Bangladesh Pharmaceutical Journal **15**(2): 171-176, 2012

Anti-inflammatory and Analgesic Activities of *Asteracantha longifolia* Nees

Md. Al Amin[1], Ishtiaque A. Chowdhury[1], K. M.M. Mahbub[1], Mafruhi Sattar[1], Masum Shahriar[1], Md. Ruhul Kuddus[2] and Mohammad A. Rashid[2]

[1]Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

Abstract

The present study was designed to evaluate the analgesic and anti-inflammatory activities of the ethanol extract of whole plant of *Asteracantha longifolia* Nees (family Acanthaceae) in mice. The analgesic activity was determined for its central and peripheral pharmacological actions using hotplate, formalin induced pain and acetic acid-induced writhing test in mice. Anti inflammatory effects were determined by ear swelling induced by croton oil, xylene-induced ear edema, leukocyte migration induced by carrageenan, cotton pellet-induced granuloma formation. Tramadol (10 mg/kg) and Ibuprofen (100 mg/kg) were used as reference analgesic agents. The crude ethanol extract showed a significant inhibition of ear swelling caused by croton oil and xylene in mice. The crude extract decreased leukocyte migration induced by carrageenan, also moderately inhibited the weight of granuloma induced by a cotton pellet, as well as the formalin-induced pain. The extract given by p.o. route, produced significant inhibition of abdominal constrictions caused by acetic acid. Moreover, the extract also showed moderate analgesic activity on the hot plate pain threshold in mice. These data demonstrated that the plant may contain bioactive compounds possessing anti-inflammatory and analgesic activities.

Key words: *Asteracantha longifolia* Nees, Acanthaceae, Analgesic, Anti-inflammatory

Introduction

Asteracantha longifolia Nees is an important medicinal herb widely distributed in Indian subcontinent and is used by local population for different medicinal purposes (Warrier *et al*., 1995). The plant is known to possess antitumor (Ahmed *et al*., 2001), hypoglycaemic (Muthulingam, 2010), antibacterial (Muhamed *et al*., 2011), free radical scavenging and lipid peroxidation activities (Vijayakumar *et al*., 2005). This plant contains a diversity of biologically active compounds such as alkaloids (Mandal *et al*., 2010), waxy substances, gum (Chopra *et al*., 2006), minerals as Ca, Mg, K, Fe, Cu, Zn, Mn, Co & Cr (Jamil *et al*., 2007) and phytosterols (Nadkarni, 2007), essential oil, a straight chain ketone (Asolkar *et al*., 2005), flavonoids, terpenoids, manganese salts, potassium chloride & sulphate and fixed oils (Dasgupta *et al*., 2007). Hence in the present study, the ethanol extract of whole plant of *A. longifolia* was examined for its anti-inflammatory and analgesic properties.

Materials and Methods

Plant material and preparation of the extract: The whole plant of *A. longifolia* was collected from Savar, Dhaka in March 2010 and was identified in Bangladesh National Herbarium, Mirpur, Dhaka (Accession number-37543). The collected materials were thoroughly washed in water, chopped, air dried at 40 ºC for a week and pulverized in electric grinder. The powdered material was extracted using 95% ethanol for 72h in a Soxhlet apparatus. The obtained extract was evaporated with the help of rotary evaporator under reduced pressure to give a residue.

Source of chemicals: All the chemicals/drugs and solvents used in this study were of analytical grade and purchased from Merck Chemicals, Square Pharmaceuticals Ltd.

