

Full Length Research Paper

Central nervous system depressant and analgesic activities of *Scutia myrtina* in experimental animal model

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The purpose of this study is to investigate the central nervous system (CNS) depressant and analgesic activities of the ethanol extract of *Scutia myrtina* (EESM) (Family: Rhamnaceae) in Swiss albino mice. To evaluate the CNS depressant activity by using the methods such as general behavior, exploratory behavior, muscle relaxant activity and phenobarbitone sodium-induced sleeping time were studied. Analgesic effect of EESM was evaluated in acetic acid induced writhing and hotplate tests. The results revealed that the EESM at the dose of 200 and 300 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), and also significantly potentiated phenobarbitone sodium-induced sleeping time. The EESM also produced significant analgesic activity in both models at the dose of 200 and 300 mg/kg. Further, the EESM potentiated the morphine and aspirin induced analgesic in mice. The results suggest that EESM exhibit CNS depressant and analgesic activity in tested animal models.

Key words: *Scutia myrtina*, central nervous system (CNS) activity, analgesic, mice, ethanol extract.

INTRODUCTION

Scutia myrtina (Rhamnaceae) is widely available in South India, especially in Kolli Hills, Tamilnadu. It is commonly known as Chimat (Hindi), a prickly shrub found throughout the hotter parts of India, East Africa, Kenya, Tanzania, and South Africa. The aerial part of the plant was used for stomach problems, salpingitis. The root and leaves of the plant is traditionally used as an antihelmintic (Kokwaro, 1976). The alcohol extract of the aerial part of the plant posses antiviral activity (Dhar et al., 1968). The root bark of *S. myrtina* is used for fever and also the

infusion of the plant is used to treat malaria. An alkaloid nitidine with potent antimalarial activity has been isolated from a Kenyan herbal remedy (Gakunju et al., 1995). In eastern Tanzania the root of this plant is used for the treatment of bilharzias, intestinal worms and fever (Chhabra et al., 1991). The leaves and root bark of the *S. myrtina* decoction is used for gonorrhoea, bilharzias, and intestinal worms in Tanzania (Hedberg et al., 1983).

Pervious report from our laboratory showed the anti-inflammatory and antimicrobial activity of petroleum ether

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and ethanol extracts of *S. myrtina* (Sambath Kumar et al., 2009). However, there are no reports on the central nervous system (CNS) activity of this plant, although decoction of *S. myrtina* was extensively used by the tribes in Kolli Hills of Namakkal District, Tamilnadu, India, to reduce mental tension and also induce sleep. Therefore, in the light of their use in traditional medicine as a sedative and antidepressant agent, the present study was undertaken for the first time to investigate CNS depressant and analgesic activities of the ethanol extract of *S. myrtina* (EEMS) in experimental animal models.

MATERIALS AND METHODS

Plant materials and extraction

The whole plant *S. myrtina* was collected in the month of December 2008 from the Kolli Hills, Tamilnadu, India. The plant material was taxonomically identified by the Botanical Survey of India, Coimbatore, Tamilnadu, India and the voucher specimen RRI/BNG/SMP-Prog/945 was retained in our laboratory for future reference.

The entire plant of *S. myrtina* was dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve number 40 and retained in sieve number 60 and stored in an airtight container for further use. The dried powder material of the plant (500 g) was defatted with petroleum ether (60 to 80°C) for 48 h in soxhlet apparatus (yield 2.75% w/w). The defatted plant material thus obtained was further extracted with ethanol for 72 h in the soxhlet. The solvent was removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotatory flash evaporator to yield (7.45% w/w) a solid residue (ethanol extract). The ethanol extracts of *S. myrtina* (EEMS) was selected for CNS depressant analgesic activity. The chemical constituents of the extract were identified by qualitative analysis followed by their confirmation by thin layer chromatography, which indicate the presence of alkaloid, flavonoids, triterpenoids and steroids.

Animals

Studies were carried out using Swiss albino mice (20 to 25 g) of either sex were used. The animals were grouped and housed in polyacrylic cages (38 × 23 × 10 cm) with not more than eight animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C) with dark and light cycle (14/10 h). 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. All procedures described were reviewed and approved by the institutional animal's ethical committee.

