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Full Length Research Paper

Anti-inflammatory activity of *Aconitum heterophyllum* on cotton pellet-induced granuloma in rats

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The anti-inflammatory activity of ethanolic root extract of *Aconitum heterophyllum* (225, 450 and 900 mg/kg p.o) has been evaluated in cotton pellet-induced granuloma in rats. The extract has reduced inflammation as evidenced by decreased weight of cotton pellet in cotton pellet-induced granuloma in rats. The results demonstrate the anti-inflammatory properties of extract and the effects were comparable to diclofenac sodium, a standard non-steroidal anti-inflammatory drug.

Key words: *Aconitum heterophyllum*, cotton-pellet, inflammation.

INTRODUCTION

Inflammation is a disease involving localized increases in number of leukocytes and a variety of complex mediator molecules including prostaglandins (Mantri and Witiak, 1994). Even though modern drugs are effective in the management of inflammation and associated conditions, but their use is often limited because of side effects (Lipsky, 1999). In recent years, there is growing realize-tion that apart from being safer, economical and easily available, herbs, phytochemicals and herbal products can influence the course of inflammatory diseases and may provides an amalgamation of nutritional substances, which help in restoring and maintaining wear and tear of tissues. Therefore, it would be rational to scientifically evaluate the traditional medicines used for their potential use in inflammatory diseases.

Aconitum heterophyllum is one such plant (family: Valeraneaceae) which is commonly known as 'Ativisha' or 'Patis' in Ayurveda; the Indian System of Medicine (Uniyal et al., 2002). It is used for the treatment of diseases of nervous system, digestive system, fever and rheumatism (Uniyal et al., 2002). It is found throughout the world and cultivated in tropic fields. Being rich in substances having potential biological significance, such as benzoylmesaconine, mesaconitine, aconitine, hypaconitine, heteratisine, heterophyllisine, heterophylline, heterophyllidine, atidine, isotisine, hetidine, hetisinone and benzoylheteratisine and other nutrients (Zhaohong et al., 2006) the plant has been reported to possess antifungal (Anwar et al., 2003), cytotoxic (Anwar et al., 2003), antiviral (Pandey et al., 2004) and immune-stimulant properties (Atal et al., 1986). Other compounds isolated from *A. heterophyllum* include flavonoids, tannins, saponins and sugars (Pelliter et al., 1968). Considering the traditional uses and demon-strated potential medicinal properties of *A. heterophyllum*, present study was undertaken to investigate its anti-inflammatory potential in cotton pellet-induced granuloma in rats.

MATERIALS AND METHODS

Collection of plant material

Fresh matured roots of *A. heterophyllum* were collected locally near Rohtak district of Haryana province of India. The identity of *A. heterophyllum* was authenticated on the basis of taxonomical characters following routine pharmacognostical studies, including organoleptic tests and macroscopic and roots of the plant used in

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present study have been kept in the departmental herbarium as voucher specimen for future references. The roots were air dried and further subjected to extraction.

Preparation of ethanolic extract of *A. heterophyllum*

The ethanolic extract of the roots of *A. heterophyllum* was prepared in accordance to previously described standard extraction procedure (Trease and Evans, 1983). The collected roots were air dried under shade at room temperature and milled to a coarse powder. The obtained dried powder was subjected to continuous extraction with 80% ethanol in a Soxhlet apparatus. The powdered root material was packed in a tumble made of Whatmann's filter paper. It was extracted with ethanol for 40 cycles. The extract thus obtained was concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature. The yield of ethanolic extract of roots of *A. heterophyllum* was 12.91%. The obtained residue was a brown color thick and sticky paste. The extract was stored in refrigerator and reconstituted in gum acacia before administration to animals. The extract was subjected to phytochemical screening for the determination of phytoconstituents in the extract following previously described procedure.

Experimental animals

Colony bred Wistar male albino rats weighing 175 - 200 g, 10 -12 weeks old were used in the present study. The study protocol and procedures were reviewed and approved by the Institutional animal ethics committee and conducted in accordance to the Indian national science academy guidelines for the use and care of experimental animals. Animals were housed in polypropylene cages (38 X 23 X 10 cm) with not more than four animals per cage under standard laboratory conditions (25 ± 2°C, relative humidity 55 ± 10%, alternating 10 h dark/ 14 h light photoperiod). They were fed commercial pellet diet and water *ad libitum*. The diet approximately contained: carbohydrate (55%), fat (5%), protein (24%), fiber (4%), calcium (0.6%), phosphorous (0.3%), moisture (10%) and ash (9%). Before treatment allocation and randomization, rats were acclimatized to the laboratory conditions for a week.

