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Toxicological profile of *Balanites agyptiaca* in albino Wistar rats

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Abstract

Background: *Balanites aegyptiaca* (BAE) is a thorny tree that grows in most arid and semi-arid areas of Africa, the Middle East and India. It is used in traditional medicine for its cardioprotective, deworming, antihelmintic, anti-infectious and antifungal properties. Excessive consumption of this plant's products can present risks of intoxication for consumers.

Objective: The aim of this study was to assess the acute and subchronic oral toxicity of the aqueous extract of *Balanites aegyptiaca* leaves and roots, to ensure the safety of its consumers.

Method: Acute toxicity assessment was carried out in accordance with the OECD experimental protocol. The plant extract was administered orally to female rats at a unique dose of 2000 mg/kg. Then the animals were observed for 14 days. For the subchronic toxicity assessment, the aqueous extract was administered orally daily to male and female rats at different doses (250, 500 and 1000 mg/kg) for 30 days. After the treatment period, the animals were sacrificed for hematological, biochemical and histopathological analyses.

Results: The results of the acute oral toxicity test showed that the aqueous extract of *Balanites aegyptiaca* leaves and roots was non-toxic at a dose of 2000 mg/kg. More so sub-chronic test revealed no abnormalities of rat signs and behaviours, in the groups of animals treated with the extract compared to the controls. A significant variation in relative heart and liver weights was observed at 1000 mg/kg. In addition, there was a significant reduction in concentrations of aspartate aminotransferase, total cholesterol, triglycerides and low-density lipoproteins in these rats. compared to the control groups. There was also a significant decrease in hemoglobin and mean corpuscular hemoglobin, with an increase in lymphocytes and white blood cells at the same dose of 1000mg/kg.

Histological examinations of the kidneys and liver showed normal renal architecture, and the liver also showed normal hepatic architecture with slight degeneration at a dose of 1000mg/kg of the extract.

Conclusion: Single administration of the 2000 mg/kg dose did not induce signs of significant toxicity in rats. However, in long-term oral treatment, safety measures must be taken. Thus, sub-chronic oral exposure of BAE to lower doses is recommended, while higher doses, of the order of 1000 mg/kg, should be avoided.

Keywords: *Balanites eagyptiaca*; Aqueous leaf and root extract; Acute toxicity; Sub-chronic toxicity

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1. Introduction

Medicinal plants are an important source of healthcare worldwide (1,2).

According to the World Health Organization (WHO), nearly 80% of the population in developing countries use plants for their primary health care (3), thanks to their availability, efficacy, affordability and fewer side effects than conventional medicines. Population growth and the scarcity of conventional medicines in developing countries contribute in part to the growing demand for traditional medicines (4).

In Africa, this demand is not only the result of the inaccessibility of modern healthcare facilities, but also of traditional medicine, which is very often considered a more appropriate method of treatment (5).

While the pharmacological effects of many plants have been proven in various laboratories, their toxicity is generally unknown. Consequently, assessing the toxicity of herbal preparations is important in determining the safety of substances with pharmacological potential. (6).

Balanites aegyptiaca (BAE) is a thorny tree belonging to the Zygophyllaceae family that grows in most arid and semi-arid areas of Africa, the Middle East and India (7). It has long been used in traditional medicine for its cardioprotective, antibacterial, febrifuge, vermifuge, antihelmintic, anti-infectious, antifungal, anti-inflammatory, stomach-ache and purgative properties (2,8). BAE contains a number of pharmacologically active compounds, notably alkaloids, coumarins, phenolics, flavonoids, triterpenes, tannins and saponosides (9-14).

The pharmacological properties of this plant have been the subject of several previous studies, but fewer have addressed the toxicity of this edible plant.

With this in mind, the present study was initiated to assess the toxicological profile of *Balanites aegyptiaca*.

2. Materials and methods

2.1. Plant material

BAE leaves and roots were harvested at the southern entrance to the city of N'Djamena in April 2022. They were shade-dried at room temperature for 14 days.

The taxonomic identification of the plant was carried out by **Dr GAIWA Daakreo** of the Faculty of Sciences at the University of Doba and authenticated using the West African dry zone tree, shrub and liana identification manual (15).

2.2. Animal material

Male and female (nulliparous), non-pregnant albino rats of wistar strains, aged 8 to 12 weeks maximum and weighing 150±10g were used to assess the acute and subchronic toxicity of aqueous extracts of BAE leaves and roots. These rats were obtained from the animal house of the Department of Pharmacy and Biomedicine at the University of N'Djamena. in order to set tolerance limits for its use.