Drugs

The following drugs were used: Diazepam (Lupin Laboratories Ltd., India), Phenobarbitone sodium (Rhône-Poulenc India Ltd., India), Morphine (M.M. Pharma, New Delhi, India), Aspirin (USV, Bombay, India), and Propylene glycol (SRL Laboratories Ltd., India). All the chemicals used in the present study are of analytical grade.

Acute toxicity in animal

For toxicity studies the test extracts in the range of doses 100 to 1600 mg/kg were administered in five groups of 10 mice respectively. The mortality rates were observed after 72 h. The LD₅₀ was determined using the graphical methods of Litchfield and Wilcoxon (1949).

General behavioral profiles

Evaluation of general behavioral profiles was performed by the method of Dixit and Varma (1976). Forty adult albino mice were divided into five groups (n = 8). EEMS was administered for the first three groups of animals at the dose of 100, 200 and 300 mg/kg (i.p.) respectively. While the last two groups were administered diazepam (5 mg/kg) as a drug control and propylene glycol (5 ml / kg) as a vehicle 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 h for the following parameters (Gupta et al., 1998; Turner, 1965).

Awareness, alertness and spontaneous activity

The awareness and alertness was recorded by visual measure of the animals' response when placed in a different position and its ability to orient itself without bumps or falls (Gupta et al., 1998). 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 scores 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 scores as (+ + + +). A similar test was performed with the same scoring, when the animals are removed from the jar and placed on a table (Gupta et al., 1998; Turner, 1965).

Righting reflex

Groups of mice were injected intraperitoneally with the test compounds. 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°C. If the animal remained on its back for 30 s, it was considered as a loss of righting reflex.

Pinna reflex

Touching the center of pinna with a hair or other fine instrument was used to test the mouse. The unaffected mouse withdraws from the irritating hair (Turner, 1965).

Grip strength

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 (Turner, 1965).

Touch response

The touch response was recorded by touching the mice with a

pencil or forceps at the various part of the body (that is, on the side of the neck, abdomen and groin).

Pain response

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

Sound response

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

Effect of phenobarbitone sodium-induced sleeping time

Mice were divided into four groups of eight in each. Animals received 40 mg/kg (i.p.) phenobarbitone sodium 30 min after the injection of EESM at the dose of 100, 200 and 300 mg/kg, and vehicle control propylene glycol (5 ml/kg).

The sleeping time was recorded, and measured as the time interval between the loss and regaining of the righting reflex (Dandiya and Collumbine, 1956).

Exploratory behavior

This was performed by (i) Y-maze and (ii) head dip tests.

Y-maze test

This was performed in the groups of 8 albino mice at 30, 60, 90 and 120 min after injection of either propylene glycol (5 ml/kg), EESM (100, 200 and 300 mg/kg), or diazepam (5 mg/kg), respectively. The mice were placed individually in a symmetrical Y-shaped runway (33 × 38 × 13 cm) for 3 min and the number of the maze with all 4 ft (an 'entry') were counted (Rushton et al., 1961).

Head dip test

Seven groups of albino mice (n = 8) were placed on top of a wooden box with 16 evenly spaced holes, 30 min after injection of the EESM (100, 200 and 300 mg/kg) vehicle (5 ml/kg propylene glycol) 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 (Dorr et al., 1971).

Muscle relaxant activity

The effect of extracts on muscle relaxant activity was studied by the (a) traction test and (b) rotarod test.

Traction test

The screening of animal was done by placing the forepaws of the mice in a small twisted wire rigidly supported above the bench top. Normally, the mice grasp the wire with the forepaws, and place at least one hind foot on the wire within 5 s when allowed to hang free. The test was conducted on seven groups of animals (n = 8) that were previously screened, 30 min after the injection of EESM (100,

200 and 300 mg/kg), diazepam (5 mg/kg) or propylene glycol (5 ml/kg) as a vehicle control. Inability to put up at least one hind foot was considered failure in the traction test (Rudzik et al., 1973).

Rotarod test

Fresh mice were placed on a horizontal wooden rod (32 mm diameter) rotating at a speed of 5 rpm. The mice which are capable of remaining on top for 3 min or more, in three successive trails, were selected for the study. The selected animals were divided into seven groups (n = 8). EESM at the dose of 100, 200 and 300 mg/kg respectively were injected intraperitoneally into Groups 1, 2 and 3. Propylene glycol (5 ml/kg) and diazepam (5 mg/kg) was given to Groups 4 and 5. Each group of animals was then placed on the rod at an interval of 30, 60, 90, 120 and 150 min. The animals that failed more than once to remain on the rotarod for 3 min were considered to have passed the test (Dunham and Miya, 1957).

