Anti-Inflammatory Activity of Extracts of Leaves of *Hygrophila spinosa* T. Anders in Chronic Animal Models of Inflammation

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ABSTRACT

Hygrophila spinosa T. Anders (Acanthaceae) is traditionally used in Indian medicine for the treatment of microbial infections, liver diseases, cancer, inflammation, rheumatism, diabetes, pain, fever etc. The aim of the present study is to evaluate the anti-inflammatory activity of chloroform and alcoholic extracts of the leaves of *H. spinosa* in chronic inflammation models in rats as our previous study revealed that these two extracts had anti-inflammatory activity in carrageenan induced paw oedema model. Anti-inflammatory activity was evaluated by cotton pellet-induced granuloma and Freund's adjuvant-induced arthritis in rats. Antioxidant activity of the extracts was revealed by their scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and a flavonoid compound (apigenin) was also isolated and characterized from the alcoholic extract of the plant. Chloroform and alcoholic extracts showed anti-inflammatory activity both in cotton pellet-induced granuloma and Freund's adjuvant-induced arthritis in a dose dependent manner. The decrease in body weight due to injection of CFA was improved significantly by the above two extracts also. Both the extracts also exhibited antioxidant activity. The results demonstrated that *H. spinosa* has anti-inflammatory activity in chronic models of inflammation which support the traditional use of *H. spinosa* in the treatment of rheumatism.

Introduction

Hygrophila spinosa T. Anders (Acanthaceae) commonly known as 'Talmakhana' in Hindi is found in water-logged areas throughout India, contains a number of phytoconstituents viz. lupeol (Shailajan and Abhishek, 2008), β- sitosterol (Tiwari et al., 1967), stigmasterol (Khare, 2007), ascorbic acid, β-carotene (Sahoo and Acharya, 2005), nicotinic acid (Sharma et al., 2002), hentriacontane (Chopra et al., 1958), luteolin, luteolin-7-rutinoside (Balraj and Nagarajan, 1982), syringic acid, vannilic acid (Daniel, 2005) etc. In our earlier studies we have reported the other constituents, traditional uses, pharmacological activities of the plant (Patra et al., 2009a); various groups of phytoconstituents in petroleum ether, chloroform, alcoholic and aqueous extracts of the leaves of the plant (Patra et al., 2009b) and also few pharmacological studies of the above extracts (Patra et al., 2008, 2009c). The aim of this study was to determine the anti-inflammatory activity of chloroform and aqueous extracts of the leaves of *H. spinosa* in chronic inflammation models in rats.

Materials and Methods

Plant material

H. spinosa plants were collected from Berhampur, Orissa, India and botanical identification was done through Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi (Voucher no. BITPcog. 463/07-08). Voucher specimen was preserved in the department for further verification.

Preparation of different extracts

The leaves were washed thoroughly, dried under shade and pulverized. The coarse powder was extracted successively with petroleum ether, chloroform and alcohol using soxhlet apparatus. The extracts were dried using a rotary vacuum evaporator and stored in a desiccator until further use.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by cotton pellet-induced granuloma and Freund's adjuvant-induced arthritis in rats.

Antioxidant activity

Antioxidant activity of the extracts was revealed by their scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical.

Isolation of compound 1 from alcoholic extract

50 gm of alcoholic extract was column chromatographed on a silica gel column (mesh 60-120) and eluted with solvent mixtures of increasing polarity: chloroform (300 ml), chloroform-acetone [(80:20, 300 ml), (60:40, 300 ml), (40:60, 300 ml), (20:80, 300 ml)], acetone (300 ml), acetone-methanol [(80:20, 300 ml), (60:40, 300 ml), (40:60, 300 ml), (20:80, 300 ml)] and methanol (300 ml). Fractions (20ml) were collected and monitored on TLC. Fractions (151 to 165) collected were pulled together as these fractions showed a single spot of same R_f value in TLC. It was evaporated in a water bath (70-80^{0}C) to afford a solid residue. The residue was dissolved in EtOH with little warming on a water bath. It was left undisturbed in refrigerator when crystals of compound **1** was obtained (yield: 0.0015% w/w).

Characterization of compound 1

The various instruments used for recording the data for compound **1** are: FTIR spectroscopy (Shimadzu, IRPrestige-21), elemental analysis (Elementar, Vario EL III), ^{1}H and ^{13}C NMR spectra (PABBO BB NMR spectrophotometer), mass spectroscopy etc. Other physicochemical characters as melting point, solubility and physical appearance were also recorded.

Statistical analysis

The results were expressed as mean ± standard error mean (SEM). Statistical analysis of the data was carried out using one way analysis of variance (ANOVA) followed by Student's t-test to determine the significant difference between the control and the treated groups. $P < 0.05$ was considered significant.

