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Acute and subchronic toxicity of the aqueous extract of *Bauhinia thonningii* (Caesalpiniaceae) Schum pods

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Abstract

Background: *Bauhinia thonningii* is traditionally used to treat bacterial infections, dysentery, and diarrhea. The present study aimed to evaluate the acute and subchronic toxicity of the aqueous extract of *B. thonningii* pods.
Methods: Acute toxicity was evaluated according to Organisation for Economic Co-operation and Development (OECD) guideline 423 in *Wistar* rats receiving single oral doses of 2000 or 5000 mg/kg of the aqueous extract. Subchronic toxicity was assessed following OECD guideline 407 by oral administration of the extract at doses of 45, 90, and 180 mg/kg body weight for 28 consecutive days in male and female rats. A satellite group was included to evaluate the reversibility of potential toxic effects. Body weight, food, and water consumption were monitored. Hematological, biochemical, and histopathological parameters were evaluated at the end of the treatment period.
Results: No mortality or severe clinical signs were observed during the acute toxicity study, indicating a median lethal dose (LD_{50}) greater than 5000 mg/kg. In the subchronic study, no significant changes in body weight were observed in treated animals compared with controls. The aqueous extract induced a significant decrease in alanine aminotransferase activity in males (P = 0.001) and females (P = 0.005). Triglycerides, total cholesterol, and HDL cholesterol levels were not significantly affected in either sex. Histopathological examination of kidney tissues revealed no treatment-related alterations.
Conclusion: The aqueous extract of *B. thonningii* pods is low-toxic under the experimental conditions of this study

Keywords: hematological parameters; oral safety assessment; Wistar rats.

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Background

Herbal medicine represents a major component of healthcare systems in Africa. Indeed, more than 80% of the African population relies on medicinal plants for the management of various health conditions [1]. This widespread use is mainly attributed to the richness of bioactive secondary metabolites and the broad spectrum of pharmacological activities exhibited by medicinal plants. However, despite their therapeutic potential, medicinal plants may also induce adverse and toxic effects, which are often related to their chemical composition, dosage, and inappropriate use [2]. Several medicinal plants traditionally considered beneficial have been reported to exhibit toxicological effects. For instance, *Peganum harmala* has been associated with hepatotoxicity and neurotoxicity, while *Markhamia lutea* and *Arisaema eunaephyllum* have been reported to induce nephrotoxicity, coma, and even death [3–5]. These findings highlight the necessity of establishing comprehensive toxicological profiles of medicinal plants to ensure their safe and rational use. *Bauhinia thonningii*, a member of the family Caesalpiniaceae, is widely distributed in several African countries, including Cameroon [6]. In traditional medicine, different parts of this plant are used for the treatment of bacterial infections, dysentery, snake bites, respiratory disorders, and diarrhea [7,8]. In particular, the pods are commonly used in traditional local practices, which justify their scientific investigation. Previous pharmacological studies have reported that the pods of *B. thonningii* possess significant antiradical and antibacterial activities [9]. Moreover, a recent study demonstrated that the leaves of this species exhibit a low toxicity profile in rats under acute and subchronic exposure conditions [10]. However, toxicological data specifically concerning the pods remain limited, despite their frequent traditional use. Therefore, the present study aimed to evaluate the acute and subchronic toxicity of the aqueous extract of *B. thonningii* pods in *Wistar* rats, to provide scientific evidence supporting their safe traditional use.

Methods

Plant material

Pods of *B. thonningii* were collected in Foumbot, West Region of Cameroon, in October 2023. Botanical identification was carried out at the National Herbarium of Cameroon by comparison with a reference specimen (No. 16987/SRF), and a voucher specimen was deposited for future reference. The collected pods were cut into small pieces and air-dried at room temperature away from direct sunlight. The dried material was then ground into a fine powder using an electric grinder. The resulting powder was used for the preparation of the aqueous extract.

