

ANTI-INFLAMMATORY ACTIVITY OF *ACACIA CONCINNA*Megha Agrawal^{1*} and Dr. Vijay Patel²¹Shri Bherul Pharmacy Institute, Indore (M.P) 452020, India.²BM College of Pharmaceutical Education and Research, Indore (M.P) 452020, India.

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ABSTRACT

The aqueous extract of the pods of *Acacia concinna* was investigated for its anti-inflammatory activity using carrageenan induced paw edema and cotton pellet granuloma models in wistar rats. A statistically significant ($p < 0.01$) dose and time dependent inhibition of edema was observed. The observed % inhibition of edema was 77.78, 69.44 and 62.04% for ibuprofen and plant extract with 100 and 200 mg/kg dose, respectively. Also, the plant extract significantly inhibited ($p < 0.01$) the granuloma induced by cotton pellets in rats in a dose-dependent manner. The % granuloma inhibition was 51.21, 26.12 and 30.59% for ibuprofen and plant extract at doses of 100 and 200 mg/kg respectively. It was concluded from the present study that extract of pods of *A. concinna* have significant anti-inflammatory activity.

KEYWORDS: Anti-inflammatory, *Acacia concinna*, Paw edema, Cotton pellet granuloma.

INTRODUCTION

Inflammation is considered as a complete cycle of events, beginning with initiation of a response and progressing through the production of the cardinal signs, to healing and restoration of tissue or organ's normal appearance and function. In certain cases, however, there seems to be no resolution, and a chronic state of inflammation occurs, which can last the rest of the person's life.^[1]

The most commonly used drugs in the treatment of inflammation are SAID's and NSAID's. Non-selective COX inhibitors show negative renal, gastric, hepatic and haematological side effects. Preferential COX-2 inhibitors including nimesulide and meloxicam also have side effects similar to that of non-selective COX inhibitors. Selective COX-2 inhibitors appear to tip the balance in favour of thromboxane, which appears to have a prothrombotic effect and raise CV risk.^[2]

Modern allopathic drugs are single-active chemical molecules that target a single pathway, while herbal medicines contain pleiotropic molecules that function in concert to target multiple elements of a complex cellular pathway.^[3] A new effective, potent, nontoxic or less toxic anti-inflammatory drug is now needed.^[4] There are medicinal plants that have anti-inflammatory therapeutic effects and have minimal to no side effects.^[5]

Acacia concinna belongs to family Fabaceae and is a medium-sized evergreen tree grows in the tropical rainforests of southern Asia with flat and dense pods up

to 5cm long with 7 seeds.^[6,7] Several saponins, including kinmoonosides A-C, triterpenoidal prosapogenols called concinnosides A, B, C, D, and E, as well as four glycosides, acaciaside, julibroside A1, julibroside A3, albiziasaponin C, and their aglycone, acacic acid, lactone, have been isolated from pods.^[8] It has antimicrobial and antibacterial properties, as well as photochemicals with antioxidant potential. It is traditionally used as a shampoo, and synthetic Ayurvedic shampoos contain it as well.^[6]

Literature survey shows that pods of *A. concinna* contain saponins.^[9,10] The anti-inflammatory and other effects of saponins from various plants are well documented.^[11-14] The numerous biological activities associated with saponins have lead to great interest in their characterization and in the investigation of their pharmacological and biological properties. This study was under taken to investigate the anti-inflammatory activity of the aqueous extract of the pods of *A. concinna*.

MATERIALS AND METHODS

Collection of plant material

The pods of *A. concinna* were collected from the market of Indore, identified and authenticated on the basis of macroscopic and microscopic characters as *A. concinna*.

Extraction of plant material

The pods were grounded to powder by using grinder and passed through mesh size 20 to get uniform powder. The aqueous extract of dried powder drug was prepared as follows.

The powdered material (100g) was suspended in distilled water that was boiled for 1hr. Extract was filtered using filtration paper No. 1 and also the marc was re-extracted by the same process twice. The collected filtrate was concentrated over a water bath.^[15]

Phytochemical screening

The above obtained extract was subjected to various qualitative tests like test for the presence of alkaloids, flavonoids, phytosterols, saponins, tannins for identification of varied phytochemical constituents with the help of literature.^[16,17]

Experimental Animals

The proposed anti-inflammatory activity of the extract was done on wistar rats weighing 150–200 g of both sexes. They were collected from animal house of BM College of Pharmaceutical Education and Research. The animals were kept in polyvinyl cages under controlled room temperature (25±2 °C) in the laboratory environment under 12 h dark and 12 h light cycle for seven days and were supplied with the normal laboratory standard food pellets and water at ease. The experimental protocol was approved by the Institutional Animal Ethical Committee of Shri Bherulal Pharmacy Institute (Approval No: 1888/PO/Re/S/16/CPCSEA/2020/02) and was strictly in accordance with the norms of Committee for the Purpose of Control & Supervision of Experiments on Animals (CPCSEA) New Delhi.

