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Research Article

ANALGESIC AND ANTI-INFLAMMATORY EFFECT OF AN AQUEOUS EXTRACT OF *DENDROCNIDE SINUATA* (BLUME) CHEW

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ABSTRACT: The study was aimed to evaluate the analgesic and anti-inflammatory effect of aqueous root extracts of *Dendrocnide sinuata* (Blume) Chew (AEDS) in Swiss albino mice and wistar rats. The animals were orally administered AEDS at doses 30 and 100 mgkg^{-1} (*p.o*). For analgesic study, acetic acid-induced Writhing test, Eddy's hot plate and Tail Flick model was performed in mice. For anti-inflammatory study, carrageen-induced paw edema study was performed in rats. In acetic acid induced model, effect of AEDS was comparable with the standard meloxicam 10 mgkg^{-1} (*i.p*). In the hot plate model, the maximum effect was observed at 30 min at a dose of 100 mgkg^{-1} (*p.o*) which was comparable with the standard Pentazocine 10 mgkg^{-1} (*p.o*), whereas in the tail flick model no significant changes were observed. In the carrageenan-induced paw edema model, administration of AEDS showed significant ($P < 0.05$) dose dependent inhibition of edema formation. AEDS was effective in both narcotic and non-narcotic models of analgesia. It also showed a significant dose-dependent increase in anti-edematogenic activity which revealed good peripheral anti-inflammatory properties of the extract.

Key words: *Dendrocnide sinuata*, Aqueous root extract**,** Analgesic, Anti-inflammatory, Carrageenan.

INTRODUCTION

Since time immemorial, mankind has made the use of plants in the treatment of various ailments. The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. About 3.5 to 4 billion people in the world rely on plants as sources of drugs (Farnsworth *et al*., 1985).

Dendrocnide sinuata (Blume) Chew is a medicinal plant used by various ethnic communities of North East India. The perusal of literature reveals that no scientific study was undertaken to scientifically validate the claims of the folklore medicine. *Dendrocnide sinuata* has been use as medicine for curing diseases by different tribal communities of North East India. The plants under the family *Urticaceae* are perennial shrubs, dull green in color, armed with minute rigid hairs or prickles. The roots of the plant have stimulant, stomachic and

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diuretic properties (Rahman *et al*., 2008). Root decoction boiled with crabs is taken for curing jaundice (Lalfakzuala *et al.,* 2006).

MATERIALS AND METHODS

Animals

Adult healthy wistar rats (100-120 gms bw) and adult albino mice (25±5 gms bw) of either sex were used in the experiment. All the animals were housed in polypropylene cages in small groups of 4-5 rats per cage. Animals had free access to standard balanced ration and clean drinking water *ad libitum,* and were maintained in standard laboratory conditions (12 : 12 h light/dark cycle at ambient temperature ranging between 12-25^{0}C). The use of experimental animal and the study protocol was duly approved by Institutional Animal Ethics Committee.

Plant collection and processing

Roots of *D. sinuata* were uprooted and collected from the college campus. The roots were washed clean and were mopped by blotting paper, and weighed. The roots were air dried in shed for a period of two weeks. On complete drying, the roots were ground to powder with a Wiley's grinder and kept in an air tight container in a deep freezer. The plant was authenticated by the regional office, Botanical Survey of India (BSI), Shillong.

Extraction

The dry powdered root of *D. sinuata* was subjected to cold aqueous extraction following the method of Manjunatha *et al.,* (2005) with some modifications. The extract obtained was further subjected to vacuum evaporation at 100^{o}C for 24 h and lyophilized for successive 24 h. Further the material was stored at -40^{o}C in deep freeze in air tight containers until use.

Phytochemistry

Phytochemical tests of the aqueous extracts were conducted as per standard procedures (Edeoga *et al.,* 2005).

Acute oral toxicity

The acute oral toxicity study of both the extracts was undertaken as per Organisation for Economic Co-operation and Development (OECD) guidelines (Protocol-425) in mice (30±10 gms bw). The animals were given extracts @ 2000 mgkg^{-1} bw. The study was done for 72 h and during this period the animals were observed for mortality and sign of abnormality. Feeding-watering as well as body weight were monitored every day.

