

Central nervous system activity of the methanol extracts of *Caesalpinia bonducella* and *Bauhinia racemosa* (Caesalpinaceae) 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 leaves of *Caesalpinia bonducella* (MECB) and stem bark of *Bauhinia racemosa* (MEBR) (Caesalpinaceae) 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 leaves of *Caesalpinia bonducella* at 100 – 200 mg/kg and stem bark of *Bauhinia racemosa* 100 – 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 MECB and MEBR exhibit CNS depressant activity in tested animal models.

Key words: *Caesalpinia bonducella*; *Bauhinia racemosa*; CNS activity; Experimental animal

INTRODUCTION

Caesalpinia (C.) *bonducella* (L.) Flem. (Caesalpinaceae) commonly known as, Nata Karanja (Hindi) is a prickly shrub found throughout the hotter regions of India, Myanmar and Sri Lanka. The twigs and young leaves of *C. bonducella* are traditionally used for the treatment of tumors, inflammation and liver disorders (Kirtikar and Basu, 1975). The leaves of *C. bonducella* showed anticonvulsant activity (Adesina, 1982). In addition, the various parts of this plant have been reported to possess multiple therapeutic

properties like antipyretic, antidiuretic, anthelmintic antibacterial (Neogi and Nayak, 1958), Antiviral (Dhar *et al.*, 1968), antiasthmatic (Gayaraja *et al.*, 1978), antiamebic and antiestrogenic (Raghunathan *et al.*, 1982). Currently, the hepatoprotective and antioxidant (Gupta *et al.*, 2003), antiinflammatory, analgesic and antipyretic activity (Gupta *et al.*, 2003) antitumor and antioxidant activity (Gupta *et al.*, 2004), antioxidant and free radical scavenging activity (Gupta *et al.*, 2004) of *C. bonducella* was reported from our laboratory.

Bauhinia racemosa (L.) Lam. (Caesalpinaceae) commonly known as Ashta (Hindi) a small crooked tree with dark scabrous bark. It is widely distributed throughout India, Ceylon, China and Timor. The bark and leaves of this plant are reported

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to be medicinally important in the traditional system of medicine and to be used extensively for the treatment of headache, fever, tumors, skin infection, disease of the blood, dysentery, and diarrhea (Kirtikar and Basu, 1975). The ethanol extract of leaves of this plant was evaluated for analgesic, anti-inflammatory, antipyretic and antispasmodic activity and reported to be active (El-Khatiba and Khaleel, 1995). The plant was screened for antiulcer activity (Akhtar and Ahmad, 1995), antimicrobial activity (Ali et al., 1995), cytotoxic, hypotensive and hypothermic activities has been reported (Dhar et al., 1968). It is also explored the potential of plant showed significant antioxidant and hepatoprotective (Gupta et al., 2004) and antitumor activity (Gupta et al., 2004) was reported from our laboratory.

However, there are no reports on the central nervous system (CNS) activity of these plants, although decoction of *C. bonducella* and *Bauhinia racemosa* are 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 activity of the methanol extracts of *Caesalpinia bonducella* (MECB) and *Bauhinia racemosa* (MEBR) in experimental animal models.

MATERIALS AND METHODS

Plant materials and extraction

The plants *C. bonducella* and *Bauhinia racemosa* (Family: Caesalpinaceae) was collected in the month of March 2003 from the Kolli Hills, Tamil Nadu, India. The plant material was taxonomically identified by Botanical Survey of India, Kolkata, India and the Voucher specimen (No. GMS-1 and 2) was retained in our laboratory for the future reference. The dried powder material of the leaves of *Caesalpinia bonducella* and stem bark of *Bauhinia racemosa* was extracted separately with methanol (Yield 8.78% and 9.25% w/w respectively) in a

soxhlet apparatus. The methanol extracts were then distilled, evaporated and dried in vacuum. The required amount of MECB and MEBR was dissolved in propylene glycol to obtain the necessary doses employed in the study.

Animals

Studies were carried out using Swiss albino mice (20 - 25 g) and Wistar albino rats (150 - 180 g) of either sex were used. They were obtained from the animal house, Indian Institute of Chemical Biology (IICB), Kolkata, India. The animals were grouped and housed in polyacrylic cages (3 × 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 University animals ethical committee.