Preparation of the aqueous extract

The aqueous extract of *B. thonningii* pods was prepared by decoction. Briefly, the pod powder was mixed with five liters of distilled water and boiled for 20 min. After cooling at room temperature, the decoction was filtered using Whatman No. 4 filter paper. The filtrate was subsequently dried in an oven at 40°C for 72 h. The dried extract was weighed, and the extraction yield was calculated (12.5%), corresponding to an extract mass of 62.5 g. The extract was stored at −20°C until use for the preparation of the different doses administered to the animals.

Experimental animals

Male and female (nulliparous) *Wistar* rats, approximately two months old and weighing an average of 110 g, were used in this study. The animals were housed in the animal facility of the Department of Animal Biology, Faculty of Science, University of Dschang, Cameroon. They were maintained under standard laboratory conditions, with natural light–dark cycles and ambient temperature. The animals had free access to standard laboratory chow and drinking water *ad libitum* throughout the experimental period.

Ethical approval

All experiments were conducted in accordance with the ethical guidelines for animal use and care as described by Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes, after approval of the research proposal by the Scientific Committee of the Department of Animal Biology, University of Dschang.

Acute oral toxicity study

The acute oral toxicity of the aqueous extract of *B. thonningii* pods was evaluated in rats according to the Organisation for Economic Co-operation and Development (OECD) guideline No. 423 (Acute Toxic Class Method) [11]. The choice of the aqueous extract was based on its widespread traditional use.

Healthy adult rats were fasted for 18 h prior to oral administration, with free access to water. The animals were randomly allocated into experimental groups using a randomization procedure. In addition, the experimental assessments were performed under blinded conditions to minimize observer bias. The animals were housed under natural environmental conditions with standard laboratory housing, including natural light/dark cycles, ambient temperature, and humidity conditions.

The aqueous extract was prepared as a solution and administered orally. The animals were divided into three groups of six rats each. The control group received distilled water at an administration volume of 1 mL/100 g body weight, while the treated groups received a single oral dose of the aqueous extract of *B. thonningii* pods at 2000 mg/kg and 5000 mg/kg body weight, respectively.

After administration, the animals were closely observed individually during the first 4 h for the occurrence of immediate clinical signs. Attention was paid to behavioral changes, fecal consistency, and mortality. Thereafter, the animals were observed daily for 14 days for delayed signs of toxicity or death.

During the observation period, body weight changes, food consumption, and water intake were recorded. Food and water intake were measured daily by calculating the difference between the quantities provided and those remaining after 24 h. At the end of the 14-day observation period, surviving animals were considered to have tolerated the administered doses.

Subchronic toxicity study

The subchronic toxicity of the aqueous extract of *B. thonningii* was evaluated to identify potential clinical, hematological, biochemical, and histopathological signs of toxicity, in accordance with the Organization for Economic Co-operation and Development (OECD) guideline 407 for repeated-dose 28-day oral toxicity studies in rodents (OECD, 2008) [12].

A total of sixty healthy adult rats (30 males and 30 females), weighing approximately 120 g, were randomly allocated into six groups of ten animals each (five males and five females per group) using a randomization procedure. The experimental assessments were conducted under blinded conditions. The animals were fasted for 18 h prior to the initiation of treatment, with free access to water. They were housed under natural environmental conditions with standard laboratory housing, including natural light/dark cycles, ambient temperature, and humidity conditions. Treatments were administered orally once daily for 28 consecutive days as follows: Groups 1 and 2 (satellite control) received distilled water (1 mL/100 g body weight). Groups 3, 4, and 5 received the aqueous solution of *B. thonningii* at doses of 45, 90, and 180 mg/kg body weight, respectively. Group 6 (satellite treated group) received the aqueous extract at a dose of 180 mg/kg body weight. The selection of doses was based on our previous studies demonstrating significant anti-Salmonella activity of *B. thonningii* pods at the same dose levels [9]. Body weight, food consumption, and water intake were recorded regularly as indicators of general health status and systemic toxicity.

Sample collection and assessment of hematological and biochemical parameters

At the end of the 28-day treatment period (Day 29), animals from Groups 1, 3, 4, and 5 were individually housed in metabolic cages for 24 h to allow urine collection, whereas animals from the satellite groups (Groups 2 and 6) were maintained under observation for an additional 14-day recovery period with free access to food and water, in order to assess the reversibility or persistence of any treatment-related effects.

