

Antinociceptive Activity of the Methanol Extract of *Balanites Aegyptiaca* (Aerial Part) in Experimental Animal Models

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ABSTRACT

Balanites aegyptiaca L. (Zygophyllaceae) has been used in a variety of folk medicines in Malaysia, Bengal, India and Myanmar, for the treatment of various complaints. The tribal people use the aqueous juice of *B. aegyptiaca* for relieving pain. Herein, the fractions and aqueous residue of the methanol extract of the aerial part of *B. aegyptiaca* were evaluated for their antinociceptive activity in mice. Two different animal models, hot-plate method and acetic acid-induced writhing test, were adopted. The present study showed that the butanol fraction of the methanol extract of *B. aegyptiaca*, at the oral doses 100 and 200 mg/kg/day, produced significant ($P < 0.001$) analgesic effects in both the tests compared to control group. No analgesic activities were observed with dichloromethane fraction and the aqueous residue. The observed results provide the scientific basis and safe folk use of this plant in treatment of mild pain, that may possibly be mediated centrally and peripherally.

Keywords - *Balanites aegyptiaca*; Traditional medicine; Pain; Analgesic.

INTRODUCTION

The use of herbal medicine is as old as human civilization. Nature has provided a complete store house of remedies to cure all ailments of mankind. Our knowledge base of medicinal plants has accumulated greatly over thousands of years, as a result of traditional trial-and-error. This has contributed significantly in the treatment and prevention of disease and has helped us improve our health.¹ In recent times, focus on plant research has intensified all over the world and large (quantities) of evidence has been collected to show the potential of medicinal plants used in various traditional systems. Pain in various cases represents the symptoms for the diagnosis of several diseases. It often has a protective function throughout history and man has used several therapies for the management of pain, some being chemically derived and others being of botanical origin such as herbal remedies. A wide variety of medicinal plants with potent analgesic activity are used in folk medicines. Therapeutic herbs are highlighted due to their wide use, and are characterized by fewer side effects.²

Balanites aegyptiaca Linn (family: Zygophyllaceae) is an evergreen thorny tree of tremendous medicinal importance. It is mainly distributed throughout the dry parts of tropical Africa and India.³ In Asia, *B. aegyptiaca* has been used in Ayurveda and in a variety of folk medicines for the treatment of different problems such as a purgative, antifertility, anti-dysenteric, epilepsy and yellow fever.⁴ *B. aegyptiaca* also has insecticidal, anthelmintic⁵ and also contraceptive activities.⁶

The search for safe and effective analgesic drugs through the evaluation of plants has had much backing and funding over the past few years; folk medicine is proving useful in documenting the plants used in its techniques with medicinal values. Hence, the pharmacological activities of *B. aegyptiaca*^{7,8} and other species of the family

Balanaceae⁹ has been the subject of a number of studies, some focusing on the antinociceptive activities, too.^{9,10}

Animal model studies have demonstrated that *B. roxburghii*⁹ has the ability to prevent mice from experiencing peripheral pains. In this regard Gaur and colleagues¹⁰ were able to demonstrate that ethanol and petroleum ether extracts of *B. aegyptiaca* induced in addition to a dose-dependent pain-reducing characteristic, it also had anti-inflammatory effect, both against exudative and proliferative. Keeping this in view, the study aimed to evaluate the analgesic activity of the methanol extract of the aerial part of *B. aegyptiaca* using two well-known different animal models in mice. The Eddy's hot-plate method and the writhing test are useful approach for the screening of potential pain-reliever agents.¹¹

MATERIALS AND METHODS

Animals

Studies were carried out using inbred male Albino mice (22-31g, $n=6$ each group). They were procured from the local animal house of the school of Pharmaceutical Sciences, Universiti Sains Malaysia (USM), Penang, Malaysia. Mice were grouped and housed in cages with no more than 6 animals per cage, and maintained under standard laboratory conditions at temperature ($25 \pm 2^\circ\text{C}$) with dark and light cycle (12:12 hr). They were allowed free access to standard dry pellet diet, and water *ad libitum*. All experimental procedures were reviewed, and approved by the Institutional Animal Ethical Committee (USM).

