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Research article

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF ROOT BARK OF *CLERODENDRUM PHLOMIDIS* IN EXPERIMENTAL MODELS OF INFLAMMATION

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ABSTRACT: The study was carried out to evaluate anti-inflammatory activity of aqueous extract of root bark of Clerodendrum phlomidis (CP) in models of acute and chronic inflammation in rats. Anti-inflammatory activity of CP was evaluated in models of acute inflammation viz. carrageenan induced rat paw oedema and acetic acid induced peritonitis in mice. The anti-inflammatory activity against chronic inflammation was assessed in model of cotton pellet granuloma in rats. The activity of CP was compared with aspirin and *Dashamoolarishta* (a multi-ingredient plant formulation containing C*lerodendrum phlomidis*) which served as positive controls. CP in the dose of 21.6 ml/kg showed significant anti-inflammatory activity (15.85 % inhibition in the carrageenan model and 50.38% inhibition in the model of chronic inflammation). In the peritonitis model, the maximum anti-inflammatory activity (27.32% inhibition was seen with the corresponding dose in mice.

The present study demonstrates anti-inflammatory activity of aqueous extract of root bark of CP and also provides a scientific basis for inclusion of CP in the *Dashamoolarishta formulation*.

Keywords: Carrageenan, Peritonitis, Cotton pellet granuloma, Dashamoolarishta, Inflammation

INTRODUCTION

The role of inflammation in diseases like osteoarthritis and rheumatoid arthritis is well known. The presently available modern medicines are not free from side effects and offer temporary relief. Thus, there is still scope to develop a safe and effective anti-inflammatory agent. The traditional systems of medicine in India offer a large variety of Indian medicinal plants. Some of the Indian medicinal plants have been claimed to have anti-inflammatory properties. But the scientific evidence for their medicinal properties for many of these plants still remains to be generated.

Clerodendrum phlomidis (CP) (Linn.) belongs to the family Verbenaceace and is found to be widely distributed in the regions of South East Asia including India, Pakistan, Sri Lanka and Burma. It is popularly known as *agnimantha* (Sanskrit), *airani* (Marathi) and *airanmool* (Hindi). The plant is reported in folk medicine to be useful in inflammation, glycosuria, pox, coryza and scrotal enlargement. The root is used as a bitter tonic and is given in the convalescence of measles (Sharma PC, et al., 2001).

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The root of CP is used in *Dashamoolarishta* which is a multi-ingredient plant based formulation prescribed for inflammatory disorders in the ancient Indian System of Ayurveda (Sharma PV,2001), (Gogte VM, 2000). *Dashamoolarishta* (DA) is used in the form of *kwath* or *arishta* since ancient times for the relief of pain and swelling related to arthritis. Dashamoola (classical Ayurvedic formulation) contains the roots of ten plants (Sharma PV,2001), (Gogte VM, 2000). Of these, five are known as Brihad panchamoola and five as laghoo panchamoola. DA contains the following plants: *Aegle marmelos corr (*Bilwa*), Clerodendrum phlomidis* (Agnimantha), *Oroxylum indicum* (Shyonak), *Stereospermum suaveolens* (Patala), *Gmelina arboria* (Gambhari), *Desmodium gangeticum* (Shaliparni), *Ureria picta desv* (Prushniparni), *Solanum indicum*(Bruhati), *Solanum xanthocarpum*(Kantakari), *Tribulus terrestris* (Gokshur). However, Ayurveda recommends root bark of CP instead of whole root for medicinal use (Gogte VM, 2000). By convention also many reputed Ayurvedic physicians use root bark of CP instead of root in the formulation of *Dashamoolarishta.*

A review of literature on *Clerodendrum phlomidis* (CP) revealed that anti-inflammatory activity of root bark of CP still remains to be scientifically investigated although it is used in traditional medicine. This study was planned to evaluate the anti-inflammatory activity of three doses of aqueous extract of root bark of CP in experimental models of acute and chronic inflammation.

