The anti-inflammatory and analgesic properties of *prosopis chilenses in rats*

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Abstract

Background: *Prosopis chilensis* is used locally in Sudan for inflammatory conditions of joints; however, literature lacks scientific evidence for anti-inflammatory effect of this plant.

Aims: To evaluate anti-inflammatory and analgesic effects of *prosopis chilenses.*

Material and Methods: Edema inhibition percent (EI %) and hot plate method were used to evaluate anti-inflammatory and analgesic effects of *Prosopis chilenses* in Wistar albino rats. Anti-inflammatory and analgesic effects of *Prosopis chilenses* were compared to indomethacin and acetylsalicylic acid respectively.

Results: Ethanolic extract of *prosopis chilensis* at a dose of 200and 100mg/kg body weight achieved peak EI% (EI% = 96.1%) and (EI% = 94.4%) three and four hours after oral dosing respectively. The maximum EI% for indomethacin was 97.0% and was recorded after 4 hours following oral administration of the drug at a dose of 5 mg/kg body weight. *Prosopis chilensis* extracts at doses of 100 and 200 mg/kg body weight significantly increased the rats' response time to hot plate compared to acetylsalicylic acid at a dose rate of 100mg/kg body weight ($P<0.05$).

Conclusion: The current results suggest potential anti-inflammatory and analgesic effects of *prosopis chilenses.* Relevance of these effects to *prosopis chilenses* phy-to-constituents was discussed.

Keywords: analgesic, anti-inflammatory, *prosopis chilenses,*

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Introduction

For centuries, medicinal plants are the basis for the treatment of various diseases. [1-3] Alternatively, synthetic drugs are dominating the market but element of toxicity that these drugs entail, cannot be ruled out. [4-6] The medicinal properties of *prosopis chilensis* (Mesquite) are well known and utilized as medicinal plant by many tribes throughout the world. [7, 8] Algarrobo (*prosopis chilensis* (Mol) Stuntz) flour is used as protein and dietary fiber source in cookies and fried chips manufacture. [10] *Prosopis pallida* has alpha-glucosidase and angiotensin converting enzyme inhibitory activities which reflect its antidiabetic and antihypertensive potential. [11] The aqueous extract of *prosopis chilensis* has soothing, astringent, and antiseptic properties. [12] In addition, the extract of the *prosopis Africana* stem is used to treat fever while barks extract is used as remedy in rheumatism and scorpion sting. [13] In Sudan, *prosopis chilensis* plants are abundant and sometimes used for inflammatory conditions of joints. However, scientific evidence is lacking apart from few reports on comparable plant. [13] This study aims to evaluate the ant-inflammatory and analgesic properties of *prosopis chilenses.*

Materials and methods

Collection and extraction of plant materials

The aerial part of the plant was collected from Omdurman–Khartoum state -Sudan; after it had been authenticated by taxonomists of Medicinal and Aromatic Plants Research Institute (MAPRI). A sample was deposited at the herbarium. The plant material was then allowed to dry at room temperature for three days. Then the plant material was coarsely powdered, and was extracted according to the method described by Shylesh and Padikkala. [14]

Animals

Sixty Wistar albino rats weighing 90-200 g were purchased, at the time of each experiment, from the animal center of MAPRI – National Center for Research (NCR) – Khartoum - Sudan. All animals had free access to food and water and were kept at room temperature 25±1 ºC, on a 12/12 light/dark cycle. Before each study, animals were submitted to fasting for at least 12 hours.

