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CNS activity of the methanol extracts of *Careya arborea* in experimental animal model

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Abstract

The aim of the present study is to investigate central nervous system (CNS) activity of the methanol extract of barks of *Careya arborea* (Myrtaceae) in Swiss albino mice and Wistar albino rats. General behavior, exploratory behavior, muscle relaxant activity and phenobarbitone sodium–induced sleeping time were studied. The results revealed that the methanol extract of barks of *C. arborea* 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), and also significantly potentiated phenobarbitone sodium-induced sleeping time. The results suggest that methanol extract of *C. arborea* exhibit CNS depressant activity in tested animal models.

Introduction

Careya arborea commonly known as wild guava belongs to the family Myrtaceae medium sized deciduous tree, dark grey exfoliating in thin strip of bark which is widely available in India, Sri Lanka, Malay and Peninsula. The plant has been extensively investigated and a number of chemical constituents from the barks, leave and seeds of the plant have previously reported which includes triterpenoids (Mahati et al., 1973), flavonoids (Gupta et al., 1975), coumarin (Basak et al., 1976; Mahato and Dutta, 1972), saponins and tannins.

Stem barks of *C. arborea* was traditionally used in the treatment of tumors, anthelmintic, bronchitis, epileptic fits, astringents, antidote to snake-venom and skin disease (Kirtikar and Basu, 1975). It was also used as remedy for diarrhea, dysentery with bloody stools and ear pain. Antipyretic, leech repellent, fish poison and antivenin activities were also reported in literature. The aqueous extract of fresh root bark used as fish poison. The tribal peoples of Kolli Hills of Tamil Nadu used the stem bark of the plant for the treatment of various tumor and liver disorders. Previous report from our laboratory showed hepatoprotective and antioxidant activity (Kumar et al., 2005a), antimicrobial and *in vitro* antioxidant activity (Kumar et al., 2006), anti-inflammatory and analgesic activity (Kumar et al., 2005b), anti-tumor and antioxidant activities of methanol extract of *C. arborea* (Kumar et al., 2008a) and N-nitrosodiethylamine-induced hepatocarcinogenesis (Kumar, 2008b).

However, there are no reports on the central nervous system (CNS) activity of this plant, although decoction of *C. arborea* 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 reported use in traditional medicine as a sedative and antidepressant agent, the present study was undertaken for the first time to investigate CNS activity of the methanol extract of *C. arborea* in experimental animal models.

Materials and Methods

Plant materials and extraction

The plant *C. arborea* (Family: Myrtaceae) stem bark was collected in March 2006 from the Kolli Hills, Tamil Nadu, India. The plant material was taxonomically identified by the Botanical survey of India, Coimbatore, Tamilnadu, India and the voucher specimen RRI/BNG/SMP-Prog/955 was retained in our laboratory for future reference. The dried powder material (500 g) of the stem bark of *C. arborea* 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 7.4% and the appearance of the extract was dried gum resin in nature. The chemical constituents of the extract were identified by qualitative analysis followed by their confirmation by thin layer chromatography, which indicate the presence of flavonoids, triterpenoids and steroids.

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, J. K. K. Nataraja College of Pharmacy, Komarapalayam, India. The animals were grouped and housed in polyacrylic cages (38 x 23 x 10 cm) with not more than eight animals per cage and maintained under standard laboratory conditions (temperature 25 $\pm$ 2°C) with dark and light cycle (14/10 hours). 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.

Drugs

The following drugs were used: Diazepam (Lupin Laboratories Ltd., India), phenobarbitone sodium (Rhone–Poulenc India Ltd., India), morphine (M.M. Pharma, New Delhi, India), aspirin (USV, Bombay, India), and propylene glycol (SRL Laboratories Ltd., India).

Acute toxicity in animal

For toxicity studies the test extracts in the range of doses 100-1600 mg/kg were administered in five groups of 10 mice respectively. The mortality rates were observed after 72 hours. The LD_{50} 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 in to five groups (n=8). Methanol extract of *Careya arborea* was administered for the first three groups of animals at the dose of 50, 100 and 200 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 hours for the following parameters.

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 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 (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. 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 (i.e. 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.

Analgesic activity

Analgesic activity was studied by a) tail immersion and b) tail flick tests.

Tail immersion test

Swiss albino mice of either sex were divided into 5 groups of eight animals each. Propylene glycol (5 mL/kg), methanol extract of *C. arborea* at the dose of 50, 100 and 200 mg/kg, and morphine (5 mg/kg) were administered intraperitoneally. The tail (up to 5 cm) was then dipped into a pot of water maintained at 55 $\pm$ 0.5°C. The time in seconds to withdraw the tail out of water was taken as the reaction time. The reading was taken after 30 min of administration of the test drugs (Ghosh, 1984).

