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Anti-inflammatory, anti-nociceptive and antioxidant activities of *Balanites aegyptiaca* (L.) Delile

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Abstract

The anti-inflammatory and anti-nociceptive activities of methanol (ME), butanol (BE) extracts and of two new saponins isolated from *Balanites aegyptiaca* bark were evaluated. The study was carried out in vivo and in vitro. The samples, extracts and pure substances, were intra-gastrically administered to animals. Two different animal models, the carrageenin-induced edema, in the rat, and acetic acid-induced writhing test in mice, were adopted.

Moreover, the antioxidant power of extracts, fractions and individual constituents from *Balanites aegyptiaca* has been evaluated in vitro, using a method based on the Briggs–Rauscher (BR) oscillating reaction.

Results obtained demonstrate that both ME or BE have a significant effect at the highest dose on the number of abdominal writhes induced by acetic acid, with a 38 and 54% inhibition respectively, but no significant difference was observed for extracts at the lowest dose and for the pure compounds compared with control animals. The same extracts exhibit a significant reduction on the rat paw edema. The inhibition produced by ME is about the same (28 ± 3% lowest dose, 32 ± 3% highest dose) after administration. A more evident effect is obtained by BE (41 ± 3% and 68 ± 6% respectively) and single saponins B1 and B2 (62 ± 5% and 59 ± 6% respectively) after oral administration. The antioxidant activity obtained seems to be in good accordance with the pharmacological results. The histological sections of rat paw confirm the antiflogistic activity of the plant extracts.

Keywords: *Balanites aegyptiaca* extracts; Saponins; Anti-inflammatory effects; Anti-nociceptive effects; Antioxidant activity

1. Introduction

Balanites aegyptiaca (L.) Delile (Zygophyllaceae) is a tropical plant used in East Africa as a component of several primitive medicinal remedies (Liu and Nakanishi, 1982; Mohamed, 1999) widely distributed along the tropical belt of Africa. The tree has many folk uses in various African countries and it is largely used as component of many popular preparations for its abortive, antiseptic, anti-malarial, anti-syphilitic and anti-viral (*Herpes zoster*) activity (Duke, 1983; Kokwano, 1976); the fruits are commonly used to purge, to remove intestinal parasites and sometimes to treat *Schistosomum japonicum* (Koko et al., 2000); the bark aqueous extract is traditionally used as anti-jaundice, while the one of fruit mesocarps is administered as oral hypoglycemic (Kamel, 1991) and seems to be effective against the *Fasciola gigantica* (Koko et al., 2000).

Phytochemical investigations on *Balanites aegyptiaca* yielded in the isolation of several classes of secondary metabolites, many of which expressed biological activities

Abbreviations: ME, methanolic extract; BE, butanolic extract; B1, Balanin 1; B2, Balanin 2; BR, Briggs–Rauscher reaction; TEAC, trolox equivalent antioxidant capacity

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such as cumarins, flavonoids and steroidal saponins (Sarker et al., 2000). From the roots and bark of *Balanites aegyptiaca* tree, several steroidal saponins, yamogenin glycosides, were isolated (Liu and Nakanishi, 1982; Pettit et al., 1991). Two furostanol glycosides and 6-methyl-diosgenin were also obtained from the fruits (Hosny et al., 1992; Kamel, 1998).

More recently five new steroidal glycosides were isolated from the roots of the plant (Farid et al., 2002).

The aim of this work was to evaluate the anti-inflammatory and analgesic activity of methanol (ME), butanol (BE) extracts and the new saponins, Balanins 1 and 2 isolated from *Balanites aegyptiaca* barks. The in vivo study was carried out using two different animal models: the carragenin-induced edema, in the rat, and acetic acid-induced writhing test in mice. The extracts, suspended in carboxymethylcellulose, were intra-gastrically administered to animals at 200 and 400 mg/kg. The animals were fasted 12 h before the experiment. As previously reported (Cook et al., 1998) a lot of plants used in traditional medicine are endowed with antioxidant power of some constituents. Therefore seemed interesting to evaluate also in vitro antioxidant capacity of *Balanites aegyptiaca* extracts and single constituents using the Briggs–Rauscher oscillating reaction method (Cervellati et al., 2001).

