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CNS Activity of the Methanol Extracts of *Sapindus emarginatus* Vahl in Experimental Animal Models

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Abstract

The aim of the present study is to investigate central nervous system (CNS) activity of the methanol extract of Pericarp of *Sapindus emarginatus* (Sapindaceae) in Swiss albino mice and Wistar albino rats. A daily dose of 100 and 200 mg/kg of the extract was administered to the animals for 15 days, after which various CNS experiments were recorded and compared with the control animals. General behavior, exploratory behavior, muscle relaxant activity cocaine induced hyperactivity and phenobarbitone sodium-induced sleeping time were studied. The results revealed that the methanol extract of Pericarp of *Sapindus emarginatus* at 100 and 200 mg/kg caused a significant reduction in the spontaneous activity (general behavioral profile), remarkable decrease in exploratory behavioral pattern (Y-maze and head dip test), a reduction in muscle relaxant activity (rotarod and traction tests), inhibition of cocaine induced hyperactivity and also significantly potentiated phenobarbitone sodium–induced sleeping time. The results suggest that methanol extract of *Sapindus emarginatus* exhibit CNS depressant activity in tested animal models.

Keywords: Sapindus emarginatus; Cocaine; CNS activity; Experimental animals.

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1. Introduction

Sapindus emarginatus Vahl family Sapindaceae is a medium-sized deciduous tree found in south india. It is commonly called as soap nut tree. This tree is 8 to 10 m high and has many branches with leaves and leaflets. Native to South India *Sapindus emarginatus* is found wild or introduced in tropical and sub-tropical regions, particularly the Indo-Malayan region. The genus *Sapindus* possesses tremendous medicinal value. Since past, it is used as emetic, tonic, astringent, anthelmintic, for asthma, colic, diarrhea, cholera, tubercular glands and paralysis of limbs. The fruit is commonly used as a remedy for hair problems and also in preparation of shampoos.

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Traditionally, *Sapindus emarginatus* is used as anti-inflammatory and antiprurutic [1]. It is used to purify the blood. The seed is in intoxicant and the fruit rind has oxytropic action. Its powder is used as nasal insufflations. *S. emarginatus* also showed strong anti-bacterial activity against the tested bacterial strains. An antifertility and antiandrogenic activity of *S. emerginatus* extract has been studied [2]. High content of saponins has been reported in the pericarp [3]. Two Pisicidal triterpenoid saponins [4], acetylated triterpene saponins, hederagenin, sweet acyclic sesquiterpene glycoside, Mukurozioside IIb [5] have been isolated from the Pericarps of *S. emarginatus.*

However, there are no reports on the central nervous system (CNS) activity of this plant; the present study was undertaken for the first time to investigate CNS activity of the methanol extract of *Sapindus emarginatus*.

2. Materials and Methods

2.1. *Plant materials and extraction*

The plant *S. emarginatus* fruit was collected in March 2007 from the Thiagarajar college campus, Madurai, Tamil Nadu, India. The plant material was taxonomically identified by the Botanical survey of India, Coimbatore, Tamilnadu, India and the voucher specimen BSI/SC/5/23/08-09/Tech 895 was retained in our laboratory for future reference. The dried powder material (500 g) of the pericarp of *Sapindus emarginatus* was extracted with 2000 ml. of methanol in a soxhlet apparatus. The methanol extract was then distilled, evaporated and dried in vacuum. The resulted extract yield was 7.45%, and the appearance of the extract was dried gum resin in nature.

2.2. *Experimental Animals*

Studies were carried out using Swiss albino mice (20 – 25 g) and Wistar albino rats (150 – 180 g) of either sex. They were obtained from the animal house, C. L. Baid Metha College of Pharmacy, Thoraippakkam, India. The animals were grouped and housed in polyacrylic cages (38×23×10 cm^3) with not more than eight animals per cage, and maintained under standard laboratory conditions (temperature 25±2^oC) with dark and light cycle (14/10 hour). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The mice were acclimatized to laboratory condition for 10 days before commencement of experiment. Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA approved the experimental protocol (IAEC ref no: IAEC/XIII/04/CLBMCP/2008-2009 dt/16-6-2008).

2.3. *Preliminary phytochemical analysis*

The Methanol extract of the Pericarp of *S. emarginatus* was subjected to preliminary phytochemical screening [6].

