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# Antinociceptive activity of methanolic extract of leaves of *Achyranthes aspera* Linn. *(*Amaranthaceae) in animal models of nociception

Chandana C Barua[1*], Archana Talukdar[1], Shameem A Begum[1], Lalit C Lahon[1], Dilip K Sarma[2], Debesh C Pathak[3] & Probodh Borah[2]

Department of [1]Pharmacology and Toxicology, [2]Microbiology and [3]Pathology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati 781 022, India.

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Antinociceptive activity of methanolic extract of leaves of *A. aspera* was studied by peripheral/non-narcotic model of nociception like acetic acid induced writhing syndrome test and central/narcotic models like hot plate and tail flick tests. The methanolic extract of the plant, administered orally (@ 300, 600 and 900 mg/kg, body weight) and the standard drug (piroxicam; 10 mg/kg body weight, po) produced significant analgesic activity in acetic acid induced writhing syndrome as compared to the vehicle treated control group. In the hot plate analgesic test, in *A. aspera* at the above doses and the standard drug treated group (morphine sulphate @ 1.5 mg/kg, ip), the duration of reaction time (sec) increased dose dependently and significantly compared to the control group. In the tail flick test, the plant extract produced dose dependant increase in reaction time which was significantly higher in the test and standard group compared to the control group. The plant possesses significant antinociceptive property as evidenced in all the animal models of nociception. It might possibly exert its effect through diverse mechanism that may involve both central and peripheral pathways. The preliminary phytochemical investigation revealed the presence of steroids, alkaloids and triterpene in the methanolic extract of leaves of *A. aspera* which may be responsible for its antinociceptive activity.

**Keywords:** *Achyranthes aspera*, Hot plate, Nociception, Tail flick test, Writhing syndrome

According to a WHO report, about 70-80% of the world's populations rely on non-conventional medicine mainly from herbal sources in their primary health care. It is specially the case in developing countries where the cost of consulting a western style doctor and the price of medication are beyond the means of most people[1]. A medicinal plant is factually any plant which in one or more of its parts contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of direct therapeutic agents. Use of medicinal plant is increasing in many countries where 35% of drugs contain natural products[2].

Most of the drugs used at present for analgesic effect are synthetic in nature, prolong use of which cause several side and toxic effects like respiratory depression, constipation, kidney damage, physical dependence as well as gastrointestinal irritation. As these drugs are not commonly available to the rural folks that constitute the major populace of the world, it is therefore essential that effort should be made to introduce new medicinal plants to develop cheaper drugs. The floral richness of the North East region cannot be neglected in context to its medicinal importance. Considering the rich diversity of this region, it is expected that screening and scientific evaluation of plant extracts for their analgesic activity may provide new drug molecule that can combat various side effects of the commercially available synthetic drugs, moreover reducing the cost of medication.

*Achyranthes aspera* Linn. locally known as Apang, is an annual, biennial, lower portion perennial erect under shrub or rather stiff herb growing up to 0.3 to 1.0 m in height[3]. It grows throughout the world in tropical and warmer regions[4,5]. Yunani doctors and local *kabiraj* use the stem, leaves and fruits as a remedy for piles, renal dropsy, pneumonia, cough, kidney stone, skin eruptions, snake bite, gonorrhea and dysentery etc[3]. Various extracts of this plant reveals presence of 27-Cyclohexyheptacosan-7-ol and 16-hydroxy-26-methylheptacosan-2-one, a long chain alcohol and 17-pentatriacontanol, alkaloid, b-sitos-

* Correspondent author
Mobile: 098640-13231
Tele fax -0361 2364941
E- mail: chanacin@gmail.com; chanacin@satyam.net.in

terol and spinasterol[3]. The plant has antibacterial[3], antitumor[6], anti-inflammatory[7,8], anti-fertility[9], abortifacient activity and increases pituitary and uterine wet weights in ovarectomised rats[10] and reproductive toxicity in male rats[11].

*Achyranthes aspera* has been extensively studied for various pharmacological properties including anti-inflammatory activity[8], but analgesic activity of the plant has not been reported so far. The study, therefore seeks to access methanolic extract of the leaves of this plant for analgesic activity in different experimental animal models.

## Materials and Methods

*Plant material*— Leaves of the plant were collected from the medicinal garden of the Department of Pharmacology & Toxicology, College of Veterinary Science, Khanapara during the month of February-June, 2008, identified by Taxonomist of NEIST, Jorhat, Assam and a voucher specimen (AAU/CVSC/PHT/01) was deposited.

