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Research Article

Toxicity assessment of a phytomedicine based on an almond extract from the fruit of *Balanites aegyptiaca* (L.) DELILE (Zygophyllaceae) intended for helminthiases care in Burkina Faso

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Abstract

Balanites aegyptiaca (L.) Delile (Zygophyllaceae) is a medicinal plant used in traditional medicine in Burkina Faso to treat helminthiasis. Using an ethnopharmacological approach, the "Institut de Recherche en Sciences de la Santé (IRSS)" has developed a phytomedicine based on an almond extract from the fruit of this plant for the treatment of helminthiasis. This study aimed to assess the acute and subacute oral toxicity and in vivo mutagenicity of phytomedicine. The acute toxicity study was carried out following OECD guideline 423 by administering a single dose of 2000 mg/kg body weight (bw) of phytomedicine orally to female mice. For subacute toxicity, OECD guideline 407 was used. Four batches of 10 rats (5 males and 5 females) were used, including a control batch and three treated batches receiving a daily oral administration of the phytomedicine at doses of 250, 500, or 1000 mg/kg bw for 28 days. The mutagenicity test was performed according to OECD guideline 474; five batches of 10 mice (5 males and 5 females) were given oral doses of 500, 1000 or 2000 mg/kg bw of phytomedicine, colchicine as a positive control at 5mg/kg bw and distilled water as a negative control. In the acute toxicity test, the LD50 was estimated at 5000 mg/kg bw. The subacute toxicity study showed no mortality or signs of toxicity in rats. Biochemical analysis revealed no significant differences between control rats and those treated with the phytomedicine for glucose, creatinine, total cholesterol, triglycerides, AST, chlorine, calcium, and potassium. On the other hand, serum levels of ALT, total protein, PO_4^{3-}, and Na^+ were significantly reduced in some treated rats. The mutagenicity test showed no change in micronucleus frequency between treated and control mice. This study showed that phytomedicine based on an extract of *B. aegyptiaca* fines would present less danger to users.

Keywords: *Balanites aegyptiaca*, acute toxicity, subacute toxicity, micronucleated erythrocytes, phytomedicine.

INTRODUCTION

Human helminths are parasites found in humans and animals' intestinal tract, urinary tract, or blood. The helminth species that cause the most morbidity in humans are schistosomes, intestinal nematodes, and tissue nematodes, including the human filariae responsible for lymphatic filariasis and onchocerciasis [1]. These intestinal parasitic infections mainly affect school-age children's health and development and slow their growth [2]. The World Health Organization (WHO) estimates that nearly 1.6 billion people worldwide are infected by helminths [3]. Most cases are observed in Latin America, Asia, and sub-Saharan Africa, including Burkina Faso [3]. According to 2022 annual statistics from Burkina Faso's Ministry of Health and Public Hygiene, more than 1.7 million people were treated for schistosomiasis [4]. Helminthiasis is, therefore, a public health problem and one of the most common infections in the poorest and most disadvantaged communities with limited access to drinking water, sanitation, and hygiene [5].

In Africa, up to 80% of the population relies on traditional medicine to meet their healthcare needs [6]. This is also the case for the populations of Burkina-Faso's Eastern, Central, and Central-Eastern regions, who use traditional

medicines for their primary healthcare. In 2022, 85% of this population reported using traditional medicine for primary healthcare [7]. The widespread use of traditional medicine is often attributed to its accessibility; it is sometimes also the only source of affordable healthcare, particularly for the world's poorest patients. Traditional medicine is also very popular in many developed countries because it is firmly integrated into more global belief systems [6].

Among the medicinal plants used in Burkina Faso to treat helminthiasis, they are *Balanites aegyptiaca* (L.) Delile (Zygophyllaceae) [2]. Using an ethnopharmacological approach, the department of "Médecine et Pharmacopée traditionnelle et Pharmacie (MEPHATRA/PH)" of the "Institut de Recherche en Sciences de la Santé (IRSS)" has developed a phytomedicine based on an almond extract from the fruit of *Balanites aegyptiaca* (*B. aegyptiaca*) for the treatment of helminthiasis [2]. Several studies have demonstrated the pharmacological properties of *B. aegyptiaca* in managing helminthiasis [8–11]. Toxicological studies have also been carried out on aqueous extracts of *B. aegyptiaca*, showing that they are harmless [12]. However, these toxicological studies have not been conducted on the phytomedicine formulated to ensure its safety before use.

This study aimed, therefore, to contribute to the safe use of *B. aegyptiaca* almond extract-based phytomedicine in managing helminthiasis in children in Burkina Faso by conducting toxicological studies.

MATERIAL AND METHODS

Drug product

The plant material of this study was a phytomedicine in syrup form derived from the kernels of *B. aegyptiaca* fruit. It was supplied by the drug production and marketing unit "U-PHARMA" IRSS. The phytomedicine consists of a blend of freeze-dried almond extract of *B. aegyptiaca*, tween 60 as a wetting agent, xanthan gum as a viscosifying and stabilizing agent, sucrose as a sweetener and sodium benzoate as a preservative [2]. It is intended to manage helminthiasis in children.