Rats were randomly selected and kept in their cages for five days prior to the first series of tests to acclimatize them to laboratory conditions. All animals (rats) were maintained under a 12/12 h light/dark cycle, at constant temperature (22±2°C) and humidity (50±15%).

The animals were housed in individual cages, fed a standard diet and given free access to water throughout the experimental period. The study was conducted in accordance with the National Institute of Heath (NIH) bioethics guidelines on the use of laboratory animals (NIH Publication No. 80-23; revised 1978) (16).

2.3. Extract preparation

Aqueous extracts of BAE leaves and roots were prepared using the infusion method. A mass of 50 g of leaf or root powder was dissolved in 450 ml of distilled water heated to 70°C for one hour. After cooling, the mixture was filtered through Whatman GF/C n°4 paper (90mm). The filtrate obtained was evaporated in a ventilated oven at 45°C until a powder was obtained. This powder, representing the aqueous extract of BAE leaves or roots, was used for further work. The extraction yield was 10.75% for the aqueous leaf extract and 11.50% for the aqueous root extract.

2.4. Acute oral toxicity test for *Balanites aegyptiaca* leaf and root extracts

Acute toxicity was assessed in accordance with OECD guideline No. 423, the sequential method using animals of the same sex (17). This method allows substances to be ranked in order of toxicity in a similar way. The experiment was conducted over a period of 14 days. To this end, 12 rats were randomly selected, weighed and divided into 4 groups of 3 rats each to determine the LD50 of aqueous extracts of BAE leaves and roots.

Rats in the normal control group were given distilled water (10mL/kg) via a stomach tube, while those in the test group were given a single oral dose of 2000 mg/kg of the aqueous extracts of BAE leaves and roots. After single administration, the animals were placed in individual cages for observation. Macroscopic symptoms observed included ptosis, pilo-erection, urinary excretion, reaction to external stimuli, stool condition and general animal behavior (aggressiveness, mobility, vocalization, convulsions,...). Mortality was assessed within 48 hours of administration. Rats were observed for 14 days to detect late signs of toxicity.

Body weights were recorded every other day for 14 days, while food and water intake was monitored daily.

2.5. Subchronic oral release assay for BAE aqueous extracts

The subchronic dependence study was carried out in accordance with OECD guideline 407 (18). Fourteen batches of five (5) rats of both sexes were used and treated as follows: batches 1 and 2 (control group) received 10 ml/100 mg body weight of distilled water and batches 3 to 12 received aqueous extracts of leaves and roots of BAE by gavage at doses of 250, 500 and 1000 mg/kg per day respectively.

2.5.1. Blood sampling

Rats were treated for 30 days, during which time body weight was measured at the end of each week. On day 31, all rats were anesthetized by intraperitoneal injection containing keta-mine (100 mg/Kg) and xylazine (12 mg/Kg) [ratio 10:1] for 1 hour. A 10 ml syringe was inserted at the base of the aorta, and the maximum amount of blood was immediately drawn and the animals checked for death (19). After collection, the blood was separated into two parts: one part of the blood for haematological analysis, collected in ethylene diamine tetra-acetic acid (EDTA)-coated tubes, and the other part of the blood collected in anticoagulant-free tubes and used for biochemical analysis.

2.5.2. Evaluation of biochemical parameters

In this study, we explored the following biochemical parameters: aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), protein, urea, creatinine, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG)).

2.5.3. Evaluation of hematological parameters

Hematological parameters analyzed included: lymphocytes , monocytes, red blood cell (RBC) count, hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH),mean corpuscular hemoglobin concentration(MCHC), platelets (PLT), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width CV (RDWCV), and red cell distribution width SD (RDWSD).

2.5.4. Organ assessment

After the animals had been sacrificed, the organs (heart, liver, spleen, lungs, kidneys) were removed and weighed, and macroscopic observations were made to identify any lesions.

2.6. Statistical analysis

Data were entered into Excel and analyzed using SPSS. All results were expressed as Means ± SEM (Standard Error on the Mean).

The One Way ANOVA test was used, followed if necessary by Tukey's post-test, to compare batch means with each other. Values of $p < 0.05$ were considered significant.

3. Results

3.1. Acute toxicity study

3.1.1. Effect of extract on rat body weight

Figure 1 shows the weight evolution of rats in the different treatment groups after a single dose of 2000 mg/kg of *Balanites aegyptiaca* aqueous leaf and root extracts. At the end of this experimental period, it was noted that the relative body weight of the animals did not vary significantly in the rats given the single 2000 mg/kg dose of the aqueous extracts of the leaves and roots of BAE, compared with the normal control group.