Urine samples collected over 24 h were used for the determination of creatinine, urea, and albumin levels. On Day 30 (Groups 1, 3, 4, and 5) and Day 43 (satellite Groups 2 and 6), animals were anesthetized by intraperitoneal administration of diazepam (10 mg/kg) followed by ketamine (50 mg/kg). This combination was used to achieve adequate surgical anesthesia, providing sedation, muscle relaxation, and analgesia, as commonly recommended in laboratory rodents for invasive procedures and blood collection [13]. Blood samples were collected by abdominal artery catheterization into ethylenediaminetetraacetic acid (EDTA) tubes for hematological analysis and into plain tubes for serum preparation.

Hematological parameters were analyzed using an automated hematology analyzer. Blood samples collected in plain tubes were centrifuged at 3,000 rotations per minute (rpm) for 15 min to obtain serum, which was stored at −20°C until biochemical analysis. Serum biochemical parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, urea, triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, total protein, and total and conjugated bilirubin, were determined. Analyses were performed using commercially available diagnostic kits according to the manufacturers' instructions.

Following blood collection, the animals were sacrificed, and vital organs, including the liver, kidneys, heart, spleen, and lungs, were carefully excised and freed from surrounding adipose tissue. The organs were then rinsed in physiological saline (0.9% NaCl) and weighed using a precision balance for the determination of relative organ weights. The left kidney and representative portions of the liver were fixed in 10% neutral buffered formalin for subsequent histopathological examination. All histological analyses were conducted under blinded conditions to minimize observer bias and ensure objective assessment.

Statistical analysis

Data were analyzed using GraphPad Prism software (version 8.4.2). Results are expressed as mean ± standard error of the mean (SEM). Prior to statistical analysis, data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. Biochemical and hematological parameters were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test. Changes in body weight, food intake, and water consumption were analyzed using two-way ANOVA followed by Bonferroni's post hoc test. When the assumptions of normality or homoscedasticity were not met, non-parametric tests (Kruskal–Wallis) were applied. Exact p-values were reported, and differences were considered statistically significant at $p < 0.05$.

Results

Acute toxicity

Effects of the aqueous extract of Bauhinia thonningii pods on animal behavior

No signs of acute toxicity such as diarrhea or mortality were observed in treated animals throughout the observation period. No mortality was recorded during the 14-day observation period, indicating that the oral LD_{50} of the aqueous extract is greater than 5000 mg/kg body weight.

Effects of the aqueous extract of Bauhinia thonningii pods on body weight during acute toxicity

Figure 1 illustrates the effects of the aqueous extract administered at doses of 2000 and 5000 mg/kg on the body weight of rats during the acute toxicity assessment. A slight, non-significant decrease in body weight was observed in animals treated with the highest dose (5000 mg/kg) compared with the neutral control group. Overall, no marked alteration in body weight gain was observed, suggesting the absence of acute systemic toxicity.

Effects of the aqueous extract of Bauhinia thonningii pods on food consumption during acute toxicity

As shown in Figure 2, food intake in rats treated with AEBT did not differ significantly from that of the control group throughout the acute toxicity study. However, from the ninth day onward, a slight but non-significant reduction in food consumption was noted in treated animals, indicating that the extract did not adversely affect feeding behavior.

Effects of the aqueous extract of Bauhinia thonningii pods on water consumption during acute toxicity

Figure 3 presents the variation in water consumption following administration of AEBT. No significant differences in water intake were observed between treated and control animals at any dose, indicating that the extract did not interfere with normal hydration behavior during the acute toxicity assessment.

Subchronic toxicity

Effects of the aqueous extract of Bauhinia thonningii pods on body weight evolution during subchronic toxicity

Changes in body weight of female and male rats treated with different doses of the aqueous extract are presented in Figure 4. No significant differences were observed between treated groups and controls throughout the experimental period. However, animals in the satellite group receiving the extract at 180 mg/kg showed a progressive weight gain during the two-week recovery period, although values remained lower than those of the satellite control group. In addition, no significant difference was observed between satellite group values and baseline body weight, suggesting partial recovery toward initial physiological conditions without complete normalization within the recovery period.