MATERIALS AND METHODS

Preparation of plant extract and authentication

The roots of *Clerodendrum phlomidis* (CP) were obtained from a known supplier of plant materials (Jadvaji Lallubhai, Dava Bazar, Mumbai) and authenticated at Agharkar Research Institute, Pune. A voucher specimen has been deposited in our laboratory for future reference. The root-barks were separated and pulverized by a mechanical pulveriser. The aqueous extract of root bark of CP was prepared using procedures described in Ayurvedic texts in which one part of powdered root bark was mixed with eight parts of water (1:8), boiled over slow fire till the mixture was reduced to one fourth of the original volume and then strained through muslin cloth (Nadkarni KM, 2002), (Tripathi B, 2001), (Sharma SK, 2000). Three doses of CP were extrapolated from the dose range of *Clerodendrum phlomidis* used for humans (60-100ml/day) as documented in Ayurvedic texts (Gogte VM, 2000),(Sharma SK, 2000). The higher and lower doses were thus obtained and a middle dose was calculated for animals taking human dose as 80ml/day.

Animals

The experiments were carried out after obtaining permission of the Institutional Animal Ethics Committee. 60 Wistar rats (180-200 g) and 30 Swiss albino mice (20-25 g) of either sex were maintained at 22 ± 3°C with 12 hourly light and dark cycle. The animals were fed with rodent chow obtained from Chakan Oil Mills, Maharashtra. Water purified by Aquaguard was provided *ad libitum*. The animals were kept fasting for 12 hrs with water provided *ad libiutum* prior to the experiments. The animals were randomly allocated to 6 groups containing 6 animals in each group for the three experiments in the study.

Drugs and Chemicals

The drugs used were *Dashamoolarishta* (DA) (Proprietary preaparation from Sandu Brothers), aspirin (Sigma Aldrich Ltd), and distilled water. Carrageenan and acetic acid were purchased from Sigma Aldrich Ltd. All the drugs were dissolved in distilled water just prior to administration. The dose of *Dashamoolarishta* was extrapolated for animals from the highest dose recommended in the Ayurvedic texts for humans (Gogte VM, 2000),(Sharma SK, 2000).

Effect of CP on acute inflammation in carrageenan induced paw edema in rats

Pedal inflammation was introduced by injecting 0.1 ml of 1% carrageenan in carboxy methyl cellulose (CMC) by Winter CA *et al* (1962) (Winter CA, et al., 1962) under the sub-plantar aponeurosis of the right hind foot of each rat. CP (16.2, 21.6 and 27 ml/kg was administered orally to three groups while another group received DA orally in the dose of 1.8 ml/kg The other two groups served as positive and negative controls and received aspirin (500 mg/kg) and distilled water (5ml/kg) respectively. The drugs were administered 1 hour prior to the experiment. The rat paw volume upto the ankle joint was measured using plethysmograph. (Techno Instruments, Lucknow) at 0 hr (just before) and 3 hrs after injection of carrageenan. Percentage edema was calculated using the following formula by the method of Mandal *et al* (2000) (Mandal SC,et al., 2000).

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$$\% \text{ Edema (E)} = \frac{\text{Paw volume at the end of 3 hours (ml)}}{\text{Basal paw volume (ml)}} \times 100$$

Percentage inhibition of rat paw edema of treatment groups with respect to the control group was calculated using the following formula.

$$\text{Percentage inhibition of edema} = \frac{Ec - Et}{Ec} \times 100$$

(Where Ec is the % edema of the control group and Et is the % edema of the test group.)

Effect of CP in the model of peritonitis using acetic acid in mice

The model of acetic acid induced peritonitis as described by Tomisawa S and Sato NL (1973) (Tomisawa S, Sato NL, 1973) was used to evaluate the effect of CP on vascular permeability. Peritonitis was induced by injecting 0.25 ml of 1.2% acetic acid intraperitoneally in mice. Different groups received CP (25, 30 and 35 ml/kg), aspirin (722.2 mg/kg), DA (2.5 ml/kg) and distilled water (5 ml/kg) orally. The animals were sacrificed three hours after injection of acetic acid by cervical dislocation. Abdominal walls were excised widely to expose the abdominal viscera. The exposed portions were irrigated with 5 ml of saline containing 0.1 mM disodium ethylene diamine tetraacetate (EDTA) to prevent clotting of washings. The samples were centrifuged for 10 minutes at 3000 rpm and the supernatant was used for determination of the protein content of the exudates using Microprotein kit (Pyrogallol red method) (Durgawale P, et al., 2005). The percentage inhibition of protein exudation was calculated.

