ORIGINAL ARTICLE

# Anti-inflammatory and analgesic activity of *Balanites aegyptiaca* in experimental animal models

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The anti-inflammatory and analgesic effect of ethanolic and petroleum ether extracts of *Balanites aegyptiaca* were evaluated in experimental animals. We have determined the anti-inflammatory and analgesic activity of ethanolic and petroleum ether extracts of dried aerial parts of *Balanites aegyptiaca* by oral administration at doses of 300 and 600 mg/kg/day of body weight to healthy animals. The extracts were studied for their anti-inflammatory activity in carrageenan-induced hind paw edema in rats and the paw volume was measured plethysmometrically at 0 and 3h after injection. The ethanolic and petroleum ether extracts were also evaluated for analgesic activity using Eddy's hot plate method and tail-flick method in albino rats. The ethanolic and petroleum ether extracts of *Balanites aegyptiaca*, significantly ($P$<0.05) reduced carrageenan-induced paw edema in rats and analgesic activity evidenced by increase in the reaction time by Eddy's hot plate method and tail-flick method in albino mice. The ethanolic and petroleum ether extracts showed a greater anti-inflammatory and analgesic effect comparative to the standard drugs, indomethacin and diclofenac sodium respectively. The present results indicated the ethanolic extract of *Balanites aegyptiaca* exhibited more significant activity than petroleum ether in the treatment of pain and inflammation.

**Key words:** *Balanites aegyptiaca*, indomethacin, inflammation, pain, paw edema

## INTRODUCTION

*Balanites aegyptiaca* Del., also known as 'desert date' in English, a member of the family *Zygophyllaceae*, is the one of the most common but neglected wild plant species of the dry land areas of Africa and South Asia.[1] The tree can grow to 6-10 meters in height, is highly resistant to stresses such as sandstorms and heat waves, and grows with minimal available moisture. The tree has thick, tough glossy leaves, spiny branches, and a double root system, and produces date-like fruits. The plants grow extensively even when neglected. One estimate is that more than 400,000 tons of Balanites fruit are produced in Sudan alone.[2] It can successfully grow in a marginal sand dune with saline and sewage water. Various parts of the Balanites tree have been used for folk medicines in many regions of Africa and Asia.[1-5] Literature has revealed antifeedent, antidiabetic, molluscicide, antihelminthic, and contraceptive activities in various *Balanites* extracts.[6-10] *Balanites aegyptiaca* is a small evergreen thorny tree found in drier parts of India. The bark, unripe fruits, and leaves of this plant are reported to have anthelmintic, antifertility, purgative and antidysentric properties.[11-13]

Phytochemical investigations on *Balanites aegyptiaca* yielded in the isolation of several classes of secondary metabolites, many of which expressed biological activities such as cumarins, flavonoids and steroidal saponins.[14] The roots and bark of *Balanites aegyptiaca* tree have several steroidal saponins, yamogenin glycosides, were isolated.[6,15] Two furostanol glycosides and 6-methyl-diosgenin were also obtained from the fruits.[16,17] More recently five new steroidal glycosides were isolated from the roots of the plant.[18] The antifeedant active saponins have been isolated from bark.[7] Aqueous suspension of dried fruits of this plant is being used as abortifacient by local herbal healers. The present study was undertaken to find out the possible actions of aerial parts of *Balanites aegyptiaca* for its anti-inflammatory and analgesic activity.

## MATERIALS AND METHODS

Indomethacin, Micro Labs, Bangalore; Carrageenan, Sigma Chemicals; USA and Diclofenac sodium, Apex Labs, Chennai; were used in the experiment. All other chemicals used were of analytical grade.

### Collection of Plant

Aerial parts of *Balanites aegyptiaca* were collected from Kuwari River, Gormi, Bhind, MP, India, in the month of July 2007. The plant was identified with the help of available literature and authenticated by Dr. S. S. Sharma, Professor, Maharana Pratap Agriculture University, Udaipur, India. A voucher specimen was

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deposited in the herbarium of department (No. 1115).

**Preparation of Extracts**

Freshly collected aerial parts of *Balanites aegyptiaca* were collected and shade-dried, powdered, and subjected to soxhlet extraction (500 g) successively with petroleum ether (60- 80°C, 2 L) and ethanol (2 L) separately and kept for seven days to get the crude extracts. The extracts were concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (50-60°C). The petroleum ether extract yield (7.56%) yellow colored mass and ethanol extract (17.24%) gave dark colored extract.

**Toxicity Studies**

The extracts were given at the doses of 300 and 600 mg/kg/day of body weight per day were selected range from 1/6 to 1/15 of $LD_{50}$ based on the preliminary study conducted at our laboratory and data are not shown in this paper.

**Animals**

Wister albino rats (120-200 g) and Swiss albino mice (20-30 g) of either sex supplied from Ravi Chand and Sons, Ahmedabad, India were used. The animals housed under standard laboratory conditions maintained at 25 ± 1°C and under 12 / 12 h light / dark cycle and fed with standard pellet diet (Gold Mohur brand, Lipton India Ltd.) and water ad libitum. Protocol was approved by the Institutional Animal Ethical Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), constituted under the directives of Ministry of Social Justice and Empowerment, Government of India.

