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Evaluation of anti-nociceptive activity of *Achyranthes aspera* linn in experimental animal models

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Abstract

Context: Evaluating analgesic activity of *Achyranthes aspera*, by comparing with standard drugs morphine and piroxicam.

Aim: To study the anti-nociceptive activity of *Achyranthes aspera* linn in experimental animal models.

Method and Material: peripheral analgesic model by writhing syndrome and central analgesic model by tail flick method. Materials required are plant leaves, healthy albino rats, drugs like morphine and piroxicam, tail flick analgesiometer

Statistical analysis used: graph pad software and ANOVA test.

Result: In acetic acid induced writhing syndrome methanolic extract was decreased 14.365 to 39.57% reduction in writhing numbers after single oral administration of 600 and 900 mg/kg body weight, respectively, which were significantly higher ($p \leq 0.001$) than control group. In the tail flick test reaction time was increased significantly ($p \leq 0.001$) from 30 to 60 minutes after single oral administration of *Achyranthes aspera* 600 & 99 mg/kg.

Conclusion: Methanolic extract of *Achyranthes aspera* was shown significant anti-nociceptive activity in peripheral and central experimental models. But it shown less activity than standard drugs.

Keywords: *Achyranthes aspera*, writhing syndrome, tail flick.

Introduction

Pain is the most common reason patient seeks medical care. According to a WHO survey more than 80% patients rely on conventional allopathic medicines to get relief from pain^[1]. But these allopathic medications showing much adverse effect along with beneficial pain alleviating effects. there is a need of herbal medicines for pain treatment in place of conventional medicines to reduce toxic effects on body mainly gastric toxicity, kidney damage and addiction. It is therefore essential that effort should be made to introduce herbal medicines which are not having any adverse effect with cheaper cost^[2]. 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, more over reducing the cost of medication.

Achyranthes aspera Linn locally known as uttarenii @rough chuff tree (English).it is an erect procumbent annual, biennial 1-2 cm in height^[3] commonly found as weed of way sides, on road sides. Although it has many medicinal properties it is particularly used spermicidal, anti-pyretic & as a cardiovascular agent. Yunani doctors local kabiraj use the stem, leaves and fruits as a remedy for pneumonia cough, asthma, piles, and renal dropsy. various extracts of this plant reveals presence of 27 cyclohexyheptacosan-7-ol and 16 hydroxy- 26- hydroxyl methyl heptacosan-2-one, a long chain alcohol and 17-penta triacontanol, alkaloid, b-sitosterol and spinasterol. The plant has anti-bacterial anti-tumor, hepatoprotective, spermicidal, anti-parasitic anti-oxidant effect.

Achyranthes aspera has been studied for various pharmacological properties, but for analgesic property very few studies 1or 2 only exist. The study, therefore seeks to methanolic extracts of the leaves of this plant for analgesic activity in different experimental animal models.

Method and Materials

Plant Material: Leaves of the plant were collected from the surrounding areas of Nandyal.

Preparation of Methanolic Extract: Leaves of *Achyranthes aspera* linn were dried in shade and powdered. About 250 g of powdered leaves was soaked in 1000 ml methanol for 72 hrs in beaker and mixture was stirred with a sterile glass rod. Filtrate was obtained after passing through what Mann filter paper no 1 for 3 times and concentrated in rotary evaporator at 50-60 until use [4]. Recovery was 6.89%.

Chemicals: Methanol and acetic acid were purchased from Merck limited Mumbai.

Animals: Healthy adult albino rats of either sex, approximately of same age, weighing between 40-60 g and adult male Sprague dawley rats weighing between 180-200 g were used for the study are supplied from central animal house SRMC, Nandyal. They were housed under controlled conditions at 25, 50% RH and kept under 10/14 h light/dark cycles with food and water ad libitum. Animals were group housed in poly propylene cages containing sterile paddy husk bedding.

Drugs Used In This Experiment: Morphine sulphate was purchased from troika pharmaceuticals ltd Gujarat India. And piroxicam from Pfizer respectively.

Animals Dosing Schedule: The animals were randomly allocated into 5 groups 6 of each.

- Group I – Control
- Group II III – Test groups – received Aspera at the dose rate of 600, 900.
- Group IV – Standard - received the standard drug piroxicam @ 10 mg/kg body weight, orally in writhing test and morphine sulphate @ 1.5 mg/ kg body weight, intra peritonially.