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 - 1,600 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.

Fifty six adult albino mice were divided in to seven groups (n = 8). MECB was administered for the first three groups of animals at the dose of 50, 100 and 200 mg/kg (i.p.) respectively. For fourth and fifth groups MEBR at the dose of 100 and 200 mg/kg were administered intraperitoneally. 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 (Turner, 1965; Gupta *et al.*, 1999).

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 (Turner, 1965; Gupta *et al.*, 1998).

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 tested it. 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 (i) tail immersion and (ii) tail flick tests.

Tail immersion test

Swiss albino mice of either sex were divided into 7 groups of eight animals each. Propylene glycol (5 ml/kg), MECB at the dose of 50, 100 and 200 mg/kg, MEBR at the dose of 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 $55 \pm 0.5^\circ\text{C}$. 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 7 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 MECB in a dose of 50, 100 and 200 mg/kg, MEBR at the doses of 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 six groups of eight in each. Animals received 40 mg/kg (i.p.) phenobarbitone sodium 30 min after the injection of MECB at the dose of 50, 100 and 200 mg/kg, MEBR at 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 (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), MECB (50, 100 and 200 mg/kg), MEBR (100 and 200 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 MECB (50, 100 and 200 mg/kg), MEBR (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 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 MECB (50, 100 and 200 mg/kg), MEBR (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., 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 seven groups (n = 8). MECB 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. While MEBR at the dose of 100 and 200 mg/kg was administered i.p. into group 6 and 7 respectively. 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 \pm 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 was considered significant.

RESULTS**Toxicity study**

The Leaf extract of MECB and stem bark extract of MEBR 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 MECB 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 MECB. The interesting finding is the significant response towards the general behavior of animals with MEBR stem bark.

Analgesic activity

The result of the analgesic activity of MECB and MEBR by tail immersion and tail flick methods is presented in Table 2. The animal treated with MECB and MEBR showed significant alteration at the dose of 100 mg/kg (3.12 ± 0.14 , 3.06 ± 0.10 s), 200 mg/kg (3.84 ± 0.14 , 3.77 ± 0.11 s) and morphine

Table 1. Effect of the MECB and MEBR on general behavioral profiles in mice

Behaviour type	MECB (mg/kg)			MEBR (mg/kg)		Diazepam	Propylene glycol
	50	100	200	100	200	(5 mg/kg)	(5 ml/kg)
Spontaneous activity	+	++	+++	++	++++	++++	-
Alertness	+	++	+++	+++	+++	+++	-
Awareness	+	++	+++	+++	+++	+++	-
Sound response	+	++	++++	++	+++	++++	-
Touch response	++	+++	++++	+++	+++	++++	-
Pain response	+	+++	+++	+++	+++	++++	-
Righting reflex	+	++	+++	+++	+++	++++	-
Pinna reflex	++	+++	+++	++++	+++	++++	-
Grip strength	++	+++	+++	++++	+++	++++	-

MECB: methanol extract of *C. bonducella*; MEBR: methanol extract of *B. racemosa*. -, no effect; +, slight depression; ++, moderate depression, +++, strong depression; +++++, very strong depression; n = 8.

Table 2. Analgesic effect of MECB and MEBR 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	2.36 ± 0.15	2.42 ± 0.14
Morphine	5 mg/kg	4.31 ± 0.19	4.58 ± 0.11
MECB	50 mg/kg	2.61 ± 0.12	2.58 ± 0.13
	100 mg/kg	3.12 ± 0.11	3.15 ± 0.06
	200 mg/kg	3.84 ± 0.14	3.85 ± 0.15
MEBR	100 mg/kg	3.06 ± 0.10	3.07 ± 0.12
	200 mg/kg	3.77 ± 0.18	3.75 ± 0.11

