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Anti-inflammatory activity of seeds of *Prosopis spicigera* in Animals

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Abstract

Prosopis spicigera Sm. (Leguminasae) is a commonly used plant in Indian traditional medicine. In the current study anti-inflammatory activity of ethanolic extract of *P. spicigera* Seeds was investigated using different animal models. The extract was also subjected to phyto-chemical analysis and their toxic potential. The anti-inflammatory activity was measured using the carrageenan-induced paw edema test. The air dried seeds of *P.spicigera* were soaked in ethanol (1:4, w/v) at the temperature of 60^{0}C for 72 h. The supernatant collected was evaporated under reduced pressure and kept at 5^{0}C until used. The extract was emulsified using 0.1% Tween -80 in normal Saline to prepare the doses of 33, 100 and 300 mg kg^{-1}, before performing each experiment. Anti-inflammatory activity in a dose-dependant manner in all assays used to the extract administered intraperitoneally exhibited significant ($p<0.05$).

INTRODUCTION

Prosopis spicigera Sm. (Leguminosae) is commonly known as Jhand or Shami [1,2,3]. Yadav R. N. et. al. (1999) use this plant for Cardenolide, and it is found basically throughout the India but abundantly in Bundelkhand region of Utter Pradesh (INDIA). It is widely used in the traditional medical practice of people living in India, Lanka and China to treatment of headache, skin diseases, ulcers, uterine trouble along with as a safe guard against miscarriage during pregnancy in women [4], laxative and used as tonic [5], used as a hair removal [6], an emulsion of the bark used as a remedy in rheumatism and scorpion sting [7] current awareness in flavour and fragrance Journal 2007 inter-science widey.com. Earlier some worker [8,9,10] have reported the presence of various biologically active constituents in this plant Jewers K.et.al., phytochemistry (1976).[10,11]

In Bundelkhand region this plant is traditionally used to treat headache, stomach ache and as anti-inflammatory drug [12]. Since its recognition to pain removal as an important chemical entity, various efforts have been made to find a suitable remedial measure, as the drugs used to reduce acid secretion have dominated the pharmacological basis of pain inflammation therapy [13,14]. Keeping in view the frequent folklore use of *P.spicigera*, the present study was carried out to determine the anti-inflammatory activity of *P.spicigera* seeds using animal models.

MATERIALS AND METHOD

Plant material and preparation of its extracts:-

P.spicigera seeds were collected locally from the region of Bundelkhand (U.P.), sun dried and grinded into powder form. The powdered seeds (300g) at 25±3^{0}C and at 80% humidity was mixed with absolute alcohol (1:4, w/v) placed in water bath at 60^{0}C for 72h and then filtered by using whatman no.1 to obtain the supernatant. The collected supernatant was then evaporated up to dryness at 100^{0}C. under reduced pressure and crude dried extract obtain, labeled as EPSS (ethanolic *Prosopis spicegera* supernatant) , was kept at 5^{0}C.

EPSS was emulsified using 0.1% Tween -80 in normal Saline to the doses of 33, 100, 300 mg kg^{-1} body weight for administration into the animals.

Preliminary phytochemical screening:

The alcoholic extract was subjected to preliminary phytochemical investigation for the presence of various phytochemical constituents [15].

Acute oral toxicity studies (LD_{50}):

The acute toxicity of alcoholic extract of *P.spicigera* seeds were determined in wister rat fasted for 3 hour. (which examined that /DEL). The highest oral dose administered was 3g / kg body weight (which was equivalent to powder crude drug 18.95g / kg of body weight). Up to 3g/kg dose levels no signs of toxicity appeared. The LD_{50} of the test extracts were calculated using AOT 425 software [16].

Oral toxicity: Not considered as toxic (DL_{50}oral/Rats > 30g/kg body weight).

Preparation of drugs and chemicals:

Ibuprofen (90mg kg^{-1}) Sigma, USA and acetylsalicylic acid (ASA) (10mg kg^{-1}) Sigma ,USA were used as reference drugs and prepared by dissolving them in distilled water (dH_2O). Other chemicals used were: Absolute alcohol, distilled water (dH_2O), acetylsalicylic acid, Ibuprofen.

Experimental animals:- six animals in each groups

Wister rats (150-180g) were used to study anti-inflammatory activity. All these animals were maintained under standard husbandry protocol and conditions (light/dark period of 12 h light /dark and temperature 25^{0}C ± 3^{0}C) with free access to food and water ad libitum and all experiments were carried out between 10 am to 5 pm daily [17].

