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

Chandana C Barua1*, Archana Talukdar1, Shameem A Begum1, Lalit C Lahon1, Dilip K Sarma2, Debesh C Pathak3 & Probodh Borah2

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

Received 26 November 2009; revised 1 April 2010

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 people1. 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 products2.

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 height3. It grows throughout the world in tropical and warmer regions4,5. 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 etc3. 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-
terol and spinasterol\(^1\). 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\(^3\), 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 Veterinary Science, Khanapara during the month of Department of Pharmacology & Toxicology, College

**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°C-60°C under reduced pressure leaving a dark brown residue which was stored in air tight container 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°C±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, intraperitoneally 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\(^11\). 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 antinociception 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. 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.

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 and liberating endogenous substances that excite pain nerve endings. 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...
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. 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. 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.

Acetylt-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. As the plant under study also contain triterpenes 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. It may also be related partly to the presence of steroids that have been shown to exert analgesic effects in animal models of nociception.

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. 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. Further pharmacodynamic investigations are required to understand the precise mechanism of antinociception exhibited by the methanolic extract of leaves of Achyranthes aspera.

Acknowledgement
The authors are grateful to Defence Research Development Organization (DRDO), Govt. of India, New Delhi for financial help and the Dean, FVSc, Kahanpara for facilities.

References
5 Prain D, Bengal plants, (Botanical survey of India, Calcutta) 1963, 655.