Effects of the aqueous extract of Bauhinia thonningii pods on food consumption during subchronic toxicity

In female rats, administration of the extract at a dose of 90 mg/kg significantly increased food consumption by 30.68% during the first week of treatment (P = 0.02) compared with controls (Figure 5). Conversely, a significant decrease in food intake of 23.37% was observed in the satellite group during the third week (P = 0.03). In male rats, significant increases in food consumption were observed at doses of 90 mg/kg (44.23%) and 180 mg/kg (55.49%) during the first week (P = 0.0009 and P < 0.0001, respectively). A significant increase of 23.27% was also recorded in the third week (P = 0.02). Additionally, during the fourth week, doses of 45 mg/kg (35.60%) and 90 mg/kg (30.54%) significantly increased food intake (P = 0.0008 and P = 0.007, respectively). In the satellite group, food consumption remained significantly higher than in the satellite control during the sixth week (19.36%, P = 0.01). Furthermore, no significant differences were observed between satellite (female and male) groups and their respective baseline values, indicating a return toward initial physiological conditions during the recovery period without statistically significant deviation.

Effects of the aqueous extract of Bauhinia thonningii pods on water consumption during subchronic toxicity

Figure 6 illustrates the variation in water consumption during subchronic exposure. In female rats, administration of the extract at 90 mg/kg significantly increased water intake during the first and second weeks (51.34% and 52.92%, respectively; P < 0.0001) compared with controls. In contrast, a significant decrease in water consumption was observed in the satellite group during the first (17.33%, P = 0.01) and fifth weeks (20.81%, P = 0.008). In male rats, the aqueous extract significantly increased water consumption during the first week at doses of 45 mg/kg (28.52%, P = 0.002) and 180 mg/kg (25.84%, P = 0.009), and during the second week at 90 mg/kg (19.93%, P = 0.02). Overall, satellite group values did not differ significantly from baseline in both males and females, indicating recovery toward initial physiological conditions during the post-treatment period.

Effects of the aqueous extract of Bauhinia thonningii pods on relative organ weights during subchronic toxicity assessment

Table 1 presents the effects of subchronic administration of the aqueous extract of *B. thonningii* pods on relative organ weights in male and female rats. In female rats, a significant increase of 70.61% (P = 0.01) in relative spleen weight was observed at the dose of 180 mg/kg compared with the control group. No significant changes were recorded in the relative weights of the liver, kidneys, heart, or lungs at any dose. In male rats, treatment with the aqueous extract at doses of 45, 90, and 180 mg/kg did not induce any significant variation in the relative weights of the examined organs compared with the neutral control group. Similarly, no significant differences were observed between the satellite treated group and the satellite control group.

Effects of the aqueous extract of Bauhinia thonningii pods on hematological parameters during subchronic toxicity assessment

The effects of subchronic administration of the aqueous extract of *B. thonningii* pods on hematological parameters in female and male rats are summarized in Tables 2 and 3, respectively. In female rats, treatment with the extract at all tested doses did not result in any significant changes in white blood cell count, lymphocytes, monocytes, granulocytes, platelet count, red blood cell count, hemoglobin concentration, or hematocrit values compared with the neutral control group. Similar findings were observed in the satellite-treated females relative to the satellite control group. In male rats, no significant variations were observed in total white blood cells, monocytes, granulocytes, or platelet counts following extract administration. However, a significant decrease of 60.78% (P = 0.01) in lymphocyte percentage was noted in the satellite-treated group (Sat AEBT 180 mg/kg) compared with the satellite control group. Additionally, a significant reduction in red blood cell count was observed in males treated with 45 mg/kg (40.49%, P = 0.02), 90 mg/kg (55.75%, P = 0.001), and 180 mg/kg (39.98%, P = 0.02) of the extract compared with the control group. Conversely, hemoglobin concentration was significantly increased at doses of 45 mg/kg (20.19%, P = 0.03) and 180 mg/kg (28.33%, P = 0.001) relative to the control group.
In the satellite group, male rats treated with 180 mg/kg of the extract exhibited a significant increase of 82.63% (P = 0.03) in red blood cell count and hematocrit values compared with the satellite control group.