Animal material

Female NMRI mice with mean body weights of 30.00 ± 1.20 g were used for acute toxicity and mutagenicity tests. Male and female Wistar rats with mean body weights of 209.00 ± 18.80 g and 168.00 ± 13.80 g, respectively, were used for the subacute toxicity test. The animal came from the "Centre International de Recherche Développement sur l'Elevage en zone Subhumide (CIRDES)" in Bobo Dioulasso. They were acclimatized to the conditions of toxicology and ecotoxicology laboratory of IRSS for two weeks (room temperature (22 ± 3^{0}C), 12 hours light/12 hours dark cycle, and 65% relative humidity).

Acute toxicity test

To assess acute oral toxicity, we followed OECD guideline 423 for the testing of chemicals toxicity class method [13]. A single dose of 2000 mg/kg of the phytomedicine was administered to a group of three mice and distilled water to a control group of three female mice. The mice were fasted 4 hours prior to receiving phytomedicine. After treatment, the mice were observed every 30 min for the first two (02) hours after administration, after which food and water were restored. The animals were then monitored daily for 14 days. This observation aimed to identify mortality and signs of toxicity, such as changes in skin, hair, eyes, and mucous membranes. The test was repeated with a 2000 mg/kg dose under the same conditions.

Subacute toxicity test

The subacute oral toxicity study was conducted in accordance with OECD guideline 407 for the testing of chemicals [14]. Four groups of 10 rats were formed (5 males and 5 females): one control group and three test groups. The test groups received the phytomedicine orally at doses of 250, 500, or 1000 mg/kg bw, respectively. Control rats received distilled water over the same period. During the test period, all rats underwent a daily clinical examination to record symptoms of intoxication, such as changes in fur, eyes, and mucous membranes, the appearance of secretions and excretions, and neuro-vegetative reactions (tear secretion, horripilation, pupil size, abnormal respiration, etc.), as well as any mortalities that may have resulted from the use of the phytomedicine.

Assessing changes in body weight, water and food consumption

All rats were weighed at the start of the test, before administration of the test substance, once a week during the 28-day study, and on the last day before sacrifice. Water consumption was assessed daily using graduated bottles, and food consumption was assessed weekly by weighing during the 28-day trial.

Biochemical analysis

At the end of the test, the rats were fasted for 12 hours, then anesthetized with Ketamine 50 mg/mL at a dose of 0.8 mL/kg. Blood was collected by cardiac puncture for biochemical tests. Analyses included blood levels of glucose (Glu), creatinine (Crea), total cholesterol (Total Chol), triglycerides (Trigly), total protein (Total prot), aspartate aminotransferase (AST), alanine aminotransferase (ALT), chlorine (Cl^-), phosphate (PO_4^3), calcium (Ca^{2+}), potassium (K^+), sodium (Na^+). Analyses were performed using Mindray 88 A spectrophotometer.

In vivo mutagenicity study

Experimental design

The mammalian erythrocyte micronucleus test was performed following OECD guideline 474 for the phytomedicinal mutagenicity evaluation [15]. Five groups of 10 mice (5 males and 5 females) were formed: a negative control group, a positive control group, and three test groups. The test groups received the phytomedicine orally at doses of 500, 1000, and 2000 mg/kg bw, respectively, for seven (07) days. The negative control received distilled water, and the positive control colchicine at a dose of 5 mg/kg body weight orally over the same study period. During the study period, all mice

underwent a daily clinical examination to record symptoms of intoxication such as changes in fur, eyes, mucous membranes, appearance of secretions and excretions, and neuro-vegetative reactions (tear secretion, horripilation, pupil size, abnormal respiration), as well as phytomedicine-related mortalities.

Slide preparation, staining and reading

At the end of the test period, blood was collected from the tail vein 24 hours after the last administration. Blood smears were taken, air-dried, fixed with methanol and stained with Giemsa diluted 1:10 for 20 min. Slides were air-dried and read under a light microscope at x1000 magnification. The proportion of micronucleated erythrocytes (Mn) and polychromatic erythrocytes (PCE) was determined by counting 1000 erythrocytes, then the frequency of micronucleated erythrocytes was calculated using the following formula:

$$\% \text{ Mn} = \frac{\text{Number of miconucleated erythrocytes counted}}{\text{Total number of erythrocytes counted}} \times 100$$

Ethical considerations

Experimental studies were carried out in accordance with international standard protocols (European Union Guidelines for the protection of animals of the CEC 86/609 Council) [16].