2.4. *Drugs*

The following drugs were used: Diazepam (Lupin Laboratories Ltd., India), phenobarbitone sodium (Rhone–Poulenc India Ltd., India), Morphine, Cocaine (M. M. Pharma, New Delhi, India), and Carboxy methyl cellulose (SRL Laboratories Ltd., India).

2.5. *Acute toxicity studies*

Albino mice weighing 22 – 25 g selected by random sampling technique were used in the study. Acute oral toxicity was performed as per OECD - 423 guidelines (acute class method) [7]. The animals were fasted overnight, provided only water after which extract was administered to the groups orally at the dose level of 5 mg/kg body weight by gastric intubation and the groups were observed for 14 days. If mortality was observed in 2 or 3 animals among 6 animals then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2,000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 hours.

2.6. *General behavioral profiles*

Evaluation of general behavioral profiles was performed by the method of Dixit and Varma [8]. Forty adult albino mice were divided in to five groups (n = 8). Methanol extract of *S. emarginatus* and 5% CMC was administered for the first four groups of animals at the dose of 50, 100 and 200 mg/kg p. o., 1 ml of 5% CMC as a vehicle control for 15 days respectively. While the last group were administered diazepam (5 mg/kg, i.p.) on the test day as a drug control. The animals were under observation for their behavioral changes, if any, at 30 min intervals in the first one hour and at the hourly intervals for the next 4 hour for the following parameters.

2.6.1. *Awareness, alertness and spontaneous activity*

The awareness and alertness was recorded by visual measure of the animal's response when placed in a different position and its ability to orient itself without bumps or falls. The normal behavior at resting position was scored as (-), little activity (+), moderate flexibility (+ +), strong response (+ + +), and abnormal restlessness as (+ + + +). The spontaneous activity of the mice was recorded by placing the animal in a bell jar. It usually shows a moderate degree of inquisitive behavior. Moderate activity was scored as (+ +) and strong activity as (+ + +). If there is little motion, the score was (+), while if the animal sleeps, the score was (–). Excessive or very strong inquisitive activity like constant

walking or running was scored as (+ + + +). A similar test was performed with the same scoring, when the animals are removed from the jar and placed on a table [9].

2.6.2. *Righting reflex*

Groups of mice were treated with the test compounds on the test day. After 15, 30 and 60 min, each mouse was placed gently on its back on an undulated surface made of white iron and kept at 30^{o}C. If the animal remained on its back for 30 s, it was considered as a loss of righting reflex.

2.6.3. *Pinna reflex*

The reflex is examined by touching the center of pinna with a hair or other fine instrument. The unaffected mouse withdraws from the irritating hair [9].

2.6.4. *Grip strength*

The grip strength test is used to assess muscular strength or neuromuscular function in rodents. It was measured by allowing the animal to grasp a pencil in the horizontal position and noting the time taken by the animal to drop the pencil on the table [9].

2.6.5. *Touch response*

The touch response was recorded by touching the mice with a pencil or forceps at the various part of the body (i.e. on the side of the neck, abdomen and groin).

2.6.6. *Pain response*

The pain response was graded when a small artery clamp was attached to the base of the tail, and response was noted.

2.6.7. *Sound response*

Swiss albino mice normally utter no sound, so that vocalization may indicate a noxious stimulus.

The righting reflex, pinna reflex, Grip strength, touch, pain and sound response were carried out to study the depressant action of the *Sapindus emarginatus* extract at various doses. The depressant action was scored as no effect (-), slight depression (+), moderate depression (++), strong depression (+++), very strong depression (++++). A trained observer unaware of the experiment assigned the score for the general behavioral studies.

2.7. *Cocaine-induced hyperactivity experiments in rats*

Male wistar albino rats were divided in to four groups of eight in each used. The animals were removed from the holding room and randomly assigned to treatment groups. Animals received either the vehicle or *S. emarginatus* (50, 100 & 200 mg/kg) for fifteen days and were placed in the activity cages. Following the 30 min of habituation period on the test day, the animals received cocaine (40 mg/kg i.p.) and were returned to the activity

cages for a further 90 min. Activity was measured as light beam interruptions per 10 min period [10].