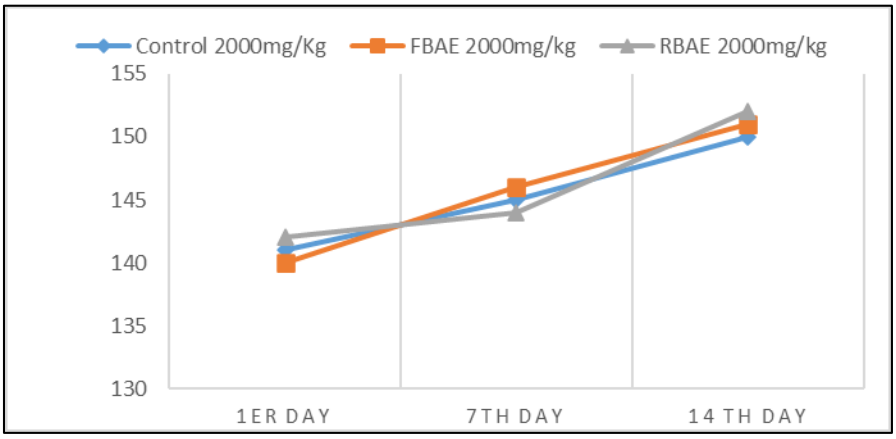

Figure 1 Effects of aqueous extracts from the leaves and roots of *Balanites aegyptiaca* on the weight gain of rats in acute trials

Each value represents the mean ± MSE, n=3. ** $p < 0.01$: statistically significant compared with normal control. FBAE: *Balanites aegyptiaca* leaves; RBAE: *Balanites aegyptiaca* roots.

3.1.2. Effects of aqueous extracts of Balanites aegyptiaca leaves and roots on some biological parameters

After 30 days of treatment of rats with aqueous extracts of BAE leaves and roots at doses of 250, 500 and 1000 mg/kg by gavage, histopathological examination of the various organs sampled showed preserved hepatic and renal architecture, with no sign of cytolysis inherent in possible toxicity. Similarly, no organ damage or fibrosis was observed in the kidneys or liver. Signs of congestion, probably due to sacrifice, were observed in both treated and control batches.

3.2. Subchronic toxicity study

3.2.1. Effect of extract on relative organ weights

Table 1 shows the variation in relative organ weights for animals subjected to the same experimental conditions. The table shows that the aqueous extract of leaves and roots at the doses used does not significantly influence the relative weights of the various organs (table1).

Table 1 Organ weights of control and BAE-treated animals

Parameters	Doses			
	Control mg/kg	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Liver	3.30 ± 0.16	3.38 ± 0.11	3.37 ± 0.22	3.55 ± 0.07
Heart	0.32 ± 0.05	0.30 ± 0.01	0.33 ± 0.02	0.39 ± 0.06
Lungs	1.17 ± 0.03	1.15 ± 0.02	1.20 ± 0.03	1.16 ± 0.03
Spleen	0.38 ± 0.03	0.35 ± 0.03	0.39 ± 0.01	0.36 ± 0.02
Kidneys	0.63 ± 0.03	0.63 ± 0.02	0.66 ± 0.00	0.65 ± 0.00

Results are expressed as mean ± SEM for n = 5 animals per group at $p < 0.05$; BAE: *Balanites eagyptiaca*

3.2.2. Effect of extract on organ histology

After 30 days of treatment of rats with aqueous extracts of BAE leaves and roots at doses of 250, 500 and 1000 mg/kg by gavage, histopathological examination of the various organs sampled showed preserved hepatic and renal architecture, with no signs of cytolysis inherent in possible toxicity. No organic lesions or fibrosis were observed in the kidneys or liver. Signs of congestion, probably due to sacrifice, were observed in both treated and control batches.

3.2.3. Effects of oral administration of aqueous extracts of BAE leaves and roots on biochemical and lipid parameters

Biochemical profile

The results in Tables 2 and 3 show that serum creatinine and serum urea activity did not vary significantly from control groups. A significant increase ($p<0.05$) in total serum protein was observed in rats in the group administered *Balanites aegyptiaca* leaf and root extract at a dose of 1000mg/Kg.

No significant differences were observed in liver function tests (ASAT and ALAT), with the exception of ALAT enzyme, which increased significantly ($p< 0.05$) at the 500mg/kg dose compared with control batches.

