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Der Pharmacia Lettre, 2009, 1 (2) 193-198
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071

Comparison of Leaf and Root Extract of *Achyranthes aspera* for Its Analgesic Activity

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Abstract

Throughout the history the man has used several forms of therapy for relief of pain, among them; medicinal herbs have gained popularity because of its wide use and less side effect. *Achyranthes aspera,* for example is a commonly used folk medicine in India for various purpose. The plant root and leaf extract were prepared by using alcohol and distilled water in proportion (50:50). The extract of *Achyranthes aspera* was examined for centrally acting analgesic activity by using hot plate method, tail flick method and acetic acid induced writhing method for peripherally acting analgesic activity. The doses administered were 200 mg/kg and 400 mg/kg. The animal that administered a dose of 400mg/kg leaf extract has shown the maximum analgesic activity. The aspirin is taken as standard drug.

Key words: Analgesic, *Achyranthes aspera*, writhing.

Introduction

The traditional natural product screening is done by testing crude extract followed by the crucial work of back tracking the active compound from the extract. The drugs used by ancient civilization were extract of plant or animal product with few inorganic salt [1]. Plants have been proven to be the primary source of biologically active natural product as their effectiveness is supported by their previous exploitation in folk medicine [2].

A vast assay of illness and medicinal complaint has always been and always will be a real part of the human condition. Plants have supplied human with cases for their ailments from relieving headache to treating heart disease. Since the time of earliest human evolution, the impact of plant-derived medicine on human history has been remarkable. Opium, snake root, digitalis, fever bark and chaulmoogra have all left their leaf print on the human time line. These medicinal plant and others are the foundation and future of the human medicine. The vast natural pharmacy of the plant medicine is accounted for the 80% of the substances that

used to cause the disease before use learned to synthesize medicinal compound in the laboratory.

Achyranthes aspera Linn. (*Amaranthaceae*) is an indigenous medicinal plant of India and is commonly used by traditional healers for the treatment of fever, dysentery, asthma, hypertension, diabetes [3, 4], cough, bronchitis and rheumatism. A decoction of the whole plant is described to have diuretic properties and the aqueous extract is given for pneumonia. The plant is commonly known as Latjira in Ayurvedic & Unani system of medicine. The root and aerial extract are used for various body ailments. The root of *A. aspera* is reported to have application in infantile diarrhea and cold [5], while dry leaves are employed against asthma [6]. Leaf extracts are reported to posse's hypoglycemic, thyroid stimulating and antiperoxidative properties [7, 8].

Materials and Methods

Plant Material:

The whole Plant of *Achyranthes aspera* was collected from Bundelkhand University campus and was identified by Dr. Tariq Hussain, Head, Taxonomy & Herbarium Divison of National Botanical Research Institute, Lucknow. A voucher specimen (No 93871) was deposited in the museum of the Department of Taxonomy. The plant washed under running water, shade dried and cut the aerial and root part separately. The dehydrate leaves powered to a fine texture and 100 g of the dried plant was repeatedly extracted with water: ethanol (50:50) solvent. The same procedure applied for root extraction.

Experimental Animal:

Albino adult male rat weighing 150-180 gm were used for tail flick method Carrageenan induced rat paw oedema, cotton pellet granuloma, male swiss albino mice weighing 18-24 gm were used for eddy's hot plate, xylene induced ear oedema. All animals supplied by Central Drug Research Institute, Lucknow. All animals were fed standard animal feed and tap water before experiment. Each experiment group constitute of six animal housed in separate cages. All experiments were carried out with the consent of Institutional Animal Ethical committee of the institute (716/02/a/CPCSEA).

