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Research Article

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Evaluation of muscle relaxant activity of aqueous extract of *Sapindus trifoliatus* (pericarp) in swiss albino mice

Jayasree. T[1], Naveen. A[1], Chandrasekhar. N[1], Sunil. M[1], Kishan. P V[1] and Jagan Rao N.[2]

[1]Department of Pharmacology, Mamata Medical College, Khammam, AP, India
[2]Department of Pharmacology, Meenakshi Medical College and RI, Kanchipuram, Tamil Nadu, India

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ABSTRACT

Sapindus trifoliate is a medium-sized deciduous tree growing wild in south India that belongs to the family Sapindaceae. The pericarp is reported for various medicinal properties. A thick aqueous solution of the pericarp is used for the treatment of hemi crania, hysteria or epilepsy in folklore medicine. As antiepileptic action is due to muscle relaxation we thought of investigating the muscle relaxant action and locomotor activity of aqueous extract of Sapindus trifoliatus (AEST) in swiss albino mice in comparison with that of diazepam. Extract was evaluated for its muscle relaxant action compared with control and standard diazepam using Rotarod and photoactometer. Fifty mice of either sex were taken and divided in to five groups of 10 each. First group was considered as control, second as standard (Diazepam), third, fourth and fifth as test group (with three different doses of AEST). All the drugs were given orally. 100 and 200 mg/kg of AEST significantly reduced the time spent by the animals on revolving rod when compared to control (P<0.01). There was dose dependent increase in muscle relaxation, maximum with 200mg/kg. The spontaneous locomotor activity with three different doses of AEST (50,100 and 200 mg p.o.) showed dose dependent decrease in locomotor activity that is 71.37%, 85.11% and 87.73% respectively when compared to control. The values are highly significant (P<0.000). In conclusion it is observed that Sapindus trifoliatus has muscle relaxant activity.

Key words: Diazepam, Muscle relaxation, Photoactometer, Rotarod, *Sapindus trifoliatus*.

INTRODUCTION

S. trifoliatus known as Rita or Aristha belongs to the family of Sapindaceae. The fruit of the plant is used therapeutically as a tonic, purgative, emetic and expectorant [1]. It also possesses anti-inflammatory and analgesic actions. It is also used as a of spermicidal, in treatment of piles, hysteria, epilepsy and anti-implantation [2]. S. trifoliatus is pungent and bitter in taste. It has emetic actions i.e. it causes vomiting and nausea and, is known to cause irritation of gastric mucosa, when administrated orally [3]. The generic name is derived from the Latin words saponis, meaning "soap," and indicus, meaning of India [4].The leaves are alternate, 15–40 cm (5.9–16 in) long, pinnate, with 14-30 leaflets, the terminal leaflet often absent. The flowers form in large panicles, each flower small, creamy white. The fruit is a small leathery-skinned drupe 1–2 cm (0.39–0.79 in) in diameter, yellow ripening blackish, containing one to three seeds. Soap nuts have historically been used in folk remedies as a mucolytic agent, emetic, contraceptive, and for treatment of excessive salivation, epilepsy, and to treat chlorosis.

Modern scientific medical research has investigated the use of soap nuts in treating migraines and epilepsy where relaxation of various muscles takes place. This study was performed to study muscle relaxant activity. The drupes (soapnuts) contain saponins which are a natural surfactant. They have been used for washing for thousands of years by native peoples in Asia as well as Native Americans[5]. Soapnuts are being considered and used for commercial use in cosmetics and detergents as well as many other products [6,7].Soapnuts have historically been used in folk remedies as a mucolytic agent[8], emetic[9], contraceptive[10], and for treatment of excessive salivation[8], epilepsy[8,11], and to treat chlorosis [8]. While they do exhibit anti-inflammatory and anti-microbial properties, the effectiveness of some of these folk-remedy treatments has not been subject to extensive scientific scrutiny. However, modern scientific medical research has investigated the use of soapnuts in treating migraines [9, 11]. in view of its antiepileptic activity which is due to its muscle relaxant action. So the study is done to evaluate its muscle relaxant action.

EXPERIMENTAL SECTION

Plant Material: The pericarp of *Sapindus trifoliatus* was obtained from a local market and was authenticated by the Professor of Botany.

Extract Preparation: The pericarp of *Sapindus trifoliatus* was dried in air, crushed to coarse powder and extracted with distilled water using Soxhlet apparatus. The extract was dried under vacuum, stored at room temperature and protected from direct sunlight.

Drugs and Chemicals: Diazepam ((Lupin Laboratories Ltd., India), 10mg/kg and normal saline (0.9% NaCl solution) were administered in the volume of 10 ml/kg. The extracts were suspended in distilled water and subjected for muscle relaxant activity using Rotarod apparatus and Actophotometer. The extracts were administered orally (p. o.) in the volume of 10 ml/kg of body weight in the doses of 50mg, 100mg and 200mg/kg

Animals: Swiss Albino mice of the either sex weighing 20-25gm were used. The animals were housed in standard cages with free access to food (standard laboratory rodent's chow) and water. The animal house temperature was maintained at 23 ± 5.0^0C with a 12-h light/dark cycle (lights ON from 0600 to 1800hrs). Permission from Institutional Animal Ethics Committee constituted for the purpose of CPCSEA Government of India was taken. The guidelines for the investigation of experiments in conscious animals were followed in all tests.