Animals and treatment: For the experiment Swiss albino mice of either sex, 6-7 weeks of age, weighing between 25-30 g, were collected from the Animal Research Laboratory in the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. Animals were

Correspondence to: Md. Al Amin; E-mail: shantopher_06@yahoo.com

maintained under standard environmental conditions temperature: (27.0 ± 1.0°C), relative humidity: 55-65% and 12 h light/12 h dark cycle and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

A. Anti-inflammatory activity of *A. longifolia*

a) Ear swelling induced by croton oil in mice: The croton oil induced ear edema test was performed as described by Zitterl-Eglseer (1997). The test samples including Ibuprofen (100 mg/kg) as a positive- control were given orally to the mice. One hour later, each animal received 15 µl of croton oil on the inner surfaces of the right ear lobe and 15 µl acetone on the inner surface of left ear lobe. Mice were sacrificed one hour after croton oil application and circular sections were taken, using a cork borer with a diameter of 3 mm, and weighed.

b) Xylene induced ear edema in mice: The xylene induced ear edema test was performed as described by Dai *et al*. (1995). The test samples including, Ibuprofen (100 mg/kg) as a positive- control were given orally to the mice. One hour later, each animal received 20 µl of xylene on the anterior and posterior surfaces of the right ear lobe. The left ear was considered as control. Mice were sacrificed one hour after xylene application and circular sections were taken, using a cork borer with a diameter of 3 mm, and weighed. The percentage of ear edema was calculated based on the weight of left ear without xylene.

c) Leukocyte migration induced by carrageenan: The carrageenan-induced peritonitis test was performed as described by Ferrandiz and Alcaraz, (1991). Mice were divided into 4 groups of 6 animals each. Group 1, the control group received normal saline, p.o., group 2, the standard group received Ibuprofen (100 mg/kg). Groups 3, 4, received ethanol extract (250 mg/kg, 500 mg/kg), 60 min before being injected with carrageenan (0.75%, 0.25 ml, i.p.). Four hours after carrageenan administration the mice were killed and 2 ml of modified PBS (Phosphate buffer saline) was injected into the peritoneal cavity. Total cell counts in the lavage fluid were performed in a Neubauer chamber. The results were expressed as (mean± SEM).

d) Cotton pellet induced granuloma formation in mice: The method of Swingle and Shideman, (1972) is used. Sterilized cotton pellets of (10 ± 1 mg) weight each was impregnated subcutaneously, one on each side of the abdomen of the animal, under light chloroform anesthesia and sterile technique. Test drugs were administered orally to male mice weighing 25-30 g in a once-daily dosage regimen for 7 days; the control group received vehicle only. The mice were sacrificed on the 8th day & removed cotton & dried 60 ºC for 24 h & weighed dry cotton weight.

B. Measurement of analgesic activity of *A. longifolia*

a) Formalin induced paw licking in mice: The method used for the study was developed by Hunskaar and Hole (1987). Mice were divided into 4 groups of 6 animals each. Group 1, the control group received normal saline, p.o., group 2, the standard group received Ibuprofen (100 mg/kg). Groups 3, 4, received ethanol extract (250 mg/kg, 500 mg/kg). After 1 h drug administration 20 µl formalin was injected into the dorsal surface of the left hind paw. The time spent for licking the injected paw was recorded. Animals were observed for the 5 min post formalin (acute phase) and for 5 min starting at 20th min post formalin (delayed phase).

b) Acetic acid induced writhing: The method according to Koster *et al*. (1959) was employed for this test. Four groups of 6 mice each were pretreated with the ethanolic extract (250 mg/kg, 500 mg/kg), Ibuprofen (100 mg/kg) and normal saline (10 ml/kg) respectively. Forty five minutes later each mouse was injected with 0.7% acetic acid at a dose of (10 ml/kg) body weight. The number of writhing responses was recorded for each animal during a subsequent 5 min period after 15 min I.P. administration of acetic acid and the mean abdominal writhings for each group was obtained.

The percentage inhibition was calculated using the formula -

$$\% \text{ Inhibition} = \frac{\text{Mean no. of writhing (control)} - \text{Mean no. of writhing (drugs)}}{\text{Mean no. of writhing (control)}}$$

c) Hot plate test: The hot plate test was used to measure response latency according to the method described by Eddy and Leimbach (1953) with slight modification. The temperature of the hot plate (model 7280; Ugo Basile, Italy) was maintained at (55 ± 2 °C). Mice were divided into 4 groups of 6 animals each. Group 1, the control group received normal saline, p.o., group 2,

the standard group received Tramadol (10 mg/kg). Groups 3 and 4 received crude extract (250 mg/kg, 500 mg/kg), and animals were placed in a Perspex cylinder on a heated surface, and the time between placement of the animal on the hot plate and the occurrence of discomfort, indicated by either licking of the paws or jumping off the surface, was recorded as response latency. Mice with baseline latencies of more than 10 s were eliminated from the study, and the cut-off time for hot plate latency was set at 15 s. The latency of discomfort was measured at 0, 30, 60, 120, and 180 min after test solution administration.

