ANALGESIC AND ANTIMOTILITY ACTIVITIES OF LEAVES OF *HYGROPHILIA SPINOSA* T. ANDERS

Arjun Patra[1,2*], Shivesh Jha[2], P. N. Murthy[1], Devdeep Roy[3] & Alakh N. Sahu[4]

[1]Royal College of Pharmacy & Health Sciences, Berhampur-760 002, Orissa, India.
[2]Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra-835 215, Ranchi, Jharkhanda, India.
[3]Master of Research Biomedical Sciences, Department of Immunology, University of Strathclyde, Glasgow, Scotland.
[4]Department of Pharmaceutics, IT, Banaras Hindu University, Varanasi-221 005, India.

Summary

Petroleum ether, choloroform, alcoholic and aqueous extracts of the leaves of *Hygrophila spinosa* T. Anders (Acanthaceae) were screened for analgesic and antimotility activities. Analgesic activity was studied by hot plate and tail flick method in thermal method, and acetic acid induced writhing test in chemical method. The choloroform, alcoholic and aqueous extracts at dose of 200 and 400 mg/kg of body weight significantly inhibited the abdominal constriction produced by acetic acid and also increased the pain threshold of mice towards the thermal source in a dose dependent manner comparable to the standard drug aspirin (100 mg/kg of body weight). Antimotility activity was studied by charcoal meal feeding method and atropine sulphate at a dose of 0.1 mg/kg (i.p.) was used as the standard drug. The extracts significantly decreased the distance travelled by charcoal meal through the gastrointestinal tract. The results suggest that the extracts exhibit analgesic activity by central as well as peripheral mechanism(s) and also have antimotility activity.

Key words: *Hygrophila spinosa*, Acetic acid induced writhing test, hot plate test, tail flick test, Antimotility.

Correspondence: Arjun Patra, Royal College of Pharmacy & Health Sciences, Berhampur-760 002, Ganjam, Orissa. Tel: +919761459749, +919359526128
E-mail: arjun.patra@rediffmail.com

Introduction

In the recent times focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. *H. spinosa* T. Anders (Acanthaceae) commonly known as 'Talmakhana' in Hindi is widely distributed throughout India, Srilanka, Burma, Malaysia and Nepal. In traditional system of medicine it is used as diuretic and for the treatment of rheumatism, jaundice, inflammation, pain, hepatic obstruction, gout, diabetes, bacterial infection etc. [1, 2]. The root showed haematinic [3], antitumor [4] and hepatoprotective activities [5]. Chemical investigation of the root exhibited the presence of greasy mass, lupeol and lupenone in petroleum ether extract. Crude petroleum ether extract, when administered (i.p.) to mice, potentiate the sedative-hypnotic action of chlorpromazine, diazepam, pentobarbitone, chlordiazepoxide and protected against strychnine-induced convulsions [6]. Roots are useful in dropsy of chronic Bright's disease, inflammation, ascites, hyperdipsia, vesical calculi, gonorrhoea, strangury, flatulence and dysentery; the seeds are refrigerant, liver tonic, aphrodisiac, diuretic, rejuvenating, lithontriptic, nervine tonic, constipating and tonic [7]. No biological activity has yet been reported on the leaves. The present study was designed to undertake the analgesic and antimotility activities of the different extracts of the leaves of *H. spinosa*.

Materials and Methods

Plant material collection and extraction:

The leaves of the plant were collected from Berhampur, Orissa, India during August to December and identified through Birla Institute of Technology, Ranchi, India. The leaves were dried under shade and coarsely powdered. The powdered plant material was successively extracted with petroleum ether, chloroform and alcohol using soxhlet apparatus. Finally the aqueous extract was prepared by decoction. The extracts were filtered through whatmann filter paper and the filtrates obtained were evaporated by using rotary vacuum evaporator to get the different extracts. The yield of petroleum ether (**HSPE**), chloroform (**HSCH**), alcoholic (**HSAL**) and aqueous (**HSW**) extracts were 3.0%, 2.4%, 14.2% and 6.2% respectively.