2.8. *Effect of phenobarbitone sodium–induced sleeping time*

Swiss albino mice were divided into four groups of eight in each. On the test day animals received 40 mg/kg (i.p.) phenobarbitone sodium 30 min after the administration of methanol extract of *S. emarginatus* at the dose of 50, 100 and 200 mg/kg and vehicle control 1 ml of 5% CMC. The sleeping time was recorded, and measured as the time interval between the loss and regaining of the righting reflex [11].

2.9. *Exploratory behavior*

This was performed by Y–maze and head dip tests.

2.9.1. *Y-maze test*

Y-maze test is used to measure the exploratory behavior in mice. This was performed in the groups of 8 albino mice at 30, 60, 90 and 120 min. after administration of either CMC, methanol extract of *S. emarginatus* (50, 100 and 200 mg/kg, p.o.) or diazepam (5 mg/kg, i.p.) respectively on the test day. The mice were placed individually in a symmetrical Y–shaped runway (33cm×38cm×13cm) for 3 min. and the number of the maze with all 4 ft (an 'entry') were counted [12].

2.9.2. *Head dip test*

The evaluation of certain components of behavior of mice such as curiosity or exploration has been attempted in Head dip test. Five groups of albino mice (n = 8) were placed on top of a wooden box with 16 evenly spaced holes, 30 min. after administration of the methanol extract of *S. emarginatus* (50, 100 and 200) mg/kg , vehicle (1 ml of 5% CMC) and diazepam (5 mg/kg) respectively. The number of times that each animal dipped its head into the holes was counted for the period of 3 min. [13].

2.10. *Muscle relaxant activity*

The effect of extracts on muscle relaxant activity was studied by the traction test and Rota rod test.

2.10.1. *Traction test*

The forepaws of the mice was placed in a small twisted wire rigidly supported above the bench top, and the screening of animal was performed for traction test. Normally the mice grasp the wire with the forepaws and place at least one hind foot on the wire without 5 s when allowed to hang free. The test was conducted on five groups of animals (n = 8) that were previously screened, on the 15th day 30 min after the administration of methanol extract of *S. emarginatus* (50, 100 and 200 mg/kg, p. o.), CMC (5%) & diazepam (5

mg/kg, i.p.) the test was carried out. Inability to put up at least one hind foot is considered as failure in the traction test [14].

2.10.2. *Rotarod test*

The test is used to evaluate the activity of drugs interfering with motor coordination. Fresh mice were placed on a horizontal wooden rod (32 mm diameter) rotating at a speed of 16 rpm (Model 7600; Ugo Basile). The mice capable of remaining on the top for 3 min or more, in three successive trails were selected for the study. The selected animals were divided into five groups (n = 8). After administration of doses on test day each group of animals was then placed on the rod at an interval of 30, 60, 90, 120 and 150 min. The animals failed more than once to remain on the rotarod for 3 min were considered as passed the test [15].

2.11. *Statistical analysis*

The results were expressed as mean ± S.E.M. Statistical analysis was carried out by using ANOVA followed by Dunnet's multiple comparison tests using Graph pad PRISM software version 4.03 (2005). The Chi-square test was used for the % muscle relaxant activity. *P*-values < 0.05 were considered significant.

3. Results

The methanol extract of *S. emarginatus* was found to be non–toxic up to the dose of 2 g/kg and did not cause any death of the tested animals. The results of the preliminary phytochemical test of methanol extract of *S. emarginatus* have been presented in Table 1. The Phytochemical tests with the methanol extract of *S. emarginatus* indicated the presence of glycosides, terpenes and, saponins.

Table 1. Preliminary phytochemical test of S. *emarginatus* methanolic extract.

S. No.	Phytochemical Tests	Results
1.	Test for Alkaloids	-
2.	Test for Carbohydrates	+
3.	Test for Proteins	-
4.	Test for Steroids	-
5.	Test for Sterols	-
6.	Test for Phenols	+
7.	Test for Flavonoids	+
8.	Test for Gums and mucilage	+
9.	Test for Glycosides	+
10.	Test for Saponins	+
11.	Test for Terpenes	+

'+' Indicates the presence of compounds; '-' Indicates the absence of compounds.

The results obtained from different experiments for effect on general behavioral profiles are presented in Table 2. The methanol extract of *S. emarginatus* affected spontaneous activity, sound and touches responses at dose of 200 mg/kg and produced moderate or slight depression relating to awareness and alertness. However, the standard drug diazepam caused a significant depression of all these responses compared with the methanol extract of *S. emarginatus*.