Experimental design

Analgesic activity

Acetic acid-induced writhing: Acetic acid - induced writhing test was performed for initial screening before the start of the experiment and the animals (mice) which showed maximum response were selected. Further the extract of *D. sinuata* was screened for analgesic activity against the colic/writhing reflex produced by irritant substance, acetic acid. Twenty four adult mice were randomly divided into 4 (four) groups having 6 (six) animals in each group. While Group I served as acetic acid control (0.7% @ 0.1 ml/10 gm bw) intraperitoneal (*i.p.*), Group III and IV received aqueous extract of *D. sinuata* @ 30 and 100 mgkg^{-1}, *per os* (*p.o*), respectively, while Group II served as standard control (Meloxicam @ 10 mgkg^{-1} (*p.o*).

Number of writhes in 15 min, 5 min after the administration of acetic acid were counted and considered for calculation of protective index as described by Somchit *et al.* (2004).

Protective index = [(control mean- treatment mean) x 100/ control mean]

Table 1. Effect of aqueous extracts of *D. sinuata* on tail flick reactions in mice.

Group	Treatment (mg/kg body weight, PO)	0 min	Reaction time (seconds)			
			30 min	60 min	90 min	120 min
I	Control	5.90±0.69	6.80 ^{b} ±0.46	6.35±0.51	7.33±0.43	6.26±0.87
II	Pentazocine(10)	5.80±0.46	10.91^{a} ±1.21	8.13±0.62	7.91±0.57	7.78±0.96
III	AEDS (30)	5.01±0.42	7.31^{b} ±0.53	7.35±0.35	6.55±0.54	7.13±0.65
IV	AEDS (100)	5.21±0.37	6.88^{b} ±0.71	7.41±0.44	6.73±0.39	7.01±0.47
F – Value		0.500 ^{NS}	7.152**	1.081 ^{NS}	0.768 ^{NS}	1.707 ^{NS}

Values are mean ± SEM, n=6 in each groups. Means bearing different superscript in a column differed significantly (P<0.05) NS - Non Significant, AEDS – Aqueous extract of *D. sinuate.*

Eddy's Hot plate: The best responding 24 (twenty four) adult mice of either sexes (7.75 ± 1.25 seconds) were selected and divided into 4 (four) groups of 6 (six) mice each. Vehicle (1% normal saline, *p.o*)) was administered to Group I, pentazocine @ 10 mgkg^{-1}, *i.p.* (Flecknell 1991) to Group II and aqueous extract of *D. sinuata* @ 30 and 100 mgkg^{-1}, *per os* **(***p.o*) to Group III and IV.

The Eddy's hot plate analgesiometer was maintained at a temperature of 55 ± 1°C. Zero, 30, 60, 120 and 180 min after the administration of test and reference compounds, animals of all the groups were individually placed on the hot plate maintained at 55± 1°C. The time taken in seconds for discomfort reaction (for paw licking or jumping) was observed and compared with pre-treatment readings (Shanmugasundaram and Venkataraman 2005).

Tail flick: Twenty four adult mice of either sex were selected and divided into 4 groups of 6 mice each. Vehicle (1% normal saline, *p.o*) was administered to Group I, pentazocine @ 10 mgkg^{-1} (*i.p*) to Group II and aqueous extracts of *D. sinuata* @ 30 and 100 mgkg^{-1} body weights to Group III and IV respectively by oral route. The tail-flick analgesiometer (Ugo Basile, Italy) was set at Infrared Radiation (IR) of 55 units and cut off period of 15 seconds to avoid tail injury. A circle of 2 centimeter radius was drawn around the point of light source, considering IR source as center of circle.

Anti-inflammatory activity

Carrageenan-induced paw edema: Twenty (20) apparently healthy rats of either sex were randomly divided into 4 (four) groups having 5 (five) rats in each group. While Group I and II served as treated carrageenan (1% w/v) control and standard meloxicam @ 10 mgkg^{-1} **(***p.o*) respectively, the remaining groups Group III and IV were treated with aqueous extracts of *D. sinuata* @ 30 and 100 mgkg^{-1} **(***p.o*), respectively. The mean increases in paw volume and % inhibition in paw volume were measured plethysmometrically at different time intervals. Percentage of oedema inhibition (% PI) was calculated with the help of above recordings (Miranda *et al*., 2001).

