ABSTRACT

Hygrophi/a 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 studies 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.

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. BITP cog. 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), acetone (300 ml), acetonemethanol (80:20, 300 ml), (60:40, 300 ml), 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 Rf value in TLC. It was evaporated in a water bath (70-80°C) to afford a solid residue. The residue was dissolved in EtOH with a 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, IR Prestige-21), elemental analysis (Elementar, Vario EL III), 1H and 13C 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.

References