Table 2 Variation in biochemical parameters of control and treated animals

Parameters	Doses			
	Control mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
Serum creatinine (mg/l)	7.27 ± 0.06	7.60 ± 0.20	7.73 ± 0.30	7.96 ± 0.04
urea (mg/L)	0.41 ± 0.084	0.42 ± 0.07	0.43 ± 0.07	0.46 ± 0.09
Total serum proteins (g/L)	42.68 ± 0.01	43.05± 0.47	43.62 ± 0.05	43.75 ± 0.26
ALAT (U/L)	35.89 ± 4.10	35.58 ± 5.11	36.22 ± 4.59	35.27 ± 5.57
ASAT (U/L)	38.84 ± 1.08	38.29 ± 1.45	38.44 ± 1.48	38.69 ± 1.11
ASAT (U/L)	38.84 ± 1.08	38.29 ± 1.45	38.44 ± 1.48	38.69 ± 1.11

The results are expressed as mean ±SD, n=animals per batch/group± SEM, *for n = 5 animals per batch*; BAE: *Balanites eagyptiaca*

3.2.4. Effects of extract on serum lipid profile.

At a dose of 1000 mg/kg, aqueous extracts of BAE leaves and roots resulted in a significant ($p < 0.05$) decrease in low-density lipoprotein (LDL) levels compared with control groups. In addition, total cholesterol (TC) and triglyceride (TG) levels decreased significantly ($p < 0.05$) compared to control batches.

Table 3 Variation in lipid parameters in control and treated animals

Parameters	**Doses**			
	Controle mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
TC (mmol/L)	1.54 ± 0.05	1.47 ± 0.04	1.24 ± 0.09	1.20 ± 0.07
TG (mmol/L)	1.17 ± 0.01	1.02 ± 0.00	1.27 ± 0.01	1.19 ± 0.02
HDL (mmol/L)	2.20 ± 0.00	2.21 ± 0.40	2.19 ± 0.21	2.22 ± 0.49
LDL (mmol/L)	0.56 ± 0.03	0.37 ± 0.05	0.31 ± 0.05	0.29 ± 0.02

The results are expressed as mean ±SD, n=animals per batch/group± SEM, *for n = 5 animals per batch*; BAE: *Balanites eagyptiaca*

3.2.5. Effect of extract on hematological profile

The results in Table 3 show the hematological profiles of animals in the control group and those treated with aqueous extracts of BAE leaves and roots at different doses (250, 500 and 1000 mg/kg). The table shows that at 500 mg/kg, there was a non-significant increase in white blood cell, RDWSD (fL) and red blood cell counts, while lymphocyte count (%) decreased significantly ($p < 0.05$) compared with the control groups.
However, non-significant decreases ($p < 0.05$) in mean corpuscular volume, mean corpuscular Hb, red cell distribution width, hematocrit and mean platelet volume were observed at the 1000 mg/kg dose. On the other hand, granulocytes and mean corpuscular hemoglobin concentration increased significantly ($p < 0.05$) compared with control batches (Table 4).

Table 4 Hematological parameters of rats treated and untreated with aqueous leaf and root extracts

Parameters	Doses			
	Control mg/kg	250 mg/kg	500 mg/kg	1000 mg/kg
White blood cells (X 103 /µL)	16.205 ± 0.91	15.730 ± 0.51	17.530 ± 1.93	17.800 ± 1.40
Lymphocytes (%)	82.650 ± 5.20	73.160 ± 11.69	85.700 ± 1.80	63.230 ± 3.0
Granulocytes (%)	15.000 ± 2.38	24.000 ± 1.27	24.300 ± 2.00	25.350 ± 2.90
Red blood cells (X 106 /µL)	6.100 ± 0.68	6.480 ± 0.12	6.760 ± 0.75	6.177 ± 0.82
Hemoglobin (g/dL)	18.000 ± 1.20	16.930 ± 1.01	18.000 ± 0.50	15.630 ± 1.63
Hematocrit (%)	28.530 ± 1.35	28.100 ± 1.25	28.050 ± 1.65	26.590 ± 1.96
Mean corpuscular volume (fL)	83.530 ± 3.05	80.950 ± 4.12	83.650 ± 3.45	82.350 ± 4.45
Mean corpuscular Hb (pg)	35.170 ± 5.44	34.470 ± 6.13	33.000 ± 5.90	34.100 ± 4.70
MCHC (g/dL)	41.850 ± 2.55	41.600 ± 1.90	51.000 ± 2.30	53.200 ± 2.01
RDWCV (%)	17.000 ± 2.20	16.570 ± 2.20	18.070 ± 1.60	16.000 ± 1.40
RDWSD (fL)	45.480 ± 2.16	44.430 ± 4.35	66.250 ± 3.85	66.850 ± 5.04
Platelets (X 103 /µL)	737.000± 60.00	600.0 ± 65.20	650.300 ± 58.80	651.300 ± 60.50
Mean platelet volume (fL)	12.700 ± 0.55	12.640 ± 0.65	12.500 ± 0.70	11.650 ± 0.71
Platelet distribution width (fL)	11.400 ± 0.80	11.000 ± 0.70	9.850 ± 1.15	10.200 ± 0.40