Statistical and data analysis

Data were calculated separately for males and females at different doses using Microsoft Excel 2016. Results were presented as mean ± standard deviation. Statistical analysis of results was performed using one-way analysis (ANOVA) with GraphPad Prism 8 software (GraphPad Software, San Diego, California, USA), followed by Dunett's multiple comparison tests. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Acute toxicity

The acute toxicity study revealed that the administration of a single oral dose of 2000 mg/kg bw of the phytomedicinal did not cause any disturbance or signs of toxicity or mortality in mice during the 14-day experimental study (Table I). According to OECD guidelines and the results obtained in Table I, the LD_{50} of the phytomedicine is estimated at 5000 mg/kg bw by the oral route. This enables us to classify this phytomedicine in category 5 of the OECD's Globally Harmonized System (GHS) classification[13] and United Nations (2017)[17], i.e., substances presenting almost no hazard to humans.

Table I: Mortality of control mice and those treated with 2000 mg/kg bw

Doses	First test (n=3)	Second test (n=3)
Control group	0/3	0/3
Test group	0/3	0/3

Subacute toxicity

Phytomedicinal effects on body weight, food and water consumption

The body weight of control rats and those treated with the phytomedicinal extract of *B. aegyptiaca* fruit are shown in Fig 1. Both the control and treated rats gained weight over the 28-day study period. However, no significant difference was observed in the weight evolution of treated rats compared to control rats. The results of average water consumption per rat and sex per day are presented in Table II. There was no significant difference between male rats treated with the phytomedicine at 250, 500, and 1000 mg/kg bw compared with respective controls. On the other hand, a significant decrease was observed in females treated with 250 mg/kg bw during some time, compared with controls. However, this difference in feed consumption was not observed at 1000 mg/kg body weight. Mean feed consumption per rat is shown in Table V. As shown in this table, there was no variation between rats treated with *B. aegyptiaca* fine phytomedicine and control rats.

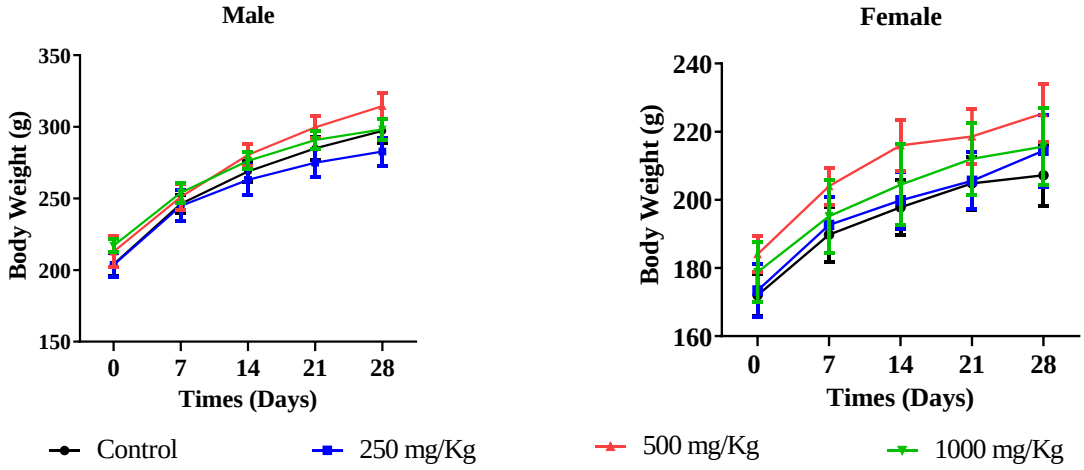

Figure 1: Body weight evolution in males and females' rats during the test period (data expressed as mean ± SD, n = 5). Treated animals are compared with a group of untreated animals.

Table II: Average daily water consumption (mL/rat/day) in control and treated rats for 28 days

Times	Sex	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Week 1	Male	62.14 ± 8.59	61.71 ± 11.50	63.86 ± 7.90	61.43 ± 9.45
	Female	45.00 ± 5.00	52.14 ± 2.67**	45.71 ± 3.45	47.86 ± 3.98
Week 2	Male	59.29 ± 5.35	56.43 ± 5.56	59.29 ± 6.73	62.14 ± 3.93
	Female	40.00 ± 2.89	45.00 ± 5.77	43.57 ± 3.78	43.57 ± 6.27
Week 3	Male	57.86 ± 6.99	52.86 ± 2.67	62.14 ± 3.93	56.43 ± 5.56
	Female	37.86 ± 3.93	44.29 ± 3.45*	37.14 ± 2.67	41.43 ± 4.76
Week 4	Male	58.57 ± 5.56	51.43 ± 4.76	63.57 ± 7.48	58.57 ± 5.56
	Female	37.14 ± 3.93	45.00 ± 5.00**	37.86 ± 3.93	42.14 ± 4.88

Data presented as mean ± SD, with n = 5; *p < 0.05; **p < 0.01 vs. control.

Table III: Average daily feed consumption (g/rat/day) in control and treated rats for 28 days

Times	Sex	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Week 1	Male	11.83	11.23	10.06	11.69
	Female	10.34	9.71	12.09	12.03
Week 2	Male	21.69	20.57	23.60	21.26
	Female	16.34	16.14	17.71	16.60
Week 3	Male	20.74	20.26	22.94	19.11
	Female	15.80	15.89	14.86	16.34
Week 4	Male	22.00	20.46	23.69	23.17
	Female	16.40	17.49	18.94	16.80

Data presented as mean, n = 5.