Cotton pellet-induced granuloma inflammation

Employing an earlier described technique (Winter and Porter, 1957) granulomatous lesions were induced by surgically implanting two cotton pellets subcutaneously in the dorsal region of the rats, one near each axilla. *A. heterophyllum* extract was administered orally. After 20 min, autoclaved sterile pellets of cotton, weighing 7 ± 1 mg each, were aseptically implanted in the interscapular distance under the skin on the previously shaved back of the rats which were anesthetized with thiopental sodium (25 mg/kg, i.p.). The rats of the control group were administered with the same volume of vehicle. The rats sacrificed on the eighth day and the pellets surrounded by granuloma tissue were dissected out carefully and dried at 70°C. Mean weight of the granuloma tissue formed around each pellet was recorded. The pellets were weighted both moist and dry. The weight of the pellets taken out from *A. heterophyllum* administered rats was compared with the weight of pellets taken out from the control group.

Drugs and chemicals

All chemicals used in present study were of analytical grade. Diclofenac sodium, a widely used non steroidal anti-inflammatory drug (NSAID) was used as standard drug. For dosing the ethanolic extract was suspended uniformly in 0.25% gum acacia and administered orally using an intragastric feeding tube.

Animal experimentation and drug treatment protocol

Rats were randomly divided into five experimental groups, each consisting of six rats and were treated as follows:

Group I: Vehicle treated control animals received gum acacia 0.25% w/v
Group II: Animals administered diclofenac sodium, 25 mg/kg, i.p.
Group III: Animals received ethanolic extract of *A. heterophyllum*, 225 mg/kg, p.o.
Group IV: Animals received ethanolic extract of *A. heterophyllum*, 450 mg/kg p.o.
Group V: Animals received ethanolic extract of *A. heterophyllum,* 900 mg/kg p.o.

Phytochemical screening

The extract was screened for various chemical constituents employing standard screening tests following conventional phytochemical screening protocols (Trease and Evans, 1983) such as alkaloids with Mayer and Dragendorff's reagent, saponins (frothing test, tannins ($FeCl_3$), glycosides (NaCl and Fehling's solution A and B), flavonoids (NaCl and HCl), phenols ($FeCl_3$ and K_3 $Fe(CN)_6$) and lipids (filter paper). The Rf value of *A. heterophyllum* was also obtained with Dragendorff reagent on thin layer chromatography to characterize the chemical constituents.

Statistical analysis

All the grouped data were statistically evaluated with SPSS 10 software. The results are expressed as the mean ± SEM. The results were analyzed for statistical significance using student-'t' test. P value of less than 0.05 was considered statistically significance.

RESULTS AND DISCUSSION

Phytochemical classes characterized in *A. heterophyllum* extract have been presented in Table 1. Preliminary phytochemical screening of extract has revealed the presence of alkaloids, glycosides, flavonoids and sterols. However, upon phytochemical screening no steroid, fixed oil, gum, terpenoids, mucilage and saponins were present in extract.

The extract has been found to reduce the weight of cotton pellet granuloma in a dose dependent manner (Table 2) in the cotton pellet induced model of inflammation in rats. The reduction in the weight of cotton pellet granuloma with different doses of extract 225, 450 and 900 mg/kg was found 17, 26 and 51% respectively, (Figure 1). However, the decrease in inflammation by *A. heterophyllum* 900 mg/kg was comparable to diclofenac sodium, which reduced the weight of cotton pellet granuloma by 64% (Figure1). In pharmacological studies, number of medicinal plants demonstrated antinociceptive and antinflammatory properties, which helped into the management of inflammatory diseases, especially,

Table 1. Phytochemical analysis of the ethanolic extract of *A. heterophyllum.*

Reaction	Chemicals	Result
Dragendorff test	Alkaloids	+
NaCl and Fehling solution A and B test	Glycosides	+
Frothing test	Saponins	-
Ninhydrin test	Proteins and amino acids	-
Saponification test	Sterols	+
Sodium chloride and hydrochloric acid test	Flavonoids	+
Gelatin and Ferric chloride test	Tannins	-
Liberman Buchard test	Steroids	-
Filter paper test	Fixed oils	-
Spot test	Gums	-
Acetic anhydride and choloroform test	Terpenoids	-
Phloroglucinol test	Mucilages	-
Potassium ferricynaide test	Phenols	+

Table 2. Changes in mean weight of cotton pellet in different groups.

Treatment	Dose (mg/kg)	Weight of dry cotton pellet (mg)	% inhibition
Control	-	120.83 ± 2.88	-
A. heterophyllum	225	99.91 ± 2.90*	17.31
A. heterophyllum	450	89.66 ± 2.90*	25.79
A. heterophyllum	900	59.33 ± 2.82*	50.89
Diclofenac sodium	25	43.66 ± 3.31*	63.86

Data represents mean ± SEM of 6 rats. *p < 0.01 vs. control.

