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Research Article

Anti-inflammatory Activity of *Portulaca quadrifida* Linn.

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ABSTRACT

Anti-inflammatory activity of the ethanolic extract of *Portulaca quadrifida* Linn. was studied in wister rats using the carrageenan induced left hind paw edema, carrageenan induced pleurisy and cotton pellet induced granuloma model. The ethanolic extract (200 mg/kg, p.o.,) produced the inhibition of carrageenan induced rat paw edema. It also showed an inhibitory effect on leukocyte migration and a reduction on the pleural exudates as well as reduction on the granuloma weight in the cotton pellet granuloma method. The results indicated that the ethanolic extract produced significant ($P<0.001$) anti-inflammatory activity when compared with the standard and untreated control.

Keywords: Anti-inflammatory; *Portulaca quadrifida*

INTRODUCTION

Portulaca quadrifida Linn. a prostrate fleshy annual or stoloniferous perennial herb with somewhat base but sometimes with simple main stems, 5-40 cm tall and generally widespread in warm countries. *Portulaca quadrifida* Linn. belongs to the family portulacaceae. It is a small diffused, succulent, annual herb found throughout the tropical parts of India. It is used as a vegetable and also used for various curative purposes. It is said to be useful in asthma, cough, urinary discharges, inflammations and ulcers. A poultice of the plant is applied in abdominal complaints, erysipelas and haemorrhoids [1]. *Portulaca quadrifida* Linn. has been reported to possess antifungal activity against *Aspergillus fumigates* and *Candida* albicans [2] and the neuropharmacological activities were reported by Syed *et al* [3]. A review of literature afforded no information on the anti-inflammatory aspects of this plant.

The enzyme, phospholipase A_2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and

leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A_2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cycloxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation [4, 5]. So the present study is therefore an attempt to assess the efficacy of this indigenous herb for its anti-inflammatory activity in rats.

MATERIALS AND METHODS

Plant material

The aerial parts of the plant were collected from the foothill of Yercaud, Salem, in the month of June 2015 and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist Dr. A. Marimuthu, Department of Botany, Government Arts College, Attur. A voucher specimen (PQM-1) has been kept in our museum for future reference. The plant parts were dried at room temperature for 10 d and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

Preparation of the extract

The powder of aerial parts of *P. quadrifida* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, alcohol, to finally chloroform: water [6]. After extraction, the extracts were concentrated under reduced pressure in tared vessel. The marc of crude drug powder was then once again subjected to successive extraction with other solvents and the extractive values were calculated with reference to the air-dried drug. The dry extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

Animals

Swiss albino mice of either sex and of approximately the same age weighing about 20-30g were used for the study. The were housed in polypropylene cages and fed with standard chow diet and water ad *libitum*. The animal were exposed to alternative cycle of 12 h of darkness and light each .Before each test, the animals were fasted for atleast 12h. The experimental protocols were subjected to the scrutinization of the institutional animal ethics committee and were cleared by the same.

Acute toxicity studies:

The acute toxicity studies were conducted as per the guidelines of OECD (guideline 423). And were observed for mortality till 48 h and the LD_{50} was calculated.

Carrageenan induced rat paw edema

Anti-inflammatory activity was assessed by the method described by Winter *et al* [7]. The rats were divided into three groups of six animals each. First group (negative control) received 1 ml of normal saline, second group (positive control) received 10 mg/kg p.o., Indomethacin and third group received ethanolic extract (200 mg/kg, p.o.,) of *P.quadrifida*, respectively. After 1 h, the rats were challenged with subcutaneous injection of 0.1 ml of 1 % w/v solution of carrageenan (Sigma chemical co, St. Louis MO, USA) into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark. The plethysmograph apparatus used for the measurement of rat paw volume was that of Singh and Ghosh [8]. The paw volume was measured immediately after injection (0 h) and followed by every hour till the 3 h after injection of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume.

Percent inhibition of inflammation was calculated using the formula, % inhibition = 100 (1-Vt/Vc), where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with test extracts.

Carrageenan induced pleurisy in rats [9, 10]

The animals were divided into three groups of six rats each as described in the carrageenan induced paw edema model and each were pretreated with ethanolic extract of *P.quadrifida* (200 mg/kg, p.o.,), Indomethacin (10 mg/kg, p.o.,) or normal saline (0.1 ml). One hour later all the animals were received 0.25 ml of an intrapleural injection of 1 % carrageenan on the right side of the thorax. The animals were sacrificed 3 h after

carrageenan injection by ether inhalation. One ml of heparinized Hank's solution was injected into the pleural cavity and gently massaged to mix its contents. The fluid was aspirated out of the cavity and the exudates were collected. The number of migrating leukocytes in the exudates was determined with Neubauer chamber. The values of each experimental group were expressed as mean ± SEM and compared with the control group.

Cotton Pellet Granuloma model

In cotton pellet granuloma model the animals were divided into three groups as described in the carrageenan induced paw edema model. The method of Penn *et al* [11] with slight modification was used. The animals were anaesthetized with pentobarbitone (30 mg/kg. s.c.). The back skin was shaved and disinfected with 70% ethanol. An incision is made in the lumbar region. Subcutaneous tunnels were formed by a blunted forceps and a sterilized, pre weighed cotton pellet was placed on both sides in the scapular region. The animals were treated with indomethacin (10 mg/kg, p.o.,) and ethanolic extract of *P.quadrifida* for 7 days. Then, the pellets were dissected out and dried until the weight remains constant. The net dry weights, i.e. after subtracting the weight of the cotton pellet were determined.

Statistical Analysis

All values were expressed as mean ±SEM. The data were statistically analyzed using one way ANOVA followed by Newman Keut's multiple range test and difference below P< 0.05 are considered as significant.