Evaluation of anti-inflammatory activity

Rat-paw edema model

The anti-inflammatory activity of ethanolic extract was studied using a modified rat paw formalin edema method as described by Abdola*et al.* [15, 16] Twenty rats weighing 90-100g, were randomly divided into four equal groups (N = 5). Group (1) and (2) were orally dosed with 200mg/kg, and 100mg/kg body weight aqueous suspension of the ethanolic extract of *prosopis chilensis*, respectively. Group (3) was orally dosed with indomethacin aqueous suspension at a dose rate of 5 mg/kg body weight. Group (4) was kept as a control group and given normal saline orally at a dose rate of 5 ml/kg body weight. Formalin was injected after 60 minutes of treatment subcutaneously in the plantar region of the right hind paw of each rat in the different experimental groups. The anti-inflammatory effect was determined after measuring the paw's thickness before the formalin injection, and then 1, 2,3,4,6, and 24h post-treatment. [15, 16] The mean response for each group was calculated using percentages of edema formation (EF %) and edema inhibition (EI %) as follows:

$$Edema\ formation\ percentage\ (EF\ \%) = \frac{Tt\_To}{\text{To}} \times 100$$

$$Edema\ inhibition\ percentage\ (EI\ \%) = \frac{EFc\_EFt}{EFt} \times 100$$

Where:

To = the thickness before formalin injection (cm)
Tt = the thickness-hours after formalin injection (cm)
Ec= edema rate of control group
Et= edema rate of treated group

The observations were statistically analyzed using analysis of variance followed by multiple comparisons via SPSS program. [15, 16]

Analgesic activity

The hot plate method was adopted. [15, 16] Twenty rats were randomly divided into four equal groups (N = 5). Group (1) and (2) were orally dosed with 200mg/kg, and 100mg/kg

body weight aqueous suspension of the ethanolic extract of *Prosopis chilensis*, respectively. Group (3) was orally dosed with acetylsalicylic acid (ASA) aqueous suspension at a dose rate of 100 mg/kg body weight as a reference drug, while group (4) was kept as a control group and given normal saline orally at a dose rate of 5 ml/kg body weight. The rats were dropped on a hot plate maintained at 55 ± 0.50°C. The response time (reaction time) was recorded at 10 and 5 minutes before and 60, 90 and 150 minutes after treatment using the Hot plate model 39 - Wagtech International Ltd. - England. The reaction time was defined as the interval from the instant the animal reached the hot plate until the moment the animal licked its feet or jumped out. Statistical analysis was determined using ANOVA followed by Dunnett's test for multiple comparisons. [15, 16]

Results

Administration of the aqueous suspension of the ethanolic extract of *prosopis chilensis* at a dose of 200 and 100mg/kg body weight achieved peak EI% three (EI% = 96.1%) and four (EI% = 94.4%) hours after oral dosing (table 1 and figure 1). Alternatively, the maximum EI% for indomethacin was 97.0% and was recorded after 4 hours following oral administration of the drug at a dose of 5 mg/kg body weight (table 1 and figure 1).

Table 2 shows analgesic activity of *prosopis chilensis* ethanolic extract and acetylsalicylic acid compared with normal saline at different time intervals. The ethanolic extracts of *prosopis chilensis* at doses of 100 and 200 mg/kg body weight significantly increased the rats' response time to hot plate compared to acetylsalicylic acid at a dose rate of 100mg/kg body weight ($P<0.05$). The peak response was recorded after 90 minutes of the oral administration of all types of treatments offered to the rats (table 2 and figure 2).

Table 1. Effects of ethanolic extract of *prosopis chilensis* on rat paws edema-formation inhibition percentage (EI %) and mean paw thickness (MPT) at different time intervals.

Extract or drug mg/kg		Time interval						
		1 Hour	2 Hours	3 Hours	4 Hours	6 Hours	24 Hours	Mean
Prosopis chilensis extract (200 mg/kg body weight)	EI %	52.9	86.5	96.1	88.1	64.9	94.2	95.1
	MPT (mm)	5.69±0.2	5.32±0.5	5.47±0.5	5.04±0.1	5.31±0.4	5.16±.0.4	5.31±0.4
Prosopis chilensis extract (100 mg/kg body weight)	EI %	60.6	69.2	71.9	94.4	69.5	80.4	71.8
	MPT (mm)	6.9±0.1	6.4±0.3	6.2±0.5	6.0±0.2	6.15±0.4	5.96±0.3	6.2±0.5
Indomethacin 5mg/kg	EI %	24.2	32.2	50.9	97.0	83.4	71.4	64.0
	MPT (mm)	8.3±0.6	7.6±0.4	6.7±0.5	5.6±0.3	5.9±0.3	6.1±0.2	6.5±1.1
Normal saline	EI %	9.0±0.7	8.5±0.4	7.7±0.1	8.2±0.4	7.7±0.3	7.2±0.2	7.68±1.2