Analgesic activity

Analgesic activity was studied by (i) Acetic acid-induced writhing response in mice and (ii) Hot plate reaction time in mice.

Acetic acid-induced writhing response in mice

Acetic acid solution (15 mg/ml) at the dose of 300 mg/kg b.w. was injected i.p. and the number of writhes during the following 30 min period was observed (Turner, 1965). Swiss albino mice of either sex were divided into 8 groups of eight animals each. Propylene glycol (5 ml/kg), EESM at the dose of 100, 200 and 300 mg/kg, and Aspirin (100 mg/kg, b.w., i.p.) and combination of different dose of EESM with morphine were administered intraperitoneally to Groups 1 to 8 respectively. A significant reduction in the number of writhes by drug treatments as compared to vehicle control animals was considered as a positive analgesic response. The percentage inhibition of writhing was then calculated and was used as standard.

Hot plate reaction time in mice

Swiss albino mice of either sex were divided into 8 groups of eight animals each. Propylene glycol (5 ml/kg), EESM at the dose of 100, 200 and 300 mg/kg, and morphine (5 mg/kg) and combination of different dose of extract with morphine were administered intraperitoneally to Groups 1 to 8 respectively. Mice were screened by placing them on a hot plate maintained at 55±1°C and the reaction time was recorded in second for licking of hind paw or jumping (Turner, 1965). The mice which reacted within 15 s and which did not show large variation when tested on four separated occasions were selected for studies. Morphine (5 mg/kg, b.w. i.p.) was used as standard.

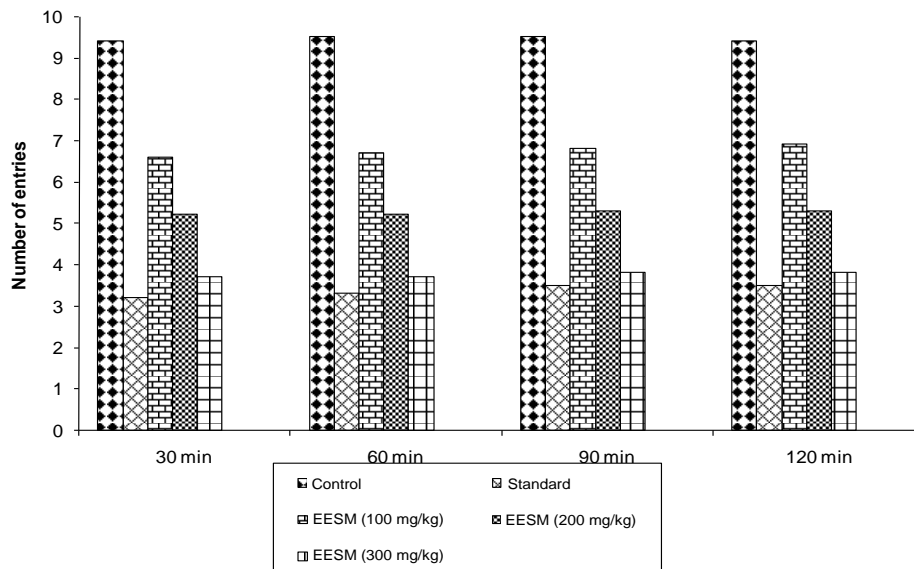
Statistical analysis

The results were expressed as mean ± S.E.M. Statistical analysis of difference between groups was evaluated by ANOVA followed by Dunnett's post hoc test. The Chi-square test used for the % muscle relaxant activity (Woodson, 1987). A value less than 0.05 were considered significant.

Table 1. Effect of the ethanol extract of *Scutia myrtina* EESM on general behavioral profiles in mice.

Behavior type	EESM (mg/kg)			Diazepam	Propylene glycol
	100	200	300	(5 mg/kg)	(5 ml/kg)
Spontaneous activity	+	++	+++	++++	–
Alertness	+	++	+++	+++	–
Awareness	+	++	+++	+++	–
Sound response	+	+++	++++	++++	–
Touch response	++	+++	++++	++++	–
Pain response	+	+++	+++	++++	–
Righting reflex	+	++	+++	++++	–
Pinna reflex	++	+++	+++	++++	–
Grip strength	++	+++	+++	++++	–

EESM: Ethanol extract of *Scutia myrtina*, –, no effect; +, slight depression, ++, moderate depression, +++, strong depression, +++++, very strong depression, n = 8.