Effect of phytomedicines on biochemical parameters in rats

The results obtained from the analysis of some biochemical parameters of control and *B. aegyptiaca* fine extract-treated rats after 28 days are shown in Table IV. These results show a decrease in serum levels of alanine aminotransferase (ALT), total protein, phosphorus ions (PO_4^{3-}), and sodium ions in some treated rats. A significant decrease in total protein was also observed in female rats treated with 250 mg/kg compared with control rats. For ALT, the decrease was significant in male rats treated at 500 and 1000 mg/kg compared with control rats. As for phosphorus, the decrease was significant in male rats treated at 1000 mg/kg compared with controls. Finally, the decrease in Na^+ was significant in females treated at 500 mg/kg compared with controls. On the other hand, there was no significant difference between control rats and those treated with *B. aegyptiaca* fine phytomedicine for parameters such as glucose, creatinine, total cholesterol, triglycerides, AST, chlorine, calcium and potassium.

Mutagenicity test

The results of the micronucleus erythrocytes mutagenicity study were presented, indicating the frequency of micronuclei in the peripheral blood of males and females mice treated with the phytomedicinal extract of *B. aegyptiaca* fines (Fig. 2). The number of polychromatic erythrocytes per 1000 erythrocytes and the PCE/NCE ratio are presented in Table VI. The results of this study show a significant increase in Mn frequency in the erythrocytes of mice treated with colchicine (positive control) compared with negative control. However, no significant difference was observed in mice treated with the phytomedicine at different doses, compared with negative control. There was also no significant difference in the number of PCE and the PCE/NCE ratio of treated mice compared with control. Micronuclei, polychromatic and normochromatic erythrocytes were observed on the slides (Fig. 3).

Table IV: Biochemical parameters of control and treated rats for 28 days

Parameters	Sex	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Glu	M	2.89 ± 0.42	3.04 ± 0.67	3.61 ± 0.77	2.89 ± 0.52
(mmol/L)	F	4.08 ± 0.89	3.82 ± 1.08	5.05 ± 0.45	3.52 ± 0.77
Creat	M	123.80 ± 27.73	110.20 ± 16.89	113.60 ± 19.92	120.20 ± 13.72
(µmol/L)	F	119.80 ± 17.68	100.00 ± 4.69	103.60 ± 14.40	114.20 ± 7.46
Total chol	M	1.72 ± 0.35	1.55 ± 0.31	1.58 ± 0.29	1.65 ± 0.29
(mmol/L)	F	1.47 ± 0.28	1.35 ± 0.29	1.45 ± 0.46	1.45 ± 0.27
Trigly (mmol/l)	M	0.26 ± 0.08	0.20 ± 0.00	0.30 ± 0.20	0.20 ±0.07
	F	0.28 ± 0.13	0.22 ± 0.05	0.28 ± 0.17	0.40 ± 0.20
AST	M	100.20 ± 10.94	75.00 ± 14.88	82.40 ± 17.56	82.00 ± 23.10
(UI/L)	F	109.80 ± 26.73	82.25 ± 41.59	85.80 ± 11.63	94.80 ± 21.21
ALT	M	36.00 ± 8.98	25.00 ± 10.39	19.40 ± 5.37*	17.20 ± 3.35**
(UI/L)	F	47.00 ± 24.48	22.00 ± 7.00	23.40 ± 2.88	33.50 ± 14.91
Total Prot	M	70.00 ± 5.39	72.60 ± 5.18	72.20 ± 2.86	72.20 ± 5.54
(g/L)	F	84.00 ± 5.87	72.25 ± 3.20*	80.40 ± 5.03	81.80 ± 8.67
Ca^{2+}	M	2.58 ± 0.07	2.59 ± 0.17	2.71 ± 0.10	2.66 ± 0.14
(mmol/L)	F	2.74 ± 0.07	2.45 ± 0.17	2.97 ± 0.08	2.78 ± 0.27
PO_4^{3-}	M	3.94 ± 0.36	3.55 ± 0.81	3.676 ± 0.25	2.93 ± 0.17**
(mmol/L)	F	3.47 ± 0.53	3.40 ± 0.25	3.16 ± 0.33	2.79 ± 0.70
Cl^-	M	106.60 ± 2.70	105.80 ± 1.92	108.40 ± 2.07	103.40 ± 2.30
(mmol/L)	F	106.00 ± 1.00	108.50 ± 3.32	106.40 ± 1.95	103,80 ± 2,49
Na^+	M	130.40 ± 4.34	129.20 ± 2.39	130.80 ± 1.64	126.80 ± 3.11
(mmol/L)	F	132.40 ± 2,51	127.50 ± 3,79	125.60 ± 3,21*	127.00 ± 5,24
K^+	M	5.96 ± 0.62	5.72 ± 1.65	5.30 ± 0.95	6.24 ± 1.04
(mmol/L)	F	5.58 ± 1.09	4.93 ± 0.57	5.36 ± 0.82	5.76 ± 0.75

Data presented as mean ± SD, with n = 5; *p < 0.05; **p < 0. 01 vs control; glucose (Glu), creatinine (Crea), total cholesterol (Total Chol), triglycerides (Trigly), total protein (Total Prot), aspartate aminotransferase (AST), alanine aminotransferase (ALT), chlorine (Cl^-), phosphorus (PO_4^{3-}) calcium (Ca^{2+}), potassium (K^+), sodium (Na^+). F = female, M = male.

