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In Vivo Antioxidant and Anti-inflammatory Effects of *Balanites aegyptiaca* (L) Del (Balanitaceae) Galls and Leaves

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Abstract: *Balanites aegyptiaca* (L.) Del. (Balanitaceae), a tropical plant is well known for its widespread uses in Burkina Faso traditional medicine. The present study aims to evaluate the anti-inflammatory effect of *B. aegyptiaca* trough the turpentine-induced inflammation. The aqueous acetone extracts of galls and leaves have been used to test the anti-inflammatory effects on adult male Wistar-Bratislava albino rats. Total leukocytes count was performed with an optical microscope (Olympus), using a Bürcker-Türk counting-chamber. The nitric oxides synthesis (NOx), the total oxidative status (TOS) and total antioxidant response (TAR) of the serum were measured using a colorimetric assay. The results have showed that all the extracts reduced significantly ($p<0.0001$) the total leukocytes and total nitrites and nitrates levels in the rats serum. The oxidative stress evaluation showed that the treatment with any dose of *B. aegyptiaca* gall and leaf extracts was significantly decreased ($p<0.0001$) the total oxidative status. Moreover, these extracts have been prevented the Turpentine oil induced inflammation by increasing the total antioxidant response. TAR increasing did correlated to TOS decrease by comparison with the inflammation group. In general, an interesting anti-inflammatory effect was found in this study with the greatest activity found in the gall extracts. *B. aegyptiaca* could then be a potential source of natural antioxidants and anti-inflammatories.

Keywords: *Balanites aegyptiaca*, Anti-inflammatory, Nitric Oxides, Oxidative Status, Burkina Faso

1. Introduction

Balanites aegyptiaca (L.) Del. (*B. aegyptiaca*), also known as 'Desert date' in English is one of the most common but neglected wild plant species of the dry land areas of Africa and South Asia [1]. Different parts of this plant are traditional used in several African folk medicines [2-4].

B. aegyptiaca is well known for its multiple pharmacological properties. Literature has revealed that the barks are anthelmintic [5], the leaves and galls have antibacterial properties [6] and the fruits are effective against *Aedes aegypti* larvae [7, 8]. The antioxidant potentialities of the barks, the galls and the leaves [2, 4, 9], analgesic activity of the leaves [10] and the anti-inflammatory activity of the galls and leaves [6] have been demonstrated *in vitro*. It is also proved that the galls and the leaves of this plant inhibit xanthine oxidase and acetylcholinesterase [4]. Anti-inflammatory activities of the aerial part from *B. aegyptiaca* have been demonstrated using the carrageenin-induced edema in the rat method [11].

To complete these pharmacological data, we undertook to

evaluate the anti-inflammatory effect of *B. aegyptiaca* trough the turpentine-induced inflammation. For that the aqueous acetone extracts of its galls and leaves have been used to test the anti-inflammatory effects on adult male Wistar-Bratislava albino rats.

2. Materials and Methods

2.1. Chemicals

Sulphanilamide (SULF), N-(1-Naphthyl) ethylenediamine dihydrochloride (NEDD), vanadium (III) chloride (VCl_3), methanol, diethylether, xylenol orange [o-cresosulfonphthalein-3,3-bis(sodium methyliminodiacetate)], ortho dianisidine dihydrochloride (3-3'-dimethoxybenzidine), ferrous ammonium sulphate, hydrogen peroxide (H_2O_2), sulphuric acid, hydrochloride acid, glycerol, trichloroacetic acid (TCA) and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). All the chemicals used were analytical grade.

2.2. Plant Materials

The galls and the leaves samples and the botanical identification of *B. aegyptiaca* have been well documented in our previous article [12].

2.3. Extraction

The aqueous acetone extracts were obtained using 50 g of dried and powdered of each sample as previous described by Meda et *al*. [12].

2.4. Experimental Design

The Wistar-Bratislava albino rats weighing 200-250 used for the experiments were bred in the Animal Facility of 'Iuliu Hatieganu' University of Medicine and Pharmacy. The study protocol has been described by Alina et *al*. [13]. Turpentine oil (6 mL/kg BW) was used to induce the inflammation by i.m. injection. The anti-inflammatory control groups treated with meloxicam (3.2 mg/kg BW) and diclofenac (20mg/kg BW) by i.p. injection [13, 14]. One group was tested by i.p. injection with galls sample (250 mg/kg BW) and three groups with leaves samples at a dose of 250, 500 and 750 mg/kg BW, respectively.

