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Anti-nociceptive and Anti-inflammatory Activities of *Wrightia arborea*

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Abstract: Anti-nociceptive and anti-inflammatory effects of methanolic extract of *Wrightia arborea* (MEWA) were examined using different models in rats. MEWA was given to rats orally upto 2000 mg kg^{-1} b.wt. for acute toxicity study and observed for 14 days. Anti-nociceptive activity was evaluated in rats against Acetic acid induced writhing (chemically induced pain) and Tail immersion method (thermally induced pain). Acute anti-inflammatory activity of MEWA was also evaluated in Formaline-induced rat paw edema model and Carrageenan-induced hind paw edema model in rats. Results demonstrated that no mortality was found upto single dose of 2000 mg kg^{-1} b.wt. in rats even after 14 days observation. In comparison to control group MEWA at 100 and 200 mg kg^{-1} b.wt. showed highly significant anti-nociceptive activity against chemically ($p<0.001$) as well as thermally ($p<0.05$ and $p<0.001$) induced pain as compared to standard drugs, indomethacin and nalbufin, respectively. In the formalin test, both the doses of 100 and 200 mg kg^{-1} of extract significantly prevented increase in volume of paw edema ($p<0.05$ and $p<0.01$) both in the neurogenic and inflammatory phases. MEWA (200 and 400 mg kg^{-1} p.o.) also significantly prevented increase in volume of paw edema in Carrageenan test ($p<0.05$ and $p<0.001$). The results suggest that MEWA has significant analgesic and anti-inflammatory potential which may be mediated by central and peripheral mechanism.

Key words: Anti-nociceptive, anti-inflammatory, writhing, tail immersion test, carageenan

INTRODUCTION

Pain and inflammation is a body defense reaction, in order to eliminate or limit the spread of injurious agents from living mammalian tissues. Inflammatory reactions are not only the local response of living tissues to injury and infection, but are also associated with the pathophysiology of various clinical conditions, such as asthma, multiple sclerosis, inflammatory bowel disease, colitis and atherosclerosis (Sarker *et al*., 2012). Chronic inflammatory diseases, one of the world's major health problems (Verma *et al*., 2012; Patrick-Iwuanyanwu *et al*., 2010), involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair (Maphosa *et al*., 2009; Suseem and Mary, 2011). Drugs which are in use presently to ameliorate this phenomenon are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well known side or toxic effects (Kaur and Jaggi, 2010; Devraj and Karpagam, 2011). Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects (Dolai *et al*., 2012). Therefore, a systematic approach should be made to find out the efficacy of plants as antinociceptive and anti-inflammatory agents since pain is one of the cardinal signs of inflammation.

Wrightia arborea (Dennst.) mabb. (Family: Apocynaceae) is a small tree or shrub, chiefly present in all the deciduous forests in Circars, Deccan and in Caenatic region. It has yellowish flowers, ovate or obovate tomentase leaves. Parts of plant are used medicinally in Ayurveda, siddha and other traditional systems of medicine for curing various ailments like menstrual disorders, renal complaints and amoebic dysentery (Jayaswal and Basu, 1965). In Thailand, the dried bark of this species is used as an antipyretic. The stem bark and root bark are believed to be useful in snake-bite and scorpion-stings (Kiritikar and Basu, 1981). Dried leaf powder used as diaphoretic, expectorant and also used in dysentery (Murugesa, 1988). The potential antibacterial activity of barks has already been reported (Khyade and Vaikos, 2011). Studies of the phytochemical on this plant have revealed the presence of unsaturated sterols, triterpenes, flavonoids, phenolic acids, alkaloids, saponin and tannins (Zaki *et al*., 1981). Yet, no systemic pharmacological studies were reported to support its use in inflammation and pain. Present study attempts to

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assess potential of the methanolic extract of *W. arborea* leaves as anti-nociceptive and anti-inflammatory agents.

MATERIALS AND METHODS

Collection and Identification of plant: The leaves of *Wrightia arborea* were collected from Hill tracts, Chittagong, Bangladesh in July 2011. The Voucher Specimen No.147 is kept for *W. arborea* in Herbarium of the Department of Botany, University of Chittagong, Bangladesh.

