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Der Pharmacia Lettre, 2011, 3(1): 442-447
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Assessment of Analgesic, Anti-pyretic and Anti-inflammatory activity of Hydro-alcoholic fraction of *Hemidesmus indicus* root in experimental animals

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ABSTRACT

Hemidesmus indicus (family:Asclepiadaceae) has been extensively used in folk medicine and well known traditional plant, mainly the root is used for the treatment of various disease. Phytochemical screening of root-bark revealed the presence of carbohydrate, tannins, saponins, triterpenoids and flavonoids. The hydro-alcoholic extract of Hemidesmus indicus (HAEHI) was investigated for analgesic (acetic acid –induced writhing response, Eddy's hot plate method), anti-pyretic (brewer's yeast induced pyrexia) and anti-inflammatory (carragenan induced rat paw oedema, cotton pellet granuloma method) activities. The treatment with the extract (100 ,200 and 300 mg/kg, p.o.) significantly inhibits writhing response, decrease the licking response in acetic acid –induced writhing response and Eddy's hot plate method respectively in dose dependent manner. A maximal effect was observed at 300 mg/kg which was comparable to10 mg of Proxicam per kg body weight i.p. The anti-pyretic effect (measured as % reduction in body temperature) was compared with paracetamol (100 mg/kg, orally). HAEHI in dose of 300 mg/kg caused significant decrease in body temperature of rats. The treatment with the extract (100,200 and 300 mg/kg, p.o.) significantly prevented increase in volume of paw oedema and formation of granulation tissue in dose dependent manner. A maximal effect was observed at 300 mg/kg which was comparable to phenylbutazone 100 mg/ kg body weight i.p. In conclusion, this study has established the analgesic, anti-pyretic and anti-inflammatory activity of Hemidesmus indicus and thus, justifies the ethnic uses of the plant.

Key words: Hemidesmus indicus, hydro-alcoholic fraction, analgesic, anti- pyretic, anti-inflammatory.

INTRODUCTION

Herbal preparations are effectively and extensively used for their medicinal properties, and have become increasingly popular worldwide [1,2].Herbal medicines generally have fewer side effects than synthetic compounds, and their effectiveness can be improved by modern pharmacological

methods[3].Hemidesmus indicus, commonly known as Anantmool or Sariva, belongs to family Asclepiadaceae and is a well-known drug in the ayurveda system of medicine. Various workers have studied the effector mechanism of H. indicus extract using different models.The methanolic extract of this plant is reported to possess antioxidant and antiulcerogenic properties. The chloroform and ethanol (95%) extract shows anti-fungal activity against Aspergillus riger.. H. indicus extract is also found to inhibit lipid peroxidation and scavenge hydroxide radicals in vitro[4-9].Accordingly, pharmacological investigations on the hydro-alcoholic extract of root bark of Hemidesmus indicus has been initiated in our laboratory and here we reported the preliminary results of analgesic, antipyretic and anti-inflammatory effects of hydro-alcoholic extract of root of Hemidesmus indicus

MATERIALS AND METHODS

2.1 plant materials:
The root of *Hemidesmus indicus* was purchased from Abirami botanicals, tuticuorin, Tamil nadu. The plant material was identified and authenticated by resident botanist, prof.Dr.s.jayaraman, plant Anatomy research centre (PARC), Chennai. A voucher specimen was submitted at C.L.Baid Metha College of Pharmacy, Chennai.

2.2 Preparation of CD roots extract:
The root was chopped to small pieces, shed dried. The root was powdered mechanically and a weighed quantity of the powder (250 g) was passed through sieve number 20 and subjected to hot solvent extraction in a shoxlet apparatus using Hydro-alcohol (ethanol 40:water 60) at a temperature of 60°-70°c. The extract was concentrated to dryness at 40°c under reduced pressure in a rotary vacuum evaporator and the residue stored in a refrigerator at 2-8°c for use in subsequent experiments.

2.3 Preliminary phytochemical screening: [10]
Preliminary phytochemical screening of root-bark revealed the presence of carbohydrate, tannins, saponins, triterpenoids and flavonoids.

2.4 Experimental animals:
Healthy inbred wistar Albino rats (120-180g) of either sex and Swiss albino mice (18-22) of either sex were obtained from animal house of C.L.Baid Metha College of pharmacy, Chennai. The animals were housed individually in polypropylene cages, maintained under standard conditions (12:12 hour light/dark cycle; 25±30°c; 35-60% humidity), the animals were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and water ad libitum. The study was approved by the Institutional Animal Ethical Committee of Committee for the Purpose of Control and Supervision of Experimentation on Animals.

2.5 Acute toxicity studies: [11]
The acute toxicity studies was done using groups of (n=10) of male albino mice (18-22g). The extract was injected intraperitoneally and mortality was recorded for 24 h.