2.5. Evaluation of Anti-inflammatory Effect

Different methods were used to assess the anti-inflammatory activity of the galls and the leaves from *B. aegyptiaca*. Total leukocytes count was performed with an optical microscope (Olympus), using a Bürcker-Türk counting-chamber and the Griess reaction was used to indirectly determine NO synthesis (NOx) of the serum using a colorimetric assay [13].

2.6. Evaluation of Antioxidant Effect

The total oxidative status (TOS) and total antioxidant response (TAR) of the serum were measured using a colorimetric assay [13]. The oxidative stress index (OSI) expressed in arbitrary unit is an indicator of the degree of oxidative stress and is calculated by the ratio of the TOS to TAR.

2.7. Statistical Analysis

The data were expressed as Mean ± Standard deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at $p<0.0001$ and linear regression) was carried out with XLSTAT 7.1.

3. Results

3.1. Anti-inflammatory Activity

To assess the anti-inflammatory activities of the *B. aegyptiaca* gall and leaf extracts, the study rats was divided into different groups: control group, inflammatory group and five (05) treated groups respectively with diclofenac, gall extracts (250 mg/kg BW) and leaf extracts (250 mg/kg, 500 mg/kg BW and 750 mg/kg BW). The base-line characteristics presented in Figure 1 show that the turpentine-induced inflammation group had a significantly higher number of total leukocytes count (Figure 1(a)) and higher rate of NOx in the serum (figure 1(b)) compared to the control and treated groups ($p<0.0001$).

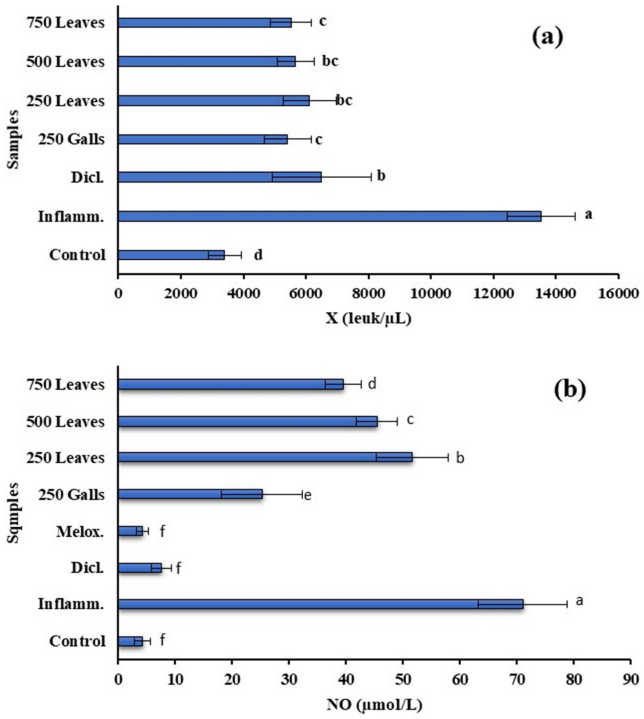

Figure 1. *Anti-inflammatory effect of Balanites aegyptiaca gall and leave extracts.*

(a): Total leukocytes count; (b): Nitric oxides; Inflamm.: Inflammation; Dicl.: Diclofenac; Melox.: Melaoxicam; 250 Galls: Gall extracts (250 mg/kg): 250 Leaves: Leaf extracts (250 mg/kg); 500 Leaves: Leaf extracts (500 mg/kg); 750 Leaves: Leaf extracts (750 mg/kg). Values are mean ± SD (n=7). Different letters indicate significant difference ($p<0.0001$).

After treating with meloxicam, diclofenac, gall extracts (250 mg/kg BW) and leaf extracts (250 mg/kg, 500 mg/kg BW

and 750 mg/kg BW), the number of total leukocytes and rate of NOx were assessed. Diclofenac highly inactivates the proliferation of immune cells ($p<0.0001$) and this inactivation drives significantly to the lowering of NOx rate in the serum ($p<0.0001$). The gall extracts (250 mg/kg BW) and leaf extracts (500 mg/kg BW and 750 mg/kg BW) reduced more the total leukocytes count than the diclofenac ($p<0.0001$). But the inactivation did not drive significantly the decrease of the production of NOx in serum of treated rats. The NOx reduction by the leaf extracts was dose-dependent, with the highest dose showing the best inhibition effect ($p<0.0001$). The gall extracts decreased twice more the NOx than the leaf extracts at a dose of 250 mg/kg BW.