**Figure 1.** Effect of ethanol extract of *Scutia myrtina* (EESM) on exploratory behaviour (Y-maze test) in mice.

RESULTS

Toxicity study

The EESM was found to be non-toxic up to the dose of 1.6 g/kg and did not cause any death of the tested animals.

Effect on general behavioral profiles

The results obtained from different experiments are presented in Table 1. The EESM affected spontaneous activity, sound and touches responses at dose of 300

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 EESM.

Exploratory behavior potentials

In Y-maze test, the animals treated with EESM at the doses of 200 and 300 mg/kg showed a marked decrease in exploratory behavior compared with control. In case of head dip test, mice treated with different dose of EESM showed marked decreases in head dip responses when compared to control (Figures 1 and 2).

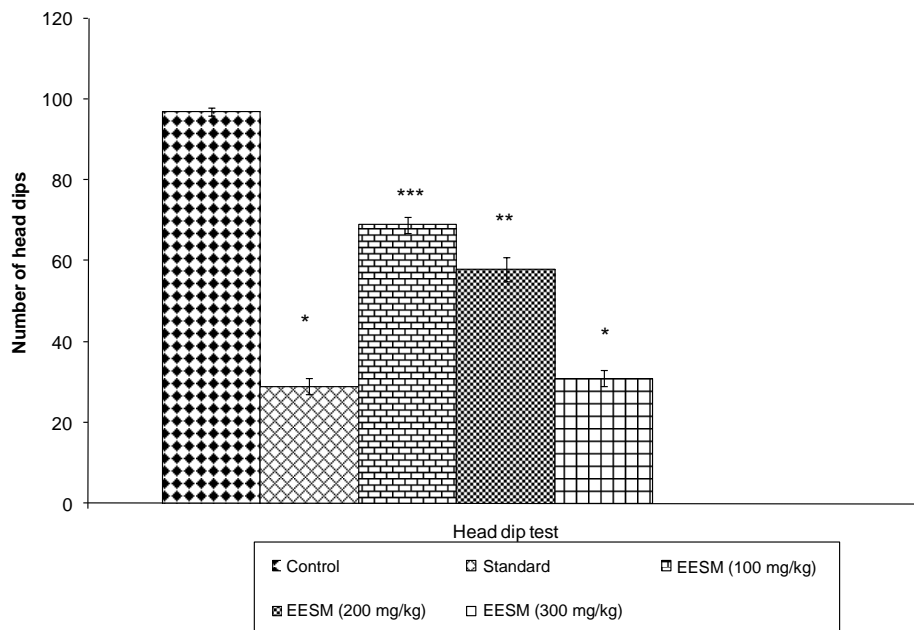


Figure 2. Effect of ethanol extract of *Scutia myrtina* (EESM) on exploratory behaviour (head dip test) in mice.

