CNS ACTIVITY OF THE METHANOL EXTRACTS OF HEARTWOOD OF TECOMA STANS IN EXPERIMENTAL ANIMAL MODEL

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Summary

The aim of the present study is to investigate central nervous system (CNS) activity of the methanol extracts of heartwood of Tecoma stans (Bignoniaceae) 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 extracts of heartwood of Tecoma stans 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 extracts of heartwood of Tecoma stans *exhibit* CNS depressant activity in tested animal models.

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Introduction

Tecoma stans commonly known as Yellow bell belongs to the family Bignoniaceae medium sized deciduous tree, dark grey exfoliating in thin strip of bark which is widely available in Kuba, Florida, Mexico and Cultivated in Indian gardens as ornamental plants for it flowers[2]. 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 Pyrindance alkaloids, Tecomanine,Iridoid glucosides,Stansioside,Plantarenaloside,Saponifiable compounds, Flavanoids,Monoterpenic alkaloids, 3', 5'-dimethoxy dihydro flavanol ,Isorhamnethin, β-carotene,zeaxanthin, Flavanone[3]. Other spp. of genus *tecoma was* traditionally used in the treatment of tumors, Antiinflammatory[4], analgesic and anti-pyretic activities were also reported in literature. 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 extracts of heartwood of Tecoma stans in experimental animal models.

Materials and Methods

Plant materials and extraction*:* The heartwood of *Tecoma stans* (Family: Bignoniaceae) was collected in March 2010 from the college of Pharmacy, Shahada, India. The plant material was taxonomically identified by the Botanical survey of India, Pune, Maharashtra, India. The heartwood *Tecoma stans* was extracted by using 3 different solvents in the following sequence of increasing Polarity I) Petroleum ether ($60\text{-}80^0$),II) Chloroform, III) Methanol. The extraction was carried out in several batches[1]. Total quantity of dried pulverized heartwood processed (1000 gm). The resulted extract yield was Brownish yellow (1.17 %w/w), Yellow- brown (2.25 %w/w), Dark reddish brown (3.61 %w/w) resp. 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, naphthoquinones, alkaloids 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, P.S.G.V.P.M's College of Pharmacy, Shahada, 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 + 2°C) 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 for10 days before commencement of experiment. All procedures described were reviewed and approved by the animal's ethical committee.

Drugs: The following drugs were used: Diazepam (Lupin Laboratories Ltd., India), morphine (M.M. Pharma, New Delhi, India), Pentazocine **(**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[16].

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 extracts of heartwood of Tecoma stans *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 hour 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 (0), little activity (1), moderate flexibility (2), strong response (3) and abnormal restlessness as (4). 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 (2) and strong activity as (3). If there is little motion, the score was (1), while if the animal sleeps, the score was (0). Excessive or very strong inquisitive activity like constant walking or running was scores as (4). A similar test was performed with the same scoring, when the animals are removed from the jar and placed on a table[15]

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^{o}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[15].

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[15].

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 (i) tail immersion and (ii) 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 extracts of heartwood of Tecoma stans *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 + 0.5ºC. 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[10].

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 extracts of heartwood of Tecoma stans *in* a dose of 50, 100 and 200 mg/kg, and morphine (1 mg/kg) were injected intraperitoneally. Propylene glycol at 5 ml/kg wasserved as control. Analgesic activity was measured after 30 min of the administration of the test and standard drug[10].

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 extracts of heartwood of Tecoma stans 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[5].

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), methanol extracts of heartwood of Tecoma stans (50, 100 and 200 mg/kg), or diazepam (5 mg/kg), respectively. The mice were placed individually in a symmetrical Y–shaped runway (33 cm x 38 cm x 13 cm) for 3 min and the number of the maze with all 4 ft (an 'entry') were counted[14].

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 extracts of heartwood of Tecoma stans (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[8].

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 second 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 extracts of heartwood of Tecoma stans *(*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[13].

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 extracts of heartwood of Tecoma stans 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[9].

Statistical analysis*:* The results were expressed as mean + S.E.M. 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 methanol extracts of heartwood of Tecoma stans *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.

Table I: Effect of methanol extract of heartwood of Tecoma stans on general behavioral profiles in mice

Behavior type	Extract (mg/kg)			Diazepam	Vehicle(5% CMC susp.)
	50	100	200	(1 mg/kg)	(1 ml/kg)
Spontaneous activity	1	2	3	4	0
Alertness	1	2	3	3	0
Awareness	1	2	3	3	0
Sound response	1	2	4	4	0
Touch response	2	3	4	4	0
Pain response	1	3	3	4	0

Righting reflex	1	2	3	4	0
Pinna reflex	2	3	3	4	0
Grip strength	2	3	3	4	0
0 no effect; 1 slight depression; 2 moderate depression; 3 strong depression; 4 very strong depression; n = 8					

Table II: Analgesic effect of methanol extract of heartwood of Tecoma stans 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)
Vehicle(5%CMC susp.)	1 ml/kg	2.42 + 0.15	2.47 + 0.14*
Morphine	1 mg/kg	4.39 + 0.19*	4.48 + 0.11*
Extract	50 mg/kg	2.67 + 0.12*	2.48 + 0.13*
Extract	100 mg/kg	3.18 + 0.11*	3.25 + 0.06*
Extract	200 mg/kg	3.92 + 0.14*	3.95 + 0.15*
Data are mean + SEM; (n = 8); *Significant difference between control group and treated group; $P<0.05$, ANOVA followed by Dunnett's post-hoc test			

Effect on general behavioral profiles*:* The results obtained from different experiments are presented in Table I. The methanol extracts of heartwood of Tecoma stans *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 extracts of heartwood of Tecoma stans .

