalcoholic extract of Sauropus androgynus has significant anticonvusant potential.

Keywords- PTZ; Picrotoxin; Diazepam; convulsion; Sauropus androgynus.

I. INTRODUCTION

Medicinal plants may serve as an alternative source for the development of new therapeutic agents, due to their biological activities. Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. Several plants used for the treatment of epilepsy in different systems of traditional medicines have shown anti-convulsant activity when tested on animal models with fewer side effects. There is no doubt that epilepsy belongs to the most encountered neurological conditions, since the disease affects approximately 1% of the population, with a high prevalence of about 0.8% in the children below the age of seven years[1]. Around 75-80% of the epileptic patients can be provided with adequate seizure control with the help of conventional antiepileptic drugs. Carbamazepine, Ethosuximide, Phenobarbital, Phenytoin and Valproate are the most frequently used conventional antiepileptics. In many cases, even multi-drug therapy is not effective and neurosurgical procedures may be indispensable. Consequently, a real need exists to develop new anticonvulsant compounds to cover seizures which are so far resistant to presently available drugs. The therapeutic failure in 20-25% of patients has stimulated intensive research on novel antiepileptic drugs or traditional drugs. Many plant derived drugs used in modern medicine systems are developed by ethno medical leads and subsequent ethno pharmacological studies. There are more than 100 drugs of known structure that are extracted from higher plants and used in allopathic medicines[2].

Several plants used for the treatment of epilepsy in different systems of traditional medicines has shown activity when tested on modern bioassays for the detection of anticonvulsant activity and many such plants remain to be scientifically investigated[3]. The physiology of convulsion involves alteration in voltage–dependent ion channels, reduction in inhibitory, i.e. GABA-mediated drive or increases in excitatory, i.e. glutamate inputs. Currently available antiepileptic drugs act by modulating these factors. However, they provide relief in only up to 75% patients with absence seizures in 85% patients with generalized tonic-clonic seizures. Also, the long term therapy leads to serious side effects. Thus, there is a constant need for newer antiepileptic drugs that can control seizures without causing significant side effects. So the Ayurvedic and indigenous system of medicines are better for the treatment of convulsion[4]. Their usage is in vogue since centuries and are quite often claimed to offer significant relief. Some of the anti-convulsant plants as well as formulations used in traditional medicines have been pharmacologically evaluated for their efficacy. However, still more numbers of plants are needed to be screened for their anticonvulsant property. One such plant is Sauropus androgynus, locally available and has been used in traditional system of medicine. It has good antioxidant property and has been proved for the treatment of inflammation, cancer, microbial infection. However, there is no scientific claim on the antiepileptic activity of the plant of Sauropus androgynus leaves. In this view, an attempt has been made to investigate the anti-convulsant role of Sauropus androgynus Linn leaves.

II. DETAILS EXPERIMENTAL

2.1. Materials and Procedures

Preliminary phytochemical screening was carried out for the hydro alcoholic extract of Sauropus androgynus Linn leaves for: Alkaloids, Glycosides, Tannins, Saponins, Carbohydrates, Flavanoids and

Steroids[5,6]. The hydro alcoholic extract of the leaves of Sauropus androgynus Linn was suspended in Tween-80 to prepare a dose of 2000 mg/kg b.w. of animal and administered 1ml/100 g b.w. of the animal. Acute toxicity study of the extract was done according to acute toxic classic method (OECD guideline 425, 2006) using albino female mice to determine the safe dose[7].

III. PENTYLENETETRAZOLE (PTZ) INDUCED CONVULSIONS

In the dose response experiment, albino mice were randomly assigned into four groups of six each.
Group I animals were treated with vehicle and groupII animals were treated with Diazepam. Group III and group IV were treated with SA 200mg/kg and SA 400mg/kg respectively for 14 days. On the 14th day, Seizures were induced to the mice with standard convulsing agents, Pentylenetetrazole (PTZ), and the animals were observed for 30 minutes for tonic convulsion episode[8] [9,10]. The time interval between PTZ-injection and occurrence of seizures can be measured. The delay of onset is calculated in comparison with the control group.