Tail flick test

Wistar strain of albino rats of either sex weighing between 150 and 180 g were selected and divided into 5 groups of six rats in each. The tail of the rat was placed on the nichrome wire of an analgesiometer and the time taken by the animal to withdraw (flick) its tail from the hot wire was taken as the reaction time. The methanol extract of *C. arborea* in a dose of 50, 100 and 200 mg/kg, and morphine (5 mg/kg) were injected intraperitoneally. Propylene glycol at 5 mL/kg was served as control. Analgesic activity was measured after 30 min of the administration of the test and standard drug (Ghosh, 1984).

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 methanol extract of *C. arborea* at the dose of 50, 100 and 200 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 a) Y-maze and b) 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), methanol extract of *Careya arborea* (50, 100 and 200 mg/kg), or diazepam (5 mg/kg), respectively. The mice were placed individually in a symmetrical Y-shaped runway (33 x 38 x 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 methanol extract of *C. arborea* (50, 100 and 200 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

Placing the forepaws of the mice in a small twisted wire rigidly supported above the bench top did the screening of animal. Normally the mice grasp the wire with the forepaws, and place at least one hind foot on the wire without 5 sec 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 methanol extract of *C. arborea* (50, 100 and 200 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 considered failure in the traction test (Rudzik et al.,

Table I

Effect of methanol extract of *Careya arborea* on general behavioral profiles in mice

Behavior type	Extract (mg/kg)			Diazepam	Propylene glycol
	50	100	200	(5 mg/kg)	(5 mL/kg)
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

1973).

Rotarod test

Fresh mice were placed on a horizontal wooden rod (32 mm diameter) rotating at a speed of 5 rpm. 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). Methanol extract of *C. arborea* at the dose of 50, 100 and 200 mg/kg respectively were injected intraperitoneally in to group 1, 2 and 3. Propylene glycol (5 mL/kg) and diazepam (5 mg/kg) was given to group 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 failed more than once to remain on the rotarod for 3 min were considered as passed the test (Dunham and Miya, 1957).

Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis of difference between groups was evaluated by ANOVA flowed by Dunnett's post hoc test. The Chi-square test used for the % muscle relaxant activity. A p value less than 0.05 were considered significant.

Results

Toxicity study

The bark extract of methanol extract of *C. arborea* 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 I. The methanol extract of *C. arborea* 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 *C. arborea*.

Analgesic activity

The result of the analgesic activity of methanol extract of *C. arborea* by tail immersion and tail flick methods is presented in Table II. The animal treated with methanol extract of *C. arborea* showed significant alteration at the dose of 100, 200 mg/kg and morphine 5 mg/kg as compared with that of control in tail flick test. It also showed that both extracts significantly enhancement of the reaction time in the tested dose of 200 mg/kg and morphine 5 mg/kg as compared to control in the tail immersion test. In both the tests the reaction time was significantly altered in a dose dependent manner.

Exploratory behavior potentials

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

Effect on muscle relaxant activity

In the traction test, the mice treated with methanol extract of *C. arborea* showed a significant failure in traction at all doses tested. The result obtained from the rotarod test, showed that methanol extract of *C. arborea*

Table II

Analgesic effect of methanol extract of *Careya arborea* on tail flick and tail immersion test in mice and rats

Treatment	Dose	Tail flick test (reaction time, s)	Tail immersion test (reaction time, s)
Propylene glycol	5 mL/kg	2.4 ± 0.1	2.5 ± 0.1[a]
Morphine	5 mg/kg	4.4 ± 0.2[a]	4.5 ± 0.1[a]
Extract	50 mg/kg	2.7 ± 0.1[a]	2.5 ± 0.1[a]
Extract	100 mg/kg	3.2 ± 0.1[a]	3.2 ± 0.1[a]
Extract	200 mg/kg	3.9 ± 0.1[a]	3.9 ± 0.1[a]

Data are mean ± SEM; (n = 8); [a]Significant difference between control group and treated group; p<0.05, ANOVA followed by Dunnett's post-hoc test

Table III

Effect of methanol extract of *Careya arborea* on exploratory behavior (Y–maze test) in mice

Experiment	Dose	Number of entries after treatment (min)			
		30	60	90	120
Propylene glycol	5 mL/kg	9.4 ± 0.8	9.4 ± 0.2	9.5 ± 0.8	9.4 ± 0.7
Diazepam	5 mg/kg	3.2 ± 0.3[a]	3.3 ± 0.1[a]	3.5 ± 0.2[a]	3.4 ± 0.3[a]
Extract	50 mg/kg	6.6 ± 0.6[a]	6.7 ± 0.5[a]	6.8 ± 0.5[a]	7.0 ± 0.6[a]
Extract	100 mg/kg	5.2 ± 0.4[a]	5.3 ± 0.4[a]	5.3 ± 0.4[a]	5.3 ± 0.5[a]
Extract	200 mg/kg	3.7 ± 0.3[a]	3.7 ± 0.3[a]	3.9 ± 0.3[a]	3.9 ± 0.3[a]

Values are the number of entries in 3 min (mean ± SEM, n = 8); [a]Significant difference between control group and treated group; p<0.05, ANOVA followed by Dunnett's post-hoc test

at 100 mg/kg (70%) and 200 mg/kg (80% respectively) significantly reduced the motor co-ordination of the tested animals (Table IV).