Preparation of extract: The collected aerial parts of plant was cleared, dried under shade at room temperature and powdered. For the extraction of phytochemicals about 200 g powder of the plant was taken in a glass jar and completely submerged with 600 mL methanol for 7 days accompanying occasional shaking and stirring. The extract was then filtered through filter paper (Double Rings filter paper 102, 11.0 cm). The filtrates was concentrated at 50°C under reduce pressure using vacuum pump rotary evaporator (STUART RF3022C, UK) to yield a solid residue 30 g and stored at 4°C in air tight containers for further studies.

Design of experiment

Experimental animal: Experiment was conducted on adult albino rats of male sex with the weights of 120-140 g procured from International Centre for Diarrheal Disease Research Bangladesh (ICDDRB). All rats were fed normal laboratory chow food containing 16% protein, 66% carbohydrate, 8% fats and water. All rats were housed at a (12:12) h light and dark cycle at 25°C and relative humidity (60-70) %.

Ethical approval: The guidelines followed for animal experiment were accepted by the institutional animal ethical committee (Zimmermann, 1983).

Drugs and chemicals: Indomethacin and Nalbufin were the gift samples from Square pharmaceuticals ltd, Bangladesh. Formalin and Acetic acid were obtained from BASF, Bangladesh. And all other reagents used were of analytical grade.

Acute toxicity test: Acute toxicity study was performed for the extract according to the acute toxic classic method as per the method of Lorke (1983). Extract was given orally to rats at the graded doses like 100, 300, 1000 and 2000 mg kg^{-1} b.wt. Immediately, after dosing, the animals were observed continuously for first four hours for behavioral change and mortality for daily upto 14 days.

Analgesic activity

Acetic acid induced writhing method: The peripheral anti-nociceptive activity of MEWA was studied using acetic acid-induced writhing model in rats (Saha and Ahmed, 2009). The abdominal writhing was induced by intraperitoneal injection of acetic acid solution (0.7%, 0.1 mL 10^{-1} g b.wt.) to each rat, a model of visceral pain. Five groups of mice (n = 6) were randomly formed. Indomethacin (10 mg kg^{-1}) was used as standard analgesic agent. MEWA was administered at 100 and 200 mg kg^{-1} b.wt. were orally administered 30 min prior to the injection of acetic acid. The number of writhing was calculated for 10 min after the application of acetic acid. The percent inhibition (% analgesic activity) was calculated by:

$$\text{Inhibition (\%)} = \{(A-B)/A\} \times 100$$

where, A is average number of writhing of the control group, B is average number of writhing of the test group.

Tail immersion method: This study is a thermal method to assess analgesia according to the method of Luiz (Di Stasi *et al.*, 1988). Rats divided in the groups of six each, were held in position in a suitable restrainer with the tail extending out. About 3-4 cm area of the tail was marked and immersed in the water bath thermo-statistically maintained at 51°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. 0.2 mL of 0.9% NaCl solution was administered to control animals, MEWA in doses of 100 and 200 mg kg^{-1} were given orally by intubation. The initial reading was taken immediately before administration of test and standard drugs (Nalbufin, 10 mg kg^{-1}) and then 30, 60 and 90 min after the administration. The criterion for analgesia was postdrug latency which was greater than two times the pre-drug average latency as reported by Janssen *et al.* (1963).

Antinflammatory activity: Formalin-induced paw edema the antinflammatory activity of MEWA was determined using the formalin test described by Sharma *et al.* (2010). Control group received 5% formalin. Twenty μL of 5% formalin was injected into the dorsal surface of the right hind paw 60 min after administration of MEWA (100 and 200 mg kg^{-1}, b.wt.) and indomethacin (10 mg kg^{-1}, b.wt.). The increase in paw diameter was measured using vernier caliper. The difference in edema of the right hind paw and the left hind paw indicates inflammation. Measurement was done immediately before and after 1-5 h following formalin injection.

Carrageenan induced paw edema: The animals were divided into five groups. Acute inflammation was produced by subplantar injection of 0.1 mL of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of the rats, 1 h after oral administration of MEWA at dose of 200 and 400 mg kg^{-1}, b.wt. The paw volume was measured at 1, 2, 3 and 4 h after the carrageenan injection using micrometer screw gauge. The difference between the two readings was taken as the volume of edema. Indomethacine suspended in 2% gum acacia was used as the standard drug.

Statistical analysis: Data were analyzed by one-way ANOVA followed by Dunnett's test. Value $p<0.05$ was considered statistically significant.