IV. PICROTOXIN INDUCED CONVULSIONS

Mice were randomly assigned into 4 groups of 6 each.
Group I : Vehicle control (0.9% saline) + Picrotoxin (3.5mg/kg).
Group II : Diazepam (4 mg/kg, i.p) + Picrotoxin (3.5mg/kg).
Group III : Test drug (200) + Picrotoxin (3.5mg/kg).
Group IV : Test drug (400) + Picrotoxin (3.5mg/kg).
On 14th day Seizures were induced into the mice with standard convulsing agents, Picrotoxin, and the animals were observed for 30 minutes for tonic convulsion episode[8,9]. The time interval between Picrotoxin -injection and occurrence of seizures can be measured.

V. RESULTS AND DISCUSSION

LD50 studies of SA were conducted in albino mice by using OECD guidelines No- 425. It was found that extract at 2000 mg/kg dose was safe; hence confirming practically it is safe in nature.
The hydro alcoholic extract prepared was subjected to phytochemical tests and the outcome of these tests revealed the presence of carbohydrate, Alkaloids, Glycosides, flavonoids, steroids and saponins.

3.1. Evaluation of anti-convulsant activity of SA on Pentylenetetrazole (PTZ) induced convulsions:

In the present study, the convulsions was successfully produced by administration of PTZ (80mg/kg), i.p. on last day of treatment and the anticonvulsant activity of SA was determined from the parameters: Onset of convulsion, Duration of convulsion and percentage of mortality.

Table 1: Effect of SA extract on PTZ induced convulsions

Groups	Treatment	Onset of convulsion (seconds)	Duration of convulsion (seconds)	%Mortality
Control	Saline	68.66± 4.38	512.66± 7.49	68.00
Standa-rd	Diazepam (4mg/kg)	No convulsion	Protected	Protected
Low dose	SA 200mg/kg	93.57± 15.22**	427.25± 10.03***	52.43*
High dose	SA 400mg/kg	114.71± 12.35***	397.66± 14.220***	38.63**

One way ANOVA followed by Dunnett's t test. All the values are Mean±SEM,. ns, $p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$ when compared with normal control.

Vehicle control (group 1) showed significant decrease in Onset of convulsion when compared to other groups. SA(200mg/kg) showed moderate significant increase (P<0.01) and SA(400mg/kg) showed highly significant increase (P<0.001)in time
of onset of convulsion when compared to vehicle treated group. Vehicle control (group 1) showed significant increase in duration of convulsion when compared to other groups. SA (200mg/kg) showed highly significant decrease (P<0.001) and SA(400mg/kg) showed highly significant decrease (P<0.001)in duration of convulsion, when compared to vehicle treated group. Vehicle control (group 1) showed significant increase in mortality percentage when compared to other groups. SA (200mg/kg) showed less significant decrease (P<0. 05) and SA (400mg/kg) showed moderately significant decrease (P<0. 01)in protective effect from convulsion, when compared to vehicle treated group .
Pentylenetetrazole was one of the most commonly used chemo-convulsant to identify anticonvulsant Activity[11]. Generally, Pentylenetetrazole test is used for screening of drugs effective in petitmal epilepsy or absence seizure[12].

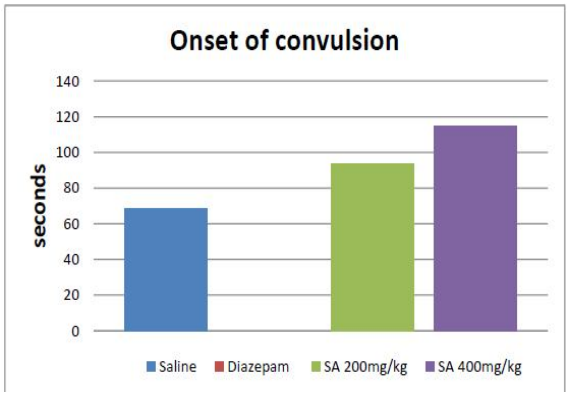

Fig 1: Effect of Diazepam and SA on Onset of convulsion.