Effect of CP in the model of chronic inflammation in rats

The effect of CP on the proliferative phase of inflammation was assessed in cotton pellet granuloma rat model as described by Winter and Porter (1957) (Winter CA, Porter CC, 1957). The rats were anesthetised using thiopentone sodium 35-50 mg/kg i.p. Sterile cotton pellets were implanted subcutaneously bilaterally in axilla through a small incision on ventral aspect of each rat under aseptic conditions. The pellets were pre-weighed (50mg ± 1 mg) and were soaked with penicillin (0.1 mg) and streptomycin (0.13 mg) just before the implantation. Different groups of animals received CP (16.2, 21.6 and 27 ml/kg), aspirin (500 mg/kg), DA (1.8 ml/kg) and distilled water (5 ml/kg) orally once daily for a period of 7 days. On 8^{th} day, the animals were sacrificed and cotton pellets were dissected out along with the surrounding granulomatous tissue. The pellets were dried in hot air oven at 60^0 C for 24 hrs and their dry weights were measured. Granuloma weight was obtained by subtracting the weight of cotton pellet on day 0 (before the experiment) from the weight of cotton pellet on eighth day (at the end of the experiment)

Statistical Analysis

The results were expressed as mean ± SD. Treatment groups were compared using ANOVA with post hoc Dunnett's test using SPSS version 14.0. A 'p' value < 0.05 was considered significant.

RESULTS

In the three models of inflammation, the positive control groups viz aspirin and DA treated groups showed significant anti-inflammatory effect. The inhibition of the inflammatory response was in the range of 21.53 %-59.27% for aspirin and 24.30 % - 41.73% for DA. The anti-inflammatory effect was found to be comparable between the two groups in all the models.

Carrageenan-induced rat paw oedema

The three doses of CP exhibited 8.41%, 15.85 % and 9.91 % inhibition of pedal inflammation in rats respectively (Table 1). CP in the dose of 21.6 ml/kg showed moderate anti-inflammatory activity (% oedema of 132.48 ± 4.99) which was significant ($p < 0.05$) as compared to the vehicle control (157.43 ± 13.59) however the effect of CP at this dose remained comparable to those of standard drugs, aspirin and DA. CP did not produce any significant anti-inflammatory activity at the other two doses tested in this model.

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Table 1: Effect of *Cp* on Carrageenan Induced Rat Paw Oedema

Group (n = 6)	Dose	Paw volume (ml of water) (mean ± SD)		% Paw oedema (Mean ± SD)	% Inhibition of paw oedema
		0 hr	3 hrs		
Distilled Water	5 ml/kg	1.45 ± 0.09	2.29 ± 0.30	157.43 ± 13.59	----
aspirin	500 mg/kg	1.42 ± 0.13	1.74 ± 0.10	123.53 ± 15.09 **	21.53
DA	1.8 ml/kg	1.37 ± 0.11	1.63 ± 0.17	119.18± 14.70***	24.3
*Cp*1	16.2 ml/kg	1.78 ± 0.11	2.56 ± 0.13	144.19 ± 11.54	8.41
*Cp*2	21.6 ml/kg	1.59 ± 0.05	2.11 ± 0.11	132.48 ± 4.99 *,NS	15.85
*Cp*3	27 ml/kg	1.65 ± 0.17	2.31 ± 0.06	141.83± 16.51	9.91

(* p< 0.05, ** p< 0.01, *** p< 0.001 *vs.* vehicle control; NS non-significant *vs.* Aspirin and Dashamoolarishta,)

Acetic acid induced peritonitis

The low (25 ml/kg) and intermediate dose (30 ml/kg) of CP showed significant anti-inflammatory activity with 18.5%, and 27.32% inhibition of peritoneal inflammation compared to vehicle control (Table 2). The anti-inflammatory activity of the intermediate dose group of CP was found to be comparable to the standard control group which received DA but not to aspirin.