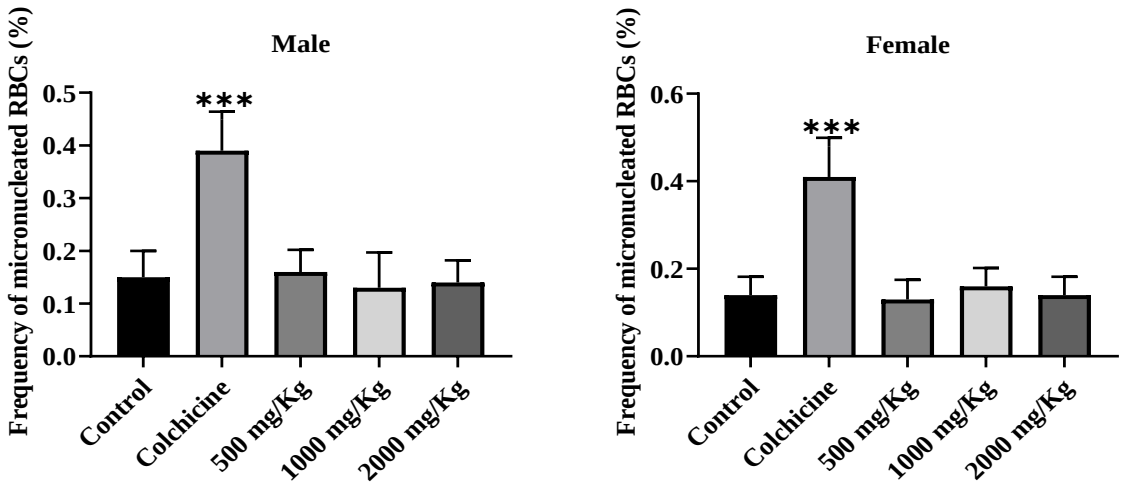

Figure 2: Frequency of micronucleated erythrocytes in peripheral blood of control and treated mice. ***p < 0.001 vs control.

Table V: Number of polychromatic erythrocytes per 1000 mouse erythrocytes in control and treated mice for two weeks

	Sex	Control	Colchicine	500 mg/kg	1000 mg/kg	2000 mg/kg
PCE	Male	17.80 ± 3.34	16.40 ± 7.02	18.60 ± 4.27	19.00 ± 7.14	15.60 ± 3.36
	Female	16.20 ± 5.58	20.20 ± 4.17	16.40 ± 4.72	17.00 ± 2.82	16.40 ± 1.81
PCE/	Male	1.81 ± 0.65	1.70 ± 0.75	1.89 ± 0.44	1.94 ± 0.79	1.58 ± 0.34
NCE (%)	Female	1.64 ± 0.58	2.06 ± 0.49	1.66 ± 0.48	1.73 ± 0.29	1.67 ± 0.18

Data presented as mean ± standard deviation, with n = 5; significant at *p < 0.05 vs control; ECN: Normochromatic Erythrocyte; EPC: Polychromatic Erythrocyte.