I. Peripheral Methods

1) Acetic Acid Induced Writhing Syndrome

The writhing syndrome is induced by intra peritoneal injection of 1% of acetic acid in rats produce within 3-10 minutes which is characterized by a wave of contraction of abdominal musculature followed by extension of hind limbs (50). in this test the plant extract, standard drug piroxicam and the normal saline was mixed with gum acacia was

administered orally 30 minutes prior to intra peritoneal injections of acetic acid (10 ml/kg of 1% v/v solution).the total number of stretching episodes for 20 minutes immediately after acetic acid injection in all the groups were recorded and anti-nociception was expressed as the present reduction in writhing numbers compare 3d between the vehicle treated control and animals are treated with methanolic extract of *Achyranthes aspera* (600,900 mg/kg per oral) and piroxicam (10 mg/kg orally)

II. Central Method

2) Screening of Analgesic Activity by Tail Flick Method

In this method analgesic meter works on the principle of observing pain threshold in the rodents before and after the drug administration so useful for differentiating between centrally acting morphine like analgesic and non-opioid analgesics, this is performed mainly to identify any pain stimulus threshold in rodents against a radiant heat and to screen analgesic drug by increasing pain threshold [6]. Tail flick is mainly mediated as a spinal reflex. In this method animals were placed into restrainer and leaving the tail exposed out sides. Then keep on the analgesic meter 1/3rd tail proximally left due to the thickened keratinized skin and then keep tail on the place made for the tail above hot wire of the analgesic meter. The time of tail flick measured and recorded before and after drug given. The cut off time is 20-30 sec for rats to avoid any further injury to the tail (for mice 15 -20 sec). The reaction time of each rat in each group was determined at 0, 30, 60 minutes following administration of test compound i.e. the extract in a dosage of 600,900 mg/kg per oral. And the test drug morphine sulphate @1.5 mg/kg intra peritonially and compared with the control.

Result

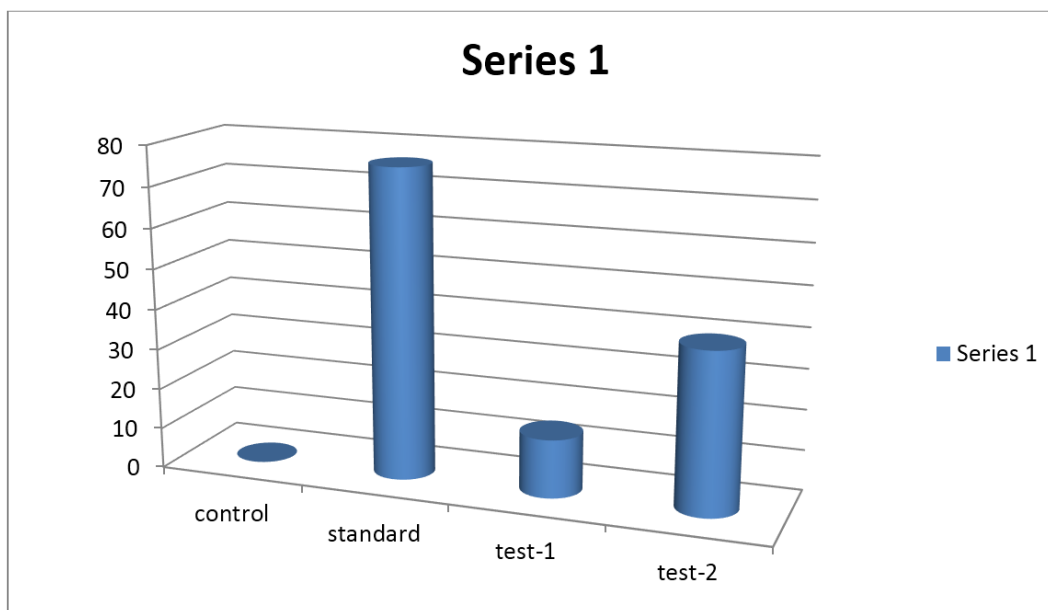
Achyranthes aspera plant extract contains alkaloids, triterpenes and steroids. The plant extract was shown some good results in both central and peripheral pain models.

I. Acetic Acid Induced Writhing Syndrome

Acetic acid was (1%) was used to produce writhes in the rats. plant extract (600,900 mg/kg), standard drug piroxicam (10 mg/kg), normal saline was given 30 minutes prior to acetic acid administered i.p after acetic acid administration total number of stretching episodes for 20 minutes immediately after acetic acid injection in all the groups was recorded [7].

Summary Data for Acetic Acid Induced Writhing Method

Group	Number of animals	Mean	Standard deviation	Standard error	Percentage % of reduction in writhes number
control	6	35.83	6.795	2.774	0
piroxicam	6	20.33	2.587	1.054	76.24
Methanolic extract (600 mg/kg)	6	31.33	4.457	1.820	14.36
Methanolic extract 9900 mg/kg)	6	25.67	3.724	1.520	39.57