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

Table 3. Effect of MECB and MEBR on exploratory behaviour (Y-maze test) in mice

Experiment	Dose	Number of entries after treatment (min)			
		30	60	90	120
Propylene glycol	5 ml/kg	9.4 ± 0.81	9.5 ± 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	3.5 ± 0.26
MECB	50 mg/kg	6.6 ± 0.57	6.7 ± 0.51	6.8 ± 0.52	6.9 ± 0.58
	100 mg/kg	5.2 ± 0.39	5.2 ± 0.43	5.3 ± 0.43	5.3 ± 0.49
	200 mg/kg	3.7 ± 0.31	3.7 ± 0.27	3.8 ± 0.27	3.8 ± 0.31
MEBR	100 mg/kg	5.9 ± 0.42	5.7 ± 0.48	6.1 ± 0.45	5.6 ± 0.47
	200 mg/kg	3.8 ± 0.23	3.5 ± 0.28	3.6 ± 0.24	3.7 ± 0.29

MECB, methanol extract of *C. bonducella*; MEBR, methanol extract of *B. racemosa*. 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.

5 mg/kg (4.31 ± 0.19 s) as compared with that of control (2.36 ± 0.15 s) in tail flick test. It is also showed the both extracts significantly enhancement of the reaction time in the tested dose of 200 mg/kg (3.85 ± 0.15 , 3.75 ± 0.11 s) and standard drug (Morphine) 5 mg/kg (4.58 ± 0.11 s) as compared to control (2.42 ± 0.14 s) 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 MECB and MEBR at the doses of 100 mg/kg (5.3 ± 0.49 , 5.6 ± 0.47) and 200 mg/kg (3.8 ± 0.31 , 3.7 ± 0.29) showed a marked decrease in exploratory behavior compared with control (9.4 ± 0.74) (Table 3). In case of head dip test, mice treated with different dose of MECB and MEBR showed marked decreases in head dip responses when compared to control (Table 4).

Effect on phenobarbitone sodium-induced sleeping time

The MECB significantly potentiates the phenobarbitone sodium-induced sleeping time in a dose dependent manner. While the MECB at 100 (85 ± 7.4 min) and 200 mg/kg (115 ± 7.3 min) dose showed much better results compared with that of MEBR at the dose of 100 mg/kg (75 ± 6.4 min) and 200 mg/kg (110 ± 8.2 min) (Table 5).

Table 4. Effect of MECB and MEBR on exploratory behaviour (head dip test)

Experiment	Dose (body weight)	Head dip test
Propylene glycol	5 ml/kg	97 ± 8.4
Diazepam	5 mg/kg	29 ± 2.3
MECB	50 mg/kg	69 ± 5.9
	100 mg/kg	58 ± 4.7
	200 mg/kg	31 ± 2.8
MEBR	100 mg/kg	70 ± 6.4
	200 mg/kg	33 ± 2.7

MECB, methanol extract of *C. bonducella*, MEBR, methanol extract of *B. racemosa*. 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.

Effect on muscle relaxant activity

In the traction test, the mice treated with MECB and MEBR showed a significant failure in traction at all doses tested. The result obtained from the rotarod test, showed that MECB and MEBR at 100 mg/kg (70 and 80%) and 200 mg/kg (70 and 60% respectively) significantly reduced the motor coordination of the tested animals (Table 6).

Preliminary photochemical tests

The results of the preliminary photochemical group test of MECB leaf and MEBR stem bark have been presented in Table 7. The phytochemical tests with

Table 5. Effect of MECB and MEBR on phenobarbitone sodium-induced sleeping time

Experiment	Dose	Sleeping time (min)
Propylene glycol	5 ml/kg	66 ± 5.9
MECB + phenobarbitone sodium	50 mg/kg	72 ± 6.2
	100 mg/kg	85 ± 7.4
	200 mg/kg	115 ± 7.3
MEBR + phenobarbitone sodium	100 mg/kg	75 ± 6.4
	200 mg/kg	110 ± 8.2

MECB, methanol extract of *C. bonducella*; MEBR, methanol extract of *B. racemosa*. 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.

the MECB indicated the presence of tannins, triterpenoids, flavonoid, alkaloids, saponins, steroids and reducing sugar. While in MEBR showed the

presence compounds namely, triterpenoids, flavonoid, tannins and steroids.

