Research Article
ISSN 0975 – 2633
Arch Pharm Sci & Res Vol 2 No 1 241 - 245 January 2010

Antinociceptive Activity OF *Abutilon indicum* (Linn) Sweet Stem Extracts

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Abstracts: The present study was designed to investigate the analgesic activity of methanolic (AIM), hydro alcoholic (AIHA) and aqueous (AIA) extracts of *Abutilon indicum* stems (Linn) in experimental models. The peripheral and central analgesic activity of different extracts (AIM, AIHA & AIA) were studied using acetic acid-induced writhing and hot plate method in healthy mice respectively. The extracts were used in the doses of 100mg/kg of body weight orally, Diclofenac (5 mg/kg, i.p.) and Pentazocine (5mg/kg, i.p.) as standard drugs in the study for comparing analgesic effects. The methanolic (AIM) extract showed a greater analgesic effect when compared with other (AIHA and AIA) extracts. The results of present study suggested that extracts of *A. indicum* posses significant analgesic activity in dose dependant manner acting peripherally, whereas feeble effect acting through centrally analgesic activity.

***Key* words:** *Abutilon indicum,* acetic acid-induced writhing, Analgesic activity, hot plate method.

INTRODUCTION

Abutilon indicum (Linn) family (Malvaceae) commonly known as 'Thuthi /Atibala' is distributed throughout the hotter parts of India[1] and used in our Traditional System of Medicine for healing various diseases. Almost all parts of Atibala are of medicinal importance and are used traditionally for the treatment of various ailments. The roots of the plant are considered as demulcent, diuretic, used in chest infection and in urethritis. The infusion of root is described in fevers as cooling medicines and is useful in stangury, haematuria and in leprosy. The leaves are effective in ulcer, for the treatment of diabetes, diuretic infection and gingivitis. Fomentation of plant materials are used to relief body pain. The decoction of the leaf is used in toothache, tender gums and internally for inflammation of bladder. In some places, juice from the leaves in combination with the liquid extract of *Allium cepa* is used to treat jaundice, and in cases of hepatic disorders [2, 3]. The bark is used as febrifuge, anthelmintics, alexeteric, astringent and diuretics. The seed are used in piles, laxative, aphrodisiac, expectorant, in chronic cystitis, gleets and gonorrhea [4, 5, 6, 7]. The leaves and seeds are crushed with water to form paste which is applied to penis to cure syphilis [8, 9, 10]. In Siddha system of medicine, it used as a remedy for jaundice, piles, ulcer and leprosy [11].The plant contains mucilage, tannins, asparagines, gallic acid and sesquiterpenes [12]. Thus the present study is therefore an attempt to asses efficacy of this indigenous herb to test the various pharmacological activity.

MATERIAL AND METHODS

Plant Material

The fresh stems of the plant *A. indicum* were collect from the wild sources of the Dhulia district of Maharashtra, in the month of June-July and was identified and authenticated by Prof. S.R. Kshirsagar, of Shri. SVPS's, Late Karmveer Dr. P.P. Ghogrey Science College, Dhule (M.S.) 425 405. A voucher Specimen was kept in the P.G. Department of Pharmacognosy, NMiMS, SPTM, Shirpur, Dhulia, for further references. The fresh dried fine powder was used for the preparation of extracts.

Preparation of Extracts

The freshly collected stems were washed, shade dried and it was treated with a mechanical pulverized for size reduction. The fine powder was collected for

preparation of extracts. The powder was successively extracted with methanol (< 60°C), methanol-water (1:1) and double distilled water using soxhlet apparatus for 72 h. The extracts were concentrated under reduced pressure using a rotary vacuum evaporator and dried at room temperature. A greenish brown, brown and dark-brown residue was obtained respectively. The percentage yields of extracts were 4.3% w/w methanolic (AIM), 1.5%w/w hydro alcoholic (AIHA) and 2.4% w/w aqueous (AIA). The extracts were kept in desiccators for further use.

Animals Used:

Inbred albino mice of either sex weighing (20-30 g) were procured from the Halfkins institute, Parel, Mumbai. The animals were kept in polypropylene cages (6-inch cage) under standard laboratory conditions. The animal house was maintained at 25 ± 2°C and relative humidity was also maintained at (50±15%).The extracts were stored at 40°C in a screw cap vial. For experimental, a dose of 100 mg/kg was calculated and the weighed amount of residue was suspended in 2% gum acacia.