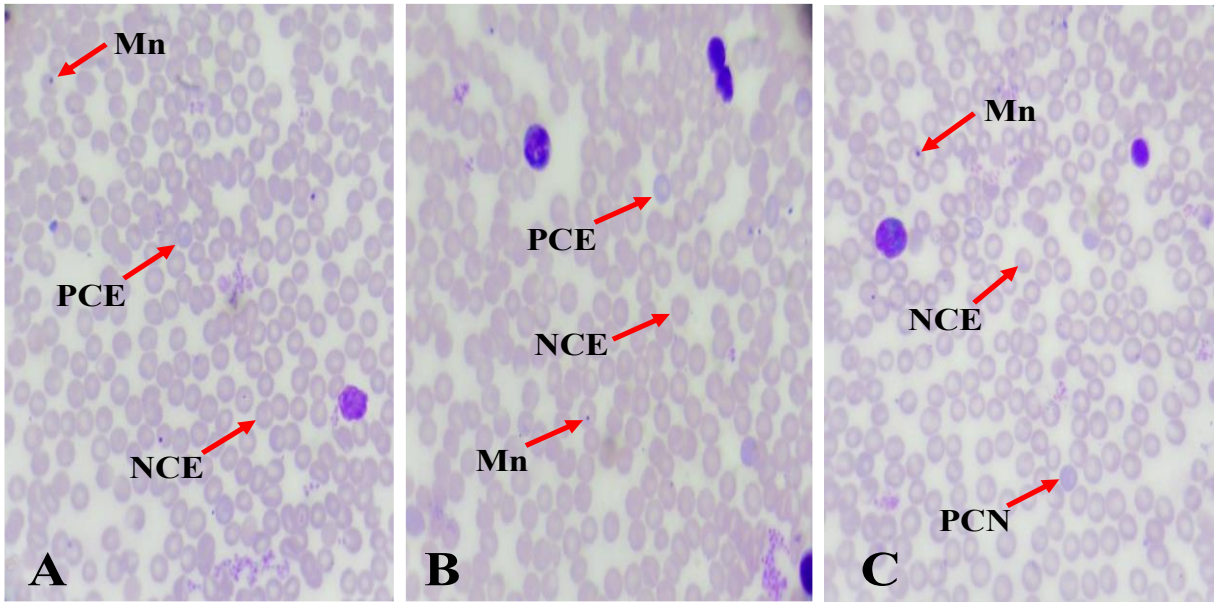

Figure 3: Microscopic observation showing micronucleated, mono, and polychromatic erythrocytes. A: negative control mice; B: positive control mice treated with colchicine; C: mice treated with phytomedicine at 2000 mg/Kg bw; MN: micronucleus; NCE: normochromatic erythrocyte; PCE: polychromatic erythrocyte.

DISCUSSION

B. aegyptiaca is known for its numerous pharmacological properties and is used in traditional medicine to treat a wide range of pathologies, including helminthiasis. The aim of this study was to evaluate the toxicity of a phytomedicine formulated from an almond extract of the fruit of *B. aegyptiaca* with a view to securing its use in the treatment of helminthiasis in children.

In acute toxicity test, a single oral dose of 2000 mg/kg to mice showed no toxicity or mortality. The LD_{50} is estimated at 5000 mg/kg body weight. This enables us to classify this phytomedicine in category 5 of substances that are virtually harmless to humans according to the OECD's Globally Harmonized System (GHS) classification. [13] and the United Nations. Our results are similar to those of other authors, who showed that the crude extract of *B. aegyptiaca* fruit at a maximum dose of 5000 mg/kg body weight administered orally to rats produced no signs of toxicity [18].

In the subacute toxicity study, no signs of toxicity or mortality were observed in rats. Weight development is used to assess the impact of chemicals on the physical and psychological state of the animals [14]. The results of this study show that there was an increase in body mass in all control rats and those treated with phytomedicine, but there was no significant difference in the weight of treated rats compared with controls. This could be explained by the fact that the phytomedicine has no adverse effect on weight gain in rats. Over 28 days, there was no significant difference in food consumption between rats treated with the phytomedicine and controls. With regard to water consumption, there was a significant decrease in females treated with 500 mg/kg body weight during some time, compared with control, but this was not dose-dependent. On the other hand, there was no significant difference between male rats treated with phytomedicinal and those treated with control. This difference has no physiological significance in that it does not affect the weight of the rats over the same period [19].

Analysis of biochemical parameters makes it possible to evaluate the various functions of the organism after administration of the phytomedicine based on an extract of the kernel of the fruit of *B. aegyptiaca* for 28 days [14].

The liver is a central organ that plays an essential role in the body's homeostasis. These include synthesis (proteins), metabolism (glucose), degradation (drugs), and excretion (bilirubin) [20]. Transaminases (AST, ALT) are the most appropriate biochemical parameters for investigating liver functions, especially metabolism.

These two enzymes are present in the cytoplasm of hepatocytes and are released in the event of hepatic cell lysis [20]. However, ALT is much more specific to the liver than AST, as it is also present in red blood cells and muscle cells and may, therefore, be elevated in hemolysis, rhabdomyolysis, or myocardial infarction [20]. After administration of the phytomedicinal to rats, biochemical analysis showed a significant decrease in ALT in rats treated at 500 and 1000 mg/kg bw compared with the corresponding controls.

On the other hand, there was no significant difference in AST level in treated rats compared to control. Other authors have shown that ethanolic extract of *B. aegyptiaca* leaves at oral doses of 100 and 200 mg/kg significantly reduced transaminases (AST, ALT) after hepatotoxicity induced in rats with carbon tetrachloride (CCl_4) [21]. On the other hand, a significant decrease in total protein was observed in females dosed at 250 mg/kg bw compared to control rats in the respective groups. This may be explained by the reduced protein synthesis functions of the liver in these rats [22].

