

Neuropharmacological Activities of *Mikania scandens* Root

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Abstract: *Mikania scandens* (L.) Willd. (Asteraceae), known as climbing hemp weed in English is a herbaceous climbing vine grown as a weed throughout the plains of the Indian subcontinent. The present study evaluated some neuropharmacological activities of hydroalcoholic extract of root from *M. scandens* (RMS) in experimental animal models. RMS (at 200 and 400 mg/kg body weight, i.p.) was evaluated for anti-nociceptive activity by acetic acid induced writhing and tail flick methods. Locomotor depressant activity was measured by means of an actophotometer. Skeletal muscle relaxant effect was evaluated by using rota-rod apparatus and sedative potentiating property by phenobarbitone-induced sleep potentiation study. The results of the present study revealed significant ($p < 0.001$) and dose dependent anti-nociceptive, locomotor depressant, muscle relaxant and sedative potentiating effects of RMS, demonstrating its depressant action on the central nervous system (CNS). From the present study, it can be concluded that the root of *M. scandens* possessed prominent depressant action on the CNS, as manifested by the important neuropharmacological activities in mice.

Key words: *Mikania scandens* • CNS Depressant • Locomotor • Muscle Relaxant • Phenobarbitone

INTRODUCTION

There is no denying that natural products have contributed significantly towards the development of contemporary therapeutics. Recently traditional medicine worldwide is being re-evaluated by extensive research works on different plant species and their active therapeutic principles. The untapped wealth of plant kingdom can represent a novel source of newer compounds with significant therapeutic activities. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and comparatively low cost. Previous authors have reported anti-nociceptive activity of several medicinal plants [1-5].

Mikania scandens (L.) Willd. (Asteraceae), known as climbing hemp weed in English, is a twining herbaceous climbing vine with long-petioled, opposite leaves and small homogamous flower-heads, grown as a common weed throughout the plains of India and Bangladesh. Traditionally, the plant has been used for some medicinal purposes in the Indian subcontinent. Aqueous leaf extracts of this plant have been used in folk medicine to treat stomach ulcers. The plant is thought to be

efficacious in the treatment of gastric problems. Traditionally its leaf juice is applied to the affected area of body in treatment of wounds and bruises. The plant is regarded as a rich source of vitamin A and C and also contains vitamin B, mikanin, friedelin, effriedinol and some sesquiterpene dilactones including mikanolide, dihydromikanolide, deoxymikanolide and scandenolide. Three diterpenic acids known as kaurenic acid, butyryloxykaurenic acid and benzoyloxykaurenic acid, stigmasterol and betasitosterin have also been isolated from this plant [6-8].

It has come to the author's notice that the rural people of Hooghly, Bardhaman and Medinipur districts of West Bengal state of India use the young leaves of this plant in management of insect bites and stings. Previous workers reported analgesic and *in vitro* antioxidant activities of *M. scandens* leaf [9]. In our previous study, we have reported some important neuropharmacological properties of aerial parts and *in vitro* anti-inflammatory effects of aerial parts, root and flower of *M. scandens* [10-12]. The present study was aimed to assess certain possible neuropharmacological activities of *M. scandens* roots in experimental rodent models.

MATERIALS AND METHODS

Plant Material: The roots of *M. scandens* were collected during October, 2011 from Gotan region of Bardhaman district of West Bengal, India. The species was authenticated by Dr. P. Lakshminarasimhan, Scientist D, at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India and a voucher specimen (CNH/44/2011/Tech. II /476) was deposited at the Pharmacognosy Research Laboratory, Bengal School of Technology, Delhi Road, Sugandha, Hooghly 712102, India. Just after collection, the plant material was washed thoroughly with running tap water and shade dried at room temperature (24-26 °C) and ground mechanically into a coarse powder.

