

PHYTOCHEMICAL, PHARMACODYNAMIC AND ANTI-INFLAMMATORY PROPERTIES OF *PROSOPIS SPICIGERA* STEM BARK

B. R. MADAN, J. L. GODHWANI, A. P. DADHICH,
R. K. SONI, S. K. GHOSAL AND O. P. MAHATMA

Department of Pharmacology, S. P. Medical College, Bikaner

Summary : The water-soluble part of the methanol extract of the bark of *Prosopis spicigera* contained sterols and reducing sugars. It significantly inhibited the inflammatory response in the rat to carrageenin, formaldehyde, 5-HT and croton oil. In the inflammation induced by formaldehyde and 5-HT, its action did not last, unlike that of betamethasone, for 24 hr period which indicates a short duration of action. It lacked analgesic, anticonvulsant and local anaesthetic activity and had no detectable action on the isolated rectus and heart of the frog and rabbit intestine.

Key Words : *Prosopis spicigera* bark extract anti-inflammatory activity carrageenin
croton oil 5-HT inflammation granuloma pouch

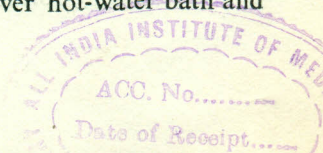
INTRODUCTION

Prosopis spicigera Linn. (N.O. *Leguminosae*, sub-family *Mimoseae*) is a tree which widely occurs in the dry and desert regions of Rajasthan, Haryana, Gujarat, Afghanistan, Baluchistan and Persia (4,10). It is known by different names in different languages—Khejra (Rajasthani), Jhand (Hindi), Jand (Punjabi), Shami (Sanskrit, Bengali and Marathi), Jammi (Telugu) and Kalisam (Tamil). Its unripe pods, known as 'Sangri' in Rajasthani language, are used for preparing curries (9).

Wagbhata (about 300 A.D.) in his classic Ayurvedic treatise described its pod as heavy, hot, dry and injurious to the hair. He repeatedly recommended the smoke of its leaves and seeds for the relief of pain and inflammation of the eyes, anal fissure and piles; further local application of powdered seeds is mentioned for the treatment of inflammatory swellings including tuberculous lymph nodes (16). Its other parts are traditionally used in snake-bite, scorpion-sting, rheumatism and to prevent abortion; its ash is rubbed over skin to remove hair (5). Recently Bhakuni *et al* (1) studied some pharmacological actions of the plant. The present report deals with its anti-inflammatory activity.

MATERIALS AND METHODS

The total bark of the stem of *Prosopis spicigera* tree was collected locally during July and August. It was dried in an incubator for about 48 hr at 45°C and finely pulverized. It was extracted with methanol in Soxhlet apparatus for three days, dried over hot-water bath and



then left overnight in a desiccator. About 6 g of dry extract in the powder form was obtained from 100 g of the bark. The dried methanol extract was mixed with distilled water and filtered. About 25% of the extract could be dissolved. The doses are expressed in term of the dried methanol extract.

The filtrate was analysed for alkaloids, polysaccharides, reducing sugars, tannins, resins and sterols by the tests described by Koch and Hanke (11). The filtrate was found positive only for reducing sugars and sterols; therefore, tests for only these two substances are described.

Test for reducing sugars : One *ml* filtrate was added to 2 *ml* distilled water and 2 *ml* Fehling's solution and was boiled for 3-5 min. Formation of red precipitate indicated the presence of reducing sugars.

Tests for sterols : (i) Hesse's reaction : One *ml* filtrate was mixed with 3 *ml* chloroform. Two *ml* concentrated sulphuric acid was poured from the side of the test tube. Development of red ring at the junction of the two layers indicated the presence of sterols.

(ii) Moleschott's reaction : One *ml* filtrate was mixed with 5 *ml* distilled water. Two *ml* concentrated sulphuric acid was poured from the side of the test tube. Development of greenish red colour changing to violet showed the presence of sterols.

(iii) Liebermann's reaction : One *ml* filtrate was mixed with 2 *ml* acetic anhydride followed by 2 *ml* concentrated sulphuric acid. Bluish colouration turning to violet indicated the presence of sterols.