Screening of Analgesic Activity:

Acetic Acid Induced Writhing Test:

The animals were kept in a temperature-controlled environment (22 ± 2^0C) with a 12 hour light dark cycle. Food and Water were freely available. Abdominal writhing were induced by intraperitoneal injection of acetic acid (0.6% of 10 ml /kg body weight). Animal were pretreated with alcoholic extract through oral route. Administration, 30 min prior to acetic acid injection and 5min thereafter the test was started. The both plant extract were tested at doses of 200, 400mg/kg orally.

Control animal received the same volume of isotonic saline solution. Acetylsalicylic acid at a dose of 100mg/kg, which is preferential dose in such studies, given orally, was used as standard for comparison. The numbers of stretching occur for 15 min after immediately the acetic acid injection was recorded. Six mice were used per group. Animals were sacrificed immediately after each 15 min experiment. The results were evaluated by calculating the mean number of stretching per group and they were represented as percentage inhibition of stretching movement with control group.

% Analgesic activity= (n′-n/n) x100

Where n is the average number of stretching of control group and n′ is the average number of stretching in test group [9].

Tail Flick Latent Period:

Male rat were trusted orally with RE200, RE400, LE200, LE400, doses of extract and aspirin to various groups of rat. The rat was held firmly to immerse its tail in a water bath maintained at the constant temperature of 58^0C. The time required for the typical reaction, a violent jerk of the tail, was recorded to assess response to noxious stimulus. The animals are allowed to adapt to the cages for 30 min before testing. The lower 5 cm portion of the tail is marked. This part of the tail is immersed in a cup of freshly filled water of exactly 55^0C. Within a few seconds the rat reacts by withdrawing the tail. The withdrawal time of untreated animals is between 1 and 5.5 sec. A withdrawal time of more than 6 sec therefore is regarded as a positive response [10].

Hot Plate reaction Time in Mice:

The mice were screened by placing them on hot plate maintained at 55 ± 1^0C and recording the reaction time in seconds for the paw licking or jumping. Only mice which reacted within 15 sec and which did not show large variation when tested on four separate occasion. Aspirin (100 mg/kg) was used as reference standard. The time for forepaw licking or jumping on the heated plate of the analgesiometer was taken as the reaction time [11].

Results and Discussion

Tail Flick Method:

The analgesic activity of aspirin, RE, LE was assessed during carrageenan oedema studies. The mean reaction time in seconds of control group at 30min, 1st, 2nd, 3rd, and 4th hour were 6.18±0.11, 6.65±0.21, 6.08±0.22, 6.38±0.29 and 6.43±0.15 respectively. Aspirin (200mg/kg) treated group showed significant analgesic activity at first to fourth hour 9.5±0.31, 13.58±00.52, 15.08±0.67 and 8.25±0.2 respectively, when compared to control. Root extract in both doses of 200mg/kg and 400mg/kg did not show significant analgesic activity at 30 min, 1st and 4th hour but showed significant analgesic activity at 2nd and 3rd hour only.

Table1. Effect of *Achyranthes aspera* extracts on tail flick latency in Rat

Treatment	Dose	Mean value of Tail Flick Latency (sec)±S.E.M.				
		30 min	1 hour	2 hour	3 hour	4 hour
Control	5ml/kg	6.18±0.11	6.65±0.21	6.08±0.22	6.38±0.29	6.43±0.15
Aspirin	200 mg/kg	6.7±0.2	9.5±0.31**	13.58±0.52**	15.08±0.67**	8.25±0.25**
Root extract	200 mg/kg	6.5±0.12	6.08±0.23	7.6±0.32*	8.65±0.50**	6.82±0.37
	400 mg/kg	5.51±0.28	7.26±0.60	9.91±0.43**	12.58±0.43**	7.39±0.55
Leaf extract	200 mg/kg	6.75±0.21	6.9±0.49	9.79±0.62**	12.41±0.79**	7.25±0.66
	400 mg/kg	6.36±0.49	8.08±0.47**	11.13±0.58**	18.25±0.21**	7.75±0.21*

n=6, The percent inhibition for each group was calculated by comparison with the control group.
Values indicate mean ± S.E.M. (ANOVA test followed by Dunnett's *t*-test). Significance variation against control at, *p<0.05, ** p<0.01

Leaf extract at the dose of 200mg/kg also showed significant analgesic activity at 2nd and 3rd hour, while failed to show any significant analgesic activity at 30 min, 1st and 4th hour. The leaf extract at a dose of 400mg/kg showed the significant analgesic activity from 1st to 4th hour (Table-1).