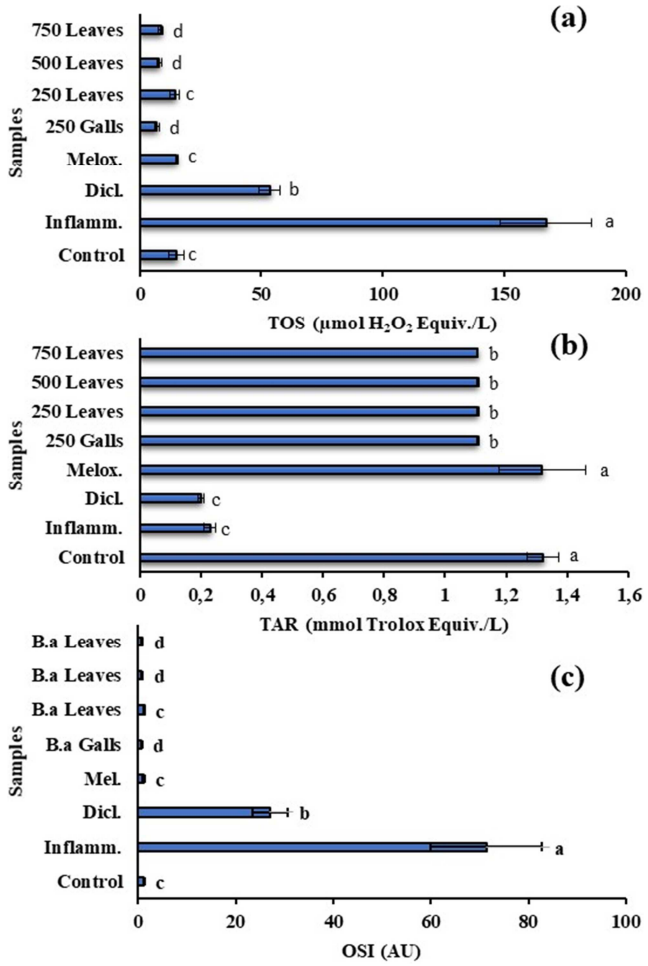

Figure 2. *Antioxidant effect of Balanites aegyptiaca gall and leave extracts.*

(A): Total Oxidative Status (TOS); (B): Total Antioxidant Response (TAR); (C): Oxidative Stress Index (OSI); Inflamm.: Inflammation; Dicl.: Diclofenac; Melox.: Melaoxicam; 250 Galls: Gall extracts (250 mg/kg): 250 Leaves: Leaf extracts (250 mg/kg); 500 Leaves: Leaf extracts (500 mg/kg); 750 Leaves: Leaf extracts (750 mg/kg). Values are mean ± SD (n=7). Different letters indicate significant difference ($p<0.0001$).

3.2. The Oxidative Stress Parameters

The serum TOS level was increased ($p<0.0001$) in the turpentine-induced inflammation group compared to the control group (figure 2 (a)). Importantly, diclofenac and meloxicam were reduced ($p<0.0001$) the TOS level. Meloxicam has reduced more TOS level than diclofenac ($p<0.0001$) and similar to control group ($p>0.0001$). The treatment with any dose of *B. aegyptiaca* gall and leaf extracts was significantly decreased the TOS level ($p<0.0001$). Similar TOS reducing level was found with the leaf extracts (250 mg/kg BW) and meloxicam ($p>0.0001$).

Moreover, the gall extracts (250 mg/kg BW) were more potent reducing than meloxicam ($p<0.0001$). These results also showed that the gall extracts are better than leaf extracts in the total oxidative stress reduction.

Turpentine-induced inflammation reduced the TAR ($p<0.0001$) compared to the control while the meloxicam treatment significantly increased the TAR (figure 2 (b)). Our results demonstrated that this decrease in the TAR has been prevented by the treatment of *B. aegyptiaca* gall and leaf extracts ($p<0.0001$). Diclofenac didn't prevent the turpentine-induced inflammation ($p>0.0001$). TAR increasing did correlated to TOS decrease by comparison with the Turpentine-induced inflammation group.