Effect on phenobarbitone sodium-induced sleeping time

Methanol extract of *C. arborea* significantly potentates the phenobarbitone sodium-induced sleeping time in a dose dependent manner. While the methanol extract of *C. arborea* at 100 and 200 mg/kg dose showed much better results (Table V).

Preliminary phytochemical tests

The results of the preliminary phytochemical group test of methanol extract of *C. arborea* stem bark have been presented in Table VI. The phytochemical tests with the methanol extract of *C. arborea* indicated the presence of tannins, triterpenoids, flavonoid, saponins and steroids.

Discussion

In the present study, the effect of methanol extract of *C. arborea* on CNS activity has been evaluated. The result indicated that the methanol extract of *C. arborea* 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 methanol extract of *C. arborea* was also evaluated in the tail immersion test as well as tail flick test for its analgesic activity. The extract effective against acute phasic pain and the effect are mediated centrally at the supraspinal level. Alternatively, the damping of this effect with high dose of extract may results from the coexistence of components with two 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. It 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 similar type of

Table IV

Effect of methanol extract of *Careya arborea* on exploratory behaviour (head dip test) and muscle relaxant activity

Experiment	Dose (body weight)	Head dip test	Traction test	Rotarod test
Propylene glycol	5 mL/kg	95 ± 8.4	0	0
Diazepam	5 mg/kg	28 ± 2.3[a]	100	100
Extract	50 mg/kg	65 ± 5.9[a]	60[a]	60[a]
Extract	100 mg/kg	56 ± 4.7[a]	70[a]	70[a]
Extract	200 mg/kg	30 ± 2.8[a]	80[a]	80[a]

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

Table V

Effect of methanol extract of *Careya arborea* on phenobarbitone sodium-induced sleeping time

Experiment	Dose	Sleeping time (min)
Propylene glycol	5 mL/kg	64 ± 5.9
Extract plus phenobarbitone sodium	50 mg/kg	70 ± 6.2[a]
	100 mg/kg	82 ± 7.4[a]
	200 mg/kg	112 ± 7.3[a]

Values are expressed as mean ± SEM, n = 8; [a]Significant difference between control group and treated group; $p<0.05$, ANOVA followed by Dunnett's post-hoc test

Table VI

Preliminary phytoconstituents present in methanol extract of *Careya arborea*

Sl. No.	Phytoconstituents	Bark extract of *Careya arborea*
1	Alkaloids	-
2	Flavonoids	+
3	Triterpenoids	+
4	Steroids	+
5	Saponins	-
6	Tannins	+
7	Reducing sugar	+
8	Amino acid	-
9	Gums	–

'–' Absence; '+' Presence

constituents present in the extract of methanol extract of *C. arborea* which is, exhibited the analgesic activity.

The effect on the CNS of the different dose of methanol extract of *C. arborea* was 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 and denote agent with depressor activity on the CNS. The sedative effect recorded here 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.

A myorelaxant effect was observed only with the higher dose of methanol extract of *C. arborea* 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 benzodiazipine, which acts as a anxiolytics (at low doses), anticonvulsants, and also produce sedation and a myorelaxant effect at higher doses (Onaivi et al., 1992). The reduction in exploratory behavior in animals treated with methanol extract of *C. arborea* 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 extract.

It has been reported that *C. arborea* contains triterpenoids, flavonoids, coumarin saponins and tannins. A number of scientific reports indicated that triterpenoids produced CNS depressant action (Chattopadhyay et al., 2003). Therefore, the presence of triterpenoids in methanol extract of *C. arborea*may be responsible for the CNS activity. Since the pharmacological profiles of the present investigation of the methanol extract of *C. arborea* was similar to that of bezodiazipine it is also possible that they might interact with benzodiazepine receptor located adjacent to the GABA receptor.

Conclusion

The use of methanol extract of *C. arborea* in folkloric medicine may be due to its CNS action and relief of pain validated by these findings.

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Ethical Issue

The study was approved by the Ethical Committee of the J. K. K. Nataraja College of Pharmacy. All animal experiments were carried out according to the law of Animal Experiments Guidelines approved by National Institute of Health (NIH).

Conflict of Interest

Authors declare no conflict of interest

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