Acetic Acid Induced Writhing Response In Mice:

The mean no of writhes induced by *Achyranthes aspera* in control was 29.33±1.20 while there was significant inhibition of writhes was observed with aspirin 100mg/kg, RE 200, RE400, LE200, LE400 mg/kg with mean values 12.33± 0.76, 22.5± 3.16, 13.33± 0.80, 13.16± 1.52 and 8.33± 0.95 respectively, except root extract at a dose of 200 mg/kg ($p<0.05$) all other treatment were found to be highly significant ($p <0.01$) (Table-2).

Table 2. Effect of *Achyranthes aspera* extracts on Acetic acid induced writhes in mice

Treatment	Dose	No of Writhes /30 min	% Inhibition of writhes
Control	5 ml/kg	29.33±1.20	
Aspirin	100 mg/kg	12.33±0.76**	57.96%
Root extract	200 mg/kg	22.5±3.16*	23.28%
Root extract	400 mg/kg	13.33±0.80**	54.55%
Leaf extract	200 mg/kg	13.16±1.52**	55.13%
Leaf extract	400 mg/kg	8.33±0.95**	71.60%

n=6, The percent inhibition for each group was calculated by comparison with the control group. Values indicate mean ± S.E.M. (ANOVA test followed by Dunnett's *t*-test). Significance variation against control at, *p<0.05, ** p<0.01

Hot Plate Test In Mice:

The control group at 30min, 60 min, 90 min, 120 min and 150 min shows hot plate reaction time in sec is 4.83±0.60, 4.54±0.45, 4.38±0.61, 4.9±0.0.63 and 5.01±0.54 respectively.

Table- 3 Effect of *Achyranthes aspera* extracts on hot plate reaction in mice

Treatment	Dose	Hot plate reaction time (in sec)				
		30 min	60 min	90 min	120 min	150 min
Control	5ml/kg	4.83±0.60	4.54±0.45	4.38±0.61	4.9±0.63	5.01±0.54
Aspirin	100mg/kg	5.66±0.58	10.2±0.58**	11.85±1.11**	20.98±1.36**	7.88±0.80
Root extract	200mg/kg	5.73±0.55	4.9±0.19	8.36±0.54*	11.18±0.79**	6.41±0.60
	400mg/kg	3.94±0.22	8.61±0.91**	11.73±1.42**	19.38±0.68**	6.38±0.45
Leaf extract	200mg/kg	5.06±0.34	6.87±0.67	9.02±0.88**	12.4±1.21**	6.73±0.92
	400mg/kg	5.3±0.46	10.75±1.37**	11.3±1.0**	22.76±2.39**	6.04±0.52

n=6, The percent inhibition for each group was calculated by comparison with the control group. Values indicate mean ± S.E.M. (ANOVA test followed by Dunnett's *t*-test). Significance variation against control at, *p<0.05, ** p<0.01

The corresponding mean volumes in aspirin (100mg/kg) treated group were 5.66±0.58, 10.2±0.58, 11.85±1.11, 20.98±1.36, and 7.88±0.80 respectively, indicating significant

analgesic activity of aspirin from 60 min onwards when compared to control. Root extract in both the doses of 200 mg/kg and 400 mg/kg had produced significant increase in hot plate reaction time in dose depended manner from 60 to 120 min. The leaf extract in both doses 200mg/kg and 400 mg/kg had also produced significant inhibition with the mean hot plate reaction time in dose dependent manner at 60 to 120 min (Table-3).