Effects of the aqueous extract of Bauhinia thonningii pods on biochemical parameters during subchronic toxicity assessment

Effects on serum transaminases and alkaline phosphatase activities

Table 4 summarizes the effects of the aqueous extract of *B. thonningii* pods on serum transaminase and alkaline phosphatase activities in rats following subchronic administration. In females, the extract induced a significant decrease in alanine aminotransferase (ALT) activity at doses of 45 mg/kg (58.24%, P = 0.0006), 90 mg/kg (37.32%, P = 0.04), and 180 mg/kg (47.88%, P = 0.005) compared with the distilled water control. A significant reduction in aspartate aminotransferase (AST) activity was also observed at all tested doses, with decreases of 43.04% (P = 0.004), 32.65% (P = 0.008), and 28.16% (P = 0.02), respectively. In males, the extract caused a significant decrease in ALT activity at doses of 45 mg/kg (37.72%, P = 0.04) and 180 mg/kg (55.03%, P = 0.001). A significant reduction in alkaline phosphatase (ALP) activity was observed at the dose of 180 mg/kg (29.63%, P = 0.01) compared with the control group. In the male satellite group (Sat AEBT 180 mg/kg), ALP activity was significantly reduced by 39.13% (P = 0.02) compared with the satellite control group.

Effects on creatinine and urea levels

The effects of the aqueous extract on serum and urinary creatinine and urea levels are presented in Table 5. In females, no significant

changes were observed in any of the evaluated renal function parameters compared with the neutral control group. In males, administration of the extract at 180 mg/kg resulted in a significant decrease in urinary creatinine by 40.02% (P = 0.0008), as well as in serum by 28.22% (P = 0.0001) and urinary urea levels by 48.74% (P = 0.03) compared with the distilled water control. In the satellite group receiving the extract (Sat AEBT 180 mg/kg), a significant increase of 63.20% (P = 0.01) in serum urea concentration was observed compared with the satellite distilled water group.

Effects on lipid profile

Table 6 presents the effects of the aqueous extract of *B. thonningii* pods on lipid profile parameters. In both females and males, no significant differences were observed in triglycerides, total cholesterol, or HDL cholesterol levels compared with the corresponding control groups.

Effects on bilirubin, serum total protein, and urinary albumin levels

The effects of the aqueous extract on total and direct bilirubin, serum total protein, and urinary albumin levels are shown in Table 7. No significant variations were observed in any of these parameters in treated animals of either sex compared with the control groups.

Effects on liver and kidney histology

Histological examination of liver sections revealed a generally preserved hepatic architecture in both male and female rats across all experimental groups (Figure 7). The portal triad structures, including the portal vein, bile canaliculi, and sinusoids, were clearly identifiable. Mild histological changes such as focal hepatocyte vacuolization, limited cytolysis, and discrete leukocyte infiltration were occasionally observed. These alterations were also present in control and satellite control groups and did not show a dose-dependent pattern. Overall, no marked hepatocellular degeneration or treatment-related liver toxicity was observed following 28 days of oral administration of the aqueous extract of *Bauhinia thonningii* pods. Histological analysis of kidney sections showed well-preserved renal architecture in both female and male rats from all experimental groups (Figure 8). Glomeruli, urinary spaces, and proximal and distal convoluted tubules appeared normal and clearly defined. Mild, non-specific histological variations were occasionally noted, including slight changes in the urinary space and tubular cell density. These observations were also present in control animals and did not follow a dose-dependent trend. No significant glomerular or tubular lesions indicative of renal toxicity were observed after subchronic administration of the aqueous extract of *B. thonningii* pods.

