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Research Article

Phytochemical, Antioxidant, and Toxicological Assessment of *Balanites aegyptiaca* Leaves Extract in Rats

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Abstract

Balanites aegyptiaca Del. (Zygophyllaceae), known as desert date, is traditionally used in the treatment of various ailments such as jaundice, intestinal worm infection, wounds, malaria, syphilis, epilepsy, dysentery, constipation, diarrhea, hemorrhoid, stomach aches, asthma, and fever, however, no toxicological evaluation of this plant has yet been documented. The purpose of this research was to determine the acute and subacute toxic effects of 50% hydroethanolic extract (HLE), methanolic extract (MLE), and aqueous extract (ALE) of *Balanites aegyptiaca* leaves extract. Standard methods were used to analyze the extracts for phytochemical constituents, DPPH and FRAP activities, total phenolic content, total flavonoid content, and total tannins. The acute toxicity was assessed using a single oral dose of 5000 mg/kg body weight (b.wt.) of the extract, whereas in the subacute study, the extracts were administered orally (at doses of 100, 250, and 500 mg/kg, b,wt.) for 28 days and signs of toxicity were observed. The effect of treatment on body weight, relative organ weight, hematological, serum biochemical indices, and histopathological examination of the liver was used to assess safety. Phytochemicals such as alkaloids, phenols, flavonoids, triterpene, tannins, and Saponins, were present in all crude extracts. The LD50 value was determined to be greater than 5000 mg/kg b.wt. In the subacute study, extract treatment had no significant effect on body weight, relative organ weight, biochemical, and hematological parameters when compared to non-treated rats at all doses. In histology, no significant hepatic lesions were found. The current study found that crude extracts of *Balanites aegyptiaca* leaves, namely, HLE, MLE, and ALE, have antioxidant properties and did not cause any harm in both acute and subacute studies of male and female rats, so using these extracts for medicinal purposes is recommended.

Keywords: Antioxidant; *Balanites aegyptiaca*; Biochemical; Phytochemical; Toxicological

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Introduction

Since the dawn of time, human beings have greatly benefited from using plant extracts to treat a variety of illnesses [1]. These extracts have also produced beneficial medications such as analgesics, antitussives, anti-hypertensives, cardiotonic, and anti-malarial [2]. For the modern discovery of novel single-molecular medicines, the knowledge of these medicinal plants used in traditional systems of medicine serves as a model.

Among the many crucial purposes, these plants have helped humanity with medicine and nourishment [1]. Plants used in herbal medicine, therefore, have a vital role to play in the preservation of well-being around the world. Herbal and animal remedies have been used by religious healers since ancient times to preserve health and cure diseases. These medicines are widely used in Africa and Asia, including India and China. Due to adverse side effects, as well as the emergence of resistance to synthetic drugs, the use of plant-derived drugs is becoming increasingly common in developed countries [3]. However, recent surveys have shown that several medicinal plants have also demonstrated adverse effects [4]. This raises questions about the possible harmful impact of chronic use of such plants. Therefore, determining the toxicological effects of many medicinal plant extracts intended for therapeutic or preclinical application is a vital part of the evaluation for safety and subsequent approval [5]. The choice of herbal products for therapy includes affordability, availability, and the perception that plants are less toxic than conventional medicines [6].

Balanites aegyptiaca has historically been used as a natural repellent for mosquito larvae in the Middle East and South [7]. Synthetic pesticide methods were regarded as inefficient in the literature. *B. aegyptiaca* reasonably effective in controlling insects. However, there is a dearth of information on its toxicity and antioxidant properties. Therefore, the purposes of this investigation were to examine the phytochemicals, antioxidant and toxicological profile of Hydroethanolic Extract (HLE), Methanolic Extract (MLE) and Aqueous Extract (ALE) of *B. aegyptiaca leaves* with the sole aim of advising on its use in humans for the treatment of different diseases.

Materials and Methods

Chemicals and reagents

Changshu Hongsheng Fine Chemicals Co. Ltd., China, supplied all the solvents used in the extraction process. All chemicals and reagents used in the study were of analytical grade.

Collection and authentication of plant materials

Balanites aegyptiaca fresh leaves were collected in January 2023 from healthy, fully grown plants in Ghana's Northern region. They were verified at University for Development Studies, School of Pharmacy and Pharmaceutical Sciences, Department of Pharmacognosy and Herbal Medicine, and a voucher specimen (UDS-SPPS/DP1/2023/L010) was deposited at the herbarium.