Plant materials

The plant growing in Penang Island, Malaysia was identified with the help of local flora and authenticated by Dr. A. Shafeen, Taxonomist, Department of Botany, School of Biological Science, Universiti Sains Malaysia. The aerial part of *B. aegyptiaca* (a Voucher number: BAAP-3-1997, deposited at the Herbarium of the same University, director: A.S.) was collected during the last week of March, 1997.

Preparation of methanol extract and fractions for antinociceptive activity

The aerial part of *B. aegyptiaca* was cut into small pieces, washed with water and then air-dried, at room temperature, for a week in the open away from direct sun light. The dried aerial part was powdered in a Wiley mill. The dried powdered materials (2 g) were macerated with 500 ml of methanol in a closed flask and were placed on a mechanical shaker for 24 hr. The liquid extract was filtered and the filtrates were evaporated under reduced pressure (Rotary evaporator, Buchi). Thereafter, the concentrated methanol extract was fractionated with dichloromethane (DCM-BAME) twice followed by 1-butanol (BOH-BAME, twice) to yield fractions of DCM-BAME (12.6%), BOH-BAME (37.8%) respectively. The remaining residue after fractionation of the methanolic extract was aqueous residue (AQR, 49.6%). The obtained fractions were stored at -20°C for subsequent evaluation of their antinociceptive activity.

Drugs and chemicals

The following drugs were used: Pentazocine (Lavoisier, France) and Diclofenac sodium (Pure pharm Ltd. Mumbai, India) were used as reference standards. All the solvents used were of analytical grade. Methanol and 2% w/v gum acacia in distilled water (Sigma Chemical Co., St. Louis, USA) were used as solvent and vehicle respectively. A bent blunted 25G needle connected to a 1 ml syringe was used for the i.p. administrations.

Drug administration

Suspensions of the DCM-BAME and BOH-BAME fractions and AQR were prepared in 2% w/v gum acacia in distilled water. Mice were divided into eight groups each has six animals. The control group received the vehicle (2% w/v gum acacia in distilled water, 0.1 ml/100 g body weight) orally (p.o) for 3 days, whereas the other tested groups received fractions and AQR at doses 100 and 200 mg/kg orally for 3 days. The positive control group were treated with either Pentazocine (10 mg/kg, i.p)⁹ or Diclofenac sodium at dose 1 mg/kg (i.p)¹⁰ for 3 days.

Acute toxicity test

For toxicity studies, the methanol extract of the aerial part of *B. aegyptiaca* was administered orally to different groups of overnight fasted healthy mice ($n=6$ each group) in the range of doses 200 to 2000 mg/kg/day. The behavioral changes and the mortality rates were observed continuously for the first three hr. Thereafter, observations were made at regular intervals for 24 hr. Further, the animals were under investigation up to a period of one week.¹² The methanol extract of *B. aegyptiaca* did not

show any mortality at 2000 mg/Kg (data not shown). Therefore, based on this preliminary study conducted at our laboratory and on others¹³ 2000 mg/Kg dose was considered as LD₅₀ cut off dose (safe dose). Thus, 1/20 and 1/10 of that dose were selected (100 and 200 mg/Kg) for these studies as sub maximal and maximal doses.¹²

Analgesic activities

Acetic acid-induced writhing response and abdominal constrictions in mice

This method was used to evaluate the possible peripheral effects of the methanol extract as an analgesic substance. The writhing acetic acid test (abdominal constriction test) was performed as previously described.¹⁴ Briefly, fractions and AQR at doses 100 and 200 mg/kg, were administered to mice 1hr before i.p. injection of 0.6% (v/v) acetic acid, at a dose of 0.1 ml/100 g body weight. 2% w/v gum acacia in distilled water was used as a negative control while the reference group treated with Diclofenac sodium, a well-known peripheral analgesic drug, as a positive control treatment. Mice ($n=6$ each group) were then placed in an observation box, and the number of writhing (a movement characterized by a wave of contraction of the abdominal musculature followed by an extension of a hind limb) that occurred between 5 and 25 min after acetic acid injection were counted. A reduction in the number of writhing as compared to the control group was considered as evidence for the analgesia, which was expressed as percent inhibition of writhing.¹⁴ The percentage inhibition of writhing was calculated using the following formula:

$$\% \text{ inhibition} = (\text{control mean} - \text{treated mean}) \times 100 / \text{control mean}$$