Electrolytes are essential for the basic functioning of life, such as maintaining electrical neutrality in cells and generating and conducting action potentials in nerves and muscles [23]. The main electrolytes are sodium, potassium, chloride, magnesium, calcium, phosphate, and bicarbonate. High or low electrolyte levels interfere with normal bodily functions and can lead to life-threatening complications [23]. Sodium is one of the essential electrolytes in extracellular fluid. It is responsible for maintaining extracellular fluid volume and regulating cell membrane potential. It is regulated in the kidneys, with major reabsorption in the proximal tubule [24]. Phosphorus is an extracellular fluid cation. It is a component of many metabolic intermediates, especially ATP and nucleotides; the kidneys are the main route of phosphorus excretion [23]. The results of our study showed some variations in ion composition. There was a significant decrease in phosphorus in male rats given 1000 mg/kg bw and in sodium in female rats given 500 mg/kg bw, compared with control rats in the respective groups. On the other hand, there were no significant differences in chlorine, calcium, and potassium ions. This could be explained by the phytomedicine's ability to stimulate the elimination of these ions or inhibit their reabsorption.

After 28 days of treatment, there was no significant difference in total cholesterol, triglyceride, and glucose levels in treated rats compared with controls. This shows that our phytomedicine does not influence lipid or glucose metabolism.

The results of this study show a significant increase in micronucleus frequency in the erythrocytes of mice treated with colchicine (positive control) compared with negative controls. However, no significant difference was observed in mice treated with different doses of the phytomedicine compared with negative controls. There was also no significant difference in the number of PCE and the PCE/NCE ratio of treated mice compared with controls. Colchicine is known as an aneugenic substance and is used as a positive control in the mammalian erythrocyte micronucleus assay [15]. It is a mitosis inhibitor that induces micronuclei in polychromatic erythrocytes during hematopoiesis in mice [25]. This effect of colchicine is due to the spindle disruption that occurs after its binding to tubulin dimers in spindle microtubules [25]. Other authors have demonstrated the antimutagenic activity of *B. aegyptiaca* fruit oil. *B. aegyptiaca* oil reduced in a dose-dependent manner (50 and 100 ppm) the percentage of micronuclei and chromosomal aberrations in hepatocytes and splenic cells infected with *Fasciola gigantica* [26]. In addition, a mixture of balanitin-6 (28%) and balanitin-7 (72%), both steroidal saponins isolated from the kernels of the *B. aegyptiaca* fruit, inhibited the growth of human cancer cell lines in vitro. The mixture (balanitin-6 and balanitin-7) showed superior antiproliferative activity to that of well-known natural anti-cancer therapeutic agents such as etoposide and oxaliplatin [27].

CONCLUSION

The aim of this study was to assess the toxicity of a phytomedicine based on an almond extract from the fruit of *B. aegyptiaca*, intended for the treatment of helminthiasis in children in Burkina Faso. Studies focused on acute and sub-acute toxicity and the micronucleus test on mammalian erythrocytes. The phytomedicinal was practically non-toxic at the doses tested in mice and rats, with an estimated oral LD_{50} of 5000 mg/kg in mice. Repeated administration of the phytomedicinal over 28 days did not affect rat body weight gain, kidney, liver, or lipid metabolism. The results of this study show that the phytomedicinal did not induce micronucleus formation in mouse erythrocytes at doses of up to 2000 mg/Kg body weight. Taken together, these results lead to the conclusion that the use of phytomedicines based on an extract of the almond fruit of *B. aegyptiaca* at tested doses would present less risk to users. However, further toxicity studies, such as reprotoxicity, need to be carried out to ensure its safety.