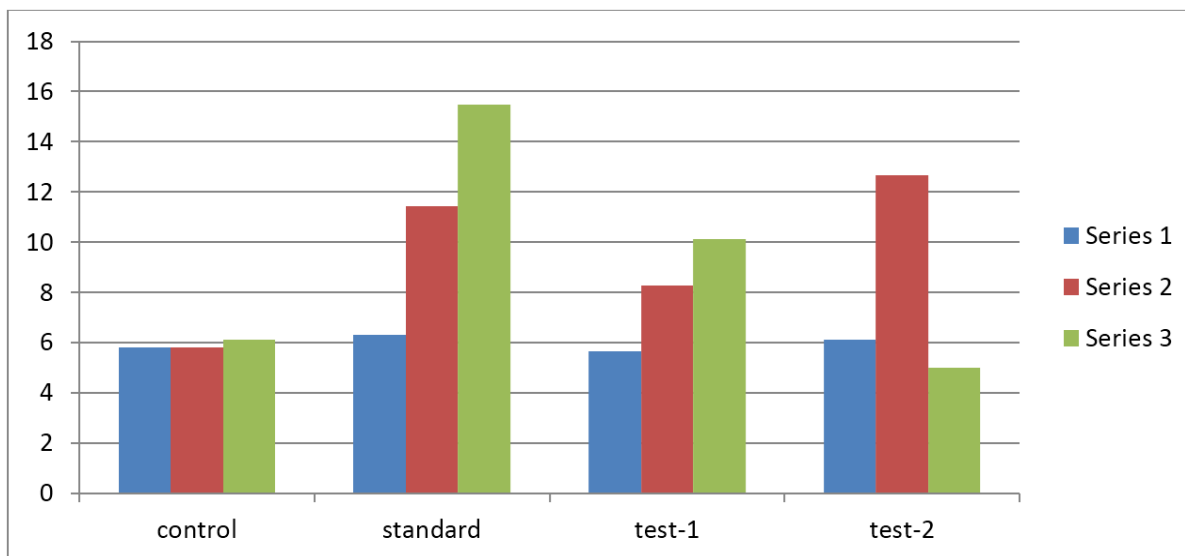


Percentage of reduction in writhes number after treatment.

2) Tail Flick Method

In the present study, tail flick method was used to evaluate the analgesic effect of *Achyranthes aspera* leaf extract in experimental animal models. In tail flick method rat reaction time was recorded before and after giving test and control group drugs.

Groups	Basal reaction time	After 30 minutes	After 60 minutes
Control	5.79	5.80	6.11
Standard	6.30	11.43	15.46
Test-1	5.60	8.26	10.12
Test-2	6.13	12.68	14.50



Comparison of tail reaction time (mean) before and after treatment with test drugs. Test and standard groups compared with control group.

Discussion

In the present study, the Ant nociceptive effect of methanolic extract of the leaves of *Achyranthes aspera* was evaluated in different experimental models of pain viz. non-narcotic like acetic acid induced writhing syndrome and narcotic model like tail flick test the results of the present study clearly demonstrated that the methanolic extract of *Achyranthes Aspera* possessed a definite dose dependent anti-nociceptive activity as absorbed by significant increase in the reaction

time in acetic acid induced writhing syndrome [8] and narcotic model like 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 ending. The intensity of Ant nociception of *Achyranthes aspera* treated group was higher than the control group in acetic acid induced abdominal constricts in rat.

NSAIDs can inhibit COX in peripheral tissues and therefore interfere with the mechanism of transduction primary afferent nociceptors. The mechanism of analgesic effect of methanolic extract of leaves of *Achyranthes Aspera* could

probably by due to blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of piroxicam and other NSAIDS.

The tail flick test is the most common test of nociception that it is based on a phasic stimulus of high intensity .the nociceptive experience is short lasting and it is well accepted that agonists of u-opioid receptors produced analgesia in acute pain models^[9]. Therefore, it is believed that substances that are effective in tail flick exert their effects predominantly through u-opioid receptors. The tail flick test is considered to selective for opioid like compounds which are centrally acting analgesic in several animals' species. The methanolic extract of *Achyranthes aspera* had anti-nociceptive activity in tail flick test that may in part be mediated by opioid receptors these findings indicate that 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, a penta cyclic tri terpenic acid present in the acid extract of the boswellia serrata gum resin is a novel highly specific inhibitor of 5-lipoxygenase 61, the key enzyme for leukotriene biosynthesis. Leukotrienes as well as peptido leukotrienes result in an increase in vascular permeability and chemotaxis of polymorph nuclear leucocytes as well as release of mediators from leucocytes, which sensitize nociceptors^[10].

As the plant under study also contain triterpene as one of its phytoconstituent, so it may act through inhibition of leukotrienes biosynthesis the presence of alkaloid in the plant extract supports the claim that this compound have anti-nociceptive 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 anti-nociceptive activity in all the animal models of nociception and possibly exerted its effect through diverse mechanism that may involve both central and peripheral path ways.

Conclusion

Methanolic extract of *Achyranthes aspera* was shown significant anti-nociceptive activity in peripheral and central experimental analgesic animal models. But it shown less activity than standard group in both peripheral and central models.

Conflict of Interest: The authors declare that they have no conflict of interest.

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