Table 2. Effect of methanolic extract of *S. emarginatus* on general behavioral profiles in mice.

Behavior type	Extract (mg/kg)			Diazepam	CMC
	50	100	200	(5mg/kg)	(5%ml)
Spontaneous activity	+	++	+++	++++	-
Alertness	+	++	+++	+++	-
Awareness	+	++	+++	+++	-
Sound response	+	++	++++	++++	-
Touch response	++	+++	++++	++++	-
Pain response	+	+++	+++	++++	-
Righting reflex	+	++	+++	++++	-
Pinna reflex	++	+++	+++	++++	-
Grip strength	++	+++	+++	++++	-

No effect (-); slight depression (+); moderate depression (++); strong depression (+++); very strong depression (++++); $n = 8$.

Methanol extract of *S. emarginatus* produced a partial inhibition of the hyperactivity induced by cocaine (40 mg/kg) in rats. This suppression by methanol extract of *S. emarginatus* was evident from 40 through 90 min after the cocaine injection and the results are tabulated in Table 3.

Table 3. Effect of Methanol extract of *S. emarginatus* on cocaine induced hyperactivity.

No. of light interruptions at an interval of 10 minutes	Experiment & Dose			
	CMC (5% ml)	Extract (50mg/kg)	Extract (100mg/kg)	Extract (200mg/kg)
10	215±2.13	175±1.63	180±0.85	165±0.74
20	170±1.63	165±1.56*	155±0.52*	145±0.58*
30	130±1.28	150±1.43*	100±0.43*	90±0.49*
40	175±1.68	125±1.18*	100±0.27*	90±0.31*
50	185±1.75	110±0.98*	110±0.99*	80±0.78*
60	160±1.56	110±0.96*	100±0.96*	80±0.68*
70	140±1.37	85±0.68*	95±0.88*	70±0.68*
80	130±1.28	65±0.58*	80±0.78*	60±0.56*
90	100±0.98	55±0.45*	70±0.63*	50±0.46*

Values are the number of entries in 3 min (mean + S.E.M., $n = 8$); *Significant difference between control group and treated group; $P<0.05$, ANOVA followed by Dunnett's Multiple comparison test.

Methanol extract of *S. emarginatus* significantly potentates the phenobarbitone sodium–induced sleeping time in a dose dependent manner. The results are interpreted in Table 4.

Table 4. Effect of methanol extract of *S. emarginatus* on phenobarbitone sodium–induced sleeping time.

Experiment	Dose	sleeping time (min)
CMC	5% 1ml	64±5.9
Extract plus Phenobarbitone sodium	50mg/kg	70±6.2*
	100mg/kg	82±7.4*
	200mg/kg	112±7.3*

Values are expressed as mean + S.E.M., n = 8;
* Significant difference between control group and treated

In Y-maze test, the animals treated with methanol extract of *S. emarginatus* at the doses of 100 mg/kg and 200 mg/kg showed a marked decrease in exploratory behavior compared with control (Table 3). In case of head dip test, mice treated with different dose of ethanol extract of *S. emarginatus* showed marked decreases in head dip responses when compared to control (Table 5).

Table 5. Effect of Methanol extract of *S. emarginatus* on exploratory behavior (Y-maze test) in mice.

Experiment	Dose	Number of entries after treatment			
		30	60	90	120
CMC	5ml/kg	9.4±0.81	9.4±0.21	9.5±0.85	9.4±0.74
Diazepam	5mg/kg	3.2±0.28*	3.3±0.12*	3.5±0.23*	3.4±0.26*
Extract	50mg/kg	6.6±0.57*	6.7±0.51*	6.8±0.52*	7.0±0.58*
Extract	100mg/kg	5.2±0.39*	5.3±0.43*	5.3±0.43*	5.3±0.49*
Extract	200mg/kg	3.7±0.31*	3.7±0.27*	3.9±0.27*	3.9±0.31*

Values are the number of entries in 3 min (mean + S.E.M., n = 8).
* Significant difference between control group and treated group; P<0.05; ANOVA followed by Dunnett's Multiple comparison test.