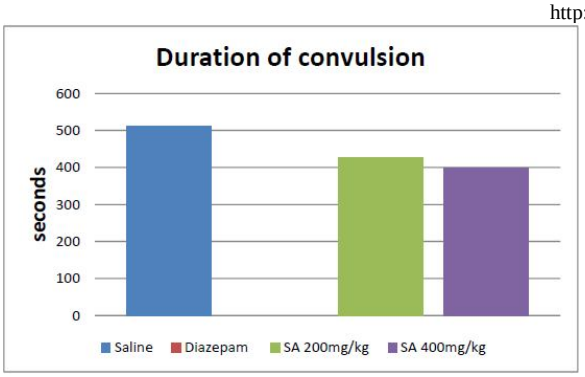

Fig 2: Effect of Diazepam and SA on Duration of convulsion.

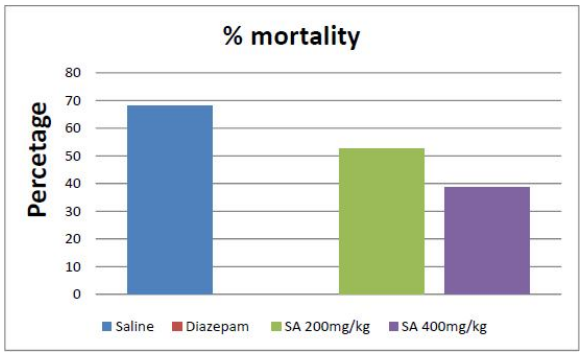

Fig 3: Effect of Diazepam and SA on percentage of mortality.

3.2.Evaluation of anti-convulsant Activity of SA on Picrotoxin induced convulsions:

In the present study, the convulsions was successfully produced by administration of Picrotoxin (3.5mg/kg), i.p.. on the last day of the treatment the anti-convulsant activity of SA was determined from the parameters like, Onset of convulsion, Duration of convulsion and percentage of mortality.

Table No 2: Effect of SA extract on Picrotoxin induced convulsions:

Groups	Treatment	Onset of Convulsion (seconds)	Duration of convulsion (seconds)	%Mortality
Control	Saline	47.12± 2.70	397.64± 5.19	72.34
Standard	Diazepam (4mg/kg)	No convulsion	Protected	Protected
Low dose	SA (200mg/kg)	75.41± 10.05***	341.33± 13.47**	59.13**
High dose	SA (400mg/kg)	103.45± 16.11***	278.08± 9.21***	35.22***

One way ANOVA followed by Dunnett's t test. All the values areMean±SEM,. ns, p>0.05, *p<0.05, **p<0.01, ***p<0.001 when compared with normal control.

Vehicle control (group 1) showed significant decrease in the onset of convulsion when compared to the other groups. SA (200mg/kg) and SA (400mg/kg) showed highly significant increase (P<0.001) in onset of convulsion when compared to vehicle treated group. Vehicle control (group 1) showed significant increase in duration of convulsion when compared to the other groups. SA (200mg/kg) showed moderate significant decrease (P<0.01) and SA (400mg/kg) showed highly significant decrease (P<0.001) in duration of convulsion, when compared to vehicle treated group. Vehicle control (group 1) showed significant increase in mortality percentage when compared to other groups. SA (200mg/kg) showed moderate significant decrease (P<0. 01) and SA (400mg/kg) showed highly significant decrease (P<0. 001) in protective effect from convulsion, when compared to vehicle treated group. GABA is the major inhibitory neurotransmitter in the brain. The inhibition of GABA by the pentylenetetrazole and picrotoxin elicits seizures. Standard antiepileptic drugs act by enhancing the inhibitory effect of GABA. Therefore it is possible that the anticonvulsant activity of the extracts in the present study may be due to the activation of GABA[13].