Table 2: Effect of *Cp* on Acetic Acid Induced Peritonitis in Mice

Group (n = 6)	Dose	Protein content of exudates (mg/dl) (mean ± SD)	% Inhibition of exudates
Distilled Water	25 ml/kg	630.35 ± 28.95	----
aspirin	722.2mg/kg	256.76 ± 26.73 ***	59.27
DA	2.5 ml/kg	373.77 ± 12.60 ***	40.70
*Cp*1	25 ml/kg	513.77 ± 62.29 **	18.50
*Cp*2	30 ml/kg	458.14 ± 96.41 ***,NS,#	27.32
*Cp*3	35 ml/kg	546.40 ± 65.51	13.32

(* p< 0.05, ** p< 0.01, *** p< 0.001 *vs.* vehicle control, NS non-significant *vs.* Dashamoolarishta, # p< 0.001 *vs.* Aspirin)

Cotton pellet granuloma

There was significant effect in reduction of granuloma formation by CP at the intermediate (21.6 ml/kg) and high dose (27ml/kg) with 50.38 % and 46.83% inhibition respectively (p< 0.01 compared to vehicle control). The extent of reduction in edema with CP appeared to be better as compared to aspirin (40.78%) and DA (41.73%) treated groups although the difference did not reach statistical significance (Table 3).

Although, there was no dose dependent increase in the anti-inflammatory effect of CP, the intermediate dose group showed better response than low and high groups consistently in all the three models.

Table 3: Effect of *Cp* on Cotton Pellet Granuloma Model

Group (n = 6)	Dose	Weight of Cotton pellet granuloma (mg) (mean ± SD)	% Inhibition
Distilled Water	5 ml/kg	197.75 ± 50.28	----
aspirin	500 mg/kg	137.5 ± 19.02*	40.78
DA	1.8 ml/kg	136.1 ± 9.00*	41.73
*Cp*1	16.2 ml/kg	163.63 ± 23.82	23.09
*Cp*2	21.6 ml/kg	123.31 ± 21.37** NS	50.38
*Cp*3	27 ml/kg	128.56 ± 17.09** NS	46.83

(*p< 0.05, ** p< 0.01, *** p< 0.001 *Vs* vehicle control, NS non-significant *vs.* Aspirin and Dashamoolarishta)

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DISCUSSION

The results of the present study demonstrate anti-inflammatory effects of aqueous extract of root bark of CP in different models of acute and chronic inflammation. CP though an ingredient of *Dashmoolarishta*, an anti-inflammatory formulation from Ayurveda, is not indicated in Ayurveda as a single drug formulation for internal use in treatment of inflammatory disorders.

There are various studies reported validating claims put forth in Ayurveda regarding use of CP in practice. For example it has been demonstrated that aqueous extract of bark of CP possessed a nootropic activity in scopolamine and diazepam induced amnesia model in aged and young mice (Joshi H,,Megeri K, 2008). Methanolic extract of roots of CP possessed both antigen specific and non specific immune response and provided an evidence of the immuno modulatory activity (Gokani RH, et al., 2007). Its antifungal activity with stem and leaf extract was studied against major human pathogenic fungi (Rajasekaran A, Ponnusamy K, 2006). Methanolic extract of leaves of CP has shown musculo-stimulant and sedative activity (Murugesan T, et al., 2000). Anti-diarrheal properties of methanolic extract was studied in rodent models (Rani S, et al., 1999). Methanolic and hexane extract of CP was shown to reduce body temperature in rats in yeast provoked elevation of body temperature (Illango K, et al., 2009).