*Preparation of methanol extract*— Leaves of *A. aspera* were dried in shade and powdered. About 250 g of powdered leaves was soaked in 1000 ml methanol for 72 h in beaker and mixture was stirred every 18 h using a sterile glass rod. Filtrate was obtained after passing through Whatman filter paper no 1 for 3 times and concentrated in rotary evaporator at 50°-60°C under reduced pressure leaving a dark brown residue which was stored in air tight container at 4°C until use. Recovery was 6.89% (w/w).

*Phytochemical screening*– Phytochemical screening of the extract was carried out by standard method[10].

*Chemicals*— Morphine sulphate and piroxicam were purchased from Sigma (Poole, UK) and Pfizer, respectively. Methanol, Acetic acid and Formalin were purchased from Merck Limited, Mumbai 400 018.

*Animals*— Healthy adult Swiss albino mice of either sex, approximately of same age, weighing between 25-30 g and adult male Sprague Dawley rats weighing between 180-200 g were used for the study. They were housed under controlled conditions at 25°±3°C, 50±5% RH and kept under 10/14 h light/dark cycles with food and water *ad libitum*. Animals were group housed in polypropylene cages containing sterile paddy husk bedding. The study was conducted after obtaining the approval of the Institutional Animal Ethics Committee. The animals were fasted for 14 h before test to achieve better drug absorption through gastrointestinal tract.

*Determination of $LD_{50}$* —The $LD_{50}$ of *A. aspera* was estimated by following up-and-down stair case method[13]. Doses were adjusted by a constant multiplication factor viz. 4 for this experiment. The dose for each successive animal was adjusted depending on the previous outcome. The acute toxicity and gross effect of crude methanolic extract of *A. aspera* was studied in albino mice by using 1/2 $LD_{50}$ dose. A total 6 male albino mice were selected for each experiment. Animals were observed hourly for 6 h and again after 24 h. The parameters for motor activity and gross effect were determined after administration of *A. aspera* orally at a dose level of 2.5 g/kg body weight.

*Dosing schedule* — The animals were randomly allocated into 5 groups of 6 each. Group I was assigned as control, group II, III and IV received *A. aspera* at the dose rate of 300, 600 and 900 mg/kg body weight orally, respectively and group V received the standard drug i.e. piroxicam @ 10 mg/kg body weight, orally in acetic acid induced writhing test and morphine sulphate @ 1.5 mg/kg body weight, intra-peritoneally in hot plate and tail flick tests.

*Acetic acid induced writhing syndrome*— The intraperitoneal injection of acetic acid results in constriction of abdominal muscle together with stretching of hind limbs known as writhing syndrome. In this test, the antinociceptive activity of crude methanolic extract of *A. aspera* leaves was studied on chemically induced pain sensation in female non-pregnant albino mice[14]. Plant extract, standard drug or the vehicle was administered orally 30 min prior to intra peritoneal injection of acetic acid (10 ml/kg of 0.7% v/v solution). Total numbers of stretching episodes for 20 min immediately after acetic acid injection in all the groups were recorded and anti-nociception was expressed as the percent reduction in writhing numbers compared between the vehicle treated control and animals pretreated with methanolic extract of *A. aspera* or piroxicam.

*Screening for analgesic activity by Eddy's hot plate*— In the hot plate test[15], mice of either sex were placed on the hot plate (Rolex) maintained at 55°±0.5°C. The time between placement on the hot plate and the occurrence of licking of the paws, shaking or jumping off from the plate was recorded as response latency. Mice with basal latency of more than 10 sec were not included in the study. The response latencies or reaction time was measured before administration (basal) and at 30 and 60 min

after administration of the test compound (300 to 900 mg/kg po) or the standard drug (morphine sulphate @ 1.5 mg/kg, ip) and compared with the control group. A cut-off reaction time was fixed at 20 sec to avoid damage to the paws.

*Screening of analgesic activity by tail flick method*— In this model, Nichrome wire analgesiometer (Rolex) was used[16]. Individually, the tail of each rat was placed over the radiant heat source of the apparatus and the tail withdrawal from the heat (flicking response) was taken as the end point. The reaction time (sec) of each rat in each group was determined at 0, 30 and 60 min following administration of the test compound (300 to 900 mg/kg po) or the standard drug (morphine sulphate @ 1.5 mg/kg, ip) and compared with the control.

*Statistical analysis*— The results were subjected to statistical analysis as per standard statistical method[17].