Results and Discussion

Croton oil is the oil prepared from the seeds of *Croton tiglium*. Externally, the oil can cause irritation and swelling. In this test, the inflammatory response is usually quantified by measuring the change in ear plug weight at a single time interval after the croton oil effect is maximal. The results obtained are summarized in Figure 1. As shown, ethanol extract at a dose of 500 mg/kg caused a significant (**p < 0.01) reduction in ear swelling (66.14%) compared to control at the time of measurement. Ibuprofen also impaired the ear swelling, but this anti-inflammatory effect was not much stronger.

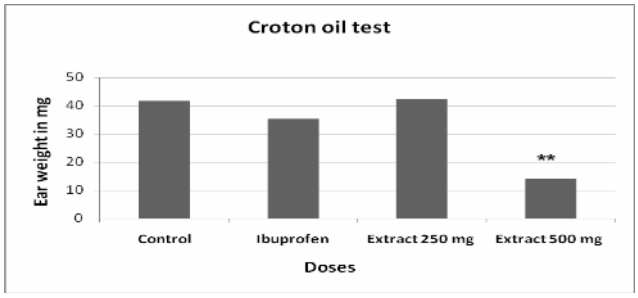

Figure 1. Graphical presentation of croton oil induced ear swelling test. Values of ear weight are (mean ±S.E.M.), (n=6) **P <0.01, significantly different from control; done by independent sample t-test

The xylene ear edema model permits the evaluation of anti-inflammatory steroids and is less sensitive to non-steroidal anti-inflammatory agents (Zaninir *et al*., 1992). The result of the xylene induced ear edema test in mice is presented in Table 1. The crude extract at a dose of 500 mg/kg produce significant inhibition of edema and this effect is stronger than the reference drug.

Table 1. Effect of ethanol extract of *A. longifolia* on xylene induced ear edema in mice.

Group	Doses (mg/kg)	Ear weight (mg)	Inhibition (%)
Control	-	182.98 ± 17.20	-
Ibuprofen	100	151.76 ± 22.09	17.06
Ethanol extract	250	166.78 ± 12.15	8.85
	500	131.96 ± 15.22**	27.88

Values of ear weight are (mean ± S.E.M.). **P< 0.01, significantly different from control; done by independent sample t-test, (n=6).

The crude ethanol extract was studied for its ability to inhibit cellular migration to the peritoneal cavity. The result of the present study was presented in the Figure 2. The ethanol extract decreased leukocyte migration to the peritoneal cavity, mainly inhibiting neutrophil and lymphocyte migration, when compared with standard (Ibuprofen). In our assays, the extract slightly reduced the vascular permeability induced by carrageenan. This suggests that the extract exerts anti-inflammatory activity, especially in the acute phase of inflammation, can also affect leukocytes function.

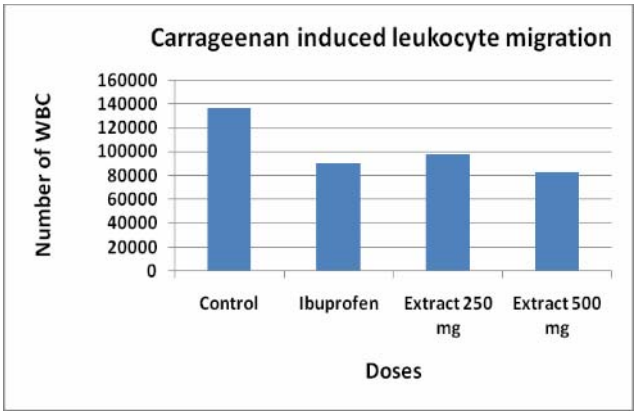

Figure 2. Graphical presentation of carrageenan induced leukocyte migration test.
Values of number of WBC are (mean ± S.E.M.), (n=6). The values are not statistically significant but reduce inflammation compare to the control; Analyzed by independent sample t-test.