Drugs:

Aspirin (Cipla Ltd., India), Atropine sulphate (Intas Pharmaceuticals Ltd., India)

Preliminary phytochemical screening:

The extracts of leaves of *H. spinosa* were subjected to Preliminary phytochemical analysis for major group of phytoconstituents [8-10]. In each test 10% (w/v) solution of the extracts were used unless otherwise mentioned for an individual test.

Animals:
Albino mice of either sex, weighing 20-25 gm were used for the study. Animals were housed in large polypropylene cages and provided with standard diets (Hindustan lever, Mumbai, India) and clean drinking water *ad libitum*.

Pharmacological studies:
Analgesic activity:
Analgesic activity of the different extracts of *H. spinosa* was determined by both chemical and thermal method. In chemical method acetic acid induced writhing response [11-13] and in thermal method both hot plate reaction time and tail flick method [14-16] were used. The animals were divided into groups as shown in Table 2. Different extracts were administered at 200 and 400 mg/kg of body weight orally, aspirin was used as standard drug at a dose of 100 mg/kg of body weight and control group received 1% (v/v) Tween 80 in water at the dose of 10 ml/kg of body weight

Acetic acid induced writhing test:
Acetic acid (0.7% v/v) was administered intraperitoneally in a volume of 10 ml/kg of body weight [17] to all the groups 60 min after the administration of the test compound. Analgesic activity was recorded by counting the number of writhes after the injection of acetic acid for a period of 10 min. A writhe is indicated by abdominal constriction and full extension of hind limb [18].

Hot plate method:
The test was performed using Eddy's hot plate maintained at a temperature of 55 $\pm 1^0$C. The basal reaction time of all animals towards thermal heat was recorded. The animals showing fore paw licking or jumping response within 6-8 secs were selected for the study. 60 min after the administration of test and standard compounds, the animals in all the groups were individually exposed to the hot plate maintained at 55 $\pm 1^0$C and the time taken for fore paw licking or jumping was taken as reaction time. Pain inhibition percentage (PIP) was calculated according to the following equation:

$$\mathbf{PIP = [(T_1 - T_0)/T_0] \times 100}$$

Where, T_1 is post drug latency and T_0 is predrug latency

Tail flick method:
Basal reaction time of all the animals to radiant heat was recorded by placing the tip of the tail on the radiant heat source [15]. The tail withdrawal from the heat (flicking action) was taken as the end point. The animals which showed flicking response within 3-5 secs were selected for the study. A cut off period of 15 secs is observed to avoid damage to the tail [19]. Tail flick latency was measured 1 hr after the drug administration and PIP was calculated as above.

Antimotility activity:
Antimotility activity was studied by determining the gastrointestinal motility of charcoal meal [20]. The animals were divided into different groups (Table 3) and the extracts were

given at a dose of 200 and 400 mg/kg of body weight. Control group received 1% v/v Tween 80 in water at a dose of 10 ml/kg of body weight and the positive control group received atropine sulphate at a dose of 0.1 mg/kg of body weight intraperitonealy. After 30 min of the drug treatment each animal was given orally 0.3 ml of charcoal meal consisting of 10% charcoal and 5% gum acacia. The animals were sacrificed after 30 min of charcoal meal treatment and the movement of charcoal meal from pylorus to caecum was measured. The distance traveled by charcoal meal was expressed in terms of percentage [21-23].

Statistical analysis:
Results were statistically analysed by student's t-test to determine the significant difference between the control and the treated groups.

Results

Preliminary phytochemical screening:
Preliminary phytochemical analysis revealed the presence of different group of phytoconstituents in different extracts of *H. spinosa* (Table 1).

Table 1. Group of phytoconstituents present in different extracts of *H. spinosa* leaf.