Anti-inflammatory activity : The anti-inflammatory activity was tested in rats of either sex weighing between 55 and 140 g.

Acute inflammation : This was produced in different groups of rats by injecting underneath the plantar aponeurosis of the hind-paw 0.1 ml of the following phlogistic agents prepared in normal saline : (i) 1% freshly prepared solution of carrageenin (The Copenhagen Pectin Factory Ltd., Denmark) (17); (ii) 3% w/v formaldehyde (B.D.H) (8); and (iii) 0.5% 5-hydroxytryptamine (5-HT) creatinine sulphate (Merck) (8). The volume of each paw was measured plethysmographically as described by Satyavati *et al.* (13). A ten *ml* syringe containing mercury was mounted on an adjustable stand. It was connected to a glass tube fixed on a scale and mounted on another adjustable stand. The ankle joint of the rat was marked with ink before immersing the paw into the syringe. The paw dipped upto the mark on the ankle joint which coincided with a prefixed line on the syringe. The level of the mercury was everytime brought to the level of this line by adjusting the height of the scale and the reading was taken. The reading was noted before and at different intervals after the injection of phlogistic agent. Thus, it was 3 hr after carrageenin and 1½, 4 and 24 hr after formaldehyde or 5-HT. The difference in the reading before and after injection of the phlogistic substance gave the difference in the paw volume. The per cent inhibition of the oedema was calculated by the following formula (7) :

$$\% \text{ anti-inflammatory effect} = \left(1 - \frac{T}{C}\right) \times 100,$$

where T and C are the mean volumes of oedema in the extract-treated and control groups respectively.

Chronic inflammation: Granuloma pouch technique of Selye (14) was used. 20 ml of air followed by 0.5 ml of 2% v/v croton oil in groundnut oil was injected into the loose connective tissue between the shoulder blades. On the 8th day, the animals were anaesthetized with ether and the pouch was opened. The volume of exudate in the pouch was measured and the dissected pouch and adrenal glands were weighed.

Conduct of the experiments: For each test-procedure, the animals were divided into 3 to 4 groups, each group consisting of 6 to 8 rats. The first group served as control and received no drug. The second and the third groups received intramuscular injection of 0.05 mg/100 g of betamethasone and 100 mg/100 g of *Prosopis spicigera* respectively. In some tests, another dose of *Prosopis spicigera* (200 mg/100 g) was also tried and this comprised the fourth group. While the drugs were given 30 min before the phlogistic agent in experiments of acute inflammation, they were administered daily for 7 days in the granuloma pouch experiments. The volume of the vehicle injected did not exceed 0.5 ml/100 g.

The results were analysed statistically using Student's 't' test.

Prosopis spicigera was also tested for: (i) analgesic activity by the rat tail method (6); (ii) anticonvulsant activity against maximal electroshock seizures in rats (15); (iii) surface anaesthetic activity by the rabbit corneal method (12) and (iv) conduction anaesthesia by rat tail-pinch method (2). The extract was given intramuscularly in doses of 100 and 200 mg/100g 30 min before the test for analgesic and anticonvulsant properties. Concentrations of the extract for local anaesthetic activity were 1% (pH 4) and 5% (pH 5.5).

Further, the extract was tested on the isolated rabbit intestine (1 and 3 mg/ml of the bath fluid), frog rectus abdominis preparation (1, 3 and 10 mg/ml of the bath fluid) and perfused frog heart (1 and 3 mg.) The techniques described by Burn (3) were used.

RESULTS AND DISCUSSION

In the present work the experimental techniques have been selected to represent acute inflammatory exudate formation (carrageenin-induced oedema), early proliferative phase (formaldehyde-induced oedema; 7) and chronic proliferative phase of inflammation (granuloma pouch technique). 5-HT-induced oedema method was also included since 5-HT is considered to be involved in the inflammatory reactions.