The cotton pellet induced granuloma test is widely used to assess the transudative and proliferative components of chronic inflammation (Winter and Porter, 1957). The weight of the wet cotton pellets correlates with transude material and the weight of dry pellet correlates with the amount of granulomatous tissue. The effects of the ethanol extract and Ibuprofen on the cotton pellet granuloma are summarized in Table 2. The ethanol extract

reduced the mass of the cotton-pellet granuloma. The highest concentrations of extracts reduced the mass to values that are comparable to those achieved with the known anti-inflammatory drug Ibuprofen.

Table 2. Effect of ethanol extract of *A. longifolia* on cotton pellet-induced granuloma formation test.

Group	Doses (mg/kg)	Weight of cotton (gm)	Inhibition (%)
Control	-	0.043 ± 0.0033	-
Ibuprofen	100	0.040 ± 0.0035	6.976
Ethanolic extracts	250	0.045 ± 0.0013	-5.14
	500	0.037 ± 0.0018	12.62

Weight of cotton pellet are (mean ±S.E.M.), (n=6). The values are not statistically significant But ethanolic extract (500) reduce cotton weight, done by independent t-tes

This observation suggests that the crude extracts of this plant have inhibitory effects on subacute inflammation (Nandal *et al*., 2009) and may have the capability to reduce the synthesis of mucopolysaccharides, collagen and the number of fibroblasts, the natural proliferative events in the granulation in tissue formation.

The formalin test was chosen to evaluate the potential analgesic effects of extracts because of its advantages over other models of pain. This test is very useful for evaluating the mechanism of pain and analgesia. Drugs such as narcotic analgesics inhibit both phases of pain in this model while peripherally acting drugs, such as aspirin or indomethacin, only inhibit the late phase (Santos *et al*., 1994). The experimental result presented in the Figure 3 showed the oral administration of ethanol extract failed to reduce the licking time of first phases. However, the extract exhibited greater effects on the second phase of the analgesic response. But this effect is not statistically significant.

These data suggests that the extract can produce analgesic action through inhibition of COX and consequently prostaglandin synthesis. Acetic acid induced abdominal constriction is a standard, simple, and sensitive test for measuring analgesia induced by both opioids and peripherally acting analgesics. This test, besides being the most appropriate antinociceptive model for opioids (Hayes *et al.*, 1987) is also commonly employed as a visceral inflammatory pain model. Table 3 shows that extract significantly reduced the number of writhing movements induced by the i.p. administration of acetic acid solution. The dose-dependent inhibition of abdominal constrictions by the ethanol extract indicates antinociceptive potential of the plant. The extract might have suppressed the synthesis of prostaglandin in the bodies of the animals.

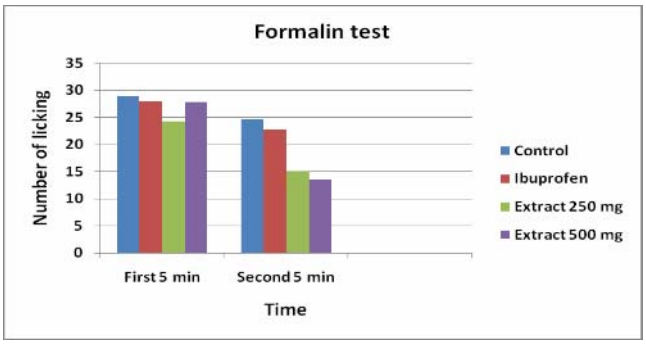

Figure 3. Graphical presentation of formalin induced pain.
Values of first 5 & second 5 minutes are (mean ± S.E.M.), (n=6). The values are not statistically significant but reduce pain compare to the control; Analyzed by independent sample t-test.