Acute toxicity study

This was carried out to determine the LD₅₀ using the method described by Lorke. It has two phases and

Change in paw thickness was considered as a measure of inflammation and percentage inhibition of edema was calculated using the following formula.

$$\% \text{ inhibition of edema} = \frac{\text{Mean edema inc. (C}_t\text{)} - \text{Mean edema inc. (T}_t\text{)}}{\text{Mean edema increase (C}_t\text{)}} \times 100$$

Where C_t = Control group at time 0, 1, 2, 3 and 4 hours

T_t = Treated groups at time 0, 1, 2, 3 and 4 hours

Cotton pellet granuloma

Wistar rats weighing 180–200 g were used. Pellets were made by cutting adsorbent cotton wool into pieces weighing 20±1 mg which were then kept in a hot air oven at 120° for 2 h for sterilization. The abdomen was shaved cleanly, swabbed with 70% ethanol and small linear incisions of about 1 cm were made in each axilla and groin under aseptic precautions under light ether anaesthesia. One on each side of the abdomen of the animal, two sterilized cotton pellets was implanted subcutaneously.^[20] The incisions were sutured and animals were kept under aseptic conditions for the entire duration of the study. Throughout the period of

thirteen mice were used to determine the LD₅₀ of the extract. In the first phase, nine mice divided into three groups each containing three mice, were administered with the extracts at doses of 10, 100, 1000 mg/kg body weight orally respectively. The second phase involving three groups of one animal each were then treated with higher doses of test substance and then observed for 24 hours for behavior as well as mortality.^[18]

Procedure

Animals were divided into four groups having six rats per group.

1. Group I (control) was treated with vehicle (normal saline, 1ml/kg body weight) only
2. Group II (reference standard) receiving standard drug ibuprofen (20 mg/kg body weight) in normal saline
3. Group III was administered with first dose of extract 100mg/kg in normal saline.
4. Group IV was administered with second dose of extract 200mg/kg in normal saline.

Carrageenan induced Paw edema in rats

Wistar rats of either sex weighing 150–200 g were used and starved overnight. Paw volumes of all rats were measured initially. All the groups were pre-treated with their respective doses orally. After 60 mins, 0.1 ml of freshly prepared carrageenan suspension (1%w/v in normal saline) was injected into the sub-plantar region of the left hind paw of each rat. The paw volume was measured with the help of plethysmometre at 0, 1, 2, 3 and 4 hours after injection of the carrageenan.^[19,20]

experimentation the food and water were allowed and the animals were maintained in clean cages.^[21] Once a daily dosage regimen was followed for 7 days to administer the test drugs orally, and the control group received vehicle. On the 8th day after implantation, rats were sacrificed and cotton pellets were removed surgically. The pellets were dissected and dried at 60° for 18 h, weighed after cooling and the dry weight was recorded. Granuloma formation extent was considered by the increment in the dry weight of the pellets. The mean weight of the cotton pellets (granuloma) for both control group and test groups was calculated.

The percentage inhibition of granuloma tissue development was calculated by using following formula.^[22]

$$\% \text{ inhibition of granuloma} = \frac{\text{Weight of pellet (control)} - \text{weight of pellet (test)}}{\text{Weight of pellet (control)}} \times 100$$

Statistical Analysis

The results were expressed as the mean \pm SEM ($n = 6/\text{group}$). Differences among data were determined using one way ANOVA followed by the Dunnett's multiple comparisons test. $p < 0.01$ was considered as statistically significant.

RESULT

Phytochemical screening

The screening revealed the presence of saponins, alkaloids, flavonoids, phytosterols, carbohydrates, glycosides and phenolic compounds.

Acute Toxicity

It was found that extract was safe at all tested doses (upto 5000mg/kg) and did not show any behavioral changes, physical lesions and mortality after 24 hrs.

