



Evaluation of antiepileptic activity of fruit pericarp of “*Sapindus Mukorossi*” in rats

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ABSTRACT

The aim of present study was to investigate antiepileptic activity of the Aqueous and Methanolic extracts of the fruit pericarp of *Sapindus mukorossi* on electrically and chemically induced seizures. The latency of tonic convulsions and the number of animals protected from tonic convulsions were noted. Both the extracts of *Sapindus mukorossi* in the Maximal Electric Shock (MES) study were not able to abolish tonic hind limb extension but significantly reduced its duration. Phenytoin and Diazepam used as references in this experiment caused significant reduction of the tonic hind limb extension phase and completely abolished this behavior. In PTZ study, both the extracts of *Sapindus mukorossi* caused significant dose-dependent anticonvulsant effect against PTZ-induced seizures by delaying the onset of myoclonic jerks and tonic convulsions in rats. It also caused profound decrease in the duration of the tonic convulsions. Diazepam and Phenytoin sodium which was used in this study as a reference anticonvulsant agent showed significant activity by delaying the onset of myoclonic jerks and tonic convulsions and decreasing the frequency and duration of tonic convulsions. In conclusion this work suggests that extract of fruits of *Sapindus mukorossi* possess antiepileptic properties that may be due to restoring the neurochemical substances in rat brain. These results support the ethno medical uses of the plant in the treatment of epilepsy. However more experimentation and experimental analysis are required for a definitive conclusion.

Keywords: Anti epileptic activity, Phenytoin, Diazepam, Tonic hind limb extension, Myoclonic jerks

INTRODUCTION

People tend to rely on traditional and other forms of complementary and alternative medicine for chronic conditions which do not respond well to conventional or modern drug treatments. Among these are neurological disorders such as anxiety, pain and epilepsy [1]. Centuries before the advent of modern medicine, synthetic chemistry and the pharmaceutical industry, virtually all medicines came from plants [2]. These medicinal plants have been an important source for the discovery of novel bioactive compounds which served and continue to serve as lead molecules for the development of new drugs [3].

Epilepsy is one of the major neurological disorders affecting approximately 0.8% of the population [4]. There has been considerable progress in the pharmacotherapy of epilepsy over the last few decades [5], including the introduction of new Antiepileptic drugs (AEDs) such as Felbamate, Lamotrigine, etc [6, 7]. However, current drug therapy of epilepsy is complicated by side-effects, teratogenic effects; long term toxicity and about a third of patients are refractory to pharmacotherapies [8, 9]. Furthermore, there is currently no drug available which prevents the development of epilepsy e.g. after head trauma and all currently available AEDs drugs are synthetic molecules.

Sapindus mukorossi, a member of the family Sapindaceae, is commonly known by several names such as soapnut, soapberry, washnut, reetha, aritha, dodan and doadni. *S. mukorossi* is used medicinally as an expectorant, emetic, contraceptive, and for treatment of excessive salivation, epilepsy, chlorosis and migraine.

S.mukorossi (*sapindus mukorossi*) is a popular ingredient in Ayurvedic shampoos and cleansers. They are used in Ayurvedic medicine for treatment of eczema, psoriasis and for removing freckles. Soapnuts have gentle insecticidal properties and are traditionally used for removing lice from the scalp [10]. Most of the phytochemical constituents of this plant have been discovered by various scientists. Among them the most explored phytoconstituents are triterpenoidal saponins of mainly three types viz oleanane, dammarane and tirucullane type [11].

Recently many of the pharmacological actions of this plant has been explored which includes the antifungal [12], hepatoprotective [13], insecticidal [14], pesticidal activity [15] and anti inflammatory activity [16].

One of the most popular activities of this plant is the contraceptive activity [17] of the saponins extracted from the pericarp of the fruits. It was found that no substantial work of *Sapindus mukorossi* was carried out for its Anti-epileptic activity. Hence the present work was undertaken to screen the Anti epileptic activity of pericarp of the fruits of *Sapindus mukorossi* in pre-clinical models.

EXPERIMENTAL SECTION

Collection and authentication of plant material

Fruits of *Sapindus mukorossi* were purchased from S.S Herbs, New Delhi. Then they are grinded coarsely and stored in air tight polyethylene bags.