2.6 Analgesic activity:
2.6.1 Analgesic activity of HAEHI evaluated by acetic acid –induced writhing response: [12,13]
Swiss albino mice were divided into 5 groups of 6 mice each. The first group was given 10 ml/kg of normal saline *i.p* and served as control, groups 2, 3 and 4 received 100,200
and 300 mg/kg body weight of HAEHI *i.p* respectively, while the fifth group was given 10 mg of Proxicam per kg body weight *i.p*. Thirty minutes later, mice in all groups were treated with

Acetic acid (0.06% 1 ml Acetic acid of 1ml per 100 g *i.p*). Five minutes after Acetic acid injection mice were placed in individual cage and the number of abdominal contractions was counted for each mouse for a period of 10 min. Percentage inhibition of writhing was calculated using the expression:

$$\text{Inhibition (\%)} = \frac{\text{mean number of writhings (control) - mean no of writhings (test) x100}}{\text{Mean number of writhing (control)}}$$

2.6.2 Analgesic activity of HAEHI was evaluated using Eddy's hot plate method: [14]
The adult wistar Albino rats (150-200g) were divided into 5 groups of 6 rats each. The first group was given 10 ml/kg of normal saline *i.p* and served as control, groups 2, 3 and 4 received 100,200 and 300 mg/kg b.w of HAEHI *i.p* respectively, while the fifth group was given 10 mg of Proxicam per kg body weight *i.p*. The Eddy's hot plate was maintained between 55-56ºC. The animals were placed on the hot plate and the time taken for licking or jumping was recorded using stop watch. The reaction was observed at 0,15,30,60 and 120 mins.

2.7 Anti-pyretic activity:
2.7.1 Anti-pyretic activity of HAEHI was evaluated by experimentally induced pyrexia in rats: [15]
The adult wistar Albino rats (150-200g) were divided into 5 groups of 6 rats each. The control group receives 10ml/kg of normal saline *i.p.* groups 2, 3 and 4 received 100,200 and 300 mg/kg body weight of HAEHI *i.p* respectively, while the fifth group was given paracetamol100 mg/ kg body weight *i.p*. 30 mins later all the groups received 12% w/v suspension of Brewer's yeast in the dose level of 1ml/100gm body weight, sub-cutaneously into the loose connective tissue between the shoulder blades. Twelve hours after the injection, the rectal temperature of each rat was measured using a digital thermometer, Only rats that showed an increase in temperature of at least 0.7°C were used for the experiments. The rectal temperature was measured at 1, 2, 3 and 5 hr after drug administration.

2.8 Anti-inflammatory activity:
2.8.1 Anti-inflammatory activity of HAEHI was evaluated by carrageenin induced paw oedema: [16, 17]
The adult wistar Albino rats (150-200g) were divided into 5 groups of 6 rats each. Carrageenin inflammation was induced by injecting 0.1ml of 1% carrageenin into the subplanter tissue of the right hind paw. The first group was given 10 ml/kg of normal saline *i.p* and served as control; groups 2, 3 and 4 received 100,200 and 300 mg/kg body weight of HAEHI *i.p* respectively, while the fifth group was given phenylbutazone 100 mg/ kg body weight *i.p* 30 min prior to the carrageenin injection. The paw volume was measured before and 3 h after carrageenin administration by the volume displacement of water-mercury column using a plethysmometer.

2.8.2 Anti-inflammatory activity of HAEHI was evaluated by cotton pellet granuloma method: [18]
The adult wistar Albino rats (150-200g) were divided into 5 groups of 6 rats each.The control group receives 10ml/kg of normal saline *i.p.* groups 2, 3 and 4 received 100,200 and 300 mg/kg body weight of HAEHI *i.p* respectively, while the fifth group was given phenylbutazone 100 mg/ kg body weight *i.p* daily for 7 days of cotton pellet implantation. Granulomata were measured by removing the cotton pellet on the 8th day and drying them at 60ºC to constant weight.

2.9 Statistical analysis:
Results were tabulated and the data was expressed as mean ± SEM. The difference between experimental group were determined using one way analysis of variance (ANOVA) followed by Dunnet test. The results were considered statistically significant if the *p*- values were 0.05 or less.

RESULTS AND DISCUSSION

The HAEHI in the dose up to 2000 mg/kg did not produce any mortality in the groups of male albino mice up to the period of 24 h after injection.

The intraperitoneal injection of acetic acid produces an abdominal writhing response due to sensitization of chemo-sensitive nociceptors by prostaglandins. Increase level of protanoids as well as lipoxygenase products have been found in the peritoneal fluid after the injection of the acetic acid. In the present study HAEHI inhibits the acetic acid induce writhing in the dose dependent manner (Table 1), The analgesic effect of the extract may therefore be due to either its action on visceral receptors sensitive to acetic acid, to the inhibition of the production of algogenic substances or the inhibition at the central level of the transmission of painful message [19].and the extract also significantly reduces the licking response in Eddy's hot plate method (Table 2).