In the inflammation group, the OSI was significantly elevated ($p<0.0001$) compared to control group (figure 2 (c)). The treatments with meloxicam and leaf extracts (250 mg/kg BW) have presented the same OSI ($p>0.0001$). The gall (250 mg/kg BW) and leaf (500 and 750 mg/kg BW) extracts were most effectives ($p<0.0001$).

4. Discussion

Inflammation is appreciated as general, nonspecific response to tissue injury in many diseases [15]. Neutrophils, macrophages, endothelial, and other cells at the site of inflammation may produce reactive oxygen species (ROS) and reactive nitrogen species, which play a modulating role in the inflammatory response [16]. The present study aims to evaluate not only the anti-inflammatory activities of *B. aegyptiaca* gall and leaf extracts trough the mechanism involving the inhibition of total leukocytes and the reduction of NOx rate in the serum but also the oxidative stress parameters via the measurement of the serum TOS and TAR from each individual rat.

This study showed that *B. aegyptiaca* gall and leaf extracts have significantly anti-inflammatory activities as well as diclofenac and meloxicam in Turpentine-induced inflammation. The total leukocytes count results obtained shown that *B. aegyptiaca* gall and leaf extracts have decreased to half the turpentine-induced inflammation in the rats and have been more effective than the diclofenac used as standard molecule. This reduction of total leukocytes induced to reduction of the nitric oxides levels in inflamed rats. Concerning the evaluation of oxidative stress parameters through TOS and TAR measurements, interesting results were also found. The gall and leaf extracts prevented considerably the turpentine-induced inflammation by reducing the levels of TOS and increasing the TAR in the serum. OSI assesses the global oxidant/antioxidant balance in the living organisms [17]. The TOS decrease and TAR increase after *B. aegyptiaca* gall and leaf extracts treatments reduced more the OSI than the

meloxicam treatment.

The antioxidant and the anti-inflammatory properties of *B. aegyptiaca* extracts have been already study *in vivo*. The fruits extract exhibited a good total antioxidant capacity [18]. The methanol and butanol extracts and isolated saponins from bark [2] as well as the ethanolic and petroleum ether extracts of dried aerial parts [10] reduced significantly the rat paw edema induced by carrageenan. Indeed, turpentine oil is like carrageenan a non-antigenic inflammatory stimulus [13]. This activity could be due to capacity of *B. aegyptiaca* extracts to reduce total leukocytes and NOx levels in the rat serum as demonstrated in our study. The inhibition of NOx synthesis is an important mechanism of anti-inflammatory effect [13, 19]. The latest explanation of antioxidant therapy failure comes from the finding that antioxidants do not inhibit oxidative stress and the associated inflammation at the same time [17]. In this study, the OSI has been well correlated to NOx and leukocytes count levels after treatment of *B. aegyptiaca* gall and leaf extracts proving that oxidative stress and inflammation are interlinked processes.

The antioxidant and anti-inflammatory activities are correlated to the phenolic content. Previous study identified some phenolic acids (gentisic, *p*-coumaric, caffeic, ferulic and sinapic) and flavonoids (hyperoside, isoquercitrin, rutosid, quercitrin, myricetol, quercetol and kaempferol) in *B. aegyptiaca* gall and leaf extracts [12].

Caffeic, *p*-coumaric, ferulic and gentisic acids, isoquercitrine, rutin, myricetin, kaempferol and quercetin are polyphenols with antioxidant and anti-inflammatory properties [20-23]. The treatment of the inflamed rats by turpentine oil using *B. aegyptiaca* galls and leaves could then be explained by the presence of these polyphenols.

Chronic inflammation can last for several months and even years and can eventually cause several diseases such as rheumatoid arthritis, atherosclerosis, asthma, heart disease, ulcerative colitis, and some cancers [24]. *B. aegyptiaca* gall and leaf extracts could then be exploited for the chronic inflammation disorders.

5. Conclusion

Aqueous acetone extracts of *Balanites aegyptiaca* galls and leaves were used to evaluate their effects against the rat turpentine-induced inflammation. The results obtained showed that all the extracts prevented the inflammation by reducing the total leukocytes count and the NOx. The TOS decrease and TAR increase after treatments with plant extracts reduced more the OSI than the meloxicam a standard anti-inflammatory molecule. *B. aegyptiaca* could then be a potential source of natural antioxidants and anti-inflammatories that could have great importance as a therapeutic agent in the prevention of inflammation, cancer, aging, rheumatism and neurodegenerative diseases.

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