Preparation of Aqueous and Methanolic extract

Coarsely powdered fruits were macerated with distilled water and methanol for seven days. After seven days liquid was decanted and filtered and concentrated under reduced temperature and pressure. Both extracts were stored at airtight container in a refrigerator at -10°C .

Preliminary Phytochemical investigation

Extracts were subjected to preliminary phytochemical screening for the presence of various phytochemical constituents [18].

Animals

Wistar rats of either sex (200-250gms) were obtained from the animal house of Krupanidhi Pharmacy College, Bangalore. All the animals were maintained in a well ventilated room and given access to feed and water *ad libitum*. All animal studies performed were in accordance with guidelines of CPCSEA and Institutional Animal Ethical Committee (CPCSEA registration number- KCP/IAEC-MPP30/2011-12).

Determination of LD₅₀ of fruit extract of *Sapindus mukorossi* :

The acute toxicity study of fruit extracts of *Sapindus mukorossi* was determined by using Wistar rats of either sex weighing 200-250gms as per OECD Guidelines No.423 [19].

Assessment of Anti-convulsant activity.

Maximal electroshock induced seizures model (MES)

Wistar rats weighing between 200-250gms were divided into six groups (n=6).

Group I animals served as control and received distilled water 0.25 ml, p.o.

Group II served as standard received Diazepam 1mg/kg, i.p.

Group III and IV were administered with the Aqueous fruit extract of *Sapindus mukorossi* at two different doses 200 mg/kg and 400 mg/kg, p.o respectively.

Groups V and VI were treated with Methanolic extract of 100 and 200 mg/kg, p.o respectively.

The treatment was continued for 15 days. On the 15th day, seizures were induced to all the groups of animals using Electro convulsimeter. A 60 Hz alternating current of 150 milliamps intensity elicited maximal electro shock (MES) seizures for 0.2 second was applied. A drop of electrolyte solution (0.9% NaCl) with Lignocaine was applied

to the corneal electrodes prior to application to the rats. This increases the contact and reduces the incidence of fatalities [20]. The observed duration of various phases of epilepsy was tabulated.

Pentylentetrazole (PTZ)-induced seizures model

Wistar rats weighing between 200-250 gm were divided into six groups of six animals each. Group I served as control and received distilled water 0.25 ml, p.o.

Group II served as standard and it received Phenytoin sodium 20 mg/kg, p.o.

Group III and IV were treated with Aqueous extract at different doses 200 and 400 mg/kg, p.o respectively.

Groups V and VI were treated with Methanolic extract of 100 and 200 mg/kg, p.o respectively.

After 30 min of administration of Phenytoin sodium and 60 min after oral administration of extracts, 60 mg/kg PTZ was injected intraperitoneally. Onset of myoclonic seizures, clonic seizures and tonic extensor was recorded. Rats that did not convulse 30 min after pentylentetrazole administration were considered protected [21].

Statistical analysis

The results of this study are expressed as mean \pm SEM. Results were analyzed by student's 't' test. In all tests the criterion for statistical significance was $p < 0.05$.

RESULTS AND DISCUSSION

Preliminary Phytochemical evaluation of extracts:

The qualitative phytochemical analysis of Aqueous and Methanolic extract showed the presence of glycosides, carbohydrates, saponins, flavonoids, tannins, phenolic compounds, proteins and amino acids, whereas Methanolic extract has also shown the presence of triterpenoids, steroids and fixed oils (Table 1).

Table 1: Preliminary Phytochemical investigation of *Sapindus mukorossi*

Sl no.	Chemical tests	Aqueous Extract	Methanolic Extract
1.	Alkaloids	-	-
2.	Carbohydrates	+	+
3.	Steroids and Sterols	-	+
4.	Glycosides	+	+
5.	Saponins	+	+
6.	Flavonoids	+	+
7.	Tannins and Phenolic	+	+
8.	Triterpenoids	-	+
9.	Proteins and Amino Acids	+	+
10.	Fixed oils	-	+

'+' represent presence and '-' represent absence of phytoconstituents.