Results

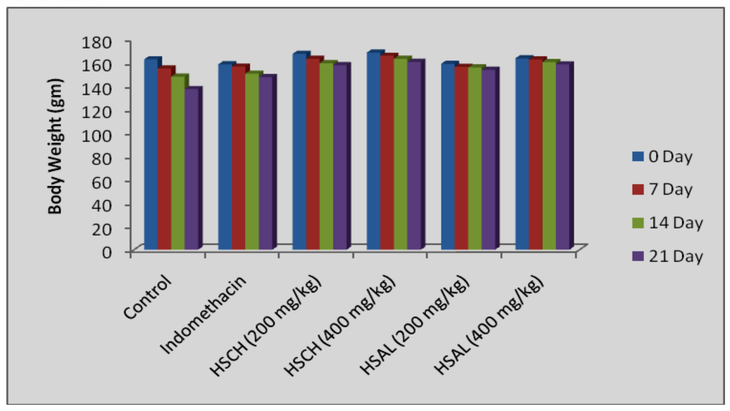

Fig. 1. Effect of different extracts of H. *spinosa* leaf on body weight of animals in CFA induced arthritic rats. HSCH, chloroform extract; HSAL, alcoholic extract

Treatment	Dose (mg/kg, p.o.)	Weight of dry granuloma (mg)	Percentage inhibition of granuloma
Vehicle (Control)	-	122.36 ± 2.32	-
Indomethacin	10	65.42 ± 1.52*	46.53*
Chloroform	200	109.65 ± 1.05*	10.38*
	400	92. 88 ± 0.98*	24.09*
Alcoholic	200	102.28 ± 1.06*	16.41*
	400	78.68 ± 0.86*	35.69*

Table 1. Effect of different extracts of *H. spinosa* leaf on cotton pellet-induced granuloma

Treatment	Dose (mg/kg)	Increase in paw volume (in ml)		Percentage inhibition	
		After 5 days	After 21 days	After 5 days	After 21 days
Control	-	0.56 ± 0.02	0.44 ± 0.04	-	-
Indomethacin	10	0.54 ± 0.02	0.35 ± 0.05*	3.57	20.45*
Chloroform	200	0.54 ± 0.02	0.40 ± 0.03	3.57	9.09
	400	0.52 ± 0.01	0.34 ± 0.02*	7.14	22.72*
Alcoholic	200	0.53 ± 0.02	0.38 ± 0.03*	5.35	13.63*
	400	0.50 ± 0.02*	0.31 ± 0.04*	10.71*	29.54*

Table 2. Effect of different extracts of *H. spinosa* on adjuvant induced arthritis

Concentration (µg/ml)	% free radical scavenging	
	Chloroform Extract	Alcoholic Extract
25	10.36±0.69	13.14±1.24
50	18.17±1.03	20.80±0.75
100	24.83±0.78	29.96±0.79
200	35.8±2.48	41.17±1.58
400	49.81±4.42	56.05±3.27

Table 3. Free radical scavenging activity of different extracts of *H. spinosa* in DPPH method

Characterization of compound 1

5,7-Dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one **(1)** i.e., apigenin (Fig. 2) was isolated as whitish yellow crystals and its elemental composition was determined to be $C_{15}H_{10}O_5$ (Anal. Calcd for $C_{15}H_{10}O_5$: C, 66.67; H, 3.73 and O, 29.60. Found: C, 66.55; H, 3.73 and O, 29.57). Melting point: 337-339^{0}C and soluble in warm EtOH. It also contains hydroxyl (Lucas reagent), phenol ($FeCl_3$ test) and ketone (2,4-dinitrophenyl hydrazene) functional groups which was found from functional group analysis (Siddiqui and Ali, 1997; Nadendl, 2005) and supported by IR spectroscopy. Phytochemical analysis (Shinoda test) of the compound confirmed its flavonoid nature (Khandelwal, 2005). IR (KBr) cm^{-1}: 3312.54 (OH), 3064.46 (CH), 2952.80 (CH), 1664.64 (C=O), 1608.15 (C=C), 1144.22 (C-O-C) etc. UV λ_{max}(EtOH): 336 nm. MS m/z: 270 (M^+) (Calcd for $C_{15}H_{10}O_5$: 270.24), 242, 220, 152 and 120. NMR spectrum of this compound superimposed with the data published in previous studies (Ersoz et al., 2002; Fathiazad et al., 2006; Breitmaier and Voelter, 1989; Harborne and Mabry, 1982). The structure was simulated using ACD/NMR program (ACD/ChemSketch-Product Version: 10.00) to obtain the chemical shifts of both proton and carbon where the experimental data matched with the simulated data.

Conclusion

In summary, our results suggest that *H. spinosa* has considerable potency in anti-inflammatory action and has prominent effects on adjuvant-induced arthritis by alleviating paw edema. Hence, the results of the present study support the traditional use of *H. spinosa* in the treatment of rheumatism.

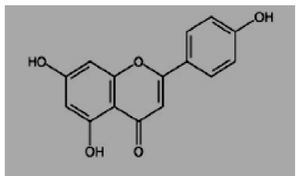

Fig. 2. Structure of compound 1

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