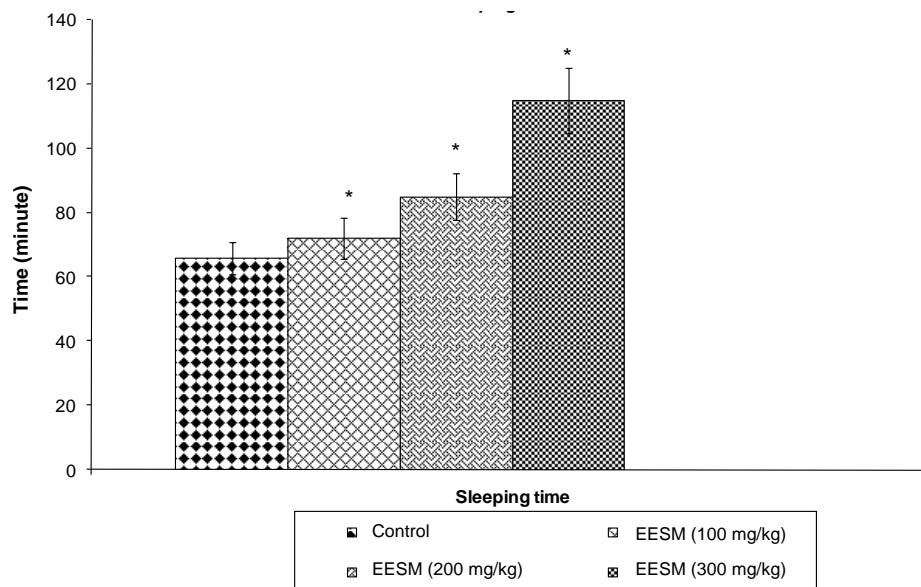


Figure 3. Effect of ethanol extract of *Scutia myrtina* (EESM) on phenobarbitone sodium induced sleeping time in mice

Effect on phenobarbitone sodium-induced sleeping time

The EESM significantly potentiates the phenobarbitone sodium-induced sleeping time in a dose dependent manner. While, the EESM at 200 and 300 mg/kg dose showed much better results (Figure 3).

Effect on muscle relaxant activity

In the traction test, the mice treated with EESM showed a significant failure in traction at all doses tested. The result obtained from the rotarod test, showed that EESM at 200 mg/kg (70%) and 300 mg/kg (80% respectively) significantly reduced the motor coordination of the tested

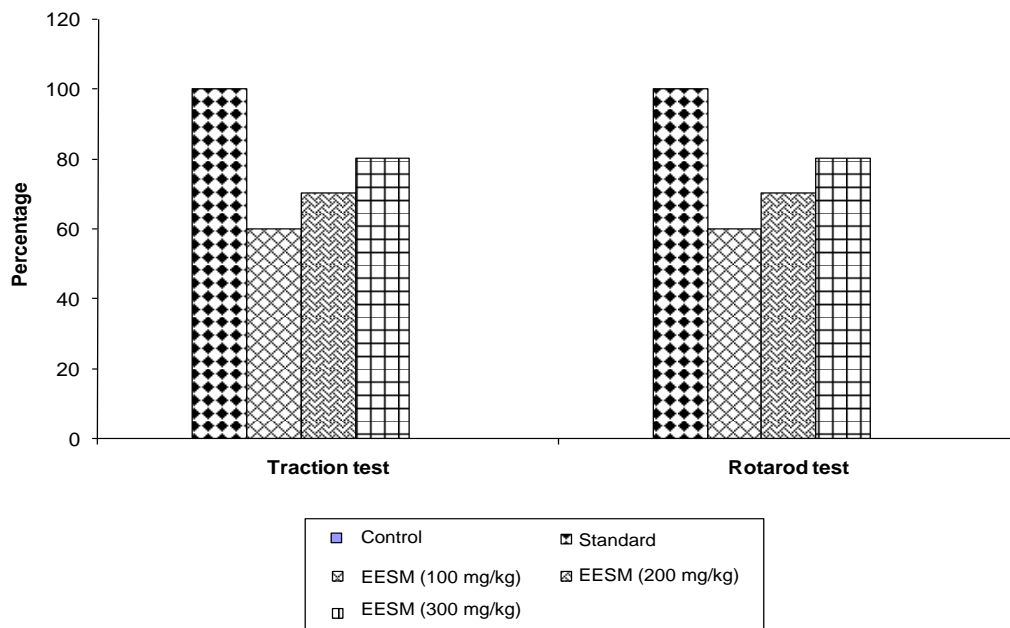


Figure 4. Effect of ethanol extract of *Scutia myrtina* (EESM) on muscle relaxant activity (Traction test and Rotarod test) in mice.