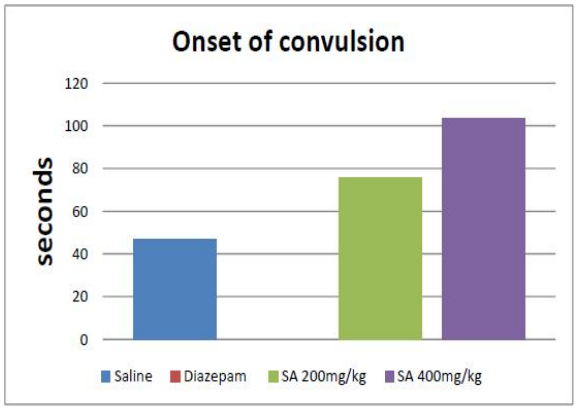

Fig 4: Effect of Diazepam and SA on Onset of convulsion.

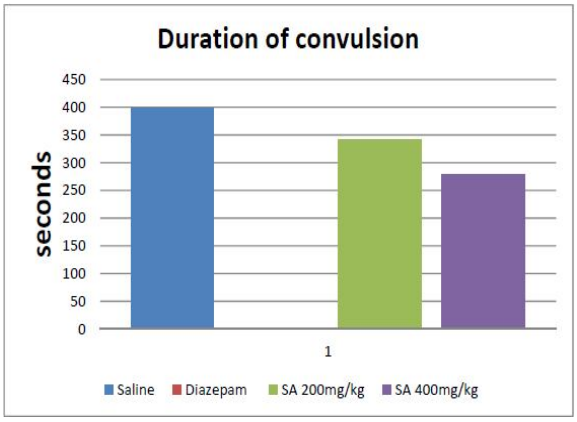

Fig 5: Effect of Diazepam and SA on Duration of convulsion

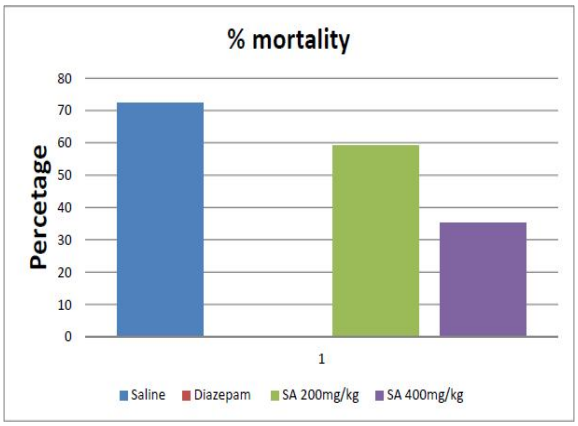

Fig 6: Effect of Diazepam and SA on percentage of mortality.

CONCLUSIONS

The investigation undertaken was aimed to study anticonvulsant activity of Sauropus androgynus, demonstrated the usefulness and beneficial effects in the treatment of CNS disorder induced by PTZ and Picrotoxin. The findings of the present study concluded that SA witnessed a dose dependent significant anticonvulsant activity.

The anticonvulsant activity was found to be more significant in high dose (SA-400mg/kg) compared to low dose (SA-200 mg/kg) in both the animal models. The anticonvulsant potential of SA in both the experimental models may be due to the presence of flavonoids, saponins and other polyphenolic compounds which are attributed for the antioxidant activity.

The exact mechanism for the anticonvulsant activity of SA is still unknown and the protection level was found to be at considerable range. Hence further studies are needed to isolate, characterize the active principles and to find out the exact mechanism responsible for its anticonvulsant activity.

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