Pakistan Journal of Pharmaceutical Sciences
Vol. 18, No.3, July 2005, pp.17-20

ANTI-INFLAMMATORY ACTIVITY OF *COLDENIA PROCUMBENS* LINN.

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Anti-inflammatory activity of the ethanolic extract of the aerial parts of *Coldenia procumbens* 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 (150 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: *Coldenia procumbens* Linn., rat paw edema.

INTRODUCTION

Coldenia procumbens Linn. (Boraginaceae) is a procumbent, deep-rooted, hairy herb, found throughout India as a weed in moist places. The powdered roots enter into a compound formulation given in leucorrhoea and menorrhagia (Kirtikar et al., 1993). Prolonged uses of both steroidal and non-steroidal anti-inflammatory drugs are well known to be associated with peptic ulcer formation (Ewart 1980). Hence, search for new anti-inflammatory agents that retain therapeutic efficacy and yet are devoid of these adverse effects are justified. There is much hope of finding active anti-rheumatic compounds from indigenous plants as these are still used in therapeutics despite the progress in conventional chemistry and pharmacology in producing effective drugs. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects and they are in line with nature, with no hazardous reactions.

The enzyme, phospholipase A_2, is known to be responsible for the formation of inflammation mediators such as prostaglandins and leukotrienes, among which leukotrienes are potent chemotactic agents causing infiltration of leucocytes to the site of inflammation leading to tissue damage probably via release of free radicals. Phospholipase A_2 converts membrane lipids 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 (Higgs et al., 1984; Vane 1971). So the present study is therefore an attempt to assess the efficacy of this indigenous herb for its anti-inflammatory activity in rats.

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MATERIALS AND METHODS

Plant material

The aerial parts of the plant were collected from the foothill of Yercaud, Salem, in the month of December 2002. The collected plant was identified and authenticated by a botanist Dr. A. Marimuthu, Department of Botany, Government Arts College, Salem. A voucher specimen (CPA-1) has been kept in our museum for future reference. The aerial parts were shade dried at room temperature for 10 d and coarsely powdered and passed through sieve No. 60.

Preparation of the extract

The powder of aerial parts of *C. procumbens* 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 (Kokate 1994). 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 dried extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

Animals

Wister rats of either sex and of approximately the same age, weighing about 150-175 g were used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for atleast 12 h. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same.

Acute toxicity studies
The animals were divided into control and test groups containing six animals each. The control group received the vehicle (1 % acacia gum) while the test groups got graded doses of different extracts orally and were observed for mortality till 48 h and the LD_{50} (Ghosh 1994) was calculated.

Carrageenan induced rat paw edema
Anti-inflammatory activity was assessed by the method described by Winter et al (1968). 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 (150 mg/kg, p.o.,) of *C. procumbens*, 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 (1968). 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
In carrageenan induced pleurisy in rats, the activity was assessed by the method described by Tomlinson et al., 1994 and Vinegar *et al.,* 1982. 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 *C. procumbens* (150 mg/kg, p.o.,), Indomethacin (10 mg/kg, p.o.,) or normal saline (1 ml). One hour later all the animals were given 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 were 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 (1963) 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 *C. procumbens* 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
Statistical analysis (Woodson 1989) was performed using student's t-test. The values are represented as mean±SEM. Level of significance was set at $P<0.001$.

RESULTS

The aerial parts of *C. procumbens* were 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 *C. procumbens* was found to be 5.6 % w/w. On preliminary phytochemical screening of the aerial parts of *C. procumbens* revealed the presence of, flavanoids, and glycosides. The LD_{50} for ethanolic extract of *C. procumbens* was found to be 1362 mg/kg.

The effect of ethanolic extract of *C. procumbens* 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 *C. procumbens* reduced the edema induced by carrageenan by 59.46 % on oral administration of 150 mg/kg, as compared to the untreated control group. Indomethacin at 10 mg/kg inhibited the edema volume by 64.86 %.

The effect of ethanolic extract of *C. procumbens* 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 *C. procumbens* (150 mg/kg, p.o.,) decreased the pleural exudates to 0.16±0.002 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 $4.53\pm0.33 \times 10^3$ cells/ml. Animals treated with the test extract and standard produced a leukocyte migration of $0.48\pm0.02 \times 10^3$ and $0.43\pm0.08 \times 10^3$ cells/ml, respectively.

Table 1
Effect of ethanolic extract of *C. procumbens o*n carrageenan induced rat paw edema

Treatment	Dose (mg/kg, p.o.)	Mean change in paw volume (ml) after 3 h	% Decrease in paw volume
Control (normal saline)	1 ml	0.37±0.001	-
Indomethacin	10	0.13±0.001*	64.9
Ethanolic extract of *C. procumbens*	150	0.15±0.001*	59.5

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

Table 2
Effect of ethanolic extract of *C. procumbens* 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 *C. procumbens*	150	0.16±0.002*	0.48±0.02*

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

Table 3
Effect of ethanolic extract of *C. procumbens* 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 *C. procumbens*	150	27.2±1.19*	53.3

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

The effect of ethanolic extract of *C. procumbens* 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 *C. procumbens* (150 mg/kg, p.o.,) decreased the granuloma weight to 27.2±1.19 mg. Treatment with indomethacin (10 mg/kg, p.o.,) produced a granuloma weight of 20.7±0.65 mg. The ethanolic extract of *C. procumbens* and Indomethacin, both inhibited the granuloma tissue formation. The inhibition of the test extract and standard drug was found to be 53.3 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 (Vinegar *et al.,* 1989). 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 *C. procumbens* 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 (Mikami *et al.,* 1983).

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 ethanolic extract of *C. procumbens*.

Thus it can be concluded that the aerial parts of the plant *C. procumbens* 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.

Based on the results of the present study it can be concluded that *C. procumbens* has potential anti-inflammatory activity in the late phase of inflammation probably induced by the bradykinin. Further studies are necessary to assess for the potential clinical use of this plant or extract or active principles, as anti-inflammatories, responsible for the pharmacological action before delineating the mechanism of action.

ACKNOWLEDGEMENT

We thank Dr. B. Jayakar, Principal, Vinayaka Mission's College of Pharmacy, Salem, for his encouragement and support throughout the work.

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Pakistan Journal of Pharmaceutical Sciences
Vol. 18, No.3, July 2005, pp.20-24

KINETICS OF COPPER(II) CATALYZEDOXIDATION OF IODIDE BY IRON(III) ORTHOPHENANTHROLINE COMPLEX IN AQUEOUS SOLUTION

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Orthophenanthroline (OPT) forms stable complex with Iron(II) at pH 3.5. In presence of Ce(IV) it forms a metal-ligand complex in which Fe is in the form of Fe^{+++} for short period when mixed in mole ratio of 1:3 (Iron: OPT). This complex is stable for not more than 30 minutes. At the end, this complex reduces itself back to iron(II)orthophenanthroline $[Fe(OPT)_3]^{+2}$ complex. In present work, this property of iron(III) complex was used to oxidize I^{-1} to I_2. Analysis of $[Fe(OPT)_3]^{+2}$ was carried out at 510 nm to monitor the reduction of $[Fe(OPT)_3]^{+3}$ complex. Copper was used as a catalyst during the described reaction to convert back $[Fe(OPT)_3]^{+2}$. The reaction increased with increase in copper concentration. The conversion of $[Fe(OPT)_3]^{+3}$ to $[Fe(OPT)_3]^{+2}$ followed first order kinetics.

Keywords: Fe(II)orthophenanthroline, Fe(III)orthophenanthroline, reduction of iodine.