Table 1: Effect of Ethanolic Extract of *P. quadrifida* on Carrageenan Induced Rat Paw Edema

Treatment	Dose (mg/kg, p.o.)	Mean increase in paw volume (ml)	% Decrease in paw volume
Control (Normal saline)	1 ml	0.37±0.001	-
Indomethacin	10	0.13±0.001*	64.9
Ethanolic extract of *P. quadrifida*	200	0.17±0.001*	54.1

**P*<0.001 when compared with control. Values are expressed as mean±SEM (n=6)

Table 2: Effect of Ethanolic Extract of *P. quadrifida* on Carrageenan Induced Pleurisy in Rats

Treatment	Dose (mg/kg, p.o.)	Pleural exudates (ml)	Leukocytes ($x10^3$ cells/ml)
Control (Normal saline)	1 ml	0.35±0.002	4.53±0.33
Indomethacin	10	0.12±0.001*	0.43±0.08*
Ethanolic extract of *P. quadrifida*	200	0.15±0.001*	0.48±0.08*

**P*<0.001 when compared with control. Values are expressed as mean±SEM (n=6)

TABLE 3: Effect of Ethanolic Extract of *P. quadrifida* on Cotton Pellet Induced Granuloma in Rats

Treatment	Dose (mg/kg, p.o.)	Granuloma wt. (mg)	% inhibition
Control (Normal saline)	1 ml	58.2±2.04	-
Indomethacin	10	20.7±0.65*	64.4
Ethanolic extract of *P. quadrifida*	200	28.8±0.73*	50.5

**P*<0.001 when compared with control. Values are expressed as mean±SEM (n=6)

RESULTS

The plant *P. quadrifida* was collected from the foothill of Yercaud, Salem, air-dried and extracted by continuous hot extraction process using soxhlet apparatus. The average percentage yield of ethanolic extract of *P. quadrifida* was found to be 3.8 % w/w. The LD_{50} was found to be 2000 mg/kg for ethanolic extract of *P. quadrifida*.

The ethanolic extract did not exhibit and toxic effects up to 1000 mg/kg when administered to mice as a single i.p. dose.

Carrageenan induced rat paw edema

The effect of ethanolic extract of *P. quadrifida* on carrageenan-induced edema in rats is shown in Table 1. The results obtained indicate that the ethanolic extract was found to have significant anti-inflammatory activity in rats. The ethanolic extract of *P. quadrifida* reduced the edema induced by carrageenan by 54.05 % on oral administration of 200 mg/kg, as compared to the untreated control group. Indomethacin at 10 mg/kg inhibited the edema volume by 64.86 %.

Carrageenan induced pleurisy in rats

The effect of ethanolic extract of *P. quadrifida* on carrageenan-induced pleurisy in rats is shown in Table 2. The volume of pleural exudates in the control group was 0.35±0.002 ml. Animals treated with the ethanolic extract of *P. quadrifida* (200 mg/kg, p.o.,) decreased the pleural exudates to 0.15±0.001 ml and treatment with Indomethacin (10 mg/kg, p.o.,) produced the exudates of 0.12±0.001 ml. The leukocyte count for the control group was found to be 4.53±0.33 X 10^3 cells/ml. Animals treated with the test extract and standard produced a leukocyte migration of 0.48±0.08 X 10^3 and 0.43±0.08 X 10^3 cells/ml, respectively.

Cotton Pellet Granuloma model

The effect of ethanolic extract of *P. quadrifida* on cotton pellet induced granuloma in rats is shown in Table 3. In this the mean weights of the cotton pellets were determined. The weight of the granuloma for the control group of animals was found to be 58.2±2.04 mg. Treatment with the ethanolic extract of *P. quadrifida* (200 mg/kg, p.o.,) decreased the granuloma weight to 28.8±0.73 mg. Treatment with Indomethacin (10 mg/kg, p.o.,) produced a granuloma weight of 20.7±0.65 mg. The ethanolic extract of *P. quadrifida* and Indomethacin, both inhibited the granuloma tissue formation. The inhibition of the test extract and standard drug was found to be 50.5 and 64. 4 %, respectively.

DISCUSSIONS

Due to the increasing frequency of intake of NSAID's and their reported common side effects, there is need to focus on the scientific exploration of herbal drugs having fewer side effects. So, there is a continuous search for indigenous drugs, which can provide relief to inflammation. The traditional medical practitioners of Kolli hills, Tamilnadu, are using this plant to cure inflammation. To give a scientific validation to this plant, an attempt was made to study the anti-inflammatory activity.

Carrageenan induced inflammation is a biphasic phenomenon [12]. The first phase of edema is attributed to release of histamine and 5-hydroxytryptamine. Plateau phase is maintained by kinin like substances and second accelerating phase of swelling is attributed to prostaglandin like substances. The knowledge of these mediators involved in different phases is important for interpreting mode of drug action.

The tests performed with the ethanolic extract of *P. quadrifida* in the pleurisy model showed that the extract behaves as an inhibitor of leukocyte migration and the formation of pleural exudates when given orally, as reported earlier [13].

In the cotton pellet granuloma model, inflammation and granuloma develops during the period of several days. This model is an indication for the proliferative phase of inflammation. Inflammation involves proliferation of macrophages, neutrophils and fibroflasts, which are basic sources of granuloma formation. Hence, the decrease in the weight of granuloma indicates that the proliferative phase was effectively suppressed by the ethanol extract of *P. quadrifida*.

Thus it can be concluded that the plant *P. quadrifida* possess significant anti-inflammatory activity in rats. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with a low toxicity and better therapeutic index.

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