Anti-inflammatory activity

Starved rats (48 h), weighing 150-180g having free access to drinking water were placed in separate single-single cages with

Table 1. Experimental groups and treatment given :

Groups (n=5)	Treatment (Dose/Kg, p. o.)
Group 1	33 mg kg^{-1} EPSS
Group 2	100 mg kg^{-1} EPSS
Group 3	300 mg kg^{-1} EPSS
Group 4	100 mg kg^{-1} Ibuprofen
Group 5	Saline

raised bottom in order to avoid cannibalism and coprophagy [18]. The rats were randomly allotted to five groups containing five animals each [17] for the anti-inflammatory activity study and received distilled water (dH_2O), 90mg kg^{-1} Ibuprofen or EPSS (33,100 and 300 mg kg^{-1}), respectively, 1h prior to subjection to the test. All of the test solutions were administered in the volume of 10 ml kg^{-1} body weight.

According to Winter et. al.(1962) each five groups containing five rats received normal Saline, Ibuprofen, EPSS (33,100 and 300 mg kg^{-1}) followed 1 h later by the administration of 0.1 ml of 1 % carrageenan suspension into the rats right hind paw . Paw volume was measured before (Vo) and 1,2,3,4 and 5 h (V_t) following the carrageenan injection using a plethysmometer (Model 7140, Ugo Basile, Italy). The inflammation was measured by volume displaced by the paw between the final volume (V_t) and the initial volume (Vo).

Mean of inflammation score for each animal was expressed by formula given below:

$$\text{Percentage of anti- inflammation} = \frac{\text{(Vt-Vo) controlled - (Vt-Vo) treated}}{\text{(Vt-Vo) controlled}} \times 100$$

Statistical Analysis:

The results are expressed as mean ± SEM. Statistical difference between means were determined by one way ANOVA followed by Dennett's post hoc test was used to analyze and compared data with P>0.05 as the limit of significance. (SEM = Standard Error of Mean).

RESULT

Preliminary phytochemical investigation:

The preliminary phytochemical screening with seeds extract of *Prosopis spicigera* revealed the presence of carbohydrates, amino acids and flavonoids in aqueous extract, alkaloids, flavonoids and tri-terpenoids in alcoholic extract and only steroids in petroleum ether extract[10].

Acute toxicity Study:

Aqueous and alcoholic extracts up to a dose of 5000 mg/kg body wt and petroleum ether Extracts up to a dose of 2500 mg/kg were found to be safe[13].

The anti-inflammatory profile of the ethanolic *P. spicigera* supernatant (EPSS)

All doses the EPSS significantly ($p< 0.05$) inhibited the development of paw edema resulted from the carrageenan administration as shown in table- II . The activity also occurred in a dose- dependent manner and started 1 h or their administration and lasted until the end of the experiment. The 100 mg kg^{-1}. Acetyl

Table 2. Effect of P.spicigera extract (EPSS) on Anti-inflammatory activity by the carrageenan-induced paw edema test in rats.

Treatment Groups (n=5)→ ---------- Mean increase In paw edema± SEM (ml)/Time Interval (h) ?	33mg kg-1 EPSS	100 mg kg-1 EPSS	300 mg kg-1 EPSS	Saline	100mg kg-1 ibuprofen
1 h	0.40±0.025*	0.14±0.025*$	0.12±0.02*	0.56±0.05	0.12±0.05*
2 h	0.38±0.025*$	0.19±0.025*$	0.16±0.02*	0.66±0.05	0.18±0.05*
3 h	0.36±0.03*$	0.23±0.025*$	0.18±0.02*	0.79±0.05	0.15±0.05*
4 h	0.31±0.03*$	0.25±0.025*$	0.16±0.02*$	0.85±0.05	0.13±0.05*
5 h	0.29±0.03*$	0.21±0.025*$	0.15±0.02*	0.83±0.05	0.10±0.05*

The volume of hind paw edema was expressed as mean ± SEM,
*Data differs significantly ($p\leq 0.05$) when compared against the normal Saline-treated group.
$Data differs significantly ($p\leq 0.05$) when compared against ASA-treated group.

salicylic acid (A S A) also shows the pattern of activity.

DISCUSSION

The present study indicated the ability of EPSS to exert anti-inflammatory activity in various animal models. The ability to inhibit chemically and thermally indicates as strong analgesics [19].The abdominal constriction induced by the acetic acid were due to the release of cyclo-Oxygenase-synthesized prostacycline [20]. Which in turn lead to inflammatory pain with in the peritoneal cavity. [13]

The results indicated that *Prosopis spicigera* extracts produced anti-inflammatory effects Possessing antisecretary ,cytoprotective and proton pump inhibition mechanism[15,19].

The present study represented the potential of *Prosopis spicigera* seeds to exert anti-inflammatory activity especially the alcoholic extract.

CONCLUSION

In conclusion, the present study indicated the potential of *Prosopis spicigera* seeds extract has a potential anti-inflammatory activity especially the alcoholic extract and thus justify the folklore uses of the plant in treating pain and inflammation related ailments.

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