## Results

Phytochemical screening of the methanolic extract of *A. aspera* revealed the presence of alkaloid, steroid and triterpenes. In acute toxicity study, there was no change in motor activity and gross behaviour during 24 h of observation and the plant extract was found to be safe up to 5 g/kg body weight, po. The low toxicity of the plant observed, suggests that the plant extract is relatively safe for consumption and did not affect any of the parameters measured.

In the acetic acid induced writhing syndrome test, there was 9.49–17.37% reduction in writhing numbers after single oral administration of *A. aspera* from 300 to 900 mg/kg, respectively, which were significantly higher ($P < 0.01$) compared to the control group. The standard drug piroxicam showed 52.66% reduction of the writhing numbers in acetic acid induced writhing syndrome test, which was however, higher than the plant extract (Fig. 1).

In the hot plate test, there was significant ($P < 0.01$) increase in reaction time in *A. aspera* (300 – 900 mg/kg) as well as morphine sulphate (1.5 mg/kg) treated group, from 0 to 60 min (Table 1). However, in the control group the duration of reaction time did not increase up to the end of the study period. Reaction time at 60 min of observation in *A. aspera* [@ 300 (7.142 sec), 600 (9.517 sec) and 900 mg/kg (10.103 sec)] as well as morphine sulphate @1.5 mg/kg (15.568 sec) treated groups was significantly ($P < 0.01$) higher compared to the control group (6.895 sec) indicating dose and time dependant antinociceptive activity of the test plant. However, the standard drug showed better analgesic activities than the plant extract.

In the tail flick test, the reaction time (sec) increased significantly ($P< 0.01$) from 30 to 60 min after single oral administration of *A. aspera* (600 and 900 mg/kg) and morphine sulphate (1.5 mg/kg, ip) from 3.54 to 5.122, 3.18 to 9.17 and 3.31 to 16.2 sec, respectively. However, with 300 mg/kg of *A. aspera* and the control group, there was no significant increase in the reaction time from 30 to 60 min of observation period (Table 1), indicating dose and time dependant analgesic activity of the plant.

## Discussion

In the present study, the antinociceptive effect of methanolic extract of the leaves of *A. aspera* was evaluated in different experimental models of pain *viz.* non-narcotic model like acetic acid induced writhing syndrome test and narcotic models like hot plate and tail flick tests. The results of the present study clearly demonstrated that the methanolic extract of *A. aspera* possessed a definite dose dependant antinociceptive activity as observed by significant increase in the reaction time in acetic acid induced writhing syndrome, hot plate and tail flick test as compared to the control group.

Acetic acid causes inflammatory pain by inducing capillary permeability[18] and liberating endogenous substances that excite pain nerve endings[19]. The intensity of anti-nociception of *A. aspera* treated group was higher than the control group in acetic acid induced abdominal constricts in mice. NSAIDs can inhibit COX in peripheral tissues and therefore, interfere with the mechanism of transduction of primary afferent nociceptors. The mechanism of analgesic effect of methanolic extract of leaves of *A. aspera* could probably be due to blockade of the effect or the release of endogenous substances that

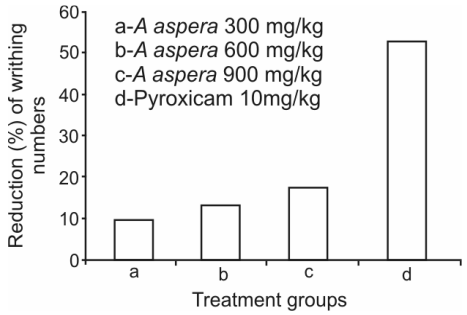


Fig. 1— Effect of the methanolic extract of *A. aspera* on percent reduction of writhing numbers compared to control in acetic acid induced writhing syndrome in female albino mice [values are mean± SE of 6 mice] ($P < 0.01$)

Table 1—Antinociceptive activity of *A. aspera* in hot plate and tail flick test
[Values are mean ± SE of 6 animals]