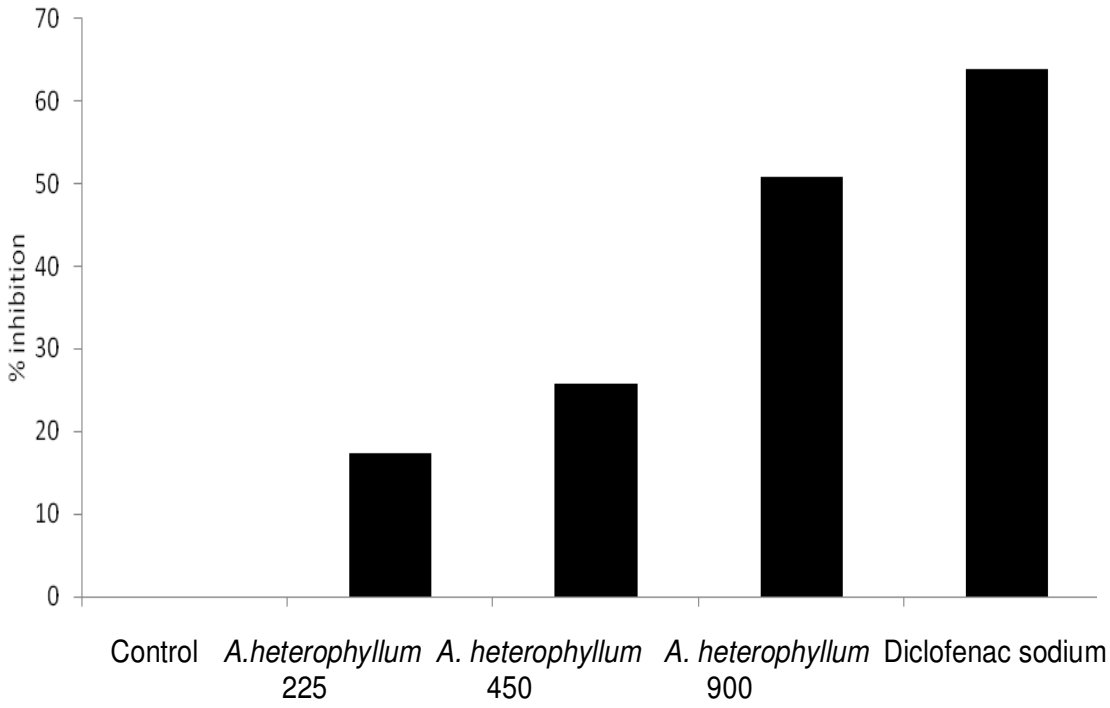

Figure 1. Percentage inhibition in mean weight of cotton pellet in different groups.

rheumatism through inhibition of synthesis of cellular prostanes (Just et al., 1998; Suleyman et al., 1991). *A. heterophyllum* is one such plant, which has been indicated in ayurvedic text and ancient literature of traditional medicines for its anti-inflammatory properties (Uniyal et al., 2002). In recent years, a number of active constituents are present in this plant, which includes alkaloids, glycosides, flavonoids and sterols (Zhaohong et al., 2006). In literature it has been reported that plants with these chemical classes of compounds possess potent anti-inflammatory effects through inhibiting prostaglandin pathways (Patwardhan and Hopper, 1992).

The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation (Winter and Porter, 1957). The weight of the wet cotton pellets correlates with transude material and the weight of dry pellet correlates with the amount of granulomatous tissue. In the present study, administration of *A. heterophyllum* extract has been observed to inhibit the weight of wet cotton pellet in a dose dependent manner and the higher dose of *A. heterophyllum* exhibited inhibition of inflammation very close to the inhibitory effect of diclofenac sodium. It is well known fact that diclofenac sodium act by inhibiting the prostaglandins synthesis at the late phases of inflammation. This effect may be due to the cellular migration to injured sites and accumulation of collagen, an important mucopolysaccharide (Smith and Dewitt, 1995). Decreasing granuloma tissue, prevention of occurring of the collagen fiber and suppression of muco-polysaccharids are indicators of the antiproliferative effect by NSAIDs. In preview of this, results of present study demonstrate that ethanolic root extract of *A. heterophyllum* has potential to inhibit sub-acute inflammation by interruption of the arachidonic acid metabolism. Present study finding supports the traditional claims and provides a scientific basis for anti-inflammatory effect of *A. heterophyllum* in inflammatory diseases.

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