Acute Toxicity Tests: Acute toxicity studies were conducted by using albino mice of either sex weighing 23-35 gm and of 90 days age. The animals were fasted overnight prior to the experimental procedure. The method of Up and Down or staircase was used to determine the dose [12, 13]. The median lethal dose of the extracts having muscle relaxant activity was determined by administering 50, 100, 200,400, 800, 1000and 2000 mg/kg body weight p. o. dose and observed for signs of behavioral, Neurological toxicity and mortality. The procedure was followed as per OECD 423 guidelines. Acute oral toxicity studies revealed the nontoxic nature of aqueous extract of *Sapindus trifoliatus*. There was no morbidity observed or any profound toxic reactions found a dose of 2000 mg/Kg p.o. which indirectly pronouns the safety profile of the plant extract.

Selection of dose for pharmacological screening*:* The aqueous extract of *Sapindus trifoliatus* was found to be non-toxic up to the dose of 2000 mg/kg and did not cause any death, therefore it is considered as safe. Hence 1/10th of this dose i.e. 200mg/kg body weight and half the $1/10^{th}$ i.e. 100mg/kg and ½ of it i.e.50mg/kg was used for the eluisidation of muscle relaxant activity.

Experimental design:
Group I – Control Rats (Normal saline 10 ml/kg)
Group II – Standard (Diazepam 10 mg/kg)
Group III – AEST 50mg/kg
Group IV – AEST 100mg/kg
Group V – AEST 200mg/kg

(i) Skeletal muscle relaxant activity (motor coordination*:* [14, 15] Mice were divided into five groups consisting of10 animals each. Group I served as control which received Normal saline 10ml/kg, Group II received standard drug Diazepam at a dose of 10mg/kg, *p.o*. Group III, IV and V received the aqueous extract of *Sapindus trifoliatus*

orally at a dose of 50, 100 and 200mg/kg. Animals remain on Rota-Rod (25 rpm) 5 min or more after low successive trials are included in the study. After the administration of control, standard and test material the fall off time from the rotating rod was noted after 2 hrs. The difference in the fall off time from the rotating rod between the control and the treated mice was taken as an index of muscle relaxation.

(ii) Locomotor activity*:* The spontaneous locomotor activity was assessed with the help of photoactometer [16]. Each animal was observed for a period of 5 min in a square closed Field arena (30 x 30 x 30 cm) equipped with 6 photocells in the outer wall. Interruptions of Photocell beams (locomotor activity) were recorded by means of a 6 digits counter.

To see the locomotor activity, the Actophotometer was turned on and each mouse was placed individually in the activity cage for 5 minutes. The basal activity score for all the animals was noted. Control normal saline, Standard Diazepam and three different doses of aqueous extract of *Sapindus trifoliatus* was given orally and after 1 hour re-testing, activity score for 5 minutes observed. The difference in the activity, before and after drug administration was noted. Percentage decrease in motor activity was calculated.

Statistical analysis

The results were expressed as mean ± SD. Statistical analysis was carried out by using ANOVA followed by Dunnet's multiple comparison tests using primer of windows McGraw –Hill software version 5.0.0.0 (2011). *P*-values < 0.05 were considered significant.

RESULTS

Actophotometer-- Test for locomotor activity

The percentage of reduction in locomotor activity with diazepam (10 mg/kg p.o.) after 1 hour is 96.06 i.e. there is highly significant (P<0.000) decrease in locomotor activity compare to control, where as three different doses of AEST (50,100 and 200 mg p.o.) showed dose dependent decrease in locomotor activity that is 71.37%, 85.11% and 87.73% respectively when compared to control. The values are highly significant (P<0.000) (Table I)

Rotarod test -- For muscle relaxation

In this test, AEST (100 mg/kg and 200mg/kg) showed highly significant reduction in the time spent by the animals on revolving rod when compared to control (P<0.01). The standard drug (Diazepam) also showed highly significant effect when compared to control (P<0.01) Low dose of AEST (50mg/kg) showed significant effect (P value < 0.05) (Table I). The result from the Actophotometer test and rotarod test showed that the extract significantly reduced the motor co-ordination of the tested animals.