| Group | Dose (µg/kg) | Hot plate test | | | Tail flick test | | |
|---|---|---|---|---|---|---|---|
| | | Reaction time (sec) | | | | | |
| | | 0 min | 30 min | 60 min | 0 min | 30 min | 60 min |
| I (Control) | 10 ml/kg | $_{A}6.56^{a}\pm0.40$ | $_{A}6.72^{a}\pm0.42$ | $_{A}6.90^{a}\pm0.28$ | $_{H}5.23^{e}\pm0.23$ | $_{H}5.57^{e}\pm0.87$ | $_{J}5.88^{e}\pm0.40$ |
| II- IV | 300 | $_{A}6.04^{a}\pm0.65$ | $_{A}6.96^{a}\pm0.45$ | $_{A}7.14^{a}\pm0.78$ | $_{H}5.47^{e}\pm0.47$ | $_{H}5.60^{e}\pm0.36$ | $_{H}5.80^{fe}\pm0.39$ |
| (*A. aspera*) | 600 | $_{A}5.87^{a}\pm0.78$ | $_{B}7.21^{a}\pm0.74$ | $_{C}9.52^{b}\pm0.60$ | $_{H}3.54^{f}\pm0.25$ | $_{J}4.53^{f}\pm0.29$ | $_{K}5.12^{f}\pm0.54$ |
| | 900 | $_{A}6.17^{a}\pm0.63$ | $_{B}8.85^{b}\pm0.71$ | $_{C}10.10^{b}\pm0.84$ | $_{H}3.18^{f}\pm0.28$ | $_{J}7.14^{g}\pm0.43$ | $_{K}9.17^{g}\pm0.64$ |
| V (Standard) | 1.5 | $_{A}6.86^{a}\pm0.34$ | $_{B}11.90^{c}\pm0.37$ | $_{C}15.57^{c}\pm0.32$ | $_{H}3.31^{f}\pm0.27$ | $_{J}6.10^{g}\pm0.34$ | $_{K}16.20^{h}\pm0.63$ |

Same superscript in column and same subscript in row indicates that the mean values differ significantly $P<0.01$

excite pain nerve endings similar to that of piroxicam and other NSAIDs.

The hot plate and tail flick are the most common tests of nociception that are based on a phasic stimulus of high intensity. The nociceptive experience is short lasting and it is well accepted that agonists of µ-opioid receptors produce analgesia in acute pain models[20]. Therefore, it is believed that substances that are effective in tail flick exert their effects predominantly through µ-opioid receptors. The hot plate test is considered to be selective for opioid- like compounds, which are centrally acting analgesics in several animal species[21]. The methanolic extract of *A. aspera* had antinociceptive activity in hot plate test that may in part be mediated by opioid receptors. These findings indicate that the methanolic extract of *Achyranthes aspera* may extort sufficiently opioid-like compounds out of the plant which are responsible for the analgesic activity of the plant.

Acetyl-11-keto-beta-boswellic acid (AKBA), a pentacyclic triterpenic acid present in the acidic extract of the *Boswellia serrata* gum resin is a novel highly specific inhibitor of 5-lipoxygenase, the key enzyme for leukotriene biosynthesis. Leukotriene as well as peptidoleukotrienes result in an increase in vascular permeability and chemotaxis of polymorphonuclear leucocytes as well as release of mediators from leucocytes, which sensitize nociceptors[22,23]. As the plant under study also contain triterpene as one of its phytoconstituent, so it may act through inhibition of leukotriene biosynthesis. The presence of alkaloid in the plant extract supports the claim that this compound have antinociceptive property since, alkaloid, flavonoids and saponins have been found in other natural products with analgesic and anti-inflammatory properties[24,25]. It may also be related partly to the presence of steroids that have been shown to exert analgesic effects in animal models of nociception[26].

The plant extract exhibited antinociceptive activity in all the animal models of nociception and possibly exerted its effect through diverse mechanism that may involve both central and peripheral pathways. *Achyranthes aspera* also possesses anti-inflammatory and anti-arthritic activity, thus supporting the rationale behind the traditional use of this plant in inflammatory condition[27]. The alcohol extract of this plant showed inhibition of oedema in carrageenan induced rat paw oedema and reduction in granuloma weight in cotton pellet granuloma model[8]. Further pharmacodynamic investigations are required to understand the precise mechanism of antinociception exhibited by the methanolic extract of leaves of *Achyranthes aspera*.

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## References

1 Chan K, Some aspects of toxic contaminants in herbal medicines, *J Chemosphere*, 52 (2003) 1371.
2 Sofowora A, *Medicinal plants and traditional medicine in Africa*, (John Wiley and Sons, New York) 1982, 105.
3 Aziz A, Rahman M, Mondal A K, Muslim T, Rahman A & Quader A, 3–Acetoxy-6-benzoyloxyapagamide from *Achyranthes aspera*, *Pharmaceutical J*, 4 (2005) 1820.
4 Hooker J D, *The flora of British India*, (L. Reeve & Co. Ltd, Kent) 1885, 730.
5 Prain D, *Bengal plants*, (Botanical survey of India, Calcutta) 1963, 655.
6 Chakraborty A, Brantner A, Mukuinaka T, Nobukuni Y, Kuchido M, Konoshima T, Tokuda H & Nishino H, Cancer chemo preventive activity of *Achyranthes aspera* leaves on Epstein - Barr virus activation and two stage mouse skin carcinogenesis, *Cancer Lett*, 177 (2002) 5.
7 Neogy N, Rathore R S, Shreshth A D & Bannerji B K, Studies on the anti-inflammatory and anti-arthritic activity of achyranthine, *Indian J Pharmacol*, 1 (1969) 47.