DISCUSSION

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

The MECB and MEBR were also evaluated in the tail immersion test as well as tail flick test for its analgesic activity. The extract effective against acute

Table 6. Percentage effect of the MEBR and MECB on muscle relaxant activity in mice

Treatment	Dose	Traction test	Rotarod test
Propylene glycol	5 ml/kg	0	0
Diazepam	5 mg/kg	100	100
MECB	50 mg/kg	60*	60*
	100 mg/kg	70*	70*
	200 mg/kg	80*	80*
MEBR	100 mg/kg	60*	60*
	200 mg/kg	80*	70*

MECB, methanol extract of *C. bonducella*; MEBR, methanol extract of *B. racemosa*. Values are the percentage animals showing a negative results; n = 8. * $P < 0.05$ compared with control (Chi-square test).

Table 7. Preliminary phytoconstituents present in MECB and MEBR

Sl. No.	Phytoconstituents	Leaf extract of <i>C. bonducella</i>	Stem bark extract of <i>B. racemosa</i>
1	Alkaloids	+	-
2	Flavonoids	+	+
3	Triterpenoids	+	+
4	Steroids	+	+
5	Saponins	+	-
6	Tannins	+	+
7	Reducing sugar	+	-
8	Amino acid	+	-
9	Gums	-	-

'-' Absence; '+' Presence.

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 result 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 (Roumy and Jean-Marie, 1998). 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 and Calixto, 1996). So it may be due to the similar type of constituents present in the extract(s) of MECB and MEBR, which is, exhibited the analgesic activity.

The effect on the CNS of the different dose of MECB and MEBR 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 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 have already been identified in certain plant extracts (Medina, 1990; Viola *et al.*, 1993; Medina and Merder, 1996).

A myorelaxant effect was observed only with the higher dose of MECB and MEBR, 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 is act as anxiolytics (at low doses), anticonvulsants, and also produce sedation and a myorelaxant effect at higher doses (Onaivi *et al.*, 1992; Tang *et al.*, 1993; Wolfman *et al.*, 1993; Davies *et al.*, 1994). The reduction in exploratory behavior in animals treated with MECB and MEBR

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 MECB and MEBR.

It has been reported that *Caesalpinia bonducella* contain flavonoid (bonducellin-A) (Purushothaman *et al.*, 1982), triterpenoid (α -amyrin, β -amyrin, lupeol and lupeol acetate) (Saeed and Sabir, 2003), diterpenoids (bonducin, bonducellpin A-C, caesaldekarin A-F, and caesalpin A-C) (Peter *et al.*, 1997; Lyder *et al.*, 1998; Peter *et al.*, 1999; Kinoshita, 2000), and steroid (β -sitosterol) (Lai *et al.*, 1977). From *Bauhinia racemosa* steroids (β -sitosterol) and triterpenoid (β -amyrin) were isolated from the stem bark (Prakash and Khosa, 1976; Anjaneyulu *et al.*, 1986). Besides these compounds, at least five flavonals (kempferol and quercetin) and two coumarin derivatives, scopoletin and scopolin were also isolated from the leaves (El-Hossary *et al.*, 2000). Stillbene (resveratrol) isolated from the heartwood of *B. racemosa* also reported (Anjaneyulu *et al.*, 1984). A number of scientific reports indicated that steroids and triterpenoids produced CNS depressant action (Subarnas *et al.*, 1993; Chattopadhyay *et al.*, 2003). Therefore, the presence of triterpenoids and steroids in MECB and MEBR may be responsible for the CNS activity.

In conclusion, the present investigation reveals that the CNS activity in the MECB and MEBR may be due to the action of either triterpenoids alone, or the combination of triterpenoids and steroids present in the methanol extracts. Since the pharmacological profiles of the present investigation of the MECB and MEBR 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 envisaged by the use of MECB and MEBR 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 activity of MECB and MEBR.