Statistical analysis

The values were expressed as mean± SEM and were subjected to statistical analysis by employing ONE WAY ANOVA test for

Table 2. Effect of aqueous extracts of *D. sinuata* on Eddy's hot plate induced pain in mice.

Group	Treatment (mg/kg bw, PO	Reaction time (Seconds)		
		0 min	30 min	60 min
I	Control	17.48±2.17	16.60^{b} ±4.12	16.95^{b} ±5.18
II	Pentazocine (10)	18.68±4.43	32.64^{a} ±3.18	49.23^{a} ±1.94
III	AEDS (30)	16.13±2.32	24.66^{ab} ±7.25	42.27^{a} ±4.23
IV	AEDS (100)	16.57±1.45	35.22^{a} ±4.34	44.56^{a} ±4.03
F - Value		5.579^{NS}	2.857*	8.100 **

Values are mean ± SEM, n=6 in each groups. Means bearing different superscript in a column differed significantly (P<0.05). NS - Non Significant, AEDS – Aqueous extract of *D. sinuate.*

meaningful and accurate comparison and interpretation between control and treatment groups using the software SPSS version 17.

RESULTS AND DISCUSSION

Phytochemical analysis

The extracts of *D. sinuata* were qualitatively analyzed for the presence of different phytochemical constituents. The extracts were found to contain terpenoids, flavonoids, saponins, tannins and cardiac glycosides in the present investigation.

The cut off LD_{50} dose was found to be 2500 mgkg^{-1} **(***p.o*), as per OECD-425 guidelines.

Analgesic effect

Acetic acid writhing test

The effect of aqueous extracts of *D. sinuata* on acetic acid induced writhing test in mice is shown in Fig.I. Administration of aqueous extracts at 30 (8.83±0.70 writhes per 15 minutes respectively) and 100 mgkg^{-1} **(***p.o*) (8.16±0.54 writhes per 15 min respectively) and 60.75 percent protection significantly reduced the acetic acid-induced writhing as compared to the vehicle control (13.16±0.40 writhes) and is comparable with the pain protection produced by the standard drug, meloxicam (7.00±0.57 writhes) which also showed 46.83 per cent protection.

Acetic acid, which is used as an inducer for writhing syndromes causes algesia by releasing of endogenous substances, which then excite the pain nerve endings; the abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins (Chen 1993, Ronaldo *et al*., 2000).

Isoeicosanoids, a family of eicosanoids isomers, are formed non-enzymatically by direct free radical based attack on arachidonic acid and related substrates (Lawson *et al*., 1999). Unlike eicosanoids, these compounds are generated on the esterified lipid in cell membrane, from which they are cleaved, presumably by phospholipases and the free iso-eicosanoids circulate in the blood. Since several iso-eicosanoids (isoprostanes) are formed which can activate the protanoid receptor, it may be speculated that they may contribute to the pathophysiology of pain and inflammatory responses in a manner insensitive to COX inhibition (Emer *et al*., 2006). Therefore, it is possible that *D. sinuata* root extract may probably exert its analgesic effect by either inhibiting the enzymatic synthesis of

Table 3. Effect of aqueous extracts of *D. sinuata* on carrageenan-induced paw oedema in rats.

Group	Treatment (mg / kg bw, PO)	Pre-treatment paw volume (CC)	Post-treatment paw volume (CC) (Percent Inhibition)			
			1 hour	2 hour	3 hour	4 hour
I	Control	0.61±0.04	1.09^{a} ±0.03	1.01±0.06	1.11^{a} ±0.02	1.17^{a} ±0.04
II	Meloxicam (10)	0.62±0.02	0.91^{b} ±0.08 (30.65)	0.79±0.02 (63.91)	0.95^{bc} ±0.02 (23.12)	0.91^{bc} ±0.01 (48.31)
III	AEDS (30)	0.61±0.03	0.92^{b} ±0.06 (13.13)	0.97±0.07 (84.53)	0.76^{d} ±0.06 (47.61)	0.73^{d} ±0.06 (66.29)
IV	AEDS (100)	0.63±0.07	0.74^{c} ±0.05 (60.58)	0.87±0.08 (25.77)	0.72^{c} ±0.11 (68.70)	0.81^{cd} ±0.04 (50.56)
F - value		3.91**	4.31**	2.07^{NS}	8.09**	14.25**