Table 1: Analgesic activity of HAEHI in acetic acid induced writhing method:

Group	Treatment (mg/kg)	No of abdominal writhings	% inhibition
I	N/saline	21±3.7	00.00
II	100	5.6±2.3	68.20
III	200	7.8±1.0	59.80
IV	300	12.2±1.5	68.20
V	Proxicam 10	10.2±2.1	45.00

Table 2: Analgesic activity of HAEHI in Eddy's hot plate method:

Group	Treatment (mg/kg)	Reaction time in minutes				
		0	15	30	60	120
I	N/saline	5.1±0.21	5.7±0.20	5.6±0.24	5.5±0.40	5.4±0.23
II	100	5.6±0.31	9.4±0.50	8.1±0.30*	7.2±0.32*	6.8±0.30*
III	200	5.6±0.25	8.1±0.20	9.0±0.54*	8.0±0.41*	7.1±0.30*
IV	300	5.6±0.24	6.4±0.20	7.5±0.20*	7.1±0.24*	6.6±0.40*
V	Proxicam10	5.6±0.21	6.2±0.20	6.8±0.30*	6.2±0.36**	5.8±0.37**

*All values are mean ±SEM, n=5-6, *P<0.05 indicates significant and **P<0.001 is more significant when compared with control.*

The widely used Brewer's yeast induced pyrexia method was employed to evaluate the possible anti-pyretic effect of the extract, in the present study extract exhibited significant anti-pyretic activity (Table 3).

Table 3:Anti-pyretic effect of HAEHI Brewer's yeast induced pyrexia method

Drug	Treatment (mg/kg)	% reduction in rectal temperature				
		1hr	2hr	3hr	4hr	5hr
Control	N/saline	16.34±0.54	19.45±0.67	20.41±0.41	22.38±0.11	23.20±0.43
Extract	100	20.21±0.42	26.03±0.61	36.52±0.43*	40.26±0.22*	46.17±0.51*
Extract	200	26.22±0.20	30.16±0.33*	42.32±0.12*	48.11±0.10*	60.57±0.14*
Extract	300	28.52±0.68*	36.43±0.23*	48.65±0.21*	57.24±0.32*	66.24±0.55*
paracetamol	100	34.56±0.82*	48.12±0.56*	58.43±0.60*	69.56±0.72*	73.35±0.52*

*All values are mean ±SEM, n=5-6, *P<0.05 indicates significant and **P<0.001 is more significant when compared with control.*

It has been widely accepted that carrageenin-induced paw oedema model is applied for the evaluation of the antioedemal effect of drugs. Recent investigation demonstrated that carrageenin oedema is effectively decreased by lipooxygenase inhibitors. In the present study HAEHI significantly inhibited carrageenin-induced paw oedema, (Table 4) it may be due to possible inhibition of lipooxygenase pathyway although such assumption obviously requires confirmation by further detailed experimentation.

Table 4: Local effect of HAEHI on carrageenin induced oedema in rats:

Drug	Intra-planter dose (0.1ml/paw)	Mean oedema volume (ml)
Control	Carrageenin 1mg	0.38±0.034
Mixture I	Carrageenin 1mg + extract 100 mg	0.168±0.042*
Mixture II	Carrageenin 1mg + extract 200mg	0.146±0.025*
Mixture III	Carrageenin 1mg + extract 300mg	0.120±0.023*
Mixture IV	Carrageenin 1mg + phenylbutazone 100 mg	0.056±0.020**

*All values are mean ±SEM, n=5-6, *P<0.05 indicates significant and **P<0.001 is more significant when compared with control.*

The widely used cotton pellet granuloma technique was employed to evaluate the possible anti-inflammatory effect of the extract on chronic inflammatory conditions; here the extract exhibited significant inhibitory activity on the formation of granulation tissue (Table 5).

Table 5: Effect of HAEHI in cotton pellet granuloma in rats

Drug	Treatment (mg/kg)	Cotton pellet granuloma: granuloma Wt (mg)
Control	N/saline	50.10±2.57
Extract	100	32.16±1.66*
Extract	200	26.18±1.102*
Extract	300	20.14±1.130**
phenylbutazone	100	14.10±0.990**

*All values are mean ±SEM, n=5-6, *P<0.05 indicates significant and **P<0.001 is more significant when compared with control.*

CONCLUSION

Based on the results of our study it can be concluded that the hydro-alcoholic extract of Hemidesmus *indicus* root posses significant analgesic, anti-inflammatory and anti-pyretic activity. Further studies have to be carried out to identify the phyto-constituent responsible for the exact and detailed mechanism of action responsible for this activity.

Acknowledgement
Authors are very much thankful to Department of pharmacology, C.L. Baid Metha college of pharmacy, Chennai, India. For providing financial assistance and for the encouragement throughout the present study.

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