Table 2. Analgesic activity of *prosopis chilensis* ethanolic extract and acetylsalicylic acid compared with normal saline at different time intervals.

Extract/drug	Mean response time (Seconds) of rats to hot plate /time interval					Response time mean±SEM
	10 minutes before treatment	5 minutes before treatment	60minutes after treatment	90 minutes after treatment	150minutes after treatment	
Prosopis chilensis (200 mg/kg body weight)	7.32	7.61	22.83	18.71	9.75	13.5±7.1
Prosopis chilensis (100 mg/kg body weight)	8.03	8.42	18.58	15.44	10.15	12.1± 5.2
Acetylsalicylic acid	8.26	7.61	12.49	11.61	9.26	9.8± 2.2
Normal saline	7.18	7.72	5.00	5.74	6.70	6.5± 1.3

Figure 1. Estimated marginal means of rat paw thickness in different studied groups at different time intervals.

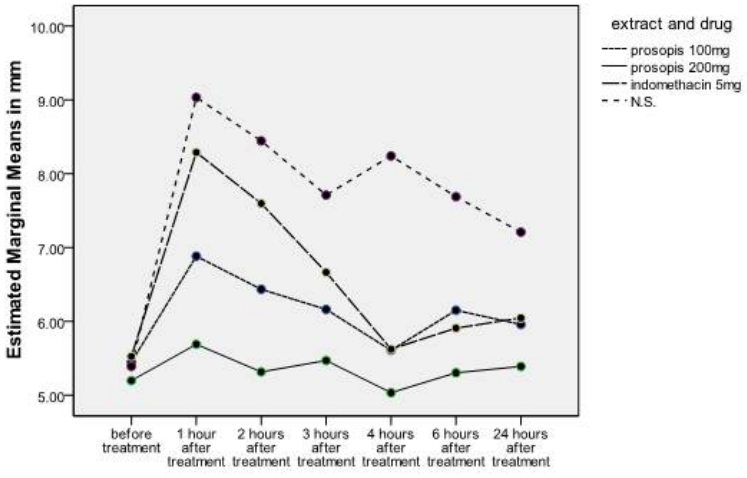
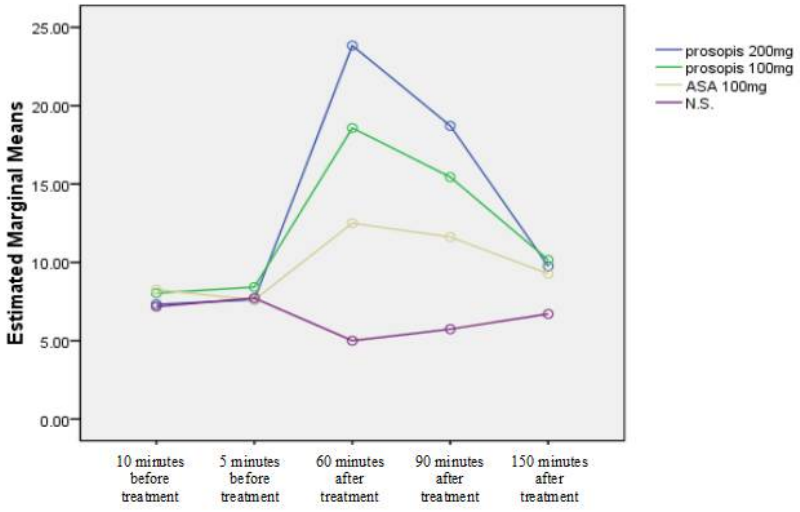


Figure 2. Estimated marginal means of response time (Seconds) of different studied groups to hot plate at different time intervals.