TABLE I: Effect of AEST on locomotor activity in actophotometer and muscle coordination in rotarod apparatus

Groups n =10	Actophotometer score in 5 min before	After 60 min of administration	% of Reduction	Time spent on revolving rod in rotarod apparatus(sec)
Group I(control) NS 10ml/kg	158.3 ± 60.89	----	0	100 ± 10.54
Group II(standard) Diazepam10mg/kg	215.7 ± 70.12	9.33 ± 8.45***	96.06	12.2 ± 3.58**
Group-III AEST 50mg/kg	165.3 ± 13.98	47.33 ± 9.13***	71.37	82.8 ± 22.14*
Group-IV AEST 100mg/kg	218 ± 25.04	33 ± 22.57***	85.11	32.3 ± 15.07**
Group-V AEST 200mg/kg	198.3 ± 76.2	14.67±14.14***	87.73	20.3 ± 2.94**

*AEST-Aqueous extract of Sapindus trifoliatus. All values are Mean±SD, n = 10, *P<0.05, **P<0.01, ***P< 0.000 when compared with control.*

DISCUSSION

Sapindus is a genus of about five to twelve species of shrubs and small trees in the Lychee family, Sapindaceae, native to warm temperate to tropical regions in both the Old World and New World. The number of species is disputed between different authors, particularly in North America where between one and three species are accepted.

The genus includes both deciduous and evergreen species. Members of the genus are commonly known as soapberries [4] or soap nuts because the fruit pulp is used to make soap.

The drupes (soap nuts) contain saponins which are a natural surfactant. They have been used for washing for thousands of years by native peoples in Asia as well as Native Americans [6]. Soap nuts are being considered [7] and used [8] for commercial use in cosmetics and detergents as well as many other products. Soap nuts have gentle insecticidal properties and are traditionally used for removing lice from the scalp. The saponins present in the fruit on acidic hydrolysis give the triterpinoids hederagenin, D-glucose, L-rhamnose and D-xylose and Arabinose [16]. The percentages of individual acids were found to be: palmitic, 4.0; stearic, 0.2; arachidic, 4.4; oleic 62.8; linoleic, 4.6; linolenic, 1.6; and eicosenoic, 22.4. The oil is composed of 0.1, 2.1, 22.0, and 75.8% trisaturated, monounsaturated disaturatd, triunsaturated, monosaturated, and triunsaturated glycerides, respectively. The special characteristic of the Sapindus mukorossi seed oil is its content of 26.3 and 26.7% triolein and eicoseno-di-oleins, respectively [17]. The pericarp of the fruit of the plant *Sapindus trifoliatus* constitutes 62% of the fruit contains, glucose, saponins and primary metabolites. The Phytochemical tests with the methanol extract of S. emarginatus indicated the presence of glycosides, terpenes and, saponins [18]. In one of the studies [19] the methanolic extract of the leaves of Rumex nepalensis showed skeletal muscle relaxant activity which is due to the presence of anthraquinone, steroids, saponins, reducing sugars and tannins in the plant extract. As Sapindus also contains some of these chemicals we can expect skeletal muscle relaxant activity with its extract.

Sapindus trifoliatus was evaluated for its effect on MES- induced seizures in rats in many studies. The onset of generalized tonic-clonic seizures and duration of extensor seizures induced by MES was delayed in pretreatment with *Sapindus trifoliatus* which could be due to its CNS depressant and sedative property [20]. Drugs with anticonvulsant activity that do not exhibit sedation or death in animal models are considered safe. Hence the effect of the extract of S. trifoliatus was evaluated for the muscle relaxant action on rotarod performance and locomotor activity test in mice.

The study on the spontaneous motor activity showed that AEST (50,100 and 200 mg/kg, p.o) has dose dependent decrease in the frequency and the amplitude of movements. The reduction *of* the spontaneous motor activity could be attributed to the sedative effect of the extract [21].The myo relaxant effect was observed even with the smaller dose (50mg) of aqueous extract of *AEST* which showed decrease in the time on the bar as detected by the rotarod test. The standard reference drug Diazepam, which acts as a anxiolytic (at low doses), anticonvulsants and also produce sedation and a myorelaxant effect at higher doses [22]. In this case Diazepam at a dose of 10mg/kg body weight showed a significant lack in motor coordination and muscle relaxant activity in animals treated with the extract. The muscle relaxation and reduced motor activity effects of AEST could be due to the interaction of isoflavonoids (chemical constituent of the plant) with the GABA/benzodiazepine receptor complex in brain [23]. In another study [24] In Rotarod motor co-ordination test, ST at 100 mg/kg, i.p. significantly ($p < 0.05–0.01$) reduced the endurance time which is in correlation with our study.

CONCLUSION

In the present study, the effect of aqueous extract of *Sapindus trifoliatus* on muscle relaxation and motor coordination has been evaluated. The result indicated that the aqueous extract of *Sapindus trifoliatus* influence the muscle coordination as evidenced in the responses on Rotarod and actophotometer. As the comparison is done with centrally acting benzodiazepine group of drug diazepam, it is assumed that the muscle relaxation and reduced motor activity effects of AEST could be due to the interaction of isoflavonoids of the plant with the GABA/benzodiazepine receptor complex in brain [22]. The muscle relaxation property has to be further evaluated which could be used as centrally acting muscle relaxant like Diazepam.

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