Preparation of extracts

The leaves were washed, dried in the shade, and milled into powder. Aqueous Extract (ALE), Methanolic Extract (MLE), and Hydroethanolic Extract (HLE) of the *Balanites aegyptiaca* leaves were obtained by suspending 100 g of powdered sample in 500 ml of distilled water, methanol, and hydro-ethanol, respectively, at room temperature with continue shaking for 24 hours. The extracts were then filtered through cotton wool and concentrated under 60°C pressure using a rotary evaporator (Buchi R205, Switzerland). They were transferred into sterile containers and freeze-dried and redissolved in normal saline at the respective doses and used for the study.

Preliminary Phytochemical analysis

Phytochemical constituents of *Balanites aegyptiaca* leaves extracts were determined using standard methods as described by Ejiofor and colleagues [8]. Alkaloids, flavonoids, triterpenes, saponins, polyphenols, and tannins were the phytochemicals examined.

In vitro Antioxidant Activity

The free radical scavenging activities of ALE, MLE, and HSE were assessed using the DPPH assay as described by Povolo and colleagues [9]. Extracts were prepared by dissolving 10 mg in 1 ml of the respective solvents followed by serial dilution to obtain a concentration range of 0.156–10 mg/ml. Moreover, a stock solution of 10 mM ascorbic acid was prepared by dissolving 2.2 mg in 1 mL distilled water while 0.5 mM DPPH was prepared using 3 mg in 15 mL absolute methanol. The absorbance of the resulting solution was determined using a spectrophotometer at 517 nm. Ascorbic acid (50 g) was used as the standard. The percentage decoloration of DPPH was used to calculate radical scavenging activity.

Determination of Total Phenolic Content (TPC)

The total phenolic content of crude extracts was assessed by the Folin Ciocalteu (FC) procedure [10], with some modifications. Approximately 0.1 g of the extract was dissolved in 5 mL of 0.3% HCl in methanol/water (60:40, v/v). The blend was allowed to stand for 5 minutes and added to 2 mL of 2 % Na_2CO_3. After 2 minutes, 50% Folin-Ciocalteau reagent (100 µl) was added to the mixture, which was then left for 30 minutes. Absorbance was measured at 750 nm using gallic acid as standard. All extracts were analyzed in triplicate. Gallic acid standards (5.0 mg/mL, 10.0 mg/mL, 15.0 mg/mL, 20 mg/ml, 25 mg/ml, and 30.0 mg/mL) were used to prepare the calibration curve. From the calibration curve, the overall phenolic content was calculated and the final results were expressed as mg GAE/100g DM.

Estimation of total Flavonoid Content (TFC)

The determination of TFC was by the colorimetric method in aluminum chloride ($AlCl_3$), using gallic acid (10-100 mg / L) as a standard [10]. Briefly, 1.5 mL of 95% methanol, 100 µL of 10% $AlCl_3$, 100 µL of 1 M potassium acetate, and 2.8 mL of distilled water were mixed with 500 µL of the 1:20 diluted and filtered extract (at the original 100 mg / mL concentration in methanol). The mixture was incubated for 40 minutes at room temperature and the absorbance was read at 415nm. The TFC was expressed as mg /g dry weight of gallic acid equivalents.

Estimation of Total Tannins (TT)

The amount of tannins in plant extracts was determined by a slightly modified Folin-Ciocalteu method [11]. Five microlitres of distilled water, 500 µL of Folin-Ciocalteu reagent, and 1 mL of 35% Na_2CO_3 solution were added to 0.5 g of sample extract and fractions. The mixture was well shaken and held for 30 min at room temperature. Gallic acid standard solutions (2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, and 20.0 mg/mL) were prepared in the same manner as previously described. The absorbance of the test and standard solutions was measured with a UV/Visible Spectrophotometer against the blank at 725 nm. From the calibration curve, the total tannin content was determined and the results expressed the tannin content in terms of mg GAE / g DM.

Ferric Reducing Antioxidant Power (FRAP) assay

The antioxidant capacity of the HLE, MLE, and ALE was determined spectrophotometrically using the procedure reported by Kalyani and colleagues [12]. The approach is based on the action of electron-donating antioxidants at low pH to reduce Fe^{3+} TPTZ complex (colorless complex) to Fe^{2+}-tripyridyltriazine (blue colored complex). The difference in absorbance at 593 nm is used to monitor this process. At 37°, 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ were mixed to make the Ferric Reducing Antioxidant Power (FRAP) reagent. Freshly made working FRAP reagent was pipetted using a 1-5 ml variable micropipette (3.995 ml) and well mixed with 5 µl of adequately diluted plant samples. After 30 minutes of incubation at 37°, a strong blue complex was generated when the ferric tripyridyl triazine (Fe^{3+} TPTZ) complex was reduced to ferrous (Fe^{2+}) form, and the absorbance at 593 nm was measured against a reagent blank (3.995 ml FRAP reagent+5 µl pure water). All determinations were made in triplicate. The calibration curve was created by graphing the absorbance at 593 nm versus various $FeSO_4$ concentrations. The $FeSO_4$ concentrations were then plotted versus the concentration of the typical antioxidant Trolox. The FRAP values were calculated by comparing the absorbance change in the test mixture to that obtained with increasing doses of Fe^{3+} and were represented as mg of Trolox equivalent per gram of sample.