Effect of pod extract of *A. concinna* pods on carrageenan induced paw edema in rats

A statistically significant ($p < 0.01$) dose and time dependent inhibition of edema was observed with aqueous pod extract of *A. concinna* at 100 mg/kg and 200 mg/kg when compared to the standard drug ibuprofen (20 mg/kg) as shown in Table 1 and Fig. 1.

Effect of pod extract of *Acacia concinna* pods on cotton pellet granuloma

The aqueous extract of pods of *A. concinna* significantly inhibited ($p < 0.01$) the granuloma induced by cotton pellets in rats in a dose-dependent manner (Table 2 and Fig. 2).

Table 1. Effect of pod extract of *A. concinna* pods on carrageenan induced paw edema in rats

Treatment	Dose (mg/kg)	Paw volume mean and (% inhibition)				
		0h	1h	2h	3h	4h
Normal saline	1	0.92	1.13 \pm 0.03	1.27 \pm 0.06	1.53 \pm 0.04	1.80 \pm 0.03
Ibuprofen	20	0.90	0.78 \pm 0.03** (30.88%)	0.68 \pm 0.03** (46.05%)	0.53 \pm 0.04** (65.22%)	0.40 \pm 0.04** (77.78%)
Extract 1	100	0.88	0.80 \pm 0.05** (29.41%)	0.75 \pm 0.04** (40.79%)	0.65 \pm 0.04** (57.61%)	0.55 \pm 0.04** (69.44%)
Extract 2	200	0.90	0.83 \pm 0.04** (26.47%)	0.72 \pm 0.03** (43.42%)	0.73 \pm 0.04** (52.17%)	0.68 \pm 0.05** (62.04%)

The results are expressed as the mean \pm SEM ($n = 6/\text{group}$). Bu using one-way ANOVA, followed by Dunnett's multiple comparisons test differences among data was found out * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$.

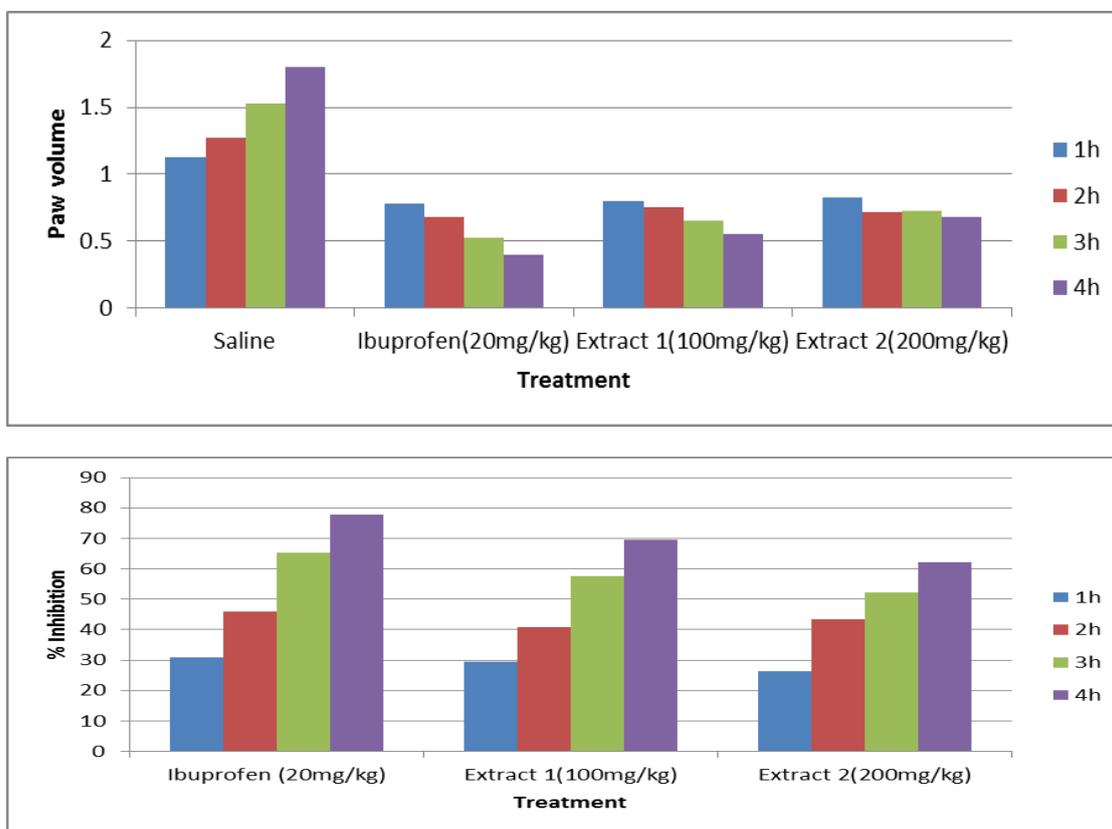
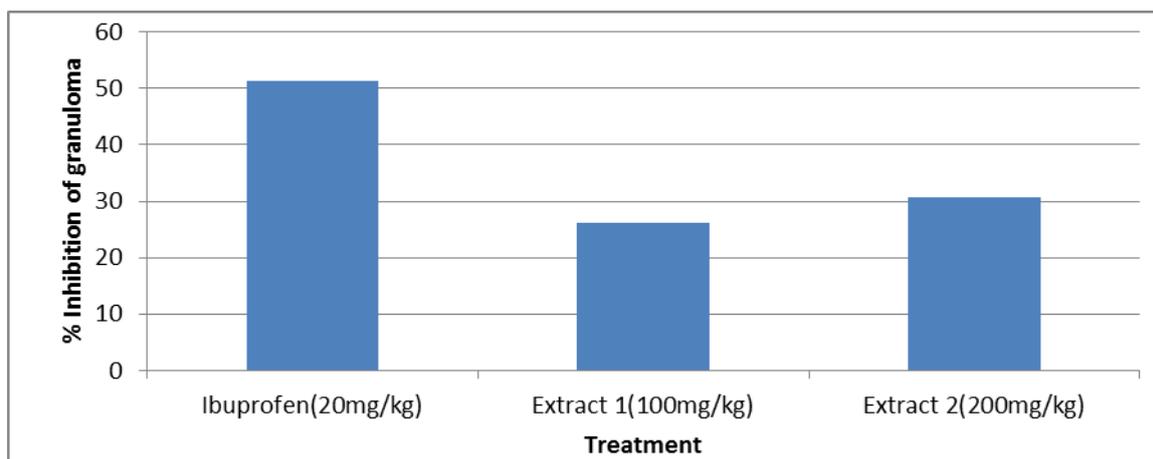