	Different Extracts			
Group of Phytoconstituents	HSPE	HSCH	HSAL	HSW
Alkaloids	-	+	+	-
Steroids	-	+	+	-
Tannins	-	-	+	+
Proteins	+	+	+	+
Flavonoids	+	+	+	-
Saponins	-	-	-	-
Mucilage	-	-	-	+
Carbohydrates	-	-	-	-
Organic acids	-	-	-	+
Fats & oils	+	+	-	-

+ indicates present and – indicates absent

Analgesic activity:
Table 2 showed that the chloroform, alcoholic and aqueous extracts reduced the number of abdominal constrictions and stretching of hind limbs induced by acetic acid in dose dependent manner. Alcoholic extract at the dose of 200 and 400 mg/kg of body weight produced about 27% and 40% of writhing inhibition respectively. The results were statistically significant ($p < 0.01$) and were comparable with standard drug aspirin, where the writhing inhibition is about 52%. Again the above three extracts increased the reaction time of the animals towards the thermal source in dose dependent manner. Alcoholic extract at a dose of 400 mg/kg of body weight produced pain inhibition percentage of 53% and 64% in hot plate and tail flick method respectively.

The results were statistically significant ($p < 0.01$) and comparable to standard drug aspirin, where the PIP was 68% and 72% in hot plate and tail flick method respectively.

Table 2. Analgesic activity of different extracts of *H. spinosa* on acetic acid induced writhing test, hot plate method and tail flick model in mice.

Group	Dose (mg/kg of body weight, p.o.)	Acetic acid induced writhing response		Hot plate method		Tail flick method	
		Writhing	% Inhibition	Reaction time (in sec)	PIP	Reaction time (in sec)	PIP
Control	-	62.8 ± 1.7	-	6.63 ± 0.2	-	3.91 ± 0.2	-
Aspirin	100	30.2 ± 1.4*	51.9	11.1 ± 0.3*	68.2	6.75 ± 0.2*	71.8
HSPE	200	61.5 ± 1.4	2.0	6.65 ± 0.2	0.3	3.95 ± 0.2	1.0
HSPE	400	59.8 ± 1.6**	4.8	6.58 ± 0.1	-	4.08 ± 0.1	4.34
HSCH	200	51.3 ± 2.0*	18.3	7.23 ± 0.1**	9.0	4.36 ± 0.2	11.5
HSCH	400	42.5 ± 1.0*	32.3	7.91 ± 0.1*	19.3	4.55 ± 0.3**	16.4
HSAL	200	46.0 ± 1.3*	26.8	8.70 ± 0.2*	31.8	5.85 ± 0.2*	48.7
HSAL	400	37.5 ± 1.5*	40.3	10.1 ± 0.2*	53.0	6.4 ± 0.1*	64.1
HSW	200	53.0 ± 1.7*	15.6	7.41 ± 0.2*	11.8	4.35 ± 0.3	11.2
HSW	400	45.2 ± 1.5*	28.0	7.83 ± 0.1*	18.1	4.68 ± 0.2**	19.7

Values are expressed as mean ± SEM (Number of animals, n=6) * indicates $p < 0.01$, ** indicates $p < 0.05$ vs. control; p.o.: per oral.

Antimotility activity:

All the extracts of *H. spinosa* produced significant antimotility activity. Alcoholic extract was most prominent and at a dose of 400 mg/kg of body weight the percentage distance traveled by charcoal meal through the gastrointestinal tract was 53.9% while in case of the standard drug atropine sulphate the percentage distance traveled by charcoal meal was 50% (Table 3).