Heavy Metal Analysis

One gram (1.0 g) of each of the samples was weighed into a 50 mL digestion tube. The sample was mixed with 1.0 mL of H_2O, 2.0 mL of conc. HCl, 5 ml of 1:1 conc. HNO3: 60% HClO4 and 2.0 mL of conc. H_2SO_4. The mixture was allowed to stand for 20 minutes. Samples were heated at 150 °C on a digestion block. Digested samples were allowed to cool, and diluted to the 50 mL mark with distilled. The digests were analyzed using an Atomic Absorption Spectrometer (Analytikjena nova 400P) for the levels of lead, copper, nickel, zinc, and iron [13].

Toxicity assessment of *Balanites aegyptiaca* leaves extract

Healthy adult male and female Sprague Dawley rats (age < 8-12 weeks: bodyweight, 150-200g (male); 120-150 g (female)) were used to evaluate the subacute toxicity studies of ALE, MLE, and HLE. Furthermore, albino mice (either sex; 20 – 30g) were used for the acute study. Animals were obtained from the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, and housed in polypropylene cages suitably lined with wood shavings. Before testing, they were acclimatized in the animal holding facility of the Department of Biochemistry and Molecular Medicine, UDS for one week, maintained under standard conditions (temperature, 25±2 °C; relative humidity 65 percent; light/dark cycle 12/12h). The animals were fed with standard rat pellet feed (Agricare, Tamale) and drinking water was supplied with stainless steel sipper tubes in clean polypropylene bottles ad libitum. They were painted solely on their tails using permanent markers for quick detection [14].

All animal experiments were performed in compliance with the recommendations of the Committee for the Monitoring and Control of Animal Experimentation [15] and protocols were reviewed and approved by a veterinarian on the research team.

Acute oral toxicity study

Albino mice of either sex were used for acute oral toxicity studies. Treated groups received the extracts orally with the aid of a feeding needle connected to a syringe at the stated doses dissolved in an inappropriate volume of sterile distilled water. Doses were selected based on the fixed-dose method [16]. The animals were observed for signs of toxicity and mortality for the first critical 4 hours and thereafter daily for 7 days. The oral median lethal dose (LD_{50}) was calculated as the geometric mean of the dose that caused 0% and 100% mortality, respectively. This was used to guide the selection of three doses (100, 250, and 500 mg/kg b.wt) for sub-chronic toxicity studies [17].

Sub-acute toxicity studies

Forty (40) male rats (150-200 g) and 40 female rats (120-150 g) were divided into twenty groups and treated for 28 days (n=4). Table 1 shows the description of groups and treatments. All animals fasted 12 hours before the first oral drug administration and had free access throughout the duration of the experiment to food and freshly distilled water. The signs of toxicity and mortality were observed in the animals. This included paw licking, respiratory distress with stretching, diarrhea, and death. At termination, all animals were sacrificed by cervical decapitation on the 29th day following overnight fasting. The animals were quickly slit at the neck and blood samples were collected into gel-activated tubes for biochemical analyses and EDTA tubes for hematological analyses. All animal experiments were performed in compliance with the recommendations of the Committee for the Monitoring and Control of Animal Experimentation.

S/N.	Group	Treatment
Male		
1	Normal (control)	Potable water p.o (1 ml/kg b.wt)
2	100 mg HLE only	100 mg/kg HLE only (per day, p.o.) for 28 consecutive days
3	100 mg MLE only	100 mg/kg MLE only (per day, p.o.) for 28 consecutive days
4	100 mg ALE only	100 mg/kg ALE only (per day, p.o.) for 28 consecutive days
5	250 mg HLE only	250 mg/kg HLE only (per day, p.o.) for 28 consecutive days
6	250 mg MLE only	250 mg/kg MLE only (per day, p.o.) for 28 consecutive days
7	250 mg ALE only	250 mg/kg ALE only (per day, p.o.) for 28 consecutive days
8	500 mg HLE only	500 mg/kg HLE only (per day, p.o.) for 28 consecutive days
9	500 mg MLE only	500 mg/kg MLE only (per day, p.o.) for 28 consecutive days
10	500 mg ALE only	500 mg/kg ALE only (per day, p.o.) for 28 consecutive days
Female		
11	Normal (control)	Potable water p.o (1 ml/kg b.wt)
12	100 mg HLE only	100 mg/kg HLE only (per day, p.o.) for 28 consecutive days
13	100 mg MLE only	100 mg/kg MLE only (per day, p.o.) for 28 consecutive days
14	100 mg ALE only	100 mg/kg ALE only (per day, p.o.) for 28 consecutive days
15	250 mg HSE only	250 mg/kg HSE only (per day, p.o.) for 28 consecutive days
16	250 mg MSE only	250 mg/kg MSE only (per day, p.o.) for 28 consecutive days
17	250 mg ALE only	250 mg/kg ALE only (per day, p.o.) for 28 consecutive days
18	500 mg HSE only	500 mg/kg HSE only (per day, p.o.) for 28 consecutive days
19	500 mg MSE only	500 mg/kg MSE only (per day, p.o.) for 28 consecutive days
20	500 mg ALE only	500 mg/kg ALE only (per day, p.o.) for 28 consecutive days