RESULTS

Acute toxicity test: The methanolic extract of *W. arborea* did not cause mortality of rats upto g kg^{-1} b.wt. during the 14 days ($p<0.05$) period. The tested dose level did not ($p>0.05$) affect the behavioral nor physiological changes of the experimental rats.

Acetic acid induced writhing test: MEWA at the doses of 100 and 200 mg kg^{-1} b.wt. and indomethacine induced a significant ($p<0.001$) decrease in the number of writhes when compared to control untreated groups. Both the doses produced significant analgesic activity and reduced the no. of writhing by 56.32 and 67.71%, respectively, whereas indomethacine caused 58.86% reduction when used as a reference drug (Table 1).

Tail immersion test: It is a thermal method, done to evaluate the anti-nociceptive effect of MEWA. The results obtained from this study shows that oral administration of MEWA significantly increased the reaction time of the animals to the thermal stimuli. MEWA at 200 mg kg^{-1} significantly increased the reaction time ($p<0.01$) from its initial value 0 to 90 min after administration and produced higher activity than nalbufin at 4th observation period (Table 2). But dose 100 mg kg^{-1} of MEWA increased the reaction time ($p<0.001$) after 60 min of observation.

Formalin-induced paw edema: The result of the anti-inflammatory studies of MEWA against acute paw edema in rats was also presented as mean±SEM in Table 3. A strong inhibition of the paw edema was observed with both the doses of MEWA and indomethacine. In the anti-inflammatory studies, the value obtained showed a significant ($p<0.05$ and $p<0.01$) reduction in the growth of edema in the hind paw of the rats from 3 to 5 h after administration.

Table 1: Anti-nociceptive effect of MEWA in Acetic acid induced writhing test

Group	Dose (mg kg^{-1} b.wt.)	Writhing No.	(%) Inhibition of writhing
Control	-	26.33±2.99	-
Indomethacin	10	10.83±1.36***	58.86
MEWA	100	11.5±1.15***	56.32
MEWA	200	8.5±1.04***	67.71

All values are expressed as Mean±SEM, (n = 6), One way Analysis of Variance (ANOVA) followed by Dunnet's test, ***$p<0.001$, significant compared to control

Table 2: Anti-nociceptive effect of MEWA in Tail Immersion test

	Tail flict time			
Treatment	0 min	30 min	60 min	90 min
Control (Vehicle)	1.0±0.11	1.1±0.12	1.15±0.05	1.25±0.11
Control (Nalbufin)10 mg kg^{-1}	1.40±0.02	2.26±0.25***	3.13±0.10***	3.06±20***
MEWA 100 mg kg^{-1}	1.14±0.63	1.2±0.75	1.26±0.80*	2.5±0.73***
MEWA 200 mg kg^{-1}	1.17±0.15	1.95±0.15***	2.36±0.72***	3.23±1.13***

All values are expressed as Mean±SEM, (n = 6), one way Analysis of Variance (ANOVA) followed by Dunnet's test *$p<0.05$, ***$p<0.01$, significant compared to control

Table 3: Anti-inflammatory effect of MEWA in formalin induced edema test in rats

	Paw edema volume (cm) in hours				
Groups	1 h	2 h	3 h	4 h	5 h
Group I (Control)	0.20±0.03	0.16±0.02	0.15±0.01	0.15±0.01	0.13±0.01
Group II (Indomethacin 10 mg kg^{-1})	0.13±0.02*	0.12±0.02	0.11± 0.01**	0.08±0.02**	0.07±0.01**
Group III (MEWA 100 mg kg^{-1})	0.18±0.02	0.13± 0.02	0.12±0.01*	0.10±0.01**	0.10±0.01*
Group IV (MEWA 200 mg kg^{-1})	0.15±0.02	0.14±0.01	0.12±0.02*	0.11±0.01*	0.08±0.01**

All values are expressed as mean±SEM, (n=6), one way Analysis of Variance (ANOVA) followed by Dunnet's test, *$p<0.05$, **$p<0.01$, significant compared to control

Table 4: Anti-inflammatory effect of MEWA in Carrageenan induced paw edema

	Paw edema volume (cm)			
Treatment	1 h	2 h	3 h	4 h
Control (vehicle)	2.60±0.11	2.65±0.12	2.76±0.05	2.82±0.11.0
Indomethacin 10 mg kg^{-1}	2.63±0.15	2.53±0.15*	2.36±0.11***	2.16±0.15***
MEWA 200 mg kg^{-1}	2.7±0.08	2.56±0.09*	2.43±0.12***	2.2±0.08***
MEWA 400 mg kg^{-1}	2.56±0.16	2.43±0.12*	2.2±0.08***	2.06±0.09***