Discussion

The present study evaluated the toxicological profile of the aqueous extract of *B. thonningii* pods following acute and subchronic oral administration in rats. In the acute toxicity study, no mortality or clinical signs of toxicity were observed at doses of 2000 and 5000 mg/kg. No changes in food or water intake were recorded. These findings suggest an LD_{50} greater than 5000 mg/kg, indicating low acute toxicity. According to OECD guideline 423, the extract can be considered practically non-toxic after single oral administration. During the subchronic toxicity study, variations in food consumption were observed in treated groups. These variations were not associated with body weight loss. Similar changes have been reported in other medicinal plants and may reflect adaptive physiological responses rather than toxicity [1].

Hematological parameters showed no marked toxic effects. A slight variation in leukocyte populations was observed in males, while females remained unaffected. These changes may indicate mild immunomodulatory activity rather than toxicity. Similar effects have been described for plant extracts rich in secondary metabolites such as flavonoids and alkaloids [14]. A decrease in urinary creatinine and urea was observed in males at the highest dose. Serum creatinine remained unchanged. The significance of this finding may reflect modulation of renal excretory function or protein metabolism. However, in toxicological interpretation, isolated biochemical variations must be interpreted with caution. In the absence of histopathological lesions, these changes are not considered indicative of nephrotoxicity. Similar discrepancies between biochemical and histological data have been reported in repeated-dose toxicity studies and are often interpreted as adaptive physiological responses rather than organ damage [15]. Renal histology confirmed the absence of structural kidney injury in all treated groups.

An increase in spleen weight was observed in females at 180 mg/kg. The spleen plays a central role in immune regulation and hematopoiesis. This variation may suggest a mild immunological response or adaptive stimulation. However, no histopathological alterations were observed. Therefore, this change alone is not sufficient to indicate toxicity. According to OECD principles, organ weight variations must always be interpreted together with histological and biochemical data [16]. Similar variations in spleen weight have been reported in repeated-dose toxicity studies of medicinal plants and are generally considered adaptive rather than pathological.

Liver biochemical analysis showed significant decreases in ALT and AST activities in treated groups. These decreases should be interpreted with caution. A reduction in transaminases does not necessarily indicate hepatoprotection in the absence of mechanistic evidence. Similar biochemical variations have been reported in toxicological studies and may reflect metabolic adaptation rather than protective effects. No significant changes were observed in bilirubin, total protein, triglycerides, or cholesterol levels [17–19].

Histopathological examination of the liver revealed mild alterations, including hepatocyte vacuolation and slight inflammatory infiltration. These changes were minimal and not associated with functional impairment. They should be interpreted as adaptive responses rather than hepatotoxicity. However, they are also insufficient to support a hepatoprotective effect. Similar mild hepatic alterations are commonly reported in repeated-dose toxicity studies of plant extracts [20].

Overall, the aqueous extract of *B. thonningii* pods did not induce severe systemic toxicity under acute and subchronic exposure conditions. The observed changes in urinary creatinine and urea, spleen weight, and liver histology appear to reflect adaptive physiological responses rather than toxic or protective effects. No evidence of hepatoprotective activity can be concluded from the present data due to the absence of mechanistic confirmation. Compared with previous studies on Bauhinia species, the extract demonstrates a generally favorable safety profile in rats.