Table 3. Effect of different extracts of *H. spinosa* on gastrointestinal motility of mice

Group	Dose (mg/kg of body weight, p.o.)	Total length of intestine (in cm)	Distance traveled by charcoal meal (in cm)	Distance traveled by charcoal meal (%)
Control	-	48.05 ± 1.22	34.15 ± 0.74	71.15 ± 1.35
At. Sul.	0.1, i.p.	47.16 ± 1.32	23.55 ± 0.85	50.02 ± 1.81*
HSPE	200	48.67 ± 1.36	33.08 ± 1.50	67.97 ± 0.93*
HSPE	400	48.05 ± 1.18	32.18 ± 1.24	66.98 ± 1.75
HSCH	200	45.16 ± 0.47	28.45 ± 0.40	62.99 ± 0.94*
HSCH	400	49.48 ± 1.11	30.85 ± 0.51	62.35 ± 1.77**
HSAL	200	52.01 ± 1.28	31.20 ± 0.88	59.98 ± 0.72*
HSAL	400	50.06 ± 1.28	27.00 ± 0.52	53.93 ± 0.91*
HSW	200	45.11 ± 0.53	28.16 ± 0.84	62.42 ± 1.80**
HSW	400	48.80 ± 1.21	29.43 ± 0.71	60.30 ± 1.05*

Values are expressed as mean ± SEM (Number of animals, n=6) * indicates $p < 0.01$, ** indicates $p < 0.05$, vs. control; p.o.: per oral; At. Sul.: Atropine sulphate.

Discussion

The analgesic activity of the different extracts of *H. spinosa* was evaluated using both chemical and thermal methods. Acetic acid induced writhing test is commonly used for evaluation of peripheral analgesic activity [24]. Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic system mediators like PGE_2 and $PGF_{2\alpha}$ as well as lipoxygenase products [25, 26]. Therefore, the results of acetic acid induced writhing test suggest that the extracts exert the analgesic activity by inhibiting the synthesis or action of prostaglandins.

Centrally acting analgesics elevate the pain threshold of mice towards heat [15, 19]. In both hot plate and tail flick method the extracts increased the pain threshold of mice towards the thermal source in dose dependent manner. Therefore, the extracts exhibit analgesic activity by central as well as peripheral mechanism(s).

Diarrhea is a very common disease and national problem in many tropical countries and the causes of 4 million deaths throughout the world annually [27]. A number of antimotility compounds like loperamide, opium alkaloids, anticholinergics etc. have been tried against diarrhea but they are certainly having side effects in prolonged use [28]. In traditional system of medicine a number of plants have been reported to be effective against diarrhea and dysentery. In the present investigation the different extracts of *H. spinosa* significantly reduced the percent intestinal transit of the charcoal meal through the gastrointestinal tract.

So it could be concluded that the different extracts of *H. spinosa* possess analgesic and antimotility activities and the alcoholic extract is most potent. However, further work is necessary to find out the active constituents responsible for these activities.

References

1. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. CSIR, New Delhi. 1986: 29.
2. Nadkarni KM. Indian Materia Medica. Popular Prakashan, Bombay, India. 1978: 667-669.
3. Gomes A, Das M, Dasgupta SC. haematinic effects of *Hygrophila spinosa* on experimental rodents. *Ind. J. Expt. Biol.* 2001; 39: 381-382.
4. Mazumder UK, Gupta M, Maiti S, Mukherjee D. Antitumor activity of *Hygrophila spinosa* on Ehrlich ascites carcinoma and sarcoma- 180 induced mice. *Ind. J. Expt. Biol.* 1997; 35: 473-477.
5. Usha K, Kasturi GM, Hemalata P. Hepatoprotective effect of *Hygrophila spinosa* and Cassia occidentalis on carbon tetrachloride induced liver damage in experimental rats. *Ind. J. Clin. Biochem.* 2007; 22(2): 132-135.
6. Mazumder UK, Gupta M, maiti S. Chemical and pharmacological evaluation of *Hygrophila spinosa* root. *Ind. J. Pharm. Sc.* 1999; 61(3): 181-183.

7. Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal Plants Used in Ayurveda. Central Council for Research in Ayurveda & Siddha, Department of ISM&H, Ministry of Health & Family Welfare, New Delhi, Government of India. 2002: 320-331.
8. Evans WC. Trease and Evan's Textbook of Pharmacognosy. 13th edition, Cambridge University Press, London. 1989: 546.
9. Khandelwal KR. Practical Pharmacognosy. 14th edition, Nirali prakashan, Delhi, India. 2005: 149-156.
10. Williamson EM, Okpako DT, Evans FJ. Selection, Preparation and Pharmacological Evaluation of Plant Material. Volume 1, John Wiley & sons Ltd., England. 1996: 15-23.
11. Ahmed F, Selim MST, Das AK, Choudhuri MSK. Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. *Pharmazie*. 2004; 59: 329-330.
12. Saha A, Masud MA, Bachar SC et al. The analgesic and anti-inflammatory activities of extracts of *Phyllanthus reticulatis*. *Pharmaceutical Biology*. 2007; 45(5): 355-359.
13. Delporte C, Backhouse N, Erazo S et al. Analgesic-antiinflammatory properties of *Proustia pyrifolia*. *J. Ethnopharmacol*. 2005; 99: 119-124.
14. Turner RA. Screening Methods in Pharmacology. Academic Press, New York. 1965: 100.
15. D'Amour FE, Smith DL. A method for determining loss of pain sensation. *J. Pharmacol. Exp. Ther*. 1941; 72: 392-402.
16. Vogel HG, Vogel WH. Drug Discovery and Evaluation. Springer-verlag Berlin heidelberg New York. 1997: 368-371.
17. Parveen Z, Deng Y, Saeed MK et al. Antiinflammatory and analgesic activities of *Thesium Chinense* Turez extracts and its major flavonoids, kaempferol and kaempferol-3-O-glucoside. *Yakugaku Zasshi*. 2007;127(8): 1275-1279.
18. Mukherjee PK. Quality Control of Herbal Drugs. 1st Edition, Business Horizon Pharmaceutical Publishers, New Delhi. . 2002: 558-560.
19. Shanmugasundaran P, Venkatraman S. Antinociceptive activity of *Hygrophila auriculata* 9Schum) Heine. *Afr J Trad CAM*. 2005; 2(1): 62-69.
20. Rouf ASS, Islam MS, Rahman MT. Evaluation of antidiarrhoeal activity *Rumex maritimus* root. *J. Ethnopharmacol*. 2003; 84: 307-310.
21. Sagar L, Sehgal R, Ojha S. Evaluation of antimotility effects of *Lanata camara* L. var. acuelata constituents on neostigmine induced gastrointestinal transit in mice. *BMC complementary and alternative medicine*. 2005; 5: 18.
22. Biswas S, Murugesan T, Sinha S et al. Antidiarrhoeal activity of *Strychnos potatorum* seed extract in rats. *Fitoterapia*. 2002; 73: 43-47.
23. Jabbar S, Khan MTH, Choudhuri MSK, Gafur MA, Ahnad K. Effects of *Semicarpus anacardium* Linn. on acute experimental diarrhea. *Ham. Med*. 1999; XLII: 48-53.
24. Gene RM, Sengura L, Adzet T, Marin E, Inglesias J. *Heterotheca inuloides*: anti-inflammatory and analgesic effect. *J. Ethnopharmacol*. 1998; 60: 157-162.
25. Derardt R, Jougney S, Delevalece F, Falhout M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. *Eur. J. Pharmacol*. 1980; 51: 17-24.

26. Dannhardt G, Kiefer W. Cyclooxygenase inhibitors-current status and future prospects. *Eur. J. Med. Chem*. 2001; 36: 109-126.
27. Abdullahi AL, Agho MO, Amos S, Gamaniel KS, Wambebe C. Antidiarrhoeal activity of the aqueous extract of *Terminalia avicennoides* roots. *Phytotherapy Research*. 2001; 15: 431-434.
28. Harrison TR. Principles of Internal Medicine. 16th edition, Macgraw Hill, New York. 2005: 224-232.