Thermally-induced pain in mice

The Eddy's hot-plate method was employed to measure the central analgesic activity of the methanol extract of *B. aegyptiaca*. This test was used to measure response latencies according to the method previously described¹⁵ with minor modifications. Briefly, for two consecutive days preceding the experiments mice (6mice/group) were placed kindly on a plate (Model-DS37) maintained at a room temperature for 4 min. each day. On third day each mouse was placed gently on 55±1°C hot plate for a maximum time of 25 sec. Animals were selected 24 hr prior to the experimentation on the basis of their normal reaction time (pain response to the hot plate to the minimum and maximum of 3-8 sec respectively). Latency to exhibit nociceptive responses such as licking forepaws, shaking or jumping off the plate was measured twice before distraction (basal) in 10 min interval (Tb = average basal responses). To avoid damage to the paw latency period of 25 sec was defined as complete analgesia. Latency time was noted at 0 (before) and 30, 60, 120 and 180 min. after the administration of vehicle, reference standard, fractions and AQR. The central analgesic drug, Pentazocine, as a positive control was administered i.p. at a dose of 10 mg/kg. Control mice orally received 2% w/v gum acacia in distilled water, 0.1 ml/100 gm. After 30 min. distraction latency time was measured (Ta = drug latency). For each mouse, the mean latency of nociceptive responses was calculated, permitting us to express the percentage of analgesia using the following formula:

$$\% \text{ of analgesia} = (Ta - Tb) \times 100 / (25 - Tb)$$

Statistical analysis

Data are expressed as a mean $\pm$ S.E.M. The obtained data following administration of each fraction and AQR at each dose and reference drug were compared with the control. The statistical comparisons for significance of difference were made by using one-way analysis of variance (ANOVA). The homogeneity of groups was verified by Tukey's post hoc test at P values of < 0.05 was taken as significant value, using GraphPad Prism (GraphPad Software Inc., version 3.0, San Diego, USA).

RESULTS

Acute toxicity study

In mice, oral administration of the methanolic extract of the aerial part of *B. aegyptiaca* did not induce any overt change in mouse behavior nor any mortality at the highest dose employed indicating that the used doses were found to be safe (data not shown). Two doses (100 and 200 mg/kg/day, p.o) were selected for the evaluation of the analgesic activity.

Analgesic studies

In the present studies the analgesic activity of the methanol extract of the aerial part of *B. aegyptiaca* was assayed in mice against two noxious stimuli (the pain was induced either thermally or chemically). Table 1 and 2 show the antinociceptive activities of the methanol extract of the aerial part of *B. aegyptiaca*.

As shown in Table 1, intraperitoneal injection of acetic acid elicited the writhing syndrome in the control mice with 51.83 ± 0.75 writhes counted in 20 min. The oral administration of the BOH-BAME fraction at the doses 100

and 200 mg/kg significantly reduced the number of acetic acid-induced abdominal constrictions in mice (51.82% and 58.85% protection, respectively; $P < 0.001$, $n=6$ each, Table 1) compared to control group. Furthermore, there was no important difference in the analgesic effects between the two doses ($P = 0.16$), indicating that the analgesic effect of BOH-BAME of *B. aegyptiaca* is not reflected in dose dependent manner. Both DCM-BAME and AQR, at 100 and 200 mg/kg, did not significantly ($P > 0.05$) protect the mice used in this study from writhing response induced by the injection of acetic acid, thus are not exhibiting peripheral analgesic effect against mild pain (Table 1). The reference drug, Diclofenac sodium, at 1 mg/kg produced 78.14% significant protective effect towards the acetic acid induced pain in this nociception model ($P < 0.001$, Table 1).