Values are mean ± SEM, n=6 in each groups. Means bearing different superscript in a column differed significantly (P<0.05) NS - Non Significant, AEDS – Aqueous extract of *D. sinuata.*

prostaglandins or by inhibiting the non-enzymatic free radical based synthesis of isoprostanes. Hassan *et al*. (2012) also showed that the ethanol extracted leaves of *Desmodium pauciflorum*, *Mangifera indica* and *Andrographis paniculata* showed significant inhibition (P<0.01) in acetic acid induced writhing response in mice. The analgesic effect of the extract may be due to its action on visceral receptor sensitive to acetic acid, inhibition of algesiogenic substances or the inhibition of transmission of painful messages at the central level (Hosoi 1999, Hassan *et al*., 2012).

Tail flick

Table 1 summarized the effect of aqueous extracts of *D. sinuata* on tail flick reaction in mice. The pre-treatment latency was 5.90±0.69, 5.80±0.46, 5.01±0.42 and 5.21±0.37 seconds in Group I, II, III and IV respectively. The latency in Group I, II, III and IV were 6.80±0.46, 10.91 ±1.21, 7.31 ±0.53 and 6.88±0.71 seconds at 30 min. At 90 min the latency were 7.33±0.43, 7.91±0.57, 6.55±0.54 and 6.73±0.39 sec. Also at 120 min the latency was 6.26±0.87, 7.78±0.96, 7.13±0.65 and 7.01±0.47 sec in Group I, II, III and IV respectively.

Administration of aqueous extracts at 30 min did not markedly alter the post-treatment latency as compared to normal Group I (6.80±0.46 seconds) but altered significantly with the Group II (10.91±1.21 seconds) pentazocine @10 $mgkg^{-1}$. However, in the subsequent readings in 60, 90 and 120 min there were no significant alteration in the post-treatment latency period between the groups as shown in the table.

Eddy's hot plate

In Eddy's hot plate, the mean reaction time in groups I, II, III and IV were 17.48±2.17, 18.68±4.43, 16.13±2.32 and 16.57±1.45 in 0 min, whilst the values at 30 min post treatment were 16.60±4.12, 32.64±3.18, 24.66±7.25 and 35.22±4.34 respectively. On the other hand the values in said groups at 60 min were

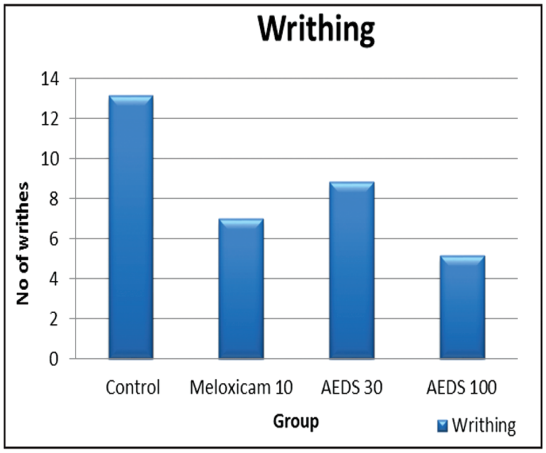

Fig. 1. Effect of aqueous extract of *D. Sinuata* on acetic acid in duced writhing test in nice.

16.95±5.18, 49.23±1.94, 42.27±4.23 and 44.56±4.03 seconds respectively. The reaction time was increased significantly (P=0.05) at 0, 30 and 60 min in groups II, III and IV compared to control untreated Group I (Table 2).