The results are expressed as mean ±SD, n=animals per batch/group± SEM, *for n = 5 animals per batch*; BAE: *Balanites eagyptiaca*

4. Discussion

Since time immemorial, many medicinal plants have been used to treat human diseases, thanks to the bioactive substances they contain. A great deal of research on BAE has already highlighted its various pharmacological properties, but very few studies have been carried out on the toxicity of these bioactive substances (20,21).

The results of acute toxicity tests showed that the aqueous extract of BAE did not significantly alter the general behavior of animals, and did not cause their death until a single dose of 2000 mg/kg. These results suggest that the extract is non-toxic and well-tolerated in female rats. Some studies have shown similar results with this same plant, which also indicated an LD50 greater than 2000 mg/Kg (27).

This high LD50 limit would indicate a wide safety margin for the use of this plant, in contrast to other plants such as Inula viscosa which, according to the authors of the work, presented an LD50 of less than 2000 mg/kg and was considered toxic (25). According to the Globally Harmonized System of Classification (GHS), the aqueous extract of *Balanites aegyptiaca* would be classified in the category of 5 substances of relatively low acute toxicity (26). These results are in line with those obtained by Sudhar and colleagues on the aqueous extract of the same plant.

extremely useful for toxicity studies, due to their sensitivity to harmful compounds and their ability to predict potential toxicity (30). Toxicity-related changes in the weight of these vital organs are often accompanied by histopathological changes (31-34).

The increase in kidney weight observed in this study could be correlated with a possible toxic effect of the plant at these doses, since the kidney is one of the main organs involved in xenobiotic metabolism (29). However, histological sections of the kidneys revealed no morphological alteration of this organ, so this hypothesis is rejected.

However, weight changes in organs such as the lungs, heart and spleen were less indicative, given their limited role in eliminating harmful substances from the body. (35,36).

In addition, biochemical analyses were carried out to assess any alterations in liver and kidney function caused by administration of the extracts tested. No significant changes in transaminase levels were observed during this study period.

These results may suggest that the aqueous extract of BAE has hepatoprotective effects, especially as no changes were observed in the histopathological study. According to several authors, the assay of these enzymes provides valuable information on the state of certain organs, notably the liver and lungs, enabling the diagnosis of certain pathologies.

Analysis of serum and urine biochemical parameters during toxicity studies provides important information on the functioning of specific body tissues (47).

Analysis of blood parameters has been shown to be relevant, providing information on hematopoietic function (assessment of myeloid lineage cells), on the occurrence of allergies (white blood cell studies) and on intravascular effects such as hemolysis (47,48).

The extracts tested caused decreases in hemoglobin, hematocrit and red blood cell levels over the course of the study. These results suggest that these extracts may induce anemia when administered subchronically. As a result, BAE aqueous extract is not recommended for people with anemic diseases such as malaria, sickle-cell anemia, diabetes, etc.(49) Similarly, BAE aqueous extract reduces blood sugar levels, creatinemia, total cholesterol, LDL-cholesterol and HDL-cholesterol. The reduction in these biochemical parameters suggests that these extracts may reduce the risk of obesity, cardiovascular disease and kidney damage (50). Similar results have been obtained with other plant extracts such as Monodora myristica (51) and Cassipourea congoensis (52).

5. Conclusion

At the end of this work, it emerged that the aqueous extract of *Balanites aegyptiaca* leaves and roots showed an LD50 greater than 2000 mg/Kg, suggesting that the aqueous extract of this plant is non-toxic and can be used safely in a single administration. However, prolonged oral administration of low doses may lead to variations in certain biochemical and haematological parameters of the organism, without causing damage to vital organs involved in xenobiotic metabolism and excretion. Consequently, safety measures must be taken before oral administration for therapeutic or other purposes. Nevertheless, it would be advisable to study the chronic toxicity of these extracts at higher doses.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Authors' contributions

DF carried out the study; **AC, DF and YMA** participated in data collection and laboratory experiments; **DF,JBO, and ABH** analyzed the data; **DF, NBH and YMA** drafted the manuscript; **MH, AC and AS** read and corrected the manuscript; **TDE, MM and HB** supervised the work; all authors read and approved the final manuscript.

Statement of ethical approval

All experiments were performed in strict compliance with established guidelines on the use of laboratory animals.

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