Table III: Effect of methanol extract of Tecoma stans on exploratory behavior (Y–maze test) in mice

Experiment	Dose	Number of entries after treatment (min)			
		30	60	90	120
Vehicle(5%CMC susp.)	5 ml/kg	9.4 + 0.81	9.4 + 0.21	9.5 + 0.85	9.4 + 0.74
Diazepam	5 mg/kg	3.2 + 0.28*	3.3 + 0.12*	3.5 + 0.23*	3.4 + 0.26*
Extract	50 mg/kg	6.2 + 0.57*	6.3 + 0.51*	6.4 + 0.52*	7.5 + 0.58*

Extract	100 mg/kg	5.4 + 0.39*	5.2 + 0.43*	5.4 + 0.43*	5.5 + 0.49*
Extract	200 mg/kg	3.5 + 0.31*	3.4 + 0.27*	3.5 + 0.27*	3.6+ 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 post-hoc test					

***Analgesic activity**:* The result of the analgesic activity of methanol extracts of heartwood of Tecoma stans by tail immersion and tail flick methods is presented in Table II. The animal treated with methanol extracts of heartwood of Tecoma stans *showed* significant alteration at the dose of 100 mg/kg, 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 extracts of heartwood of Tecoma stans at the doses of 100 mg/kg and 200 mg/kgshowed a marked decrease in exploratory behavior compared with control (Table III). In case of head dip test, mice treated with different dose of methanol extracts of heartwood of Tecoma stans *showed* marked decreases in head dip responses when compared to control (Table IV).

Table IV: Effect of methanol extract of Tecoma stans on exploratory behaviour (head dip test) and muscle relaxant activity

Experiment	Dose (body weight)	Head dip test	Traction test	Rotarod test
Vehicle(5%CMC susp.)	5 ml/kg	94 + 8.4	0	0
Diazepam	1 mg/kg	29 + 2.3*	100	100
Extract	50 mg/kg	66 + 5.9*	55*	55*
Extract	100 mg/kg	57 + 4.7*	65*	65*
Extract	200 mg/kg	31 + 2.8*	75*	75*

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 post-hoc test; **Muscle relaxant activity:** Values are the percentage animals showing a negative results; n = 8; *$p< 0.05$ compared with control (Chi-square test)

Effect on muscle relaxant activity*:* In the traction test, the mice treated with methanol extracts of heartwood of Tecoma stans *showed* a significant failure in traction at all doses tested. The result obtained from the rotarod test, showed that methanol extracts of heartwood of Tecoma stans *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*:* Various extract of heartwood of *Tecoma stans significantly* potentates the phenobarbitone sodium–induced sleeping time in a dose dependent manner. While the methanol extracts of heartwood of Tecoma stans *at* 100 and 200 mg/kg dose showed much better results (Table V).

Table V: Effect of methanol extract of Tecoma stans on phenobarbitone sodium–induced sleeping time

Experiment	Dose	Sleeping time (min)
Vehicle(5%CMC susp.)	5 ml/kg	64 + 5.9
Extract plus phenobarbitone sodium	50 mg/kg	71 + 6.2*
	100 mg/kg	81 + 7.4*
	200 mg/kg	110 + 7.3*

Values are expressed as mean + S.E.M., n = 8; *Significant difference between control group and treated group; $p<0.05$, ANOVA followed by Dunnett's post-hoc test

Preliminary phytochemical tests*:* The results of the preliminary phytochemical group test of methanol extracts of heartwood of Tecoma stans have been presented in Table VI. The phytochemical tests with the methanol extracts of heartwood of Tecoma stans *indicated* the presence of tannins, triterpenoids, flavonoid, saponins and steroids.

Discussion

In the present study, the effect of methanol extracts of heartwood of Tecoma stans on CNS activity has been evaluated. The result indicated that the methanol extracts of heartwood of Tecoma stans 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 extracts of heartwood of Tecoma stans 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[6] and also steroidal constituents[11]. So, it may be due to the similar type of constituents present in the extract of methanol extracts of heartwood of Tecoma stans which is, exhibited the analgesic activity.

The effect on the CNS of the different dose of methanol extracts of heartwood of Tecoma stans 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[7]. 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 extracts of heartwood of Tecoma stans 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[12]. The reduction in exploratory behavior in animals treated with methanol extracts of heartwood of Tecoma stans 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 *Tecoma stans* contains triterpenoids, flavonoids, coumarin saponins and tannins. A number of scientific reports indicated that triterpenoids produced CNS depressant action[12]. Therefore, the presence of triterpenoids in methanol extracts of heartwood of Tecoma stans may be responsible for the CNS activity. Since the pharmacological profiles of the present investigation of the methanol extracts of heartwood of Tecoma stans was similar to that of bezodiazipine it is also possible that they might interact with benzodiazepine receptor located adjacent to the GABA receptor. Therefore, the use of methanol extracts of heartwood of Tecoma stans 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 phytoconsituents that are responsible for CNS depressant activity of methanol extracts of heartwood of Tecoma stans .

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