Table 3. Effect of ethanol extract of *A. longifolia* on acetic acid induced writhing test.

Group	Doses (mg/kg)	Number of writhing	Inhibition (%)
Control	-	24.20 ± 2.84	-
Ibuprofen	100	17.83 ± 3.11	26.32
Ethanolic extracts	250	20.17 ± 4.22	16.69
	500	11.17 ± 3.60*	53.84

Number of writhing values are (mean ±S.E.M.). *P < 0.05, significantly different from control; done by independent t-test, (n=6).

Hot plate test is commonly used to assess narcotic analgesics, other centrally acting drugs, including sedatives and muscle relaxants or psychotomimetics have shown activity in this test. However, in contrast to the effect for morphine, indomethacin and other NSAIDs have no effect according to the hot-plate test (Yamamoto and Nozaki-Taguchi, 1996). Table 4 shows the results of the hot plate test. The crude extract increased the reaction time to the thermal stimulus. The highest nociception inhibition of thermal stimulus was exhibited by alcoholic extract at a dose of 250 mg/kg, which was comparable to the Tramadol.

Table 4. Effects of ethanol extract of *A. longifolia* on latency to hotplate test.

Group	Doses (mg/kg)	-30 min	+30 min	+60 min	+120 min	+180 min
Control	-	5.88 ± 1.14	8.76 ± 1.77	7.78 ± 1.12	10.54 ± 1.73	8.92 ± 1.64
Tramadol	10	8.07 ± 1.30	11.34 ± 0.97	11.44 ± 1.14*	12.09 ± 1.33	8.71 ± 1.29
Ethanolic extracts	250	8.38 ± 1.93	11.56 ± 1.22	12.60 ± 1.10**	11.86 ± 1.14	6.86 ± 2.20
	500	5.24 ± 0.41	5.94 ± 1.37	6.46 ± 1.46	7.72 ± 1.62	7.12 ± 0.65

Values are mean ±SEM, (n = 6);
-30 min means 30 minutes before drug administration, +30, +60 indicates 30, 60 minutes after drug administration
*: $p<0.05$, **: $p<0.01$ significantly different from control; Analyzed by independent sample t-test.

Conclusion

The results demonstrated significant analgesic and anti-inflammatory properties of the ethanol extract of *A. longifolia* which were similar to, and in some cases better than, those of the positive controls. These indicated that this plant could be a potential source for discovery of newer analgesic and anti-inflammatory "leads" for drug development. Present study finding supports the traditional claims and provides a scientific basis for anti-inflammatory effect of *A. longifolia* in inflammatory diseases.

References

Ahmed, S., Rahman, A., Mathur, M., Athur, M. and Sultana, S. 2001. Antitumor promoting activity of *Asteracantha longifolia* against experimental hepatocarinogenesis in rats. *Food Chem Toxicol*. **39**, 19-28.

Asolkar, L.V., Kakkar, K. K. and Chakre, O.J. 2005. Second supplement to glossary of Indian medicinal plants with active principles, Part I, NISCAIR, CSIR, New Delhi. p. 362.

Chopra R. N., Nayar S. L. and Chopra I. C., 2006. *Glossary of Indian Medicinal Plants*, NISCAIR, CSIR, New Delhi. **29**, 324-325.

Dai, Y., Liu, L. H. and Kou, J. P. 1995. Anti-inflammatory effect of aqueous extract of Wu-HU-Tang. *China Pharm. Uni*. **6**, 362-364.

Dasgupta, N. De. B. 2007. Antioxidant activity of some leafy vegetables of India: A comparative study. *Food Chem*. **101**, 471-474.

Eddy, N. B. and Leimbach, D. 1953. Synthetic analgesics. II. dithienylbutenyl and dithienylbutylamines. *J. Pharmacol. Exp. Ther*. **107**, 385–393.