Table 1: Showing the Grouping and Treatment of Animals.

Effect of Treatment on the Bodyweight of Animals

During treatment, individual body weights of all animals were recorded on the first day (D_o) and the end of every fourth day (D_4, D_8, D_{12}, D_{16}, D_{20}, D_{24} and D_{28}). Using the formula, the percent change in body weight was calculated

$$\%\ Change\ in\ Body\ Weight = \frac{Weight\ n - Weight\ o}{Weight\ o} 100$$

Where, Weight n is the weight on D_4, D_8, D_{12}, D_{16}, D_{20}, D_{24} and D_{28} and Weight o is the weight on the first day (D_o).

Effect of Extract on Organ Weight in Rats

The liver, kidney, the heart, stomach, spleen, lung, testes (male), and uterus (female) were collected and rinsed in a buffered saline solution, dried on tissue paper, grossly observed, and weighed to obtain the Absolute Organ Weight (AOW). The Relative Organ Weight (ROW) of each organ was calculated from the formula:

$$ROW = \frac{AOW}{Body\ Weight\ as\ at\ Sacrifice} 100$$

Effect of treatment on Haematological Parameters of rats

An automatic hematological analyzer (Sysmex XS-1000i) was used to analyze the hematological profile of animals. The following were evaluated; erythrocytes, leukocytes, hemoglobin, and platelets.

Effect of Treatment on some Biochemical Parameters

Blood samples in activated gel tubes were allowed to clot and centrifuged at 1500 x g for 15 min to obtain blood serum. The following biochemical parameters were assessed: ALT, AST, ALP, albumin, and bilirubin using an automated biochemistry analyzer (ADVIA 2400, Siemens Healthcare) with reagents.

Histopathological Studies

The liver was surgically removed, fixed in 10% buffered formalin (pH 7.4), and histologically processed. Five microliter sections were cut and stained with hematoxylin-eosin (HE). The tissues were examined microscopically and photomicrographs were taken.

Statistical Analysis

All statistical data were presented as mean ± SEM and analyzed using GraphPad Prism for Windows version 8.0 (GraphPad Software, San Diego, CA, USA) with a one-way analysis of variance test followed by Tukey Multiple Comparison Test, at the significance of $p < 0.05$.

Results

Preliminary Phytochemical screening

As shown in table 2, preliminary phytochemical screening of HLE, MLE, and ALE revealed the presence of major phytochemical groups.

Phytochemicals	HLE	MLE	ALE
Alkaloids	+++	++	++
Phenols	+++	+	++
Flavonoids	+++	++	+
Triterpene	++	+	++
Tannins	+++	++	++
Saponins	++	+++	+++

Table 2: Phytochemical constituents of B. aegyptiaca leave extracts.

Key: Present in low concentration (+); Present in moderate concentration (++); Present in high concentration (+++).

In vitro DPPH scavenging activity of crude extracts

The DPPH radical scavenging activity of crude extracts is shown in table 3. When compared to standard ascorbic acid, HLE had the highest free radical scavenging capacity.

Sample/ Standard	EC50 (mg/ml) n = 3	P value
Hydro (HLE)	0.62 ± 0.07 *	0.00
Methanol (MLE)	3.17 ± 0.04 **	
Aqueous (ALE)	1.22 ± 0.05*	
Ascorbic acid	0.43 ± 0.07*	

Table 3: DPPH scavenging activity (EC50) values of standard ascorbic acid and extracts.

Values having different superscripts (**) other than (*) in a column are statistically differ significantly, $p < 0.00$.

TPC, TT, TFC and FRAP activity of crude extracts

Table 4 shows the total contents of phenols, tannins, flavonoids, and FRAP activity of crude extracts. HLE recorded the highest contents of phenols, tannins, flavonoids, and FRAP when compared to ALE and MLE.