**Determination of Anti-inflammatory Activity**

The albino rats of either sex were divided into six groups of six animals each. Group I received 0.2 ml of 2% w/v carboxy methyl cellulose suspension orally for seven days as a control group, Group II received 300 mg/kg body weight of ethanolic extract of *Balanites aegyptiaca* (EEBA-I) orally for seven days, Group III received 600 mg/kg body weight of ethanolic extract of *Balanites aegyptiaca* (EEBA-II) orally for 7 days, Group IV received 300 mg/kg body weight of petroleum ether extract of *Balanites aegyptiaca* (PEEBA-I) orally for seven days, Group V received 600 mg/kg body weight of petroleum ether extract of *Balanites aegyptiaca* (PEEBA-II) orally for seven days and Group VI received 10 mg/kg of body weight of indomethacin intraperitoneally for seven days as a standard drug. Acute inflammation was induced in all groups by injecting 0.1 ml of 1% w/v carrageenan into the sub-plantar region of the right hind paw of rats. On the seventh day, paw volume was measured 1h prior to carrageenan injection using plethysmometer and at 0 and 3h after the carrageenan injection.[19] Mean increase in the paw volume was measured and percentage inhibition was calculated [Table 1].

Percentage of inhibition = 100 (1-Vt / Vc)

Where, Vc= Edema volume in control and Vt= Edema volume in test / standard compound.

**Determination of Analgesic Activity**

*Analgesic activity by tail flick method:* The albino mice were divided into six groups of six animals each. Group I received 0.2 ml of 2% w/v carboxy methyl cellulose suspension orally for seven days as a control group, Group II received 300 mg/kg body weight of ethanolic extract of *Balanites aegyptiaca* orally for seven days, Group III received 600 mg/kg body weight of ethanolic extract of *Balanites aegyptiaca* orally for seven days, Group IV received 300 mg/kg body weight of petroleum ether extract of *Balanites aegyptiaca* orally for seven days, Group V received 600 mg/kg body weight of petroleum ether extract of *Balanites aegyptiaca* orally for seven days and Group VI received 1 mg/kg of body weight of diclofenac sodium intraperitoneally for seven days as a standard drug. The reaction time was recorded using tail flick analgesiometer at 0, 30, 60, 120 and 180 minutes time interval after the drug administration.[20] The temperature was maintained at 50-55°C and data are represented in [Fig. 1].

*Analgesic activity by Eddy's hot plate method:* The method as described by Turner RA, 1965 was adopted.[20] Mice were divided into six groups of six animals each and drug treatments were given as per tail-flick method. Animals were placed on the Eddy's hot plate maintained at 55±1°C. The reaction time in control and treated animals was recorded at 0, 30, 60, 120 and 180 minutes after the treatment and data are represented in [Fig. 2].

**Statistical Analysis**

Results were expressed as Mean ± SEM, statistical

**Table 1: Effect of *Balanites aegyptiaca* on Carrageenan-induced Paw Edema**

| Treatment | Dose (mg/kg) | Mean paw volume in ml | | | | | | Percent inhibition |
|---|---|---|---|---|---|---|---|---|
| | | 0 min. | 15 min. | 30 min. | 60 min. | 120 min. | 180 min | |
| Control | 2% CMC | 0.79±0.04 | 1.05±0.10 | 1.32±0.16 | 1.69±0.12 | 1.77±0.11 | 1.48±0.04 | — |
| EEBA-I | 300 | 0.78±0.11 | 1.17±0.18 | 1.41±0.10 | 1.35±0.10 | 0.83±0.14 | [a]0.71±0.20 | 55.03 |
| EEBA-II | 600 | 0.73±0.12 | 0.83±0.15 | 0.91±0.12 | 0.82±0.16 | 0.67±0.17 | [a]0.51±0.15 | 65.54 |
| PEEBA-I | 300 | 0.77±0.10 | 1.11±0.11 | 1.29±0.15 | 1.62±0.13 | 1.59±0.11 | [a]1.41±0.19 | 04.73 |
| PEEBA-II | 600 | 0.74±0.15 | 1.15±0.18 | 1.41±0.10 | 1.58±0.10 | 1.46±0.14 | [a]1.37±0.12 | 07.43 |
| Indomethacin | 10 | 0.72±0.08 | 0.79±0.10 | 0.85±0.10 | 0.74±0.10 | 0.55±0.17 | [a]0.28±0.04 | 81.08 |

n= 6, Values are expressed as Mean ± SEM, [a]*P < 0.05* when compared with control group