Drugs and Chemicals: Morphine sulphate, diazepam, chlorpromazine hydrochloride and phenobarbitone sodium were from Sigma-Aldrich Corporation, St. Louis, MO, USA. Ibuprofen was from Perk Indus Pharmaceuticals, Faridabad, India. All the other chemicals were of analytical grade obtained commercially. Doubled distilled water from all-glass still was employed throughout the study.

Preparation of Extract: The powdered plant material (50 g) was extracted with 50% aqueous ethanol (400 ml) by boiling under reflux for 90 minutes. The extract was filtered and evaporated to dryness to yield the dry extract (RMS, yield: 8.35%). The dry extract was kept in a refrigerator until use. Preliminary phytochemical studies were performed on RMS as per reported method [13].

Experimental Animals: Adult Swiss albino mice of either sex weighing 20 ± 2 g were used in the present study. The mice were grouped and housed in polyacrylic cages (38×23×10 cm) with not more than three animals per cage and maintained under standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$, relative humidity 48%, with dark/light cycle 12/12 h). They were allowed free access to standard diet and water *ad libitum*. The mice were acclimatized to laboratory conditions for 7 days before commencement of the experiments. All experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee, Bengal School of Technology.

Evaluation of Anti-nociceptive Activity

Acetic Acid-Induced Writhing Test: The prescreened animals were divided into four groups ($n = 6$). The first

group of animals (which served as control) received normal saline 5 ml/kg body weight i.p. The second and third groups received RMS at the doses of 200 and 400 mg/kg body weight i.p., respectively. The last group of animals (which served as reference) received ibuprofen at the dose of 50 mg/kg body weight i.p. Thirty minutes after administration of normal saline, ibuprofen and test extracts acetic acid was administered (1 % v/v, 0.2 ml, irrespective of body weight, i.p.) to all groups. The onset and number of writhing were noted during a period of 15 minutes [10]. The mean writhing scores in each group were calculated and expressed the percentage of protection using the following formula:

$$(\text{Control mean} - \text{Treated mean} / \text{Control mean}) \times 100 \%$$

Tail Flick Test: Before treatment the basal reaction time for each mouse to radiant heat (Analgesimeter, Techno) was determined by placing the tip of the tail on the radiant heat source (nichrome wire). The strength of the current passing through the naked nichrome wire was kept constant at 6 Amps. The tail withdrawal time in seconds from the heat (flicking response) was considered as nociceptive end point. Any mouse failing to withdraw its tail within 2 to 4 seconds was excluded from the study.

The prescreened animals (reaction time: 2-4 seconds) were divided into three groups ($n = 6$). The first two groups received the RMS at the doses of 200 and 400 mg/kg body weight i.p., respectively. The last group (which served as reference) received morphine sulfate at a dose of 5 mg/kg body weight s.c. The reactions of all groups of mice were determined after 5, 15, 30, 60 and 90 minutes of treatment by tail flick method similarly as mentioned above and the latency times (in seconds) were recorded. Each animal served as its own control. The mean reaction times for each group were calculated [14].

Evaluation of Locomotor Activity: The central nervous system (CNS) depressant activity of RMS was evaluated by studying locomotor activity of mice using an actophotometer (Techno, India). The mice were divided into three groups ($n = 6$). The equipment was turned on and animals of each group were placed individually in the activity cage for 10 minutes and the basal activity score of all the animals were monitored and recorded. Then, the first two groups received the RMS at the doses of 200 and 400 mg/kg body weight i.p., respectively. The last group (which served as reference) received chlorpromazine hydrochloride at the dose of 3 mg/kg body weight i.p.

The animals were again tested for activity scores similarly 30 and 60 minutes after these treatments. Each animal served as its own control [15]. Percent reduction in motor activity was calculated for each animal by using the following formula:

$$\% \text{ reduction in motor activity} = (W_a - W_b/W_a) \times 100 \%$$

Where, W_a and W_b are the mean activity scores before and after treatment, respectively.