As mentioned in the introduction, the rationale behind this work was to scientifically authentify and confirm the traditional, folk and preliminary claims of *Achyranthes aspera* for its anti-inflammatory and analgesic activities. The present study was also planned to make a step ahead in the direction of increasing the numbers of marketable drugs with traditional background with modern & scientific standards. The *A. aspera* belonging to family *Amaranthaceae* was reported to posses many activities, as mentioned in the plant profile, which include the preliminary antiarthritic and anti-inflammatory activity [12,13] However, there is no report found regarding the analgesic activity and the anti-inflammatory effect of different parts i.e. leaf and root of the plant. Keeping in view search / screen the natural product to find out the novel anti-inflammatory and analgesic drug. We have evaluated the hydro alcoholic extract of leaves and roots of mature *Achyranthes aspera* in models of acute and sub acute inflammation in albino rats, simultaneously animal were also have been observed for the analgesic activity.

Literature survey indicates that there are no such reports regarding analgesic activity of *A. aspera*. The analgesic effect of extract was tested in three different models of analgesia; the acetic acid induced writhing test, hot plate test in mice and tail flick test in rat. In analgesic study aspirin in all models showed significant activity, corroborate the earlier reports. Considering the finding of the present study, though root and leaf extract can produce analgesia, there utility as an analgesic in therapeutics may require confirming the mechanism in relieving different types or all types of pain and the phytochemical responsible for the activity. With respect to the writhing test, the research group of Deraedt *et al*. (1980) [14] described the quantification of prostaglandin by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid. They found high levels of prostaglandins $PGE_{2\alpha}$ and $PGF_{2\alpha}$ during the first 30 min after acetic acid injection. Nevertheless, it was found that the intraperitoneal administration of acetic acid induces the liberation not only prostaglandin but also of the sympathetic nervous system mediators [15-17]. Thus the result obtained for writhing test using acetic acid are to similar to those obtained for the edematogenic test using carrageenan, since root and leaf extract of *A. aspera* at the doses of 200 and 400 mg/kg was effective in inhibiting the acetic acid induced writhing in mice. Therefore, an anti-inflammatory substance may also be involved in the peripheral analgesic activity. The other models of analgesic where pain was induced by means of heat and analgesia produced by the high dose of extracts may be due to their capacity to penetrate in to CSF may explain the probable analgesic activity form 1^{st} to 3^{rd} hour. Noxious heat may stimulate and augment calcium dependent calcitonin gene-related peptides (CGRP) release from desheathed sciatic nerve in a long linear manner and a threshold between 40^0 and 42^0C. The increases were 1.75 fold at 42^0C, 3.8 fold at 45^0C and 29.1 fold at 52^0C [18]. Heat stimuli are transduced by calcium dependent non-selective cation current via nitric oxide-cyclic GMP signaling pathway rather than by membrane damage [19]. Further bradykinin, ATP and many other inflammatory mediators sensitize the heat-activated current in neurons mediators activated *phospholipase C* led to metabolism of PIP_2 and inhibit the inhibitory effect of PIP_2.[20]. It is difficult to explain why in 4^{th} hour onwards the analgesic activity got diminished, it may be probably due to glucocorticoids which might decreases the threshold of electrical excitation of brain *i.e.* stimulate brain excitability.

If the present finding could be extrapolated to clinical situation then there may be possibility to develop to one of the novel anti-inflammatory and analgesic drug from a treasure of our traditional system of medicine. However further studies such as phytochemical investigation, their structure elucidation, molecular pharmacological studies to find out specific receptor oriented mechanism, biochemical investigation and measurement of plasma levels of different hormones and other substances are needed.

Acknowledgements

The authors wish to thanks Taxonomy & Herbarium Division of National Botanical Research Institute, Lucknow, India and also thankful to Bundelkhand University, Jhasi, India, for providing facilities.

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