However there is a single report that leaves of CP possess anti-inflammatory activity in model of hind paw edema in rats (Gomathi A, et al., 2008). But the part which is commonly used by Ayurvedic practitioner and manufacturers of ayurvedic formulations is root bark of CP. Hence the present study was carried out, which to our knowledge, first time reported anti-inflammatory activity of root bark of CP. The literature search revealed that Ayurveda recommends root bark of CP instead of whole root for medicinal use (Gogte VM, 2000). Hence it was felt that the activity of CP may be missed if root of CP is used instead of root bark. For comparison as a positive control, we found that formulation containing only CP is not available in the market. DA, the multi-ingredient formulation was the only formulation available containing CP and was chosen as the positive control because it is commonly used.

In carrageenan induced rat paw oedema, the initial phase of inflammation seen at the 1st hour is attributed to the release of histamine, prostaglandins and serotonin (Winter CA, Porter CC,1957).,(Brooks, P.M, Day, R.O,1991) (Nantel, F, et al., 1999) The second accelerating phase of swelling is due to the release of prostaglandin, bradykinin and lysozyme. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal antiinflammatory agent (Furst DE, Ulrich RW, 2009). CP was administered 1 hour before carrageenan but the repeat measuring of paw volume was done after 3 hours. CP was found to prevent paw edema. But as no readings were taken during the 3hours period, it is difficult to conclude whether CP inhibited first phase or second phase. It can be only postulated that CP inhibits the release of some of the mediators of inflammation. As prostaglandins are common to both the phases, it is possible that CP acts by inhibiting prostaglandins like modern anti-inflammatory medicines.

Prostaglandins and other mediators affect vascular permeability. Increase in vascular permeability contributes to shift of plasma, migration of inflammatory cells to the site and exudate formation. To evaluate effect of CP on vascular permeability, the next model of acute inflammation i.e. acetic acid induced peritonitis in mice was used. The amount of protein exudates in the peritoneal fluid was estimated which provided objective measure for anti-inflammatory activity of CP. Maximum anti-inflammatory activity was seen with the intermediate dose which was comparable to DA although it was less than that of aspirin treated group. Thus CP showed a significant anti-inflammatory activity in both the models of acute inflammation which was consistent at the intermediate dose corresponding to 21.6 ml/kg in rats and 30 ml/kg in mice. In order to assess the anti-inflammatory effect of CP against proliferative phase of inflammation in which tissue degeneration and fibrosis is evident (Kumar V, et al., 2004); the widely used cotton pellet granuloma model was employed. The maximum anti-inflammatory activity was seen with intermediate dose (21.6ml/kg) of CP which was comparable to that of aspirin and DA. The results of cotton pellet granuloma test were consistent with the results of acute inflammation models.

During the study we noticed that in spite of using 3 different doses of CP, dose response curve could not be determined. No dose dependent effect was observed for the three doses tested. The aqueous extract of root bark of CP showed consistent anti-inflammatory properties mainly at the intermediate dose in all the models. As discussed earlier, the response at this dose was comparable to the reference drugs in all the models.

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However, in acute model both lower and higher dose failed to exhibit anti-inflammatory effect. In these models only a single dose was given. When administered for a longer time in the model of chronic inflammation, high dose of CP showed an anti-inflammatory response. But response observed with intermediate dose of CP was higher, though not statistically. Repeated administration of CP may be needed to reach the concentration that can exert anti-inflammatory effects as seen in the chronic model. Therefore, it can be inferred that treatment with CP may be beneficial when CP is administered for a longer duration of time for chronic inflammation. However, in this model the plateau in the anti-inflammatory activity is seen when higher dose is used.

The results of the present study thus pointed out that CP exerts anti-inflammatory activity over a very narrow range of doses. Further studies in clinical settings are needed to confirm the dose specificity observed in our study. To conclude, this preliminary study demonstrated for first time the anti-inflammatory activity of aqueous extract of root bark of CP using basic experimental models of acute and chronic inflammation. It would be too early to recommend use of CP alone based on results of one preliminary study. A decision whether to use it alone to replace or substitute *dashamoolarishta* can be taken following further large scale experimental and clinical studies. Further studies can be planned in other models of inflammation like arthritis and also to explore mechanism of action of CP. Nevertheless, the findings of the present study provide the scientific basis for inclusion of CP in *dashamoolarishta.*

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