Evaluation of Muscle Relaxant Activity: The muscle relaxant activity of RMS was evaluated by studying neurological deficit of mice using rota-rod apparatus (Techno, India). The mice were divided into three groups ($n = 6$). The equipment was turned on and adjusted to appropriate speed (20 rpm). Animals of each group were placed individually on the rotating rod and the fall off time of all the animals were recorded when the mouse falls from the rotating rod. Then the first two groups received the RMS at the doses of 200 and 400 mg/kg body weight i.p., respectively. The last group (which served as reference) received diazepam at the dose of 4 mg/kg body weight i.p. The animals were again tested for determination of fall off time similarly 30 and 60 minutes after these treatments. Each animal served as its own control [10, 16]. Percent reduction in fall off time was calculated for each animal by using the following formula:

$$\% \text{ reduction in fall off time} = (W_a - W_b/W_a) \times 100 \%$$

Where, W_a and W_b are the mean fall off times before and after treatment, respectively.

Table 1: Effect of RMS on acetic acid induced writhing in mice.

Treatment	Dose	Onset of writhing (seconds)	Writhing scores [§]	% Protection
Normal saline	5 ml/kg	270.0± 45.83	75.33 ± 6.49	-
RMS	200 mg/kg	420.0 ± 91.70*	30.33± 8.37*	59.74
RMS	400 mg/kg	710.0 ± 112.69*	20.66 ± 7.42*	72.57
Ibuprofen	50 mg/kg	586.54 ± 23.78*	32.51 ± 4.70*	56.84

[§]Data are expressed as mean ± SEM ($n = 6$). * $p < 0.001$ compared with saline control.

Table 2: Effect of RMS on tail flick test in mice

			Reaction time (seconds) [§]				
			After treatment				
Treatment	Dose (mg/kg)	Before treatment	5 min	15 min	30 min	60 min	90 min
RMS	200	3.88 ± 0.39	7.24 ± 0.22*	9.36 ± 0.40*	>10* [¶]	>10* [¶]	>10* [¶]
RMS	400	2.27 ± 0.58	7.20 ± 1.53*	8.23 ± 0.63*	>10* [¶]	>10* [¶]	>10* [¶]
Morphine sulphate	5	1.60 ± 0.28	3.83 ± 0.30*	8.16±0.30*	9.67±0.33*	>10* [¶]	>10* [¶]

[§]Data are expressed as mean ± SEM ($n = 6$); * $p < 0.001$ compared with control (mice before treatment). [¶]A cut off time of 10 seconds was taken as maximum anti-nociceptive response to avoid tail damage due to heat.

Evaluation of Phenobarbitone-Induced Sleep Potentiation:

The animals were divided into four groups ($n = 6$). The first group (which served as control) received normal saline 5 ml/kg body weight i.p. The second and third groups received the RMS at the doses of 200 and 400 mg/kg body weight i.p., respectively. The last group (which served as reference) received chlorpromazine hydrochloride at the dose of 3 mg/kg body weight i.p. Thirty minutes after these treatments, all the animals received phenobarbitone sodium at the dose of 20 mg/kg i.p. Immediately after phenobarbitone administration, each animal was kept in an individual cage under observation. The latency to the loss of righting reflex (i.e., onset of action or induction time in minutes) and the time required to recover righting reflex or awakening (i.e., duration of action or sleeping time in minutes) for each group were recorded [10, 17].

Statistical Analysis: All data are presented as the mean ± standard error of mean (SEM). The results were analyzed for statistical significance by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparisons test of significance. P values less than 0.001 were considered as statistically significant.

RESULTS

The results of anti-nociceptive activity i.e., acetic acid induced writhing are summarized in Table 1. The RMS showed significant ($p < 0.001$) inhibition of onset and number of writhes, at both test doses as compared with control group in a dose dependent manner.