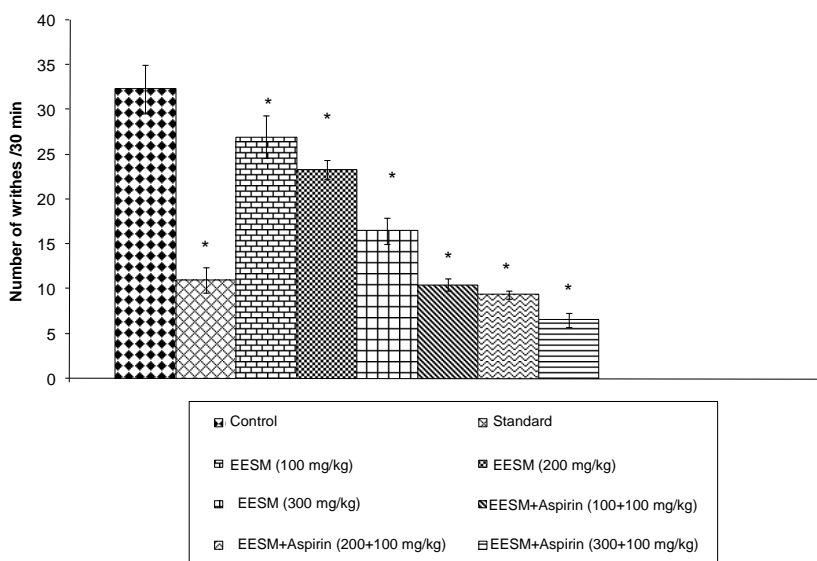


Figure 5. Effect of ethanol extract of *Scutia myrtina* (EESM) on acetic acid induced writhing in mice.

animals (Figure 4).

Analgesic activity

Acetic acid-induced writhing in mice

Analgesic effects induced by different doses of EESM on

the writhing test in mice are shown in Figure 5.

EESM at the dose of 100, 200 and 400 mg/kg, b.w. and aspirin 100 mg/kg, b.w. exhibited significant ($P < 0.01$) inhibition of the control writhes at the rate of 20.6, 36.7, 53.4 and 66.7% respectively in the acetic acid-induced writhing. In addition, EESM at the different doses also potentiated (71.4, 77.0 and 84.0%) the aspirin-induced analgesia.

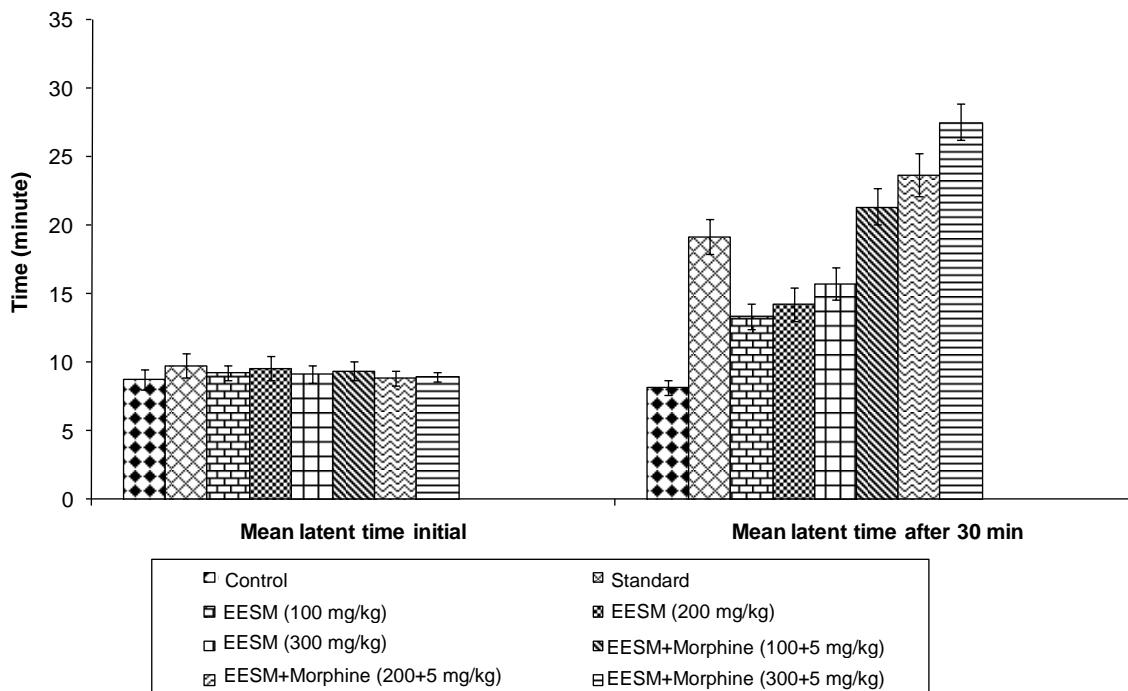


Figure 6. Effect of ethanol extract of *Scutia myrtina* (EESM) on hot plate reaction time in mice.

Hot plate reaction time in mice

As shown in Figure 6, the EESM produce significant ($P < 0.01$) analgesic activity at all the tested doses. Additionally, EESM at different doses potentiated the analgesic activity of morphine (5 mg/kg., b.w.).