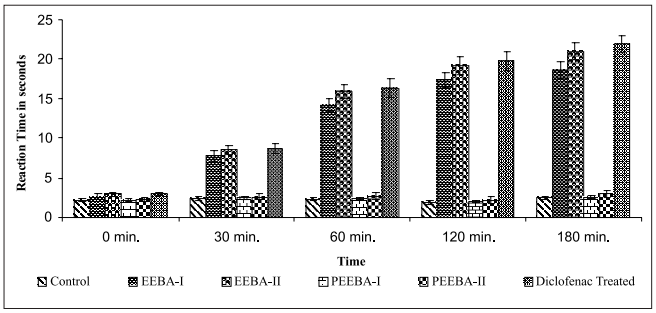


**Figure 1:** Analgesic activity of *Balanites aegyptiaca* by tail-flick method. n= 6, Values are expressed as Mean ± SEM, *P < 0.05* when compared with control group

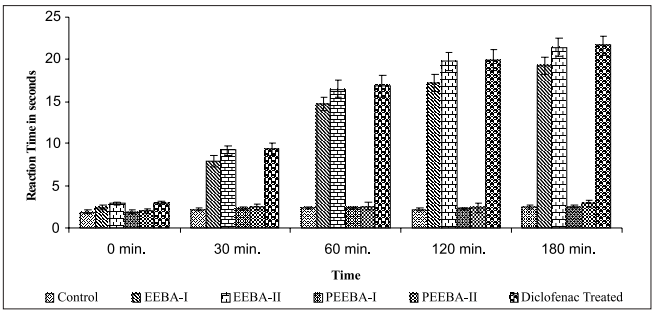


**Figure 2:** Analgesic activity of *Balanites aegyptiaca* by Eddy's hot plate method. n= 6, Values are expressed as Mean ± SEM, *P < 0.05* when compared with control group

significance was calculated by applying *t- test*. *P*<0.05 was considered as significant.

## RESULTS

In the present study, carrageenan-induced Paw edema method shows the result given in Table 1. Ethanolic extract of *Balanites aegyptiaca* at 300 mg/kg body weight per day (EEBA-I) when given orally as a suspension the paw volume were reduced by 55.03% whereas in case of ethanolic extract of *Balanites aegyptiaca* at 600 mg/kg body weight per day (EEBA-II) shows 65.54% inhibition after 3h which indicate that effect of ethanolic extract of *Balanites aegyptiaca* is reflected in dose dependent manner. Both EEBA-I and EEBA-II showed inhibitory effect on carrageenan-induced paw edema thus, exhibiting anti-inflammatory effect against acute inflammation.

In case of petroleum ether extract of *Balanites aegyptiaca* at 300 mg/kg body weight per day (PEEBA-I) reduced the paw volume 04.73% and petroleum ether extract of *Balanites aegyptiaca* at 600 mg/kg body weight per day (PEEBA-II) exhibited 07.43% reduction in paw volume after 3h so petroleum ether extract of *Balanites aegyptiaca* does not possess significant anti-inflammatory activity when compared with control and Indomethacin treated animals [Table 1]. It may be due to absence of flavonoid in the petroleum ether extract.

For the determination of analgesic activity, we used two methods i.e. tail-flick method and Eddy's hot plate method. [Figs. 1 and 2] shows; the analgesic activity profile of EEBA-I and EEBA-II showed significant (*P*<0.05) analgesic activity when compared with control as well as standard drug but in case of PEEBA-I and PEEBA-II don't exhibit significant analgesic activity when compared with control and Diclofenac treated animals. Thus EEBA-I and EEBA-II extracts exhibited marked central analgesic effect as evidenced by significant increase in reaction time when compared to the control.

## DISCUSSION

Inflammation has different phases the first phase is caused by an increase in vascular permeability, the second one by infiltrate of leucocytes and the third one by granuloma formation. We determined anti-inflammatory activity by using inhibition of carrageenan-induced inflammation which is one of the most feasible methods to screen anti-inflammatory agents. The development of carrageenan-induced edema is bi-phasic; the first phase is attributed to the release of histamine, serotonin and kinins and the second phase is related to the release of prostaglandins and bradykinins.[21-25] We observed that EEBA-I and EEBA-II showed significant inhibition against carrageenan-induced paw edema in the dose dependent manner but in case of PEEBA-I and PEEBA-II failed to possess anti-inflammatory effect may be due to absence of flavonoid in the petroleum ether extract.[26] This response tendency of the extract in carrageenan-induced paw edema revealed good peripheral anti-inflammatory properties of the ethanolic extract. This anti-inflammatory effect of EEBA-I and EEBA-II may be due to the presence of flavonoids. It has been reported that a number of flavonoids possess anti-inflammatory[27] and analgesic[28] activities. Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase and reported to produce anti-inflammatory effects.[29] Since, prostaglandins are also involved in the pain perception; inhibition of their synthesis might be the possible reason for the analgesic activity of the ethanolic extract. The presence of flavonoid identified might be responsible for the analgesic and anti-inflammatory activities in ethanolic extract.

Thus, it is concluded that the ethanolic extract of aerial parts of *Balanites aegyptiaca* produces significant analgesic and anti-inflammatory activities in dose dependent manner.

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