8 Vetrichelvan T & Jegadeesan M, Effect of alcohol extract of *Achyranthes aspera* Linn. on acute and sub-acute inflammation, *Phytother Res*, 17 (2003) 79.

9 Prakash A O, Potentialities of some indigenous plants for anti-fertility activity, *Int J Crude Drug Res*, 24 (1996) 24.

10 Workineh S, Eyasu M, Legesse Z & Asfaw D, Effect of *Achyranthes aspera* L. on fetal abortion, uterine and pituitary weights, serum lipids and hormones, *African Health Sci*, 6 (2006) 112.

11 Sandhyakumary K, Boby R G & Indira M, Impact of feeding ethanolic extracts of *Achyranthes aspera* Linn. on reproductive functions in male rats, *Indian J Exp Biol*, 40 (2002) 1309.

12 Harborne J B, Phytochemical methods, in *Guide to modern techniques of plant analysis,* 2nd edition, (Chapman and Hall, India) 1991, 653.

13 Bruce R D, An up- and -down procedure for acute toxicity testing. *Fundam Appl Toxicol*, 5 (1985) 157.

14 Koster R, Anderson M & De Beer E J, Acetic acid analgesic screening, *Federation Proceedings*, 18 (1959) 412.

15 Eddy N B & Leimbach D, Synthetic analgesics: II, Dithienylbutenyl and dithienylbutylamines, *J Pharmacol Exp Ther*, 107 (1953) 393.

16 Turner R A, Analgesics, In *Screening methods in pharmacology*, (Academic Press, New York, London) 1965.

17 Snedecor G W & Cochran W G, *Statistical methods*. 8th edition, (Oxford and IBH Publishing Co, New Delhi) 1994.

18 Amico- Roxus M, Caruso A, Trombadore S, Scifo R & Scapagini U, Gangliosides antinociceptive effects in rodents, *Arch Int Pharmacodyn Ther*, 272 (1984) 117.

19 Raj P P, Pain mechanism, in *Pain medicine: A comprehensive review*, edited by Raj P P, 1st edition, (Mosby- year book, Missouri) 1996, 23.

20 Shuanglin H, Osamu T & Hiroshi I, Intrathecal endomorphin-I produces antinociceptive modulated by alpha 2- adrenoceptors in the rat tail flick, tail pressure and formalin test, *Life Sci*, 6 (2000) 204.

21 Janssen P A J, Niememgeers C J & Dony G H, The inhibitory effect of fentanyl and other morphine –like analgesics on the warm water induced tail withdrawal reflex in rats, *Arzneimittelforschung*, 13 (1963) 507.

22 Boden S E, Schweizer S, Bertsche T, Dufer M, Drews G & Safayhi H, Stimulation of leukotriene synthesis in intact polymorphonuclear cells by 5-lipoxygenase inhibitor 3-oxo-tirucallic acid, *Mol Pharmacol*, 60 (2001) 273.

23 Jain N K, Kulkarni S K & Singh A, Role of cystenyl leukotrienes in nociception and inflammatory conditions in experimental animals, *Eur J Pharmacol*, 423 (2001) 92.

24 Fernanda L B, Victor A K, Amelia T H & Elisabetsky E, Analgesic properties of Umbellatine from *Psychotria umbellate*, *Pharmaceutical Biol*, 44 (2002) 56.

25 Onasanwo S A & Elegbe R A, Anti-nociceptive and anti-inflammatory properties of the leaf extracts of *Hedranthera barteri* in rats and mice, *African J Biomed Res*, 9 (2006) 117.

26 Calixto J B, Beirith A, Ferreira J, Santos A R, Cechinel Filho V & Yunes R A, Naturally occurring anti-nociceptive substances from plants, *Phytother Res*, 14 (2000) 418.

27 Gokhale A B, Damre A S, Kulkami K R & Saraf M N, Preliminary evaluation of anti-inflammatory and anti-arthritic activity of *S. lappa, A. spaciosa* and *A. aspera*, *J Ethnopharmacol*, 31 (1991) 57.