Table 3: Effect of RMS on locomotor activity in mice

Treatment	Dose (mg/kg)	Motor activity scores (in 10 minutes) [§]				% Reduction in motor activity	
		Before treatment	After treatment		After 30 min	After 60 min	
			30 min	60 min			
RMS	200	438.50 ± 5.50	182.50 ± 52.50*	50.50 ± 41.50*	58.38	88.48	
RMS	400	400.0 ± 47.98	17.0 ± 4.50*	6.0 ± 1.53*	95.75	99.00	
Chlorpromazine HCl	3	394.01 ± 22.08	102.37 ± 11.05*	71.14 ± 8.23*	74.02	81.94	

[§]Data are expressed as mean ± SEM ($n = 6$); * $p < 0.001$ compared with control (mice before treatment).

Table 4: Effect of RMS on muscle relaxant activity in mice

Treatment	Dose (mg/kg)	Fall off time (seconds) [§]			% Decrease in fall off time	
		Before treatment	After treatment		After 30 min	After 60 min
			30 min	60 min		
RMS	200	19.29 ± 1.64	7.99 ± 2.15*	3.60 ± 1.51*	58.58	81.34
RMS	400	19.0 ± 10.06	4.0 ± 1.73*	1.91 ± 0.95*	78.95	89.95
Diazepam	4	15.23 ± 2.14	4.01 ± 0.76*	2.83 ± 0.45*	73.67	81.42

[§]Data are expressed as mean ± SEM ($n = 6$); * $p < 0.001$ compared with control (mice before treatment).

Table 5: Effect of RMS on phenobarbitone induced sleeping time in mice

Treatment	Dose	Onset of action [§] (minutes)	Duration of action [§] (minutes)
Normal saline	5 ml/kg	57.50 ± 0.12	58.50 ± 0.12
RMS	200 mg/kg	8.33 ± 1.20*	87.33 ± 3.71*
RMS	400 mg/kg	7.0 ± 2.08*	92.0 ± 1.15*
Chlorpromazine HCl	3 mg/kg	15.85 ± 2.05*	148.65 ± 2.83*

[§]Data are expressed as mean ± SEM ($n = 6$); * $p < 0.001$ compared with saline control.

The results of tail flick test are shown in Table 2. RMS at the both doses exhibited significant ($p < 0.001$, after 5 to 90 minutes) and dose dependent steady increase in reaction time of mice more than 10 minutes, observed at the both doses. Peak analgesic effect was observed at 30-90 minutes indicating maximum increase in reaction times.

In locomotor activity study, it was found that RMS significantly ($p < 0.001$) depressed the locomotor activity in mice in a dose and time dependent fashion. The activities decreased as time approached to 60 minutes. The results are summarized in Table 3.

In muscle relaxant study, the RMS at both doses significantly ($p < 0.001$) and dose dependently decreased the fall off time in mice demonstrating its skeletal muscle relaxant property. The effect was most prominent after 60 minutes of administration (Table 4).

The results of phenobarbitone sodium induced sleeping time are presented in Table 5. Here, RMS pretreatment at all doses exhibited significant ($p < 0.001$) and potentiation of phenobarbitone-induced sleeping time in mice by hastening the onset of sleep (non-dose dependent) and delaying recovering the animals from sleep (dose dependent) as compared with the vehicle control group.

DISCUSSION

The anti-nociceptive activity of RMS was evaluated by acetic acid induced writhing and tail flick methods in mice to assess peripheral (non-narcotic) and central (narcotic) analgesic activities respectively [18]. The present results clearly indicated that the RMS had significant peripheral as well as central anti-nociceptive actions revealing the involvement of the central nervous system (CNS) in anti-nociception. This implies that the RMS exerted analgesic activity interfering with the peripheral as well as central mechanisms for the transmission of painful messages in mice.