Acetic acid induced writhing:

The method described by Collier et al. (1968) was used[13]. The peripheral analgesic activity was evaluated by acetic acid induced writhing method. The mice were divided into five groups of six mice each. The writhing syndrome was elicited by an intra-peritoneal injection of 0.6% acetic acid at the dose of 0.1 ml/10 g body weight. Group wise the animals received dose of extracts (AIM, AIHA and AIA) of test drug orally, (100mg/kg). The grouping of animals as follows.

Group I: Control group treated with 0.6% v/v acetic acid (0.1 ml/10 g body weight, i.p.)

Group II: Test group treated with AIM (100mg/kg body weight, orally), and after 20min, treated with 0.6% v/v acetic acid i.p.

Group III: Test group treated with AIHA (100 mg/kg body weight, orally), and after 20min, treated with 0.6% v/v acetic acid i.p.

Group IV: Test group treated with AIA (100 mg/kg body weight, orally), and after 20min treated with 0.6% v/v acetic acid i.p.

Group V: Test group treat with Diclofenac Sodium (5 mg/kg body weight, i.p.), and after 20 min 0.6% v/v acetic acid i.p

The onset and frequency of writhing response were observed. The severity of pain response (writhing) was assessed by counting of the number of wriths (constriction of abdomen, turning of trunk and extension of hind legs) in mice. Number of wriths per animal was counted during 20 min. series beginning 5 min. after the injection of acetic acid. Analgesic activity was calculated as percentage (%) maximum possible effect (MPE) using the following relation. The observations are recorded in table 1.

The significance of results was calculated by Dunnett's Multiple Test.

Hot plate method:

The central analgesic activity of *A. indicum* extracts were studied by using hot-plate method in mice. Albino mice weighing 25-30 g were divided in five groups of 5 each. The hot plate was maintained at 55±1°C. Animal were placed in to a glass cylinder of 24 cm diameter on the heated surface and the time between placement and licking the paws or jumping was recorded as response latency. The reaction time was recorded for control mice and animals treated with pentazocine (10mg/kg i.p) at 0, 15, 30, 60 and 90 min. The test was terminated at 15 sec. to prevent tissue damage.

$$\% \text{ MPE} = \frac{\text{Mean of in control} - \text{Mean of writhes in test}}{\text{Mean of writh in control}} \times 100$$

Group I: Control group treated with 2% gum acacia in normal saline

Group II: Test group treated with AIM (100mg/kg body weight)

Group III: Test group treated with AIHA (100 mg/kg body weight)
Group IV: Test group treated with AIA (100 mg/kg body weight)
Group V: Test group treat with Pentazocine (10 mg/kg body weight, i.p.),
The percentage analgesia was calculated using the following formula:

$$\text{Percentage of analgesia} = \frac{\text{Tc - Tt}}{\text{Ct - Tc}} \text{ X } 100$$

Where, Tc = Reaction time in control mice, Tt = Reaction time in treated mice and Ct = Cut-off time.

Stastistical Analysis:

The statistical analysis were performed by analysis of variance (ANOVA) test followed by Dunnett's comparison test. P values, < 0.001, were considered significant. (Peripherally) and P value, < 0.05 were consider significant (Centrally).

RESULT

The analgesic activities of different extracts of *A. indicum* determined by using Acetic acid induce writhings method and Eddy's hot plate methods.

Acetic acid-induced writhing in mice

The results in Table 1 demonstrated that the extracts (AIM, AIA, AIHA), when administered at the dose of 100 mg/kg orally caused an inhibition on writhing response induced by acetic acid. It was observed that the percentage of wriths in methanolic Extract of stem (54.44 %) was higher as compared with Aqueous Extract (47.87 %) and Hydro-alcoholic (37.19 %). However, the standard drug, Diclofenac (5mg/kg i.p.) exhibited about 66.40 % inhibitions.

Hot plate method:

The results in Table 2 demonstrated that the extracts (AIM, AIA, AIHA), when administered at the dose of 100 mg/kg orally caused an inhibition. It was observed that the percentage of inhibition after 90 min in Aqueous Extract (22.27 %) was higher as compared with Hydro-alcoholic (12.70 %) and Methanolic Extract of stem (0.06 %). However, the standard drug, Pentazocine (10mg/kg i.p.) exhibited about 15.62 % inhibitions. The extracts exhibited feeble central analgesic effect as evidenced by significant increase in reaction time when compared to the control group. The results were also comparable to the standard drug, Pentazocine.