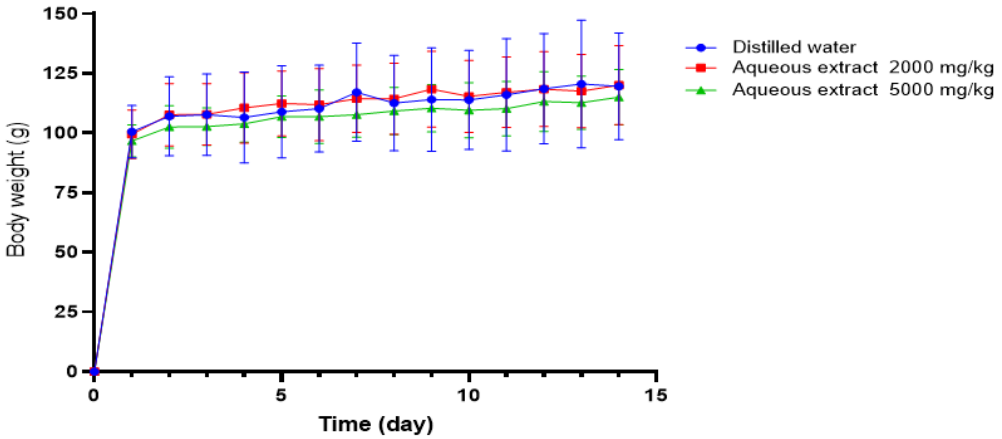

Figure 1. Effect of the aqueous extract of *Bauhinia thonningii* pods on body weight variation during the acute toxicity assessment.
Values are expressed as mean ± SEM (n = 6).

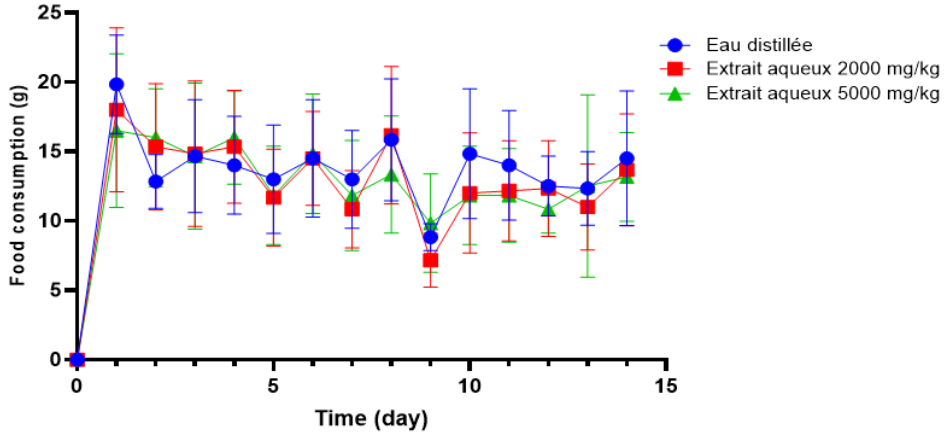

Figure 2. Effect of the aqueous extract of *Bauhinia thonningii* pods on food consumption during the acute toxicity assessment.
Values are expressed as mean ± SEM (n = 6).

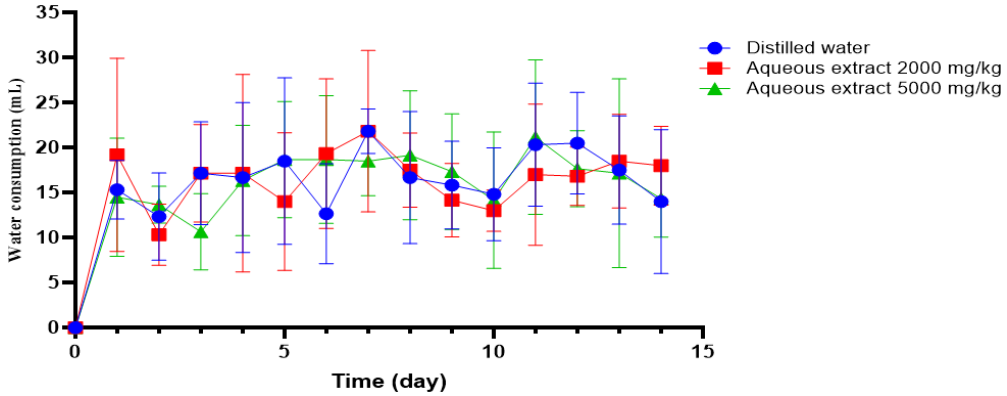

Figure 3: Effect of the aqueous extract of *Bauhinia thonningii* pods on water consumption during the acute toxicity assessment.
Values are expressed as mean ± SEM (n = 6).

