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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF *PHOLIDOTA ARTICULATA* EXTRACTS

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ABSTRACT

Evaluation of anti-inflammatory property of *Pholidota articulata* whole plant with preliminary phytochemical profile of the extracts has been carried out in this part of work. The dried plant material was packed in Soxhlet apparatus and extracted successively with pet-ether to de-fat the drug, petroleum-ether was removed from the powdered defatted drug, which was then extracted with chloroform and methanol as increasing polarity. All the extracts were screened for anti-inflammatory activity using carrageenan induced paw edema. The toxicity and phytochemical screening were done using standard procedure. Alkaloids, flavonoids, phenolic compounds, glycosides carbohydrates, proteins, gums and amino acids have been determined by preliminary phytochemical tests. The acute toxicity study of various extracts of *Pholidota articulata* was conducted and dose of 3000 mg kg-1 body weight fixed for anti-inflammatory activity.

KEYWORDS: *Pholidota articulata*Orchidaceae Anti-inflammatory activity, Indomethacin.

INTRODUCTION

Inflammation is considered as a primary physiologic defence mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of this chronic illness. Although it is a defence mechanism, the complex events and mediators involved in the inflammatory reaction can easily be induced[1]. The side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses[2]. Therefore, the development of newer and more potent anti-inflammatory drugs with lesser side effects is necessary. The genus *Pholidota* (Orchidaceae) belongs to the tribe coelogyneae, and comprises 55 species with a distrubution from tropical asia to tropical australia and china. Among them 9 species in India. Commonly distributed from submontane to montane Himalaya. The genus *pholidota* are epiphytic herbs generally grown on rocks and trees[3]. At present, the pharmaceutical sector in India is making use of 280 medicinal plant species, of which 175 are found in the IHR[4]. The plants of the genus *pholidota* are used traditionally for medicinal purposes. The whole plant has long been used as a remedy for acute or chronic bronchitis, toothache, treatment of dysentery, infections, asthma, bronchitis, eczema and duodenal ulcer[5].

MATERIALS AND METHODS

Collection of Plant material

Pholidota articulata (Orchidaceae) whole plants were collected from the Ukhimath, Distt- Rudraprayag, Uttarakhand, in September-October 2014. The plant was authentic and identified by Dr. C. S. Rana, Department of Botany and the voucher specimen number is GUH **4325**. H. N. B. Garhwal (A Central University) Srinagar Garhwal, Uttarakhand India.

Preparation of Plant Extracts

The collected plant material was then shade dried and coarsely powdered. The powdered plant material (3000g) was subjected to the hot method of extraction using Soxhlet extractor. The extraction process was carried out using various solvents viz., pet-ether, chloroform and methanol with the increasing polarity. The obtained extracts were filtered and evaporated to dryness under reduced pressure in rotary vacuum evaporator.

Phytochemical Screening

The extracts were subjected to preliminary phytochemical investigation to identify various phytoconstituents i.e., alkaloids, steroids, carbohydrates, flavonoids, phenolics, tannins, saponins, glycosides, amino acids etc. present in whole part of plant by using standard tests[6,7].

Acute toxicity studies

The acute oral toxicity studies were performed to study the acute toxic effects and to determine minimum lethal

dose of the plant extracts. Swiss albino mice of either sex weighing 18-25 g were used for the study. The pet-ether, chloroform and methanol extracts were administered orally to different groups of overnight fasted mice at the doses of 1000, 2000, and 3000 mg kg-1 body weight. After the administration of extracts, animals were observed continuously for the first 3 h for any toxic manifestation. Thereafter, observations were made at regular intervals for 24 h. Further the animals were under investigation up to a period of one week[8]. This study showed that, drug is safe up to the dose of 3000 mg kg-1 for all the extracts.

Experimental design

Thirty experimental animals were randomly selected and divided into five groups denoted as Group I, Group II, Group III, Group IV and Group V, consisting of 6 Wister rats in each group. Each group received a particular treatment i.e. control, standard and the three doses of the extract. Prior to any treatment, each rat was weighed properly and the dose of the test samples and control material was adjusted accordingly. Group III to Group V received the crude extracts orally at the dose of 300 mg kg-1 body weight. Group II received intraperitoneal administration of Indomethacin at a dose of 10 mg kg-1 - body weight as standard for anti-inflammatory study, while Group I was kept as control giving 2% gum acacia solution in normal saline water. Similarly thirty-six animals were selected and divided into six groups denoted as Group I, Group II, Group III, Group IV, Group V and Group VI consisting of 6 Wister rats in each group. Each group received a particular treatment i.e. normal control, diabetic control, standard and the three doses of extracts. Prior to any treatment, each rat was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Group IV to Group VI received the crude extracts orally at the dose of 300 mg kg-1- body weight. Group III received intraperitoneal administration of Glibenclamide at the dose of 5 mg kg-1 -body weight as standard drug for anti-diabetic study, while Group II was kept as diabetic control giving 2% gum acacia solution in normal saline water.