Preliminary phytochemical tests

The results of the preliminary phytochemical group test of EESM are shown in Table 2. The phytochemical tests with the EESM indicated the presence of, triterpenoids, flavonoids, alkaloids, saponins, tannins and steroids.

DISCUSSION

In the present study, the effect of EESM on CNS activity has been evaluated. The result indicated that the EESM 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 (Johnson et al., 1970). The reduction of pinna reflex may be due to blocking synapses of the afferent pathway (Scholfield, 1979).

The effect on the CNS of the different dose of EESM

produced a significant increase in the hypnotic effect induced by the phenobabitone, in a dose dependent manner, thus suggesting a profile sedative activity. It should be emphasized that the method employed for this assay is considered as a very sensitive way denote agent with depressor activity on the central nervous system (Carlini, 1973). The sedative effect recorded here may be related to an interaction with benzodiazepines and related compounds that bind to receptors in the CNS that has already been identified in certain plant extracts (Viola et al., 1993; Medina, 1990; Medina and Merder, 1996).

A myorelaxant effect was observed only with the higher dose of EESM 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 act as a anxiolytics (at low doses), anticonvulsants, and also produce sedation and a myorelaxant effect at higher doses (Onaivi et al., 1992; Tang et al., 1993; Davies et al., 1994; Wolfman et al., 1993). The reduction in exploratory behavior in animals treated with EESM is similar with the action of other CNS depressant agents. A significant lack in motor coordination and muscle relaxant activity was also noted in animals treated with the EESM. The EESM was also evaluated in the acetic acid-induced abdominal writhing, as well as hotplate method for its

Table 2. Preliminary phytoconstituents present in ethanol extract of *Scutia myrtina* EESM.

S/N	Phytoconstituents	Ethanol extract of <i>Scutia myrtina</i>
1	Alkaloids	+
2	Flavonoids	+
3	Triterpenoids	+
4	Steroids	+
5	Saponins	-
6	Tannins	+
7	Reducing sugar	+
8	Amino acid	-
9	Gums	-

'-' Absence; '+' Presence.

analgesic activity. The extract effective against acute phasic pain and the effect are mediated centrally at the supraspinal level (Wong et al., 1994). Alternatively, the damping of this effect with high dose of extract may results from the coexistence of components of this extract, which may block pain inhibition pathways of the brain. Such a mode of action is proposed for opioid analgesic such as morphine (Roumy and Jean-Marie, 1998). It is also reported that the inhibition of pain could arise not only from the presence of opioids and/ or opiodiomimetics but could also arise from the presence of phenolic constituents (De Campos et al., 1997) and also steroidal constituents (Miguel et al., 1996). So it may be due to the presence of phenolic and steroidal constituents present in the extract of EESM which is, exhibited the analgesic activity.

In acetic acid-induced abdominal writhing which is the visceral pain model, the release of arachidonic acid by the processor via cyclooxygenase and prostaglandin biosynthesis plays a role in the nociceptive mechanism (Meade et al., 1986). Results of the present study show that all the doses of the EESM produced significant analgesic effect and this effect may be due to inhibition of the synthesis of the arachidonic acid metabolite. In addition, EESM potentiates the analgesic activity of aspirin.

The hot plate test has been found to be suitable for evaluation of centrally acting analgesics. The validity of this test has been shown even in the presence of substantial impairment of motor performance (Plummer et al., 1999). The findings of this study indicate that the EESM may be centrally acting.

In the preliminary phytochemical screening of the EESM showed the presence of triterpenoids, flavonoids, alkaloids, steroids and tannins. A number of scientific reports indicated that triterpenoids produced CNS depressant action (Chattopadhyay et al., 2003; Subarnas et al., 1993). Therefore, the presence of triterpenoids in EESM may be responsible for the CNS activity. Since the

pharmacological profiles of the present investigation of the EESM were similar to that of benzodiazepine, it is also possible that they might interact with benzodiazepine receptor located adjacent to the GABA receptor. Therefore, an envisage of it by the use of EESM in folkloric medicine may be due to its CNS action and relief of pain validated by our findings. However, further investigation is underway to determine the exact phytoconstituents that are responsible for CNS depressant and analgesic activity of EESM.

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