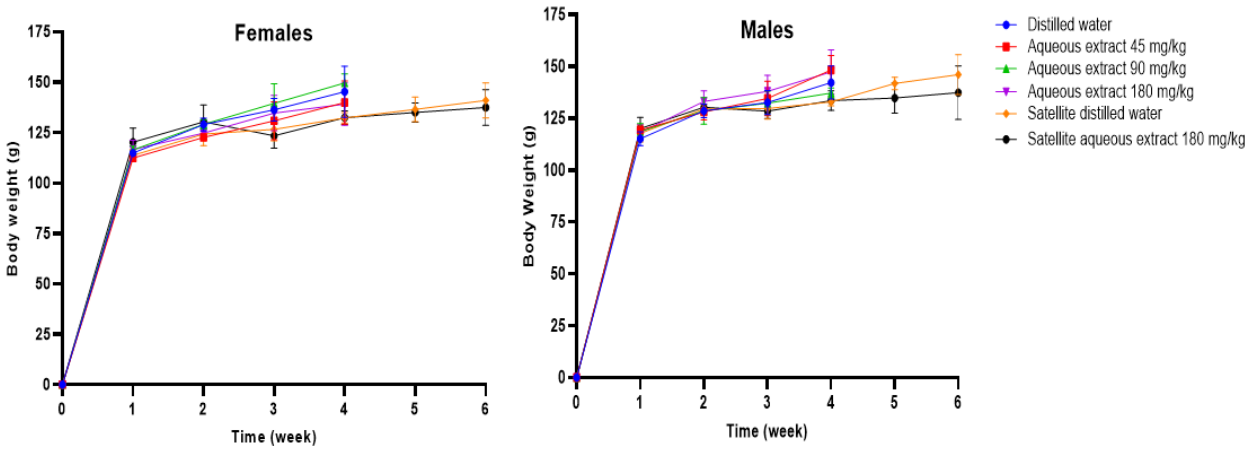

Figure 4. Effect of subchronic oral administration of the aqueous extract of *Bauhinia thonningii* pods on body weight evolution in rats.
Values are expressed as mean ± SEM (n = 5 per sex).

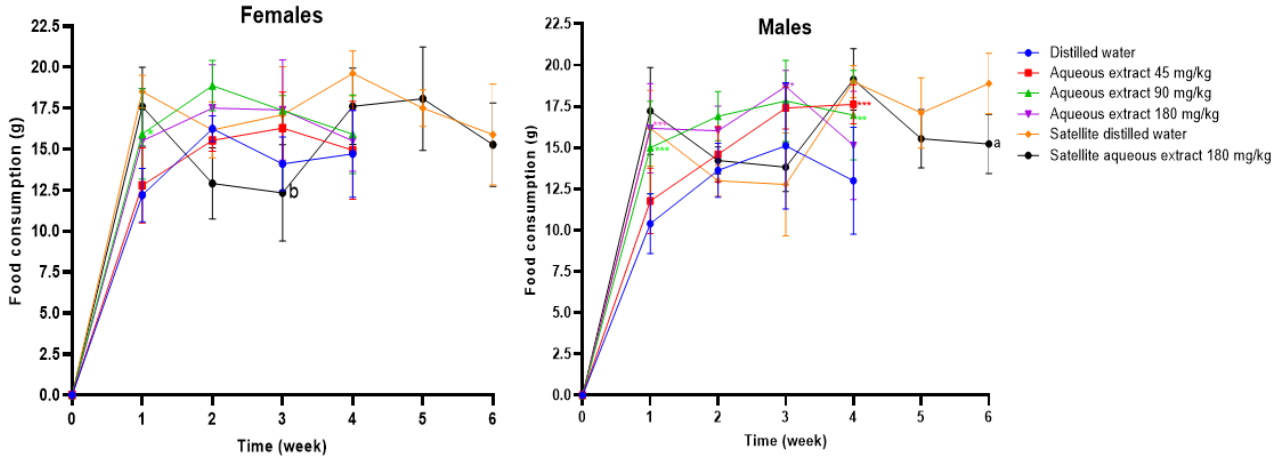

Figure 5. Effect of the aqueous extract of *Bauhinia