Determination of LD₅₀ of fruit extract of *Sapindus mukorossi* :

Aqueous extract of *Sapindus mukorossi* did not show the toxic symptoms up to the dose of 2000 mg/kg body weight. All the test animals survived for 14 days without showing any toxic symptoms. Based on this 1/5th i.e. 400 mg/kg (High dose) and 1/10th i.e. 200 mg/kg (Low dose) were selected for further pharmacological screening.

Methanolic extract of *Sapindus mukorossi* did not show the toxic symptoms up to the dose of 1000 mg/kg body weight. All the test animals survived for 14 days without showing any toxic symptoms. Based on this 1/5th i.e. 200 mg/kg (High dose) and 1/10th i.e. 100 mg/kg (Low dose) were selected for further pharmacological screening.

Assessment of Anticonvulsant Activity of fruit extract of *Sapindus mukorossi*

MES model

Diazepam treated group significantly ($p < 0.001$) reduced the mean time of tonic hind limb flexion and clonus, whereas it totally prevented the development of tonic hind limb extension and stupor.

A comparison of mean duration of tonic hind limb flexion of control group with other groups indicate that low dose and high dose of aqueous extracts showed statistically significant protection ($p < 0.05$ and $p < 0.001$). Similar results were observed with Methanolic extracts at both doses ($p < 0.001$).

A comparison of mean duration of tonic hind limb extension of control group with test groups indicate that there is no significant decrease in the mean time of tonic hind limb extension when treated with aqueous extracts. Methanolic extract at both doses also exhibited weak activity in abolishing tonic hind limb extension.

Analysis of results compared with control suggests that there is a decrease in the mean time of clonus in Aqueous extract treated groups with a significant value of $p < 0.01$. Aqueous extract at both doses showed good response in comparison with Methanolic extract. High dose of Methanolic extract at 200 mg/kg did not show significant activity.

A comparison of mean duration of stupor with control indicates that there is a decrease in the mean time stupor in both Aqueous and Methanolic extract of *Sapindus mukarossi* which is statistically significant ($p < 0.01$). All these data indicate that both Aqueous and Methanolic extract exhibited weak anti convulsant activity in MES model as shown in Table 2.

Table 2: Effect of *Sapindus mukarossi* fruit Aqueous and Methanolic extract on MES induced convulsions

Groups/ Parameters	Tonic hind limb Flexion (Sec)	Tonic hind limb extension (Sec)	Clonus (Sec)	Stupor (Sec)
Group-I Control	3.33±0.5	11.83±0.95	13.83±1.04	104.66±12.45
Group-II Diazepam 1 mg/kg	1.33±1.12***	0.00	3.83±0.55***	0.00
Group-III AESM 200 mg/kg	2.0±0.48*	11.16±0.83	7.83±1.21**	66.83±4.48**
Group-IV AESM 400 mg/kg	0.83±0.50***	11.33±1.0	6.33±0.95**	54.83±3.88**
Group-V MESM 100 mg/kg	0.66±1.21***	10.00±3.8	11.00±2.97*	64.50±4.90**
Group-VI MESM 200 mg/kg	0.33±1.22***	10.00±4.48	14.16±3.1	62.66±5.68**

Data expressed as mean±SEM. n=6, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (compared with control).
AESM- Aqueous extract of *Sapindus mukarossi*, MESM- Methanolic extract of *Sapindus mukarossi*.

The Aqueous and Methanolic extracts of *Sapindus mukarossi*, were not able to abolish tonic hind limb extension at all the doses used in this study but significantly reduced the duration of the tonic hind limb flexion, clones and stupor. Tonic hind limb extension is the universal feature of maximal electroshock in mice, rats, rabbits, cats, monkeys and human. Also, abolishing hind limb extension indicates the ability of testing material to inhibit or prevent seizure discharge within brainstem seizure substrate. All the currently available drugs that are clinically effective in the treatment of generalized tonic seizures are effective in MES model. Both the extracts of *Sapindus mukarossi* in this study were unable to abolish tonic hind limb extension but significantly reduced its duration. Diazepam used in this experiment caused significant reduction of the tonic hind limb extension phase and completely abolished this behavior. Reduction in the duration of flexion, clonus and stupor but inability to completely abolish hind limb extension indicated weak anticonvulsant activity in MES model but suggested strongly the presence of anticonvulsant compounds in the extract.