Anti-inflammatory activity

The anti-inflammatory activity of *Pholidota articulata* was studied using acute (Carrageenan induced paw edema) model of inflammation. This model is based on the principle of release of various inflammatory mediators by carrageenan. Edema is due to carrageenan in the rat paw as biphasic event. The initial phase is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome[9,10]. Subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from plasma extravasations, increased tissue water and plasma protein exudation along with neutrophil extravasations, these results due to the reaction of arachidonic acid[11]. The first phase begins immediately after injection of carrageenan and diminishes in two hours. The second phase begins at the end of first phase and remains through third hour up to 5 h.

The carrageens an induced rat paw edema by known technique[11]. The animals were housed in cages under standard laboratory conditions. They had free access to standard diet and water. The animals were divided into 5 groups of six animals each and fasted for 12 h before the experiment. The initial right hind paw volume of the rats were measured using a plethysmometer and then 0.5 ml of 1% w/v carrageen an solution in normal saline was subcutaneously injected into the sub plantar region of the right hind paw. The volume of right hind paw was measured at 0, 1, 2, 3 and 5 h after carrageen an injection and the paw volume was determined. The data were expressed as paw volume (ml) and compared with the initial hind paw volume of each rat. Co solvent (2% gum acacia solution, p.o), various extracts of *Pholidota articulata* as suspension in 2% gum acacia solution (p.o) and indomethacin (10 mg kg-1)[10] was administered 30 min before carrageenan injection. The group received co solvent was treated as control. The hind paw volume was measured plethysmometrically before and after the carrageenan injection, at hourly intervals for 5 hours.

RESULTS AND DISCUSSION

The results obtained in the carrageenan-induced edema test are shown in table 1. The bark extracts of *Pholidota articulata* (300 mg kg-1) administered 30 min before the injection of carrageenan caused a significant and dose dependent inhibition of increase in paw edema. In the carrageenan test, the maximum inhibition elicited by the methanol extract (33.18%) was comparable to that of indomethacin (10 mg kg-1; p.o; 67.72%). Pet-ether extract showed a poor inhibition while Chloroform extract showed a moderate inhibition of increase in paw edema in rats when compared to standard Indomethacin.

Table 1: Anti-inflammatory activity of *Pholidota articulata* whole extracts by Carrageenan induced paw edema in rats.

Table1: Anti-inflammatory activity of *Pholidota articulata* extracts by Carrageenan induced paw edema in rats

Groups	Dose (mg kg^{-1})	Increase in paw edema(cm) at time T(h)					
		0h	1h	2h	3h	5h	% inhibition
Chloroform	300	0.55±0.01	0.55±0.03	0.51±0.02	0.47±0.07	0.42±0.05	20.75
Methanol	300	0.55±0.03	0.54±0.01	0.46±0.04	0.41±0.02	0.35±0.01	33.18
Petroleum Ether	300	0.56±0.06	0.54±0.05	0.52±0.01	0.46±0.04	0.41±0.03	19.96
Indomethacin	10	0.55±0.04	0.46±0.01	0.41±0.04	0.33±0.05	0.18±0.02	67.72

The Inflammation is a reaction of living tissues towards injury. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. Carrageenan has been widely used as a noxious agent; it can able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity[12]. This phlogistic agent, when injected locally into the rat paw, produced a severe inflammatory reaction, which was discernible within 30 min. The development of edema induced by carrageenan corresponds to the events in the acute phase of inflammation, mediated by histamine, bradykinin and prostaglandins produced under an effect of cyclooxygenase[13]. This anti-inflammatory effect may be due to the inhibition of any inflammatory mediators by the glycosides or steroids[14]. present in the extract. From evaluation, it has been concluded that the Pet-ether, chloroform and methanol extracts showed significant reduction of inflammation as compared to Indomethacin.

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