DISCUSSION

The analgesic activities of 'Atibala' Stem Extracts (ASE) were studied for central (narcotic) and peripheral (non-narcotic) activities. The analgesic activity of ASE against acute pain was moderate as compared to potent inhibitory activity of Diclofenac. Dicolfenac offer relief from pain by suppressing the formation of pain substances in the peripheral tissues, where prostaglandins and bradykinin were suggested to play an important role in the pain process [14]**.** Therefore, it is likely that ASE might suppress the formation of these substances or antagonize the action of these substances and thus exerts its analgesic activity in acetic acid-induced writhing test and in Randall-Selitto assay. In the present study, ASE (100 mg/kg) increased the reaction time in hot-plate test, suggesting its central analgesic activity. However, it showed moderately centrally-mediated analgesic effect in hot plate. Presences of flavonoids were reported in *abutilon indicum* species [15,16,17] and flavonoids are known to inhibit prostaglandin synthetase [18]. Since prostaglandins are involved in pain perception and are inhibited by flavonoids, it could be suggested that reduced availability of prostaglandins by flavonoids of ASE might be responsible for its analgesic effect. The writhing induced by chemical substances is due to sensitization of nociceptors by prostaglandins. This test is useful for evaluation of mild analgesic non-steroidal anti-inflammatory compounds [19, 20](Ferreira and Vane, 1974; Berkenkopf

Table1: Effect of AIM, AIHA and AIA on acetic acid induced writhing in mice

Treatments	Dose (mg/kg)	Mean No. of Wriths ± SEM (20 min)	Percentage Inhibition of Wriths
Control	Saline (i.p.) 1ml /100mg	129.5±13.925	_
Methanolic extract of stem	100 (orally)	59± 3.337 * *	54.44%
Hydro-alcoholic extract of stem	100 (orally)	81.33±4.055 * *	37.19%
Aqueous extract of stem	100 (orally)	67.5±2.742 * *	47.87%
Diclofenac sodium	5 (i.p.)	43.5±1.765 * *	66.40%
One way ANOVA , F- 23.19, df- 29, Sum of square - 32812, * * P < 0.001			

Values expressed as mean ±SEM, n= 6 in each group. Statistical significant test with control was done by ANOVA. *P < 0.001.

Table2: Effect of AIM, AIHA and AIA on thermic stimulus induced (Hot plate test) in mice.

Treatment	**Reaction time in second at different interval**						
	Dose mg/kg	**0 min**	**15 min**	**30 min**	**60 min**	**90 min**	**after 90 min % inhibition**
Control	Saline (i.p.) 1ml/100mg	2.106 ±0.398	2.468 ±0.829	3.080 ±0.883	1.740 ±0.452	4.502 ±0.728	–
AIM	100 (orally)	5.280 ±0.403* *	6.468 ±0.551**	3.332 ±0.859	4.808 ±0.644	5.206 ±0.855	0.06 %
AIA	100 (orally)	1.720 ±0.208	2.024 ±0.298	2.066 ±0.489	2.082 ±0.416	2.164 ±0.278*	22.27 %
AIHA	100 (orally)	6.220 ±0.327* *	5.006 ±0.985*	1.878 ±0.173	2.656 ±0.295	3.168 ±0.350	12.70%
Pentazocine	10 (i.p.)	1.810 ±0.252	3.620 ±0.205	5.322 ±1.732	7.120 ±1.698 **	2.862 ±0.220	15.62 %
Degree of freedom (D.F) - 24, * P < 0.01, * * P < 0.05, **Sum of Square** – 61.174 **F** – 5.111,							

Values expressed as mean ±SEM, n= 5 in each group. Statistical significant test with control was done by ANOVA. *P < 0.01, **P< 0.05.

and Weichmann, 1988).AIM, AIHA and AIA showed significant inhibitory activity on the writhing response induced by acetic acid when compared to control. Furthermore, the extracts (AIM, AIHA and AIA) did not show any significant difference form that of the control with respect to the resistance to heat. The results obtained rather suggest that the extracts possessed an analgesic activity and the mode of action might involve a peripheral mechanism.

CONCLUSION

According to the present data, AIA, AIHA and AIM extracts of *A. indicum* reduce the pain response in mice to acetic acid injection. The extracts did not show any influence in the hot-plate response in mice, which is a spinal motor reflex sensitive to opoid like agent. The results thus indicated that the analgesic activity was attributing by acting peripherally. The AIM extracts posses more significant analgesic activity by increasing reaction time compared to AIHA and AIA extracts. Thus, it can be concluded that all the extracts of stem of *A. indicum* produced significant analgesic activities in dose dependent manner acting through peripheral mechanism.

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