All values are expressed as mean±SEM, (n = 6), one way Analysis of Variance (ANOVA) followed by Dunnet's test, *p<0.05, ***p<0.01, significant compared to control

Carrageenan induced paw edema: In the carrageenan-induced edema test both the doses of the extract significantly ($p<0.05$ and $p<0.001$) inhibited the edema compared with the control from 2 h after carrageenan injection. The extract at 400 mg kg^{-1} of body weight produced higher activity than indomethacin (Table 4).

DISCUSSION

The analgesic properties and ant-inflammatory effect of methanolic extract of *W. arborea* leaves were investigated in this study. The anti-nociceptive study of MEWA showed its suppressing ability of reflex and behavioral responses to the applied painful stimuli in both peripheral (non-narcotic) and central (narcotic) type pain models in rats. Acetic acid induced writhing test was used for detecting both central and peripheral analgesia as intraperitoneal administration of acetic acid in rat releases prostaglandins and sympathomimetic system mediators like PGE_2 and $PGF_{2\alpha}$ (Ribeiro *et al*., 2000) as well as lipoxygenase products (Dhara *et al*., 2000). Inhibitory effect on the writhing response of MEWA strongly suggest that the extract possesses peripheral analgesic activity and its mechanism of action may be mediated through inhibition of local peritoneal receptors or arachidonic acid pathways, involving cyclo-oxygenases and/or lipoxygenases. Again tail immersion tests are most sensitive to centrally acting analgesics indicating narcotic involvement (Besra *et al*., 1996) and are more sensitive to opioid ì receptors (Abbott and Young, 1988) (Furst *et al*., 1988). Moreover the effectiveness of analgesic agents in the tail flick pain model is highly correlated with human pain relief (Park *et al.,* 2010). In this model, MEWA in both the doses increased the pain threshold significantly during the period of observation and this indicates the involvement of a higher center.

In the Anti-inflammatory studies, the Formaldehyde induced edema is believed to be a multimediated phenomenon that liberates diversity of mediators which could be in two phases, the first being the release of serotonin and histamine while the second after the 1 h is mediated by prostaglandins (Dharmasiri *et al*., 2003). The cyclooxygenase products and the continuity between the phases are provided by kinins (Adedapo *et al*., 2008; Ageel *et al*., 1986). In this study, both the doses of MEWA showed a significant reduction from the 3rd h, in the growth of edema in the hind paw of the rats. Previous studies have shown significant correlations among cytokine production (induced by TNF-α), COX-2 protein expression and PG synthesis in the paw tissues of rats in which edema was invoked by intraplantar injection of formalin (Nantel *et al*., 1999; Beutler and Cerami, 1989). These results suggest that MEWA played a role in the anti-inflammatory activities in the model of Formalin-induced paw edema of Rats through the inhibition of TNF-α and COX-2 level.

Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation and exhibits a high degree of reproducibility (Winter *et al*., 1962). Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Carrageenan-induced edema is a biphasic response. The first phase (inflammation) is mediated through the release of histamine, serotonin and kinins (upto 2 h) whereas the second phase (edema) is related to the release of prostaglandin and slow reacting substances which peak at 3 h (Vinegar *et al*., 1969). MEWA produced significant inhibition of carrageenan-induced paw edema. Both the doses may be showing its effect through inhibition in production of prostaglandin as the result of pre-treatment of MEWA is effective in the late phase of inflammation which has been reported because of prostaglandin primarily. Phytochemical analysis of *W. arborea* revealed the presence of alkaloids, flavonoids, triterpene, phenolic acids and steroids (Zaki *et al*., 1981; Khyade and Vaikos, 2011). Of these, flavonoids and saponins are well known for their ability to inhibit pain perception. Flavonoids also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation (Oweyele *et al*., 2005). Flavone and its methoxy derivatives exhibited significant dose-dependent analgesic activity (Thirugnanasambantham *et al*., 1990).

CONCLUSION

On the basis of results obtained from the present study, it can be concluded that the plant extract possesses remarkable analgesic and anti-inflammatory activities. Present work was a preliminary effort which will require further detailed investigation including characterization of active compounds and requires preformulation studies for development of a potential dosage form.

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