Prosopis spicigera in doses of 100 and 200 mg/100 g shared the efficacy of betamethasone, 0.05 mg/100 g, in significantly reducing carrageenin-induced inflammation (Table I) and in inhibiting the exudative and granulation tissue response to croton oil as well as in decreasing the weights of adrenal glands (Table II). In formaldehyde-induced-inflammation, the extract was

TABLE I: *P. spicigera* extract in inflammation induced by carrageenin, formaldehyde and 5-HT in rat paw

Compound	Dose mg/100g	After 1½ hr.		% inhibition of oedema	After 4 hr		% inhibition of oedema	After 24 hr		% inhibition of oedema
		Mean paw volume (ml ± S.E.)			Mean paw volume (ml ± S.E.)			Mean paw volume (ml ± S.E.)		
		Control	Treated		Control	Treated		Control	Treated	
<i>*Carrageenin-induced paw oedema</i>										
Extract	100	2.5 ± 0.1	0.7 ± 0.2	72 ^b						
Extract	200	2.5 ± 0.1	0.5 ± 0.08	80 ^b						
Betamethasone	0.05	2.5 ± 0.1	0.9 ± 0.1	64 ^b						
<i>Formaldehyde-induced paw oedema</i>										
Extract	100	2.6 ± .14	1.8 ± .3	30 ^d	4.6 ± .5	2.8 ± .3	39 ^c	5.6 ± .9	5.5 ± .8	2 ^e
Extract	200	2.6 ± .14	1.8 ± .3	30 ^d	4.6 ± .5	2.2 ± .28	52 ^c	5.6 ± .9	4.6 ± .51	8 ^e
Betamethasone	0.05	2.6 ± .14	1.5 ± .3	42 ^c	4.6 ± .5	2.1 ± .5	54 ^c	6.0 ± .9	4.2 ± .7	24 ^d
<i>5HT—induced paw oedema</i>										
Extract	100	6.25 ± .25	3.96 ± .31	36 ^b	5.5 ± .4	3.2 ± .07	40 ^b	1.16 ± .16	3.06 ± .36	72 ^a
Extract	200	6.24 ± .25	5.04 ± .54	19 ^e	5.5 ± .4	3.2 ± .43	41 ^b	1.16 ± .16	2.08 ± .54	79 ^a
Betamethasone	0.05	4.6 ± .3	1.9 ± .7	59 ^b	2.5 ± .1	1.0 ± .3	60 ^b	.24 ± .09	0.04 ± .03	83 ^b

*measured 3 hr after carrageenin injection.

a— indicates increase in oedema

c— P less than .01.

e— P more than .05.

b— P less than .001

d— P less than .05.

TABLE II : *P. spicigera* extract on croton oil-induced granuloma pouch and adrenal glands weight of the rat.

Compound	Dose mg/100g	Exaduate volume (ml \pm S.E.)			Granulation tissue wt (g \pm S.E.)			Adrenal gland wt (mg \pm S.E.)		%inhibition
		Control	Treated	%inhibition	Control	Treated	%inhibition	Control	Treated	
Extract	100	0.84 \pm .1	0.4 \pm .08	52 ^a	1.85 \pm .1	1.10 \pm .15	40 ^a	14 \pm 1.0	10 \pm 1.0	28 ^c
Betamethasone	0.05	1.57 \pm .12	0.3 \pm .06	81 ^a	2.62 \pm .2	0.94 \pm .10	64 ^a	20 \pm .7	6.5 \pm 0.7	67 ^b

a— P less than .01

b— P less than .001

c— P less than .05

effective at both the dose-levels and after 1½ and 4 hr but not after 24 hr (Table 1). In 5-H-T induced oedema, 100 mg/100 g of the extract was effective after 1½ and 4 hr but 200 mg/100 g was effective only at 4 hr (Table I). The anti-inflammatory effect of the extract did not persist for 24 hr indicating a short duration of action. On the other hand, betamethasone (0.1 mg/100 g) could check the inflammatory response to formaldehyde and 5-HT over the 24 hr period.

Prosopis spicigera extract did not exhibit analgesic, local anaesthetic or anticonvulsant activity. It had no apparent action on rabbit intestine, frog rectus and frog heart.

Thus the results obtained indicate that the water-soluble part of methanol extract of *Prosopis spicigera* exhibits anti-inflammatory actions. They appear to be consistent with some clinical uses of this plant mentioned in the Ayurvedic System. Bhakuni *et al.* (1) found that 50% ethanol extract of the stem is not active in several pharmacodynamic experiments and that its LD50 in mice by ip route is over 1 g/kg. It would be of interest to further investigate the anti-inflammatory activity of its purified fractions.

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