Ferrandiz, M. L. and Alcaraz, M. J. 1991. Anti-inflammatory activity and inhibition of arachidonic acid metabolism by flavonoids. *Agents Actions.* **32**, 283-288.

Hayes, A. G., Sheehan, M. J. and Tyers, T.B. 1987. Differential sensitivity of models of antinociception in the rat, mouse and guinea-pig to –μ- and κ-opioid receptor agonists. *Br. J. Pharmacol.* **91**, 823-832.

Hunskaar, S. and Hole, K. 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory *Pain*. **30**, 103-114.

Jamil, A., Shahid, M., Khan, M. H. and Ashraf, M. 2007. Screening of some medicinal plants for isolation of antifungal proteins and peptides. *Pak. J. Bot.* **39**, 211-221.

Koster, R., Anderson, M. and De-Beer, E.J. 1959. Acetic acid analgesic screening. *Fed. Proc.* **18**, 412-417.

Mandal, S., Dutta, G. K. and Nath, S. 2010. Qualitative phytochemical screening of *Hygrophila spinosa* plant extract. *Vet. World.* **3**, 367-368.

Muhamed M. H., Doss, A., Dhanabalan, R., and Venkataswamy, R. 2011. In-vitro antimicrobial effects of some selected plants against bovine mastitis pathogens. *Hygeia. J. D. Med.* **3**, 71-75.

Muthulingam, M. 2010. Antidiabetic efficacy of leaf extracts of *Asteracantha longifolia* (Linn.) Nees. *Int. J. Pharm. Biomed. Res.* **1**, 28-34.

Nadkarni, A. K. 2007. Indian Materia Medica, Popular Prakashan, Mumbai. **1**, 668.

Nandal, S., Dhir, A., Kuhad, A., Sharma, S. and Shopra, K. 2009. Curcumin potentiates the anti-inflammatory activity of cyclooxygenase inhibitors in the cotton pellet granuloma pouch model. *Meth. Find. Exp. Clin. Pharmacol.* **3**, 89-93.

Santos, A. R. S., Filho, V. C., Niero, R., Viana, A. M., Moreno, F. N., Campos, M. M., Yunes, R. A. and Calixto, J. B. 1994. Analgesic effects of callus culture extracts from selected species of *Phyllantus* in mice. *J. Pharm. Pharmacol.* **46**, 755-759.

Saxena, H. O., and Brahmam, M. 1995. The Flora of Orissa, Capital Business Services & Consultancy, Bhubaneswar. 3rd edn., pp. 1578-1580.

Vijayakumar, M., Govindarajan, R., Shriwarkar, A., Kumar, V., Rawat, A., Mehrotra, S. and Pushpangadan, P. 2005. Free radical scavenging and lipid peroxidation inhibition potential of *Hygrophila auriculata*. *Nat. Prod. Sci.* **11**, 22-26.

Warrier, P. K., Nambiar, V. P. K. and Ramankutty, C. 1995. Indian Medicinal Plants: A Compendium of 500 Species. **4**, 100-102.

Winter, C. A. and Porter, C. C. 1957. Effect of alterations in the side chain upon anti-inflammatory and liver glycogen activities of hydrocortisone esters. *J. Am. Pharm. Assoc. Am. Pharm. Assoc (Baltim)*. **46**, 515-519.

Yamamoto, T. and Nozaki-Taguchi, N. 1996. Analysis of the effects of cyclooxygenase COX-1 and COX-2 in spinal nociceptive transmission using indomethacin, a non-selective COX inhibitor, and NS-398, a COX-2 selective inhibitor. *Brain. Res.* **739**, 104-110.

Zaninir J. C., Medeiros Y. S., Cruz A. B., Yunes R. R. A. and Calixto, J. B. 1992. *Phytother. Res.* **6**, 1.

Zitterl-Eglseer, K., Sosa, S., Jurenitsch, J., Schubert-Zsilavecz, M., Della Loggia, R., Tubaro, A., Bertoldi, M. and Franz, C. J. 1997. *Ethnopharmacol.* **57**, 139-144.