PTZ model

Table 3 shows the effect of administration of different doses of *Sapindus mukarossi* Aqueous and Methanolic extract on the seizure threshold for the onset of myoclonic jerks, clones and tonic extensor produced by PTZ in rats.

Treatment with PTZ (Group II) has prevented myoclonic seizures, clonic seizures and tonic extensor. Aqueous extracts at both doses increased the onset of myoclonic seizures significantly ($p < 0.05$) while only high dose of Methanolic extract has shown significant increase in onset of myoclonic seizures ($p < 0.01$).

Both high doses of Aqueous and Methanolic extracts showed increase in onset of time for clonic seizures when compared with control ($p < 0.001$).

Aqueous plant extracts at both doses showed a significant increase ($p < 0.01$) in seizure threshold for the onset of tonic extensor as compared to the vehicle treated groups. Higher dose of the Methanolic extract increased the PTZ seizure threshold for the onset of tonic extensor phase compared to low dose. This establishes the role of extract in

preventing seizure propagation. Drugs which increases the threshold for the onset of myoclonic jerks and tonic extensor are known to prevent seizure generation and propagation.

Table 3: Effect of *Sapindus mukarossi* fruit Aqueous and Methanolic extract on PTZ induced convulsions

Groups/ Parameters	Myoclonic jerks (Sec)	Clonic seizure (Sec)	Tonic extensor (Sec)
Group-I (Control)	37.00±5.5	44.16±9.44	60.83±4.33
Group-II Phenytoin 20 mg/kg	0.00	0.00	0.00
Group-III AESM 200 mg/kg	45.50±6.4*	33.66±5.5*	93.33±6.0**
Group-IV AESM 400 mg/kg	48.00±6.8*	57.83±7.1**	93.33±4.3**
Group-V MESM 100 mg/kg	37.00±7.36	33.00±10.05*	87.66±8.54**
Group-VI MESM 200 mg/kg	43.83±5.08*	60.00±4.92**	94.16±6.19***

Data expressed as mean±SEM. n=6, * p <0.05, ** p <0.01, *** p <0.001 (compared with control). AESM-Aqueous extract of *Sapindus mukarossi*, MESM- Methanolic extract of *Sapindus mukarossi*

The ability of an agent to prevent or delay the onset of tonic and tonic-clonic convulsion induced by PTZ in animals is an indication of anticonvulsant activity. In this study, the extracts of *Sapindus mukarossi* caused significant dose-dependent anticonvulsant effect against PTZ-induced seizures by delaying the onset of myoclonic jerks and tonic convulsions in rats. It also caused profound decrease in the duration of the tonic convulsions. Anticonvulsant activity in PTZ-induced seizures identifies compounds that can raise seizure threshold in brain. Phenytoin sodium which was used in this study as a reference anticonvulsant agent showed significant activity by delaying the onset of myoclonic jerks and tonic convulsions and decreasing the frequency and duration of tonic convulsions.

The results indicate that both Aqueous and Methanolic extracts of *Sapindus mukarossi* exhibited a poor anticonvulsant activity in MES model and fairly good anticonvulsant effect in PTZ model. Retrospective reports reveal that the phytoconstituents such as triterpenoids and saponins are responsible moieties in most of the plants for their Anticonvulsant activity [22, 23]. Indeed, the Methanolic extract of fruit of *sapindus mukarossi* has shown the positive test for its presence of triterpenoids and saponins. Hence these moieties in the fruit extract of *Sapindus mukarossi* may be responsible for the exhibited Anticonvulsant activity of the plant.

CONCLUSION

This work suggests that extracts of fruits of *Sapindus mukarossi* possess Antiepileptic properties that may be due to presence of flavonoids and saponins in Aqueous extract and presence of terpenoids in Methanolic extract. These results support the ethno medical uses of the plant in the treatment of epilepsy. However more experimentation and experimental analysis are required for a definitive conclusion.

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