Using hot-plate method, it was also shown that administration of BOH-BAME (100 and 200 mg/kg) produced significant increase in latency response to thermal noxious stimuli compared to control mice (27.39% and 29.32% protection, respectively, $P < 0.001$, $n=6$ each, Table 2). The maximum effect of the BOH-BAME fraction was observed at the dose 200 mg/kg, at 120 min, which showed a value of 9.98 ± 0.21 seconds. In this model, the standard drug Pentazocine (10 mg/kg) significantly increased the response time of mice to pain by 50.95% ($P < 0.001$, Table 2) compared to control mice. Pentazocine showed maximum effect at 30 min with value of 14.36 ± 0.46 seconds (Table 2). In contrast, DCM-BAME fraction and AQR at 100 and 200 mg/kg in addition they failed to exhibit peripheral analgesic effect was too failed to exhibit central analgesic effect ($P > 0.05$, Table 2).

Table 1: Effects of DCM-BAME, BOH-BAME fractions and the aqueous residue of the methanol extract of the aerial part of *B. aegyptiaca*, and Diclofenac sodium against acetic acid-induced writhing in mice.

Treatment	Dose (mg/kg, p.o) ^a	Writhing ^b	Inhibition (%)
Control	0.1 ml/100 g	51.83 ± 0.75	-
Diclofenac sodium	1, (i.p.)	11.33 ± 0.56	78.14 *
DCM-BAME	100	49.83 ± 0.83	7.72
DCM-BAME	200	49.33 ± 0.76	4.82
BOH-BAME	100	24.97 ± 0.49	51.82 *#
BOH-BAME	200	21.33 ± 0.88	58.85 *#
AQR	100	49.50 ± 0.56	4.50
AQR	200	50.17 ± 1.05	5.79

^aAdministered 30 min before 0.6% v/v acetic acid injection (10 ml/kg, i.p.).

^bCounted for 20 min, starting 5 min after acetic acid injection.

Data are mean (6 mice per group) $\pm$ SEM.

* $p < 0.001$ vs. control, # $P < 0.001$ vs. diclofenac sodium treated group. ANOVA followed by Tukey's test.

Table 2: Effects of DCM-BAME, BOH-BAME fractions and the aqueous residue of the methanol extract of the aerial part of *B. aegyptiaca*, and Pentazocine against thermally induced pain by hot-plate method.

Treatment ^a	Response time (sec.) at				
	0 min	30 min	60 min	120 min	180 min
Control (0.1 ml/100 g)	3.62 ± 0.04	3.27 ± 0.06	3.11 ± 0.03	3.25 ± 0.04	3.39 ± 0.07
Pentazocine (10mg/kg)	3.31 ± 0.04	14.36 ± 0.46 (50.95)*	13.26 ± 0.45 (45.87)*	11.37 ± 0.76 (37.16)*	9.11 ± 1.02 (26.74)*
DCM-BAME (100 mg/kg)	3.72 ± 0.33	3.08 ± 0.17 (-3.01)	3.38 ± 0.17 (-1.60)	3.23 ± 0.10 (-2.30)	3.45 ± 0.24 (-1.27)
DCM-BAME (200 mg/kg)	3.19 ± 0.12	3.41 ± 0.27 (1.01)	3.58 ± 0.29 (1.79)	3.87 ± 0.03 (3.12)	3.08 ± 0.21 (-0.50)
BOH-BAME (100 mg/kg)	3.68 ± 0.21	8.47 ± 0.39 (22.47)*#	9.39 ± 0.26 (26.78)*#	9.52 ± 0.44 (27.39)*†	7.41 ± 0.69 (17.50)*
BOH-BAME (200 mg/kg)	3.75 ± 0.16	8.84 ± 0.43 (23.95)*#	9.86 ± 0.25 (28.75)*#	9.98 ± 0.21 (29.32)*‡	7.52 ± 0.55 (17.74)*
AQR (100 mg/kg)	3.88 ± 0.35	3.26 ± 0.28 (-2.94)	3.56 ± 0.35 (-1.52)	3.45 ± 0.18 (-2.04)	3.59 ± 0.28 (-1.37)
AQR (200 mg/kg)	3.36 ± 0.24	3.44 ± 0.33 (0.37)	3.63 ± 0.34 (1.25)	3.73 ± 0.39 (1.71)	3.26 ± 0.14 (-0.46)

^aAdministered 30 min before thermal stimuli at 55±1°C.