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REFERENCES

- Adesina SK. (1982) Studies on some plants used as anticonvulsant in amerindian and african traditional medicine. *Fitoterapia* **53**, 147-162.
- Akhtar AH, Ahmad KU. (1995) Anti-ulcerogenic evaluation of the methanol extract of some indigenous medicinal plants of Pakistan in aspirin ulcerated rats. *J. Ethnopharmacol.* **46**, 1-6.
- Ali MS, Azhar I, Amtul Z, Ahmad VU, Usmanghani K. (1995) Antimicrobial screening of some Caesalpiniaceae. *Fitoterapia* **70**, 299-304.
- Anjaneyulu ASR, Reddy AVR, Reddy DSK, Rose SP, Cameron TS. (1986) Racemosal : A novel tetracyclic phenol from *Bauhinia racemosa*. *Tetrahedron* **42**, 2417-2420.
- Anjaneyulu ASR, Reddy AVR, Reddy DSK, Ward RS, Adhikesavalu D, Cameron TS. (1984) A new dibenzo (2,3-6,7) oxepin derivative from *Bauhinia racemosa*. *Tetrahedron* **40**, 4245-4252.
- Carlini EA. (1973) Farmacologia prática sem Aparelhagem. Sarvier, Editora de Livros medicos Ltda, São Paulo, pp. 145-197.
- Chattopadhyay D, Arunachalam G, Mandal SC, Bhadra R, Mandal AB. (2003) CNS activity of the methanol extract of Malloatus (Geist) Muell Arg. Leaf : an ethnomedicine of Onge. *J. Ethnopharmacol.* **85**, 99-105.
- Dandiya PC, Collumbine H. (1956) Studies on *Acorus calamus* (L.) some pharmacological action of the volatile oil. *J. Pharmacol. Exp. Ther.* **125**, 353-359.
- Davies MF, Onaivi ES, Chen SW, Maguire PA, Tsai NF, Loew GH. (1994) *Pharmacol. Biochem. Behav.* **49**, 47-56.
- De Campos RPO, Santos ARS, Vaz ZR, Pinherio TR, Pizzolatti MG, Filho VC, Monache FD, Yunes RA, Calixto JB. (1997). Antinociceptive propertoies of the hydroalcoolic extract and preliminary study of a xanthone isolated from *polgaya cyparissias*. *Life Sci.* **61**, 1619-1630.
- Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Roy C. (1968) Screening of Indian plants for biological activity. *Indian J. Exp. Biol.* **6**, 232-247.
- Dixit VK, Varma KC. (1976) Effects of essential oil of leaves of *Blumea lacera* DC on central nervous system. *Indian J. Pharmacol.* **18**, 7-11.
- Dorr M, Stienberg H, Tomkiewicz M, Joyee D, Porosolt RD, Summerfield A. (1971) Persistence of dose related behavior in mice. *Nature* **231**, 121-123.
- Dunham NW, Miya TS. (1957) A note on simple apparatus for detecting neurological deficit in rats and mice. *J. American Pharmacol.* **46**, 208-209.
- El-Hossary GA, Selim MA, Sayed AE, Khaleel AE. (2000) Study of the flavonoid content of *Bassia muricata* and *Bauhinia racemosa*. *Bull. Fac. Pharm. Cairo Univ.* **38**, 93-97.
- El-Khatiba AS, Khaleel AE. (1995) Evaluation of some pharmacological properties of different extract of *Bauhinia racemosa* leaf and *Bassia muricata* whole plant. *Bull. Fac. Pharm. Cairo Univ.* **33**, 59-65.
- Gayaraja S, Shinde S, Agarwal SL. (1978) Antiasthmatic properties of *Caesalpinia bonduc* leave. *Indian J. Pharmacol.* **10**, 86-89.
- Ghosh MN. (1984). Fundamental of Experimental Pharmacology 2nd ed. Scientific Book Agency, Calcutta, p. 153.
- Gupta M, Mazumdar UK, Sambath Kumar R, Gomathi P, Rajeshwar Y, Siva kumar T. (2004) Screening of antioxidant and antimicrobial activities of *Caesalpinia bonducella* Flem., leaves (Caesalpiniaceae), *Orient. Pharm. Exp. Med.* **4**, 197-209.
- Gupta M, Mazumder UK, Bhawal SR. (1999) CNS activity of *Vitex negundo* Linn. in mice. *Indian J. Exp. Biol.* **37**, 143-146.
- Gupta M, Mazumder UK, Das S. (1998) Effect of leaf extract from *Clerodendron colebrookianum* on CNS function in mice. *Indian J. Exp. Biol.* **36**, 171-174.
- Gupta M, Mazumder UK, Sambath Kumar R. (2003) Hepatoprotective and antioxidant role of *Caesalpinia bonducella* on paracetamol-induced liver damage in rats. *Nat. Prod. Sci.* **9**, 186-191.
- Gupta M, Mazumder UK, Sambath Kumar R, Siva Kumar T. (2003) Studies on antiinflammatory, analgesic and antipyretic properties of methanol extract of *caesalpinia bonducella* leaves in experimental animal models. *Iran. J. Pharmacol. Ther.* **2**, 30-34.
- Gupta M, Mazumder UK, Sambath Kumar R, Siva