Thermal stimuli causes pain by stimulation of nociceptive receptors and transmitted over intact neural pathways. The hot plate test has been regarded as one of the best methods to study on central nociceptive effect of a compound / extract (Pini *et. al*., 1997 and Barua *et al*., 2010). The thermal stimulus is also described as an acute, non-inflammatory nociceptive stimulus as it causes direct stimulation of the nociceptors (sensory receptors) without causing any inflammatory-mediated nociception. In this present study, mice treated with aqueous extract @ 30 and 100 $mgkg^{-1}$ **(***p.o*) of *D. sinuata* showed tolerant to the thermal stimulus than the normal control animals. The analgesic activity was comparable to pentazocine, an opioid analgesic whose analgesic activity is mediated through central route. The analgesic effect of opioids arise from their ability to directly inhibit the ascending transmission of nociceptive information from the spinal cord dorsal horn and to activate pain control circuits that descends from the mid brain via the rostral ventromedial medulla to the spinal cord dorsal horn (Howard and Huda, 2006). The aqueous extract of *D. sinuata* had antinociceptive activity in hot plate test that may in part be mediated by opioid receptors. These findings indicate that the aqueous extract of *D. sinuata* may extort sufficiently opioid like compounds out of the plants which are responsible for the analgesic activity of the plant.

Since, aqueous extract of *D. sinuata* could increase the latency period in mice which was comparable to those mice receiving pentazocine, it may explain the possibility of involvement of a centrally mediated analgesic activity.

Anti-inflammatory effect

The anti-inflammatory effect of aqueous extracts of *D. sinuata* monitored up to 4 h post-treatment in carrageenan–induced rat paw edema model is shown in Table 3. The percent inhibition of rat paw edema after 1 h interplanter administration of carrageenan in Groups II, III and IV were 30.65, 13.13 and 60.58% respectively.

From the table it was observed that there were no significant responses between the groups at the end of 2 h. However, the anti-inflammatory activity of the meloxicam treated group II (63.91%) was shown to increase from the previous readings (30.65%). At 3 h post-treatment, the aqueous extract (30 and 100 $mgkg^{-1}$ respectively) revealed that there was a dose dependant (47.61% and 68.70% respectively) inhibition of the rat paw edema induced by carrageenan as shown in the table. At the end of 4 h, there was still increase in

anti-inflammatory effect in all the treated groups, *viz.* meloxicam @ 10 $mgkg^{-1}$ **(***p.o*) (48.31%), aqueous extract 30 $mgkg^{-1}$ **(***p.o*) (66.29%), 100 $mgkg^{-1}$ with 50.56% inhibition of edema.

It was observed that the aqueous root extract of *D. sinuata* showed dose-dependent increase in anti-edematogenic activity by suppressing carrageenan-induced rat paw edema at different phases of its development. Carrageenan-induced acute vascular response occurs in 3 phases with involvement of histamine and serotonin; kinins; and prostaglandins in phase 1, 2 and 3, respectively (Di Rosa *et al.,* 1971; Larsen and Hanson 1983; Vane and Booting 1987; Brooks and Day 1991). Anti-inflammatory activity of meloxicam and aqueous extracts may be attributed to their involvement in suppressing the cascade of acute vascular response with suppression of all 3 phases. The aqueous root extract of *D. sinuata* at the dose 100 $mgkg^{-1}$ **(***p.o*), possess significant ($P<0.05$) inhibition against carrageenan-induced paw edema in rats. This response tendency of the extract in carrageenan-induced paw edema revealed good peripheral anti-inflammatory properties of the extract.

D. sinuata revealed the presence of flavonoids and saponins. This anti-inflammatory effect of aqueous root extract of *D. sinuata* may be due to the presence of flavonoids (Gaur *et al*., 2009; Kavimani *et al*., 2000) and saponins (Lacaille-Dubois and Wagner 1996). Flavonoids are known to inhibit the enzyme prostaglandin synthase, more specifically the endoperoxidase (Ramaswamy *et al*., 1985) and which was reported to produce anti-inflammatory effects (Alcaraz and Jimenez, 1988).

Significant anti-inflammatory activity was observed in carrageenan-induced edema model. Since prostaglandins are involved in swelling and are inhibited by flavonoids (Clarke and Clumby 1975), it could be suggested that reduced availability of prostaglandins by flavonoids of *D. sinuata* might be responsible for its anti-inflammatory effect.

CONCLUSION

It can be concluded from this study that the aqueous root extract of *D. sinuata* possess significant central and peripheral analgesic activities and also capable of inhibiting inflammatory pain. The clinical applications of these findings must await further studies. Although the mechanism involved was not determined in the present study. This is likely to be focus on the forthcoming studies. This could help in creating mass awareness regarding the need for conservation of such plants and also in the promotion of ethno-medico knowledge within the northeast region of India.

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