Acetic acid-induced writhing is chemically induced nociception by intraperitoneal injection of dilute acetic acid solution to mice. The chemical agents can produce nociceptive reactions in mice. Intraperitoneal injection of phenyl para quinone, bradykinin or dilute acetic acid (1-3 % v/v) produces pain reaction that is characterized as writhing response. Constriction of abdomen, turning of trunk (twist) and extension of hind limbs (at least one) are considered as writhing reaction to chemically induced pain. Acetic acid induced writhing test is also known as a visceral pain model nociception. Several mediators like

kinins, acetylcholine, substance P, calcitonin-gene-related peptide and different prostaglandins (PGs) take part in visceral pain model nociception and transmission of the nociception from the viscera. In this test, both central and peripheral analgesics can be detected. Analgesics of narcotic (central) e. g. morphine, pentazocin, pethidine and non-narcotic (peripheral) type, e. g. aspirin, ibuprofen, indomethacin can inhibit the writhing response in mice [18, 19].

The tail flick test is thermally induced nociception model where radiant heat is used as a source of pain. Here, radiant heat (through a hot nichrome wire) is applied to the tail of mice and the withdrawal of tail from the radiant heat source (hot nichrome wire) is considered as flicking response to thermally induced pain. The flicking reaction which is the end point of this test may be mediated as a spinal reflex. Analgesics of only narcotic (central) type, e. g., morphine, pethidine, pentazocine etc., can increase the tail flick latency period indicating anti-nociception [18, 20].

Most of the centrally acting analgesics have certain CNS depressant effects. The locomotor activity was evaluated to assess the CNS-depressant property of RMS on the motor activity in mice. Most of the centrally active analgesic agents influence the locomotor activities in human beings and rodents mainly by reducing the motor activity because of their CNS depressant property [21]. Locomotor activity is considered as an index of wakefulness or alertness of mental activity and a decrease may lead to calming and sedation as a result of reduced excitability of the CNS [22]. The results of the present study showed significant influence in locomotor activity of mice by RMS treatment demonstrating decrease in locomotor activity and hence indicating its CNS depressant property in mice.

In muscle relaxant evaluation, the RMS-induced decrease in fall off time was due to the loss of muscle grip implying skeletal muscle relaxation [18]. Demonstration of marked muscle relaxant effect by the rota-rod study indicated that RMS induced neurological deficit accompanied with taming or calming effect in mice, thereby further supporting its CNS-depressant effect.

Barbiturates are putative sedatives inducing sleep in human beings and animals by depressing the CNS [23]. Phenobarbitone, although a long acting barbiturate, at lower doses it can serve as short to intermediate acting barbiturate [10, 17]. In the present study, in the saline control group of mice, phenobarbitone (20 mg/kg) produced intermediate onset and duration of sleep as indicated by the loss of righting reflex (inability to

maintain posture) and awakening or regaining righting reflex subsequently [19]. RMS pretreatment remarkably reduced the sleep induction time (onset of sleep) in mice; however, here the observed effect was not dose dependent. It markedly and significantly prolonged the duration of sleep in phenobarbitone-induced mice in a dose dependent manner. Potentiation of phenobarbitone induced sleeping time by RMS indicated the anxiolytic or sedative property of RMS, thereby confirming its CNS depressant role in mice. The CNS depressant role of RMS corroborated its central anti-nociceptive action in mice.

Preliminary phytochemical analysis revealed the presence of alkaloids, saponins, polyphenolics and carbohydrates in RMS. Polyphenols are well known natural products known to possess several notable biological properties [24]. Many flavonoids and neuroactive steroids were found to be ligands for the GABA_A receptors in the CNS; which led to the assumption that they may act as benzodiazepine-like molecules (which act through GABA_A receptor) [25] The presence of putative polyphenolic compounds could provide the chemical basis of its neuropharmacological effects in mice.

From the present series of experiments, it can be concluded that the roots of *M. scandens* possessed promising centrally and peripherally mediated anti-nociceptive, locomotor depressant, skeletal muscle relaxant and sedative potentiating effects in the experimental rodent models demonstrating its prominent depressant action on the central nervous system, as manifested by these important neuropharmacological properties in Swiss albino mice. Purification of the plant extract and further definitive studies may reveal the exact mechanisms and constituents behind the observed neuropharmacological activities of *M. scandens* root.

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