In the traction test, the mice treated with methanol extract of *S. emarginatus* showed a significant failure in traction at all doses tested. The result obtained from the Rota rod test, showed that methanol extract of *S. emarginatus* at 100 mg/kg (70%) and 200 mg/kg (80% respectively) significantly reduced the motor co-ordination of the tested animals (Table 6).

Table 6. Effect of Methanol extract of *S. emarginatus* on exploratory behavior (head dip test) and muscle relaxant activity.

Experiment	Dose	Head dip test	Traction test	Rotarod test
CMC	5ml/kg	95 ± 8.4	0	0
Diazepam	5mg/kg	28±2.3*	100	100
Extract	50mg/kg	65±5.9*	60*	60*
Extract	100mg/kg	56±4.7*	70*	70*
Extract	200mg/kg	30±2.8*	80*	80*

Exploratory behavior: Values are the number of head dips in 3 min (mean + S.E.M), (n=8);
*Significant difference between control group and treated group; p<0.05, ANOVA followed by Dunnett's Multiple comparison's test;
Muscle relaxant activity: Values are the percentage animals showing a negative results; n = 8;
*p<0.05 compared with control (Chi-square test).

4. Discussion

In the present study, the effect of methanol extract of *S. emarginatus*on CNS activity has been evaluated. The result indicated that the methanol extract of *S. emarginatus* influence the general behavioral profiles, as evidenced in the spontaneous activity, righting reflex, pinna reflex, grip strength and pain responses. Reduction of awareness and depressant action may be due to the action of the extract on CNS. Reduction of pinna reflex may be due to blocking synapses of the afferent pathway.

The effect on the CNS of the different dose of methanol extract of *S. emarginatus* has produced a significant increase in the hypnotic effect induced by the phenobarbitone, in a dose dependent manner, thus suggesting a profile sedative activity. It is emphasized that the method employed for this assay is considered as a very sensitive way and denote agent with depressor activity on the CNS. The sedative effect recorded in this study may be related to an interaction with benzodiazepines and related compounds that bind to receptors in the CNS and have already been identified in certain plant extracts.

The myorelaxant effect was observed only with the higher dose of methanol extract of *S. emarginatus* which resulted in an increase in the number of falls and a decrease in the time on the bar as detected by the rotarod test. The intensity of reduction in exploratory behaviors in the treated animal groups which reflects the same line of action like the standard reference drug benzodiazepine, which acts as a anxiolytic (at low doses), anticonvulsants and also produce sedation and a myorelaxant effect at higher doses [16]. The reduction in exploratory behavior in animals treated with methanol extract of *S. emarginatus* is similar with the action of other CNS depressant agents in Y-maze test. A significant lack in motor coordination and muscle relaxant activity was also noted in animals treated with the extract.

Administration of cocaine to rats, which releases both dopamine and noradrenalin, causes a cessation of normal 'ratty' behavior (exploration and grooming), and the appearance of repeated 'stereotyped' behavior (rearing, gnawing and so on) unrelated to

external stimuli. These effects are prevented by dopamine antagonists and by destruction of dopamine-containing cell bodies in the midbrain, but not by drugs that inhibit the noradrenergic system. The cocaine-induced motor disturbances in rats probably reflect hyperactivity in the nigrostriatal dopaminergic system. Numerous studies have demonstrated that dopamine antagonists prevent the hyperactivity following cocaine administration in mice and rats. For example, haloperidol and clozapine in a dose-dependent manner attenuated the locomotor effects of cocaine [17]. SCH23390, the selective D1 antagonist, also significantly suppressed the locomotor response produced by cocaine. These data suggest that both D1 and D2 receptors are involved in cocaine-induced hyperactivity [17-18]. In the present study, methanol extracts of *S. emarginatus* produced a partial reduction in the hyperactivity produced by cocaine.

It has been reported that *S. emarginatus* contains triterpenoids & saponins. A number of scientific reports indicated that triterpenoids produced CNS depressant action [19]. Therefore the presence of these active constituents in the methanol extract of *S. emarginatus* may be responsible for the CNS activity. Since the pharmacological profiles of the present investigation of the methanol extract of *S. emarginatus* was similar to that of dopamine it is also possible that they might interact with dopamine receptor.

However, further investigation is underway to determine the exact phytoconstituents that are responsible for CNS depressant activity of methanol extract of *S. emarginatus* and the receptors involved for the execution of the activity.

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