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REFERENCES

1. Muller R, Wakelin D., Worms and Human Disease. 2nd ed. CABI, 2002; P 312.
2. Ouedraogo S, Sidiki T, Jules Y, Aristide T, Traore T, Marius L, et al., Formulation and Evaluation of a Syrup Based on *Balanites aegyptiaca* L. Delile, Journal of Pharmaceutical Research International, 2010; 23: 1-9. https://doi.org/10.9734/JPRI/2018/42857
3. Organisation mondiale de la santé, Rapport annuel des statistiques sanitaires mondiale, 2002; p. 119.
4. Ministère de la santé et de l'Hygiène Publique Burkina Faso, Annuaire des statistiques 2022, 2023 ; p. 390.
5. Organisation mondiale de la Santé, Lutte contre les helminthiases chez les enfants d'âge scolaire, Guide à l'intention des responsables des programmes de lutte, 2012; 2em éd. Genève. P. 76.
6. Organisation mondiale de la santé, Stratégie de l'OMS pour la médecine traditionnelle pour 2002-2005, 2002; p. 65
7. Ouoba K, Lehmann H, Zongo A, Pabst JY, Semdé R., Prevalence of traditional medicines use and adverse events: A population-based cross-sectional survey in Burkina Faso, European Journal of Integrative Medicine, 2022; 51: 102129. https://doi.org/10.1016/j.eujim.2022.102129
8. Guissou IP, Ouedraogo S, Sanfo A, Some N., Etude de l'effet antihelminthique des amandes de *Balanites aegyptiaca* (L) del (balanitacaea), Burkina medical, 1997; 32-37.
9. Koko WS, Abdalla HS, Galal M, Khalid HS., Evaluation of oral therapy on *Mansonial Schistosomiasis* using single dose of *Balanites aegyptiaca* fruits and praziquantel, Fitoterapia, 2005; 76(1):30-34. https://doi.org/10.1016/j.fitote.2004.08.003
10. Koko WS, Galal M, Khalid HS., Fasciolicidal efficacy of *Albizia anthelmintica* and *Balanites aegyptiaca* compared with albendazole, Journal of Ethnopharmacology, 2000; 71(1-2):247-252. https://doi.org/10.1016/S0378-8741(00)00172-0
11. Anto F, Aryeetey ME, Anyorigiya T, Asoala V, Kpikpi J., The relative susceptibilities of juvenile and adult *Bulinus globosus* and *Bulinus truncatus* to the molluscicidal activities in the fruit of Ghanaian *Blighia sapida*, *Blighia unijugata* and *Balanites aegyptiaca*, Annals of Tropical Medicine and Parasitology, 2005; 99(2):211-217. https://doi.org/10.1179/136485905X24229
12. Gamde M, Wali U, Garba A, Dansy A, Ayuba H., Histopathological and biochemical effects of Aqueous Fruit Extract of *Balanite aegyptiaca* on Selected Organs of Mice, International Journal of Human and Health Sciences (IJHHS), 2023; 07:54-59. http://dx.doi.org/10.31344/ijhhs.v7i1.497
13. OECD, Test No. 423: Acute Oral toxicity - Acute Toxic Class Method | Guidelines for the Testing of Chemicals, Section 4: Health Effects. OECD 2002 (Ed), Paris, France.
14. OCDE, Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents | OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. OECD 2008 (Ed); Paris, France.
15. OCDE, Test No. 474: Mammalian Erythrocyte Micronucleus Test. OECD 2016 (Ed); Paris, France.
16. Union Européenne, Directive 2010/63/UE du Parlement européen et du Conseil du 22 septembre 2010 relative à la protection des animaux utilisés à des fins scientifiques Texte présentant de l'intérêt pour l'EEE, 2010; p. 79.
17. Organisation des Nations Unis, Système général harmonisé de classification et d'étiquetage des produits chimiques (SGH), 7em ed. Genève, 2017; P. 553.
18. Sudhakar K, Gowrishankar N, Shankar M, Suvetha K, Chinna EM., Evaluation of anti-ulcer activity of methanolic extract of *Balanites aegyptiaca* L. bark, International Journal of Phytopharmacology, 2013; 4:308-310.
19. Traesel GK, de Souza JC, de Barros AL, Souza MA, Schmitz WO, Muzzi RM, et al., Acute and subacute (28 days) oral toxicity assessment of the oil extracted from *Acrocomia aculeata* pulp in rats, Food and Chemical Toxicology, 2014; 74:320-325. https://doi.org/10.1016/j.fct.2014.10.026
20. Goirand F, Roman S., Les fondamentaux de la pathologie digestive Enseignement intégré – Appareil digestif, Elsevier Masson, 2014; p. 288.
21. Suky TMG, Parthipan B, Kingston C, Mohan VR, Soris PT., Hepatoprotective and antioxidant effect of *Balanites aegyptiaca* (L.) DEL against CCl_4 induced hepatotoxicity in rats, International Journal of Pharmaceutical Sciences and Research, 2010; 887-892. http://dx.doi.org/10.13040/IJPSR.0975-8232.2(4).887-92
22. Gosselin S., Ramaiah L., and Earl L., Clinical chemistry in toxicity testing: scope and methods, General, Applied and Systems Toxicology, 2009; Wiley, Hoboken, NJ, USA, 1–35. https://doi.org/10.1002/9780470744307.gat040
23. Shrimanker I, Bhattarai S., Electrolytes.Treasure Island (FL), 2023; StatPearls Publishing.
24. Lacour B., Physiologie du rein et bases physiopathologiques des maladies rénales, Revue Francophone des Laboratoires, 2013; 2013(451):25-37. https://doi.org/10.1016/S1773-035X(13)71993-2
25. Ghaskadbi S, Vaidya VG., Studies on modulation of the effects of colchicine by l-cysteine using bone marrow of Swiss mice, Mutation Research/Genetic Toxicology, 1991; 260(2):181-185. https://doi.org/10.1016/0165-1218(91)90006-8
26. Al Ashaal HA, Farghaly AA, Abd El Aziz MM, Ali MA., Phytochemical investigation and medicinal evaluation of fixed oil of *Balanites aegyptiaca* fruits (Balantiaceae). Journal of Ethnopharmacology, 2010; 127(2):495-501. https://doi.org/10.1016/j.jep.2009.10.007
27. Gnoula C, Mégalizzi V, De Nève N, Sauvage S, Ribaucour F, Guissou P, et al., Balanitin-6 and -7: diosgenyl saponins isolated from *Balanites aegyptiaca* Del. display significant anti-tumor activity in vitro and in vivo, International Journal of Oncology, 2008; 32(1):5-15. https://doi.org/10.3892/ijo.32.1.5