- Kumar T, Vamsi MLM. (2004) Antitumor activity and antioxidant status of *Caesalpinia bonducella* against Ehrlich ascites carcinoma in Swiss albino mice. *J. Pharmacol. Sci.* **94**, 177-184.
- Gupta M, Mazumder UK, Sambath Kumar R, Siva Kumar T. (2004) Antitumor effect of *Bauhinia racemosa* against Ehrlich ascites carcinoma with reference to lipid peroxidation and antioxidant system in Swiss albino mice. *Acta Pharmacol. Sin.* **25**, 1070-1077.
- Gupta M, Mazumder UK, Sambath Kumar R, Sivakumar T, Gomathi P. (2004) Antioxidant and hepatoprotective effect of *Bauhinia racemosa* against paracetamol and carbontetrachloride induced liver damage in rata. *Iran. J. Pharmacol. Ther.* **3**, 12-20.
- Johnson ES, Roberts MHT, Stranghan DW. (1970) Amino acid induced depression of cortical neuron. *Br. J. Pharmacol.* **38**, 659.
- Kinoshita T. (2000) Chemical studies on the philippine crude drug calumbibit; the isolation of new cassane diterpenes fused with alpha. *Chem. Pharm. Bull.* **48**, 1375-1377.
- Kirtikar KR, Basu BD. (1975) Indian Medicinal Plants. Vol 2, 2nd ed, India : Dehradun; Bishen Singh Mahendra Pal Singh, p. 842-844, 894-895.
- Lai MS, Shameel S, Ahmad VU, Ushmanghani K. (1977) Chemical constituents of *Caesalpinia bonduc*. *Pakistan J. Sci. Ind. Res.* **40**, 20-22.
- Litchfield JT, Wilcoxon F. (1949) A simplified method of evaluating dose effect experiments. *J. Pharmacol. Exp. Ther.* **96**, 99-133.
- Lyder DL, Peter SR, Tinto WF et al. (1998) Minor cassane diterpenoids of *caesalpinia bonduc*. *J. Nat. Prod.* **61**, 1462-1465.
- Medina JH. (1990) Natural benzodiazepines in the Brain. Possible biological roles. *Comunicacioes Biologicas* **8**, 217-234.
- Medina JH, Merder M. (1996) Flavonoids neuroactivos; una nueva familia de ligandos especificos del receptor 'A' benzodiazepinas con acciones ansioliticas, In Calixto JB, Yunes R, Lima -Nogueira TCM (eds). XIV Simposio de Plantas Mediciniais do Brsil. Florianópolis -SC September, p. 17-20, 23.
- Miguel OG, Calixto JB, Santos, ARS, Messana I, Ferrari F, Fuho VC, Pizzolatti MG, Yunes RA. (1996) Chemical and preliminary analgesic evaluation of Geraniin and Furosin isolated from *phyllanthus sellowianus*. *Planta Med.* **62**, 192-197.
- Neogi NC, Nayak KP. (1958) Biological investigation of *Caesalpinia bonducella* F. *Indian J. Pharmacol.* **20**, 95-100.
- Onaivi ES, Maguiri PA, Tsai NF, Davies MF, Locu GH. (1992) Comparison of behavioral and central BDZ binding profile in three rat lines. *Pharmacol. Biochem. Behav.* **43**, 825-831.
- Peter SR, Tinto WF, Lean S, Reynolds WF, Yu M. (1997) Bonducellpins A-D new cassane furanoditerpens of *Caesalpinia bonduc*. *J. Nat. Prod.* **60**, 1219-1221.
- Peter SR, Tinto WF, Lean S, Reynolds WF, Yu M. (1999) Cassane diterpenes from *Caesalpinia bonducella*. *Phytochemistry* **47**, 1153-1155.
- Prakash A, Khosa RL. (1976) Chemical studies on *Bauhini racemosa*. *Curr. Sci.* **45**, 705.
- Purushothaman KK, Kalyani K, Subramaniam K, Shanmughanathan SP. (1982) Structure of bonducellin-A new homoisoflavone from *Caesalpinia bonducella*. *Indian J. Chem.* **21**, 383-386.
- Raghunathan K, Mitra R, Karanja. (1982) In : Raghunathan K, Mitra R., editors. *Pharmacognosy of Indigenous Drugs*. Part-I, Central Council for Research in Ayurveda and Siddha, New Delhi, India, pp. 484-510.
- Roumy M, Jean-Marie Z. (1998) Neuropeptide FF pain and analgesia. *Eur. J. Pharmacol.* **345**, 1-11.
- Rudzik AD, Hester JB, Tang AH, Staw RN, Friis W. (1973) The Benzodiazepines, Raven Press, New York, pp. 285-297.
- Rushton R, Steinberg H, Tinson C. (1961) Modification of the effects of an amphetamine barbiturate mixture by the past experience of rats (Y-shaped runway). *Nature* **192**, 533-535.
- Saeed MA, Sabir AW. (2003) Irritant potential of some constituents from seeds of *Caesalpinia bonducella* (L) flaming. *J. Asian Nat. Prod. Res.* **5**, 35-41.
- Scholfield CN. (1979) Leptazol antagonizes the post-synaptic actions of gamma-amino butyric acid. *Br. J. Pharmacol.* **67**, 433-444.
- Subarnas A, Tadano T, Oshima Y, Kisara K, Ohizmi Y. (1993) A possible mechanism of antidepressant activity of β -amyrin palmitat from *Lobelia inflata* leaves in the forced swimming test. *J. Pharm. Pharmacol.* **45**, 545-550.
- Tang TG, Code RA, Himes CS. (1993) Antagonism of hypothermia produced by benzodiazepine related compounds by U-78875 in mice. *Eur. J. pharmacol.* **236**, 1-5.