2. Material and methods

2.1. Plant material and isolation of the compounds

The bark of *Balanites aegyptiaca* (L.) Delile was kindly gifted from Dr. M. Shayoub of Pharmaceutics Department of Khartoum University (Sudan) and a voucher specimen was deposited at the same Department.

Powdered bark (55 g) was extracted with methanol at room temperature for 4 h (ME). The residue obtained after solvent removal under vacuum (7 g; 12.75%) was suspended in methanol/water (1:9) and defatted with petroleum ether. The hydroalcoholic mixture was then extracted three times with butanol (50 mL). The butanol extracts were combined and after solvent removal a residue (5 g) was obtained (BE).

Two grams of extract BE were chromatographed on silica gel 60 column (100 g, particle size: 0.015–0.040 mm) using chloroform/methanol/water in different ratios (10:5:1; 10:6:1 v/v). Fractions were collected on the basis of their chromatographic behaviour in eight groups (F1–F8).

Further chromatographic steps on silica gel plates (Merck cat.5715) yielded in the isolation of compounds 1 and 2 from F6 and F7 respectively and of three known phenolic compounds, vanillin, vanillic acid and *N-trans*-feruloyltyramine from F2.

2.2. Animal procedures

Male Sprague–Dawley rats (Harlan, Italy), weighting 160–190 g, were housed under controlled conditions, 12 h light:12 h dark cycle, $22 \pm 2\,^{\circ}C$, $55 \pm 5\%$ humidity. They were divided into groups of three and kept in plastic cages. Food and water were supplied ad libitum. The animals were fasted for the night before the experiment.

Male mice (Harlan, Italy), weighting 20–26 g, were housed under controlled conditions, 12 h light:12 h dark cycle, $21 \pm 2\,^{\circ}C$, $55 \pm 5\%$ humidity. They were divided into groups of five or six and kept in plastic cages. Food and water were supplied ad libitum.

Procedure and animal comfort were controlled by the University Veterinary Service of Bologna.

2.3. Anti-nociceptive test

The response to intra-peritoneal injection of 1% acetic acid, i.e. contraction of the abdominal muscle and elongation of the hind limbs, was induced by the method proposed by Koster et al. (1959). The mice (groups of six) were pre-treated intra-gastrically with morphine (7.5 mg/kg), the extracts suspended in carboxymethylcellulose (200 and 400 mg/kg), the new Balanins B1 and B2 and carboxymethylcellulose (1 mL/kg). The extracts, the pure compounds, the vehicle and morphine were administered 20 min before the intra-peritoneal injection of 1% acetic acid (10 mL/kg). The number of abdominal writhes was counted over periods of 5 min, starting 5 min after the injection of acetic acid till 20 min (5–10, 10–15, 15–20 min). The anti-nociceptive activity was evaluated in terms of writhes inhibition percentage.

2.4. Anti-inflammatory test

A volume of 50 μl of 3% (w/v) carragenin suspension in physiologic saline solution were injected into the subplantar region of one hind paw of the rat (Winter et al., 1962). The samples at different doses (200, 400 mg/kg for ME and BE while only 200 mg/kg for B1 and B2) were intra-gastrically administered suspending the extracts in carboxymethylcellulose; indomethacin (100 mg/kg) and carboxymethylcellulose (1 mL/kg) were administered 30 min before the carrageenin injection to the rats (five for group).

Paw volume was measured immediately before injection (time 0) and after 0.5, 1.5, 2.5 and 3.5 h using a plethysmometer (Ugo Basile, Como, Italy).

2.5. Antioxidant activity test

Antioxidant activity of extracts, fractions and saponins from *Balanites aegyptiaca* was measured using the chemical in vitro method reported by Cervellati et al. (2001) which is based on the inhibitory effects by ROS scavengers on the oscillations of the Briggs–Rauscher (BR) reaction. The BR system (Briggs and Rauscher, 1973) consists of hydrogen peroxide, acidic iodate, malonic acid,