Extract	HLE	MLE	ALE
Total Phenol (mgGAE/g)	20.04 ± 2.34^{a}	10.35 ± 4.74^{b}	18.76 ± 3.21^{a}
Total Tannin (mgGAE/g)	17.13 ± 4.11^{a}	10.43 ± 3.84^{b}	11.33 ± 2.84^{b}
FRAP(µmol Fe(II)/g)	32.81 ± 3.95^{a}	30.05 ± 2.61^{a}	31.11 ± 3.82^{a}
Total Flavonoid	16.34 ± 3.16^{a}	8.43 ± 4.71^{b}	10.23 ± 1.54^{b}

Table 4: TPC, TT, TFC, and FRAP activity of crude extracts.

Values with different superscripts along the row are significantly different ($p<0.05$).

Heavy Metal

Table 5 shows the presence of heavy metals in both raw plant materials, HLE, MLE, and ALE. Iron (Fe), zinc (Zn), and copper (Cu) concentrations were low in the raw leaves, but they were Below the Detection Limit (BDL) of 0.0001 ppm in all crude extracts.

Sample	Concentration (mg/L)				
	Fe	Zn	Ni	Cu	Pb
Leaves Raw Powder	0.005 ± 0.0001	0.006 ± 0.0002	BDL	0.005 ± 0.0002	BDL
HLE	BDL	BDL	BDL	BDL	BDL
MLE	BDL	BDL	BDL	BDL	BDL
ALE	BDL	BDL	BDL	BDL	BDL

Table 5: Metal content in raw leaf powder and crude extracts.

Acute Toxicity Study

In the acute toxicity investigations, no death was reported within 12 hours of continuous monitoring, nor after 24 hours. There was also no fatal effect discovered after administering the extract for 28 days. Morphological characteristics (hair, skin, eyes, and nose) appeared normal. There was no drooling, diarrhoea, lethargy, or unusual behaviour. The LD50 could be estimated to be ≥5000 mg/kg, indicating that it is safe.

Subacute Toxicity Study

During 28 days, rats were given HLE, MSE, or ALE at doses of 100, 250, and 500 mg/kg b.wt. via oral gavage with no fatalities. Throughout the study, no rats (male or female) displayed any visible morbidity or clinical signs of poisoning, such as changes in skin and hair, eyes, respiration rate, autonomic (salivation, sweating, and piloerection), or stereotypical behavior.

Effect of treatment on body weight

Body weight increased in both the control and all treatment groups, both male and female, as shown in figures 1-3.

Effect of treatment on relative organ weight

There were no significant differences in the relative organ weights of the rats for both sexes after the administration of HLE, MLE, or ALE (Table 6).

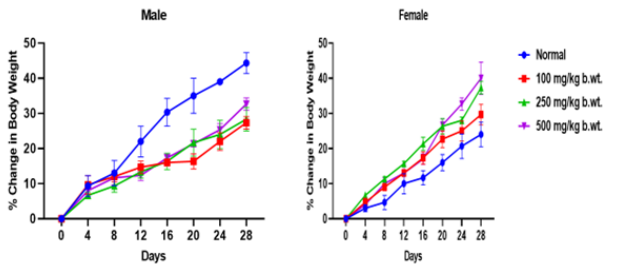

Figure 1: Effect of HLE on percent change in body weight of male and female animals.

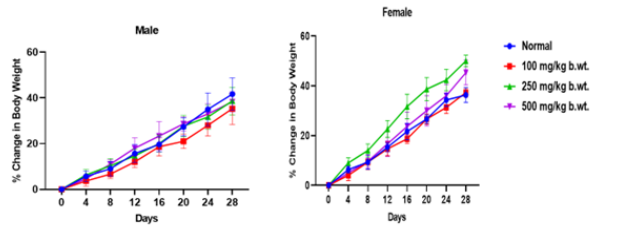

Figure 2: Effect of MLE on percent change in body weight of male and female animals.

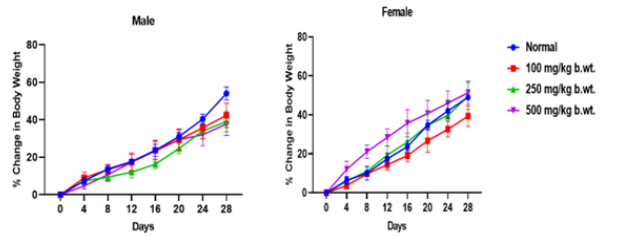

Figure 3: Effect of ALE on percent change in body weight of male and female animals.