- Turner RA. (1965) Screening Methods in Pharmacology. Academic Press, New York, pp. 26-35.
- Viola H, Wolfman C, Levi De Stein M, Wasowski C, Peòà C, Medina JH, Paladini AC. (1993) Isolation and pharmacological activity of benzodiazepine receptors ligands from *Tilia* spp. In Niemeyer H, Dajas F, Silveira R, R. Medina J (Eds). Book of abstracts First symposium Trends In Natural Products Research. Prospects For Pharmacological and Agrochemical applications, LANBIO, Asuncion, Paraguay, August, 6-9, pp. 87-88.
- Wolfman C, Viola H, Levi DE, Stein M, Wasowski C, Peòà C, Medina, JH, Paladini AC. (1993) Chrysin (5-7 DI-OH-flavone), a naturally occurring lignad for benzodiapine receptor, with anticovulsant properties. In: Niemeyer H Dajas F, Silveria R, Medina J (Eds). Book of abstract. First symposium. Trends In Natural Products Research. Prospects For Pharmacological and Agrochemical Application. LANBIO, Asuncion, Paraguay, August, 6-9, pp. 89.
- Wong CH, Day P, Yarmuch J, WU W, Zbuzek UK. (1994) Nifedipine-induced analgesic after epidural injections in rats. *Anaesth. Analgesia* **79**, 303-306.
- Woodson RF. (1987) Statistical method for the analysis of bio medical data, probability and mathematical statistics. Wiley, Chichester, pp. 313-316.