Data are expressed as mean response time ± S.E.M of six mice in each group. Percentages of analgesia are in parentheses. ANOVA followed by Tukey's multiple comparison test. * P<0.001 vs. control group. † P<0.05, ‡ P<0.01, # P<0.001 vs. Pentazocine treated group.

DISCUSSION

Among several traditional claims, the value of the aerial part of *B. aegyptiaca* in pain has been emphasized only in literature. Hence, the present findings might give a scientific authentication to the traditional claims.

In this study, by using two animal models of nociception activity it was found that BOH-BAME fraction of the methanol extract of the aerial part of *B. aegyptiaca* was effective in producing significant analgesic activity. The antinociceptive tests used herein involved both thermal stimulus (hot-plate) and chemical visceral nociceptive stimuli (acetic acid). These types of pharmacological studies were respectively used to evaluate the central and peripheral antinociceptive activities. It has been indicated that it is essential to use more than one test to confirm the analgesic activity, as it has been shown that some false-positive activity can be observed with agents that are not normally considered as analgesics.¹⁶

The present study showed that BOH-BAME fraction has protective effects against the nociception to the injected acetic acid-induced writhes and to thermal pain stimuli, the most feasible methods to screen analgesic agents. The increase in the response time of the mice on the hot plate following administration of BOH-BAME indicated good central analgesic properties. However, the suppression of writhing by using BOH-BAME suggests peripherally mediated analgesic activity based on the association of the model with stimulation of peripheral receptors especially the local peritoneal receptors at the surface of

cells lining the peritoneal cavity.^{17,18} Nonetheless, since opioid receptors are also present in the periphery^{19,20}, the possibility of the extracts acting on peripheral sites to cause anti-nociceptive effects has been partially ruled out. Therefore, it can be inferred that the extract may act *via* central and peripheral mechanisms.

These findings are in agreement with the observations by others¹⁰ who did evaluate the pain-reducing activities of the family *Zygophyllaceae*. Studies by Gaur et al.,¹⁰ have shown that the ethanol and the petroleum ether extracts of *B. aegyptiaca* displayed greater analgesic effects in rats against mechanical and thermal stimulation, indicating the induction of central analgesic effects. Whereas, the studies by Dubey et al.,²¹ on nociception, have showed that the analgesic effects for the *B. aegyptiaca* in mice treated intragastrically with methanol and butanol extracts were induced peripherally. Nonetheless, the similarity in mechanisms of actions seen in this study and others^{10, 21} indicated that the genus *Balanites* exhibited antinociceptive activities and these analgesic effects are species and extract-form independent. Furthermore, it might be suggestive for the presence of several pharmacologically active compounds.

Further research would be of interest to investigate and to explain the exact pathway that may be involved in the pharmacological mechanism of this analgesic effect. On this basis, our present findings indicated that, the extract evaluated has analgesic effect arising from both CNS and

peripheral actions since it showed significant effects on both thermal and chemical pain stimuli. Such an efficacy on these two stimuli is characteristic of centrally acting analgesics, such as morphine, which inhibits inflammatory and non-inflammatory pains.²² It has been reported that a number of flavonoids²³ and saponins^{21,24} possess analgesic and anti-inflammatory activities. Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase and reported to produce anti-inflammatory effects.²³ Similarly, saponins have been shown to inhibit prostaglandin synthesis.²⁵ Accordingly, since, prostaglandins are involved in the pain perception, inhibition of their synthesis might be the possible reason for the analgesic activity of the BOH-BAME fraction. Therefore, since several studies have shown that the broad spectrum of bioactivity of this plant has usually been ascribed to the components of its flavonoids²⁶ and saponins²¹ it seems likely that the *B. aegyptiaca* analgesic properties, observed in the present mice study, attributed to one of the/these active constituents.

Thus, as far as the analgesic effects are concerned our findings have highlighted the analgesic activities of *B. aegyptiaca* as a potential natural pain-reducer compound for mild pain use.

CONCLUSION

From the above studies it can be concluded that the methanol extract of the aerial part of *B. aegyptiaca* possess promising analgesic properties. This effect may be beneficial for the management of several unpleasant health condition, pain included.

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