	Relative liver weight (g)	
	Male	Female
Normal	3.42 ± 0.34	4.04 ± 1.10
HLE		
100 mg/kg	3.11 ± 0.12	4.23 ± 1.27
250 mg/kg	3.47 ± 0.44	4.09 ± 0.87
500 mg/kg	3.21 ± 0.51	4.45 ± 1.35
MLE		
100 mg/kg	3.08 ± 1.03	4.33 ± 0.47
250 mg/kg	3.71 ± 0.34	4.36 ± 1.68
500 mg/kg	3.21 ± 0.34	4.18 ± 1.07
ALE		
100 mg/kg	3.28 ± 0.67	4.12 ± 1.06
250 mg/kg	3.34 ± 1.38	4.27 ± 1.27
500 mg/kg	3.59 ± 1.87	4.26 ± 1.80

Table 6: Effect of treatment on relative liver weight in male and female animals.

Values are expressed as mean ± SEM (n=5).

Effect of treatment on hematological parameters

There was no significant difference between the control and treated rats in all hematological parameters for both males and females as shown in table 7.

	Erythrocyte (1012/L)	Leukocyte (109/L)	Hemoglobin (g/L)	Platelet (uL)
MALE				
Normal	6.32 ± 2.12	5.04 ± 1.10	10.26 ± 2.20	836.15 ± 20.32
HLE				
100 mg/kg	6.15 ± 0.34	5.33 ± 1.24	9.98 ± 3.23	788.63 ± 35.71
250 mg/kg	6.22 ± 1.10	5.09 ± 0.24	10.23 ± 2.36	804.51 ± 27.46
500 mg/kg	6.61 ± 1.34	5.25 ± 0.30	11.04 ± 2.17	768.64 ± 26..96
MLE				
100 mg/kg	6.08 ± 1.14	5.33 ± 0.47	9..86 ± 2.43	779.52 ± 30.65
250 mg/kg	6.71 ± 1.22	5.36 ± 1.68	9.77 ± 2.84	863.11 ± 23.87
500 mg/kg	6.21 ± 1.32	5.18 ± 1.47	9.69 ± 2.36	764.23 ± 28.98
ALE				
100 mg/kg	6.38 ± 1.04	5.12 ± 2.14	11.33 ± 3.61	860.38 ± 38.63
250 mg/kg	6.54 ± 1.38	5.27 ± 1.42	10.50 ± 2.54	786.36 ± 36.16
500 mg/kg	6.19 ± 1.87	5.26 ± 1.53	11.14 ± 2.36	796.56 ± 53.89
FEMALE				
Normal	5.70 ± 1.62	6.52 ± 2.04	11.74 ± 1.47	784.48 ± 17.68
HLE				
100 mg/kg	5.95 ± 1.32	6.13 ± 1.34	11.81 ± 2.64	812.54 ± 43.26
250 mg/kg	5.57 ± 0.89	6.43 ± 0.96	10.34 ± 2.87	833.11 ± 40.72
500 mg/kg	5.78 ± 1.37	6.06 ± 1.37	11.22 ± 3.65	806.65 ± 32.35
MLE				
100 mg/kg	5.63 ± 1.03	6.33 ± 2.78	10.35 ± 2.28	768.25 ± 24.56
250 mg/kg	5.38 ± 1.63	5.78 ± 1.68	11.15 ± 1.36	795.14 ± 20.67
500 mg/kg	5.37 ± 0.34	6.34 ± 2.74	10.24 ± 2.47	783.69 ± 36.25
ALE				
100 mg/kg	5.67 ± 0.67	6.12 ± 3.12	11.33 ± 2.98	796.32 ± 35.87
250 mg/kg	5.74 ± 1.38	6.27 ± 2.36	11.21 ± 0.78	796.14 ± 30.14
500 mg/kg	5.36 ± 2.31	6.26 ± 1.25	11.58 ± 2.73	783.42 ± 28.27

Table 7: Effect of *B. aegyptiaca* crude extracts on hematological parameters.

Values are expressed as mean ±SEM (n=5). Superscript "a" is significant at P<0.05-0.01 compared with the normal group.

Effect of treatment on biochemical parameters

Table 8 show the effects of treatment on some biochemical markers to detect the state of some organs. No significant difference was observed in the biochemical parameters.

	ALT (U/L)	AST (U/L)	ALP (U/L)	Albumin (g/dL)	Bilirubin (µmol/L)
MALE					
Normal	62.81 ± 2.54	188.32 ± 8.34	140.25 ± 8.36	16.84 ± 2.61	4.23 ± 1.24
HLE	60.46 ± 7.32	184.17 ± 23.24	137.42 ± 4.35	14.12 ± 3.64	4.65 ± 1.34
100 mg/kg	65.37 ± 4.87	185.64 ± 17.87	130.36 ± 6.10	16.23 ± 4.18	4.57 ± 0.45
250 mg/kg	70.31 ± 5.20	190.64 ± 14.66	143.21 ± 7.69	17.16 ± 4.02	4.11 ± 1.10
500 mg/kg					