Figure 1: Effect of pod extract of *A. concinna* pods on carrageenan induced paw edema in rats.

Table 2: Effect of pod extract of *A. concinna* pods on cotton pellet granuloma.

Treatment	Dose (mg/Kg)	Granuloma wt. after drying (mg)	Inhibition (%)
Normal saline	1	97.00±1.83	-
Ibuprofen	20	47.33±2.15**	51.21
Extract 1	100	71.66±2.78**	26.12
Extract 2	200	67.33±2.29**	30.59

The results are expressed as the mean \pm SEM ($n = 6/\text{group}$). By using one-way ANOVA, followed by Dunnett's multiple comparisons test differences among data was found out * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$.

**Figure 2: Effect of pod extract of *A. concinna* pods on cotton pellet granuloma.**

DISCUSSION

In this study, the aqueous extract of *A. concinna* pods exerted inhibitory effect on carrageenan-induced paw edema in rats starting from the first hour after administration. Therefore, the inhibitory effect in the first phase may be due to the suppression of the histamine signaling by the mast cell stabilizing effect and direct inhibition of histamine H_1 receptor and histidine decarboxylase gene transcriptions. The anti-edematous activity of the extract persisted in the second phase with the maximal effect observed at 4 hours. This could be explained by the possible inhibition of the release and/or action of kinin and prostaglandin. Curtailment of the maintenance phase indicates that the extract had inhibited bradykinin release and/or its vascular permeability promoting action.

There's also evidence that compounds that prevent edema formation by carrageenan may also stop cyclooxygenases from functioning. Based on this reports, the inhibitory effect could also be mediated via this mechanism.

In cotton pellet granuloma model, the aqueous extract of *A. concinna* at doses of 100 and 200 mg/kg, effectively inhibited the development of granulomatous tissues as compared to control group. The extract may act by inhibiting cellular migrations involved in this inflammation model, reducing the number of fibroblasts, preventing angiogenesis and synthesis of collagen and mucopolysaccharide.

CONCLUSION

It was concluded from the present study that extract of pods of *A. concinna* have significant anti-inflammatory activity. The observed effects of *A. concinna* pods are difficult to assign to any single chemical moiety. Since *A. concinna* pods are rich in saponins. The mechanism of anti-inflammatory activities may be due to the presence of saponins in the pod.

However, further research is needed to isolate the compound responsible for these activities and to assess the potential mechanism underlying them.

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