Discussion

According to the findings of this study, *prosopis chilensis* extract exhibits effective anti-inflammatory and analgesic activities. In contrast to analgesic action of *prosopis chilensis*, the anti-inflammatory property of this plant seems to be dose related. Both ethanolic extract of *prosopis chilensis* and indomethacin achieved EI % more than 90% at the same time interval following oral administration of the same dose rate i.e. 100 mg/kg body weight. Increasing the oral dose of ethanolic extract of *prosopis chilensis* to 200 mg/kg body weight achieved EI % comparable to indomethacin at a dose of 5 mg/kg body weight but with earlier onset of action. Although ethanolic extracts of *prosopis chilensis* significantly increased the rats' response time to hot plate compared to acetylsalicylic acid, the peak response was recorded after the same time interval in all types of treatments offered to the rats.

Prosopis chilensis, as many mesquite species, contains flavonoids, the seeds contain saponins, the bark contains tannins, and the leaves contain alkaloids. [13] The anti-inflammatory activity of many plants has been attributed to their sterol and flavonoid contents. [17] Flavonoids like gossypin, [18] isolated from the flowers of *Hibiscus vilfolius*, and hypolactic-8-glucoside, isolated from the aerial part of *Sideritis mugronensis* [19] have been shown to possess anti-inflammatory activity. The flavonoid fraction of Cathaedulis was also reported to produce significant anti-inflammatory and anti-ulcer activities. [20] At the beginning of the current decade, Ayanwuyi and Yaro investigated analgesic and anti-inflammatory effects of *prosopis Africana* extract using acetic acid-induced writhing assay and carrageenan-induced inflammation in rats. [13] Similar results of *prosopis chilensis* in the present study, were reported for *prosopis Africana* extract, which showed significant anti-inflammatory activity after three hours of oral dosing. Ayanwuyi and Yaro preliminary phytochemical screening revealed that the presence of flavonoids, saponins, cardiac glycosides, tannins and alkaloids in *prosopis Africana* may explain its therapeutic potentials.

Pain due to inflammation is usually alleviated by non-narcotic anti-inflammatory analgesics, which interfere with release of hyper-algesic inflammatory mediators. [21] Centrally acting drugs such as opoids inhibit both neuropathic and inflammatory phases equally. [22] Previous reports on analgesic activity of *prosopis chilensis* are deficient; however, a recent investigation conducted by Muzammil *et al* used tail immersion test to assess analgesic effects of a similar plant named *prosopis cineraria.* [23] Tail immersion test consists of a thermal stimulus and a response time used to evaluate central anti-

nociceptive activity. According to Muzammil *et al*, the inhibitory effect of *prosopis cineraria* extract needs 30 minutes to two hours to be initiated, which is comparable to the maximum analgesic response time for rats treated with *prosopis chilensis* in the present study. However, further researches are required to explain weather analgesic action of *prosopis chilensis* is due to central inhibition of pain or modification of hyper-algesic inflammatory mediator's release.

In conclusion, the present study confirms anti-inflammatory and analgesic therapeutic potentials of *prosopis chilensis*. The anti-inflammatory action of this plant was proved to be comparable to the indomethacin, although maximum anti-inflammatory effect of *prosopis chilensis* extract may be achieved earlier at a dose rate of 200 mg/kg body weight. Alternatively, ethanolic extracts of *prosopis chilensis* has significantly increased analgesic effect compared to acetylsalicylic acid, however, the time needed for maximum analgesia was comparable in both types of treatments. Further studies are needed to detect other mechanisms for anti-inflammatory and analgesic effects of *prosopis chilensis* together with potential side effects of this plant.

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