MLE 100 mg/kg 250 mg/kg 500 mg/kg	66.35 ± 6.37 64.98 ± 6.58 67.11 ± 5.14	183.58 ± 17.35 181.39 ± 13.54 186.36 ± 15.00	146.11 ± 28.15 153.25 ± 12.36 142.39 ± 11.45	16.23 ± 5.67 16.67 ± 5.36 16.96 ± 4.82	4.50 ± 1.38 3.96 ± 0.33 4.53 ± 1.98
ALE 100 mg/kg 250 mg/kg 500 mg/kg	62.28 ± 5.17 61.36 ± 7.35 65.02 ± 8.25	189.46 ± 18.14 176.87 ± 15.85 187.26 ± 19.71	146.84 ± 9.27 140.93 ± 15.78 148.85 ± 11.20	15.37 ± 5.67 15.99 ± 3.06 16.10 ± 5.98	5.31 ± 1.45 5.03 ± 1.66 5.12 ± 2.71
Female					
Normal	63.68 ± 5.08	182.45 ± 10.63	147.15 ± 7.34	15.44 ± 3.57	5.10 ± 2.64
HLE 100 mg/kg 250 mg/kg 500 mg/kg	65.35 ± 4.82 65.11 ± 5.37 64.24 ± 4.92	186.19 ± 9.24 188.07 ± 11.74 191.35 ± 8.12	146.25 ± 8.42 142.38 ± 12.32 138.11 ± 10.74	15.23 ± 4.61 15.87± 2.57 15.03± 3.42	5.99 ± 1.35 5.84 ± 1.07 5.25 ± 2.10
MLE 100 mg/kg 250 mg/kg 500 mg/kg	63.64 ± 8.68 62.87 ± 5.81 64.11 ± 5.98	184.67 ± 13.36 188.12 ± 16.81 186.98± 14.47	149.22 ± 6.17 142.37 ± 6.87 145.81 ± 7.44	15.11 ± 4.18 16.63 ± 3.10 15.74 ± 4.25	5.71 ± 1.35 5.22 ± 2.35 5.00 ± 1.12
ALE 100 mg/kg 250 mg/kg 500 mg/kg	65.32 ± 6.25 63.18 ± 7.10 66.84 ± 5.87	187.65 ± 15.87 187.14 ± 10.25 181.87 ± 7.50	140.37 ± 9.26 148.69 ± 7.37 150.11 ± 6.98	16.68 ± 6.85 15.97 ± 5.96 17.06 ± 6.41	5.62 ± 2.76 5.13 ± 1.69 5.89 ± 3.10

Table 8: Effect of *B. aegyptiaca* crude extracts on biochemical parameters.

Values are expressed as mean ±SEM (n=5). Superscript "a" is significant at P<0.05-0.01 compared with the normal group.

Effect of treatment on liver histology

Figure 4 shows the effect of HLE, MLE, and ALE on liver histology. No major pathological alteration was observed in the gross and histopathological examination of the liver after treatment with extracts.

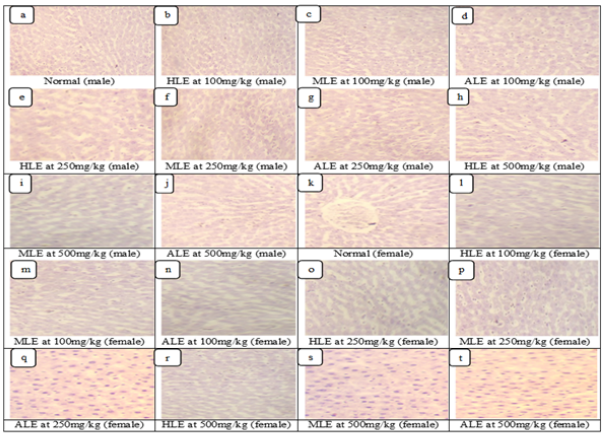

Fig. 4: Photomicrographs of the liver from rats administered orally for 28 days. Photomicrograph (a) – (t) shows normal hepatocytes with no observable lesion. (H&E X 400). HLE (Hydroethanolic crude extract of *Balanites aegyptiaca* leaves); MLE (Methanolic crude extract of *Balanites aegyptiaca* leaves); ALE (Aqueous crude extract of *Balanites aegyptiaca* leaves)

Figure 4: Photomicrographs of the liver from rats administrated orally for 28 days.

Discussion

As a complement to synthetic prescription medicines, the medicinal plants and the elements they contain have been used to cure a variety of diseases in many different countries [17]. To assure their safety, scientific information on the choice of acceptable doses for animals, including humans, must be provided.

Systematic research on the harmful effects of herbal plants is necessary. No toxicological studies on the hydroethanolic, methanolic, or aqueous leaf extracts of *B. aegyptiaca* have been carried out, despite the fact that the current literature has demonstrated their medicinal purposes [18]. Without knowledge of their potentially harmful effects, they may have long-term negative effects on science. As a result, the current study offers first-hand information on the safety assessment and antioxidant profile of ALE, MSE, and HLE. Preliminary phytochemical screening of *B. aegyptiaca* leaf extracts revealed the presence of alkaloids, polyphenols, flavonoids, triterpenes, tannins, and saponins. Indeed, several studies, notably by Wufem and colleagues [19], found a strong link between the existence of these chemicals and their antioxidant effects. These phytochemicals, as seen in earlier research, have pharmacological effects. Tannin-rich plants are utilized in the treatment of several illnesses and are known to have anticancer and hypolipidemic properties [20]. Numerous studies have shown that flavonoids, which are important polyphenols, are excellent at scavenging free radicals [21,22]. Alkaloids are also known to control hormonal activity and oxidative stress to guard against infections [23]. In addition, it was discovered that alkaloids, flavonoids, and saponins shielded the liver and kidney from carbon tetrachloride-induced damage in rats by reducing oxidative stress [24-26].

Additionally, triterpenoids have been found to have anti-inflammatory, insecticidal, sedative, and cytotoxic properties [27]. These phytochemicals are thought to have a variety of therapeutic effects in combination. A DPPH scavenging experiment was used to assess the potential of the different extracts to stabilize free radicals. When compared to ascorbic acid, HLE observed the highest free radical scavenging capacity (0.62 ± 0.07). These results were further supported by TPC, TT, TFC, and FRAP values. Plant-derived total phenols can suppress the development of cancer and are powerful antioxidants [27].

Heavy metal concentrations have been reported in various therapeutic plants, which may explain some of the associated toxicities [28]. The extract was tested for heavy metals in this regard. Iron, zinc, lead, nickel, and copper were not detected in the crude extract; however, iron, zinc, and copper were found in low concentrations in the raw powdered sample. Toxicity testing is the only scientific basis for verifying the safety of plant-based medications in traditional disease treatment. It has also been proposed that all-natural therapeutic products be subjected to the same safety tests as new scientific medicines [29]. The subacute toxicity research produced data that may be utilized to make choices about the safe amount of dosage, target organ toxicity, and possibly adverse implications on animal models. The impact of HLE, MSE, and ALE in rats was studied in this study at dosages of 100, 250, and 500 mg/kg body weight over 28 days in both sexes.

Weight loss in treated animals is one metric of toxicity [30]. Weight increases were observed in the treated animals when they were fed, suggesting that the extracts were not harmful at different dosages of administration. Animals lose their appetite as a result of abnormalities

in glucose, protein, or fat metabolism [30]. Furthermore, there were no significant variations in relative organ weights between treated and untreated animals, indicating that the extracts were not harmful at the varied dosages supplied. In general, hematological indicators are excellent instruments for evaluating toxicity in animals. This is significant since eating hazardous or foreign substances might change the predicted range of values [31]. The hematological parameters in most of the treated animals were non-significantly different from those of the controls, indicating that these extracts are non-toxic in animals at various dosages. The liver also plays an important part in the metabolism of foreign substances, lipids, carbohydrates, and proteins. As a result, both the liver and the kidney are prone to drug-induced harm [32]. This has made it easier to assess the safety of plant extracts about these two organs, especially given the ongoing global rise in liver and kidney illness. Some xenobiotics can harm the liver and kidneys [32]. There were no significant variations in any of the biochemical parameters when compared to the control.

ALT is a superior indicator for assessing liver harm, and the absence of substantial increases at all dosages shows that the extract may not be toxic to the liver. High amounts of ALP and AST in the blood could indicate liver failure, whereas low levels indicate normal liver function [33]. The extract's safety is supported by non-significant variations in ALT, ALP, and AST levels between treated and control animals. The non-significant changes in albumin and bilirubin levels are also strong evidence of the therapies' likely non-detrimental impact on the liver.

Histological examination of tissue, particularly the liver, following therapy, is another method for determining the safety of drug-induced toxicity [34]. The liver's gross and histological evaluation revealed no significant abnormal changes. In the acute toxicity investigation, the Lethal Dose (LD_{50}) of 5000 mg/kg b.wt reveals that HLE, MSE, and ALE are safe to employ under acute situations.

Conclusion

The current work on *B. aegyptiaca* leaf extracts revealed its phytochemical constituents with medicinal properties. It is calculated that the LD_{50} value was greater than 5000 mg/kg body weight. Based on the biochemical characteristics of this investigation, HLE, MLE, and ALE are all safe at orally delivered dosages. Extracts were also demonstrated not to cause liver problems. Histopathological examination of the liver revealed no observable lesions after treatment with extracts at all doses.

Data Availability

The data used to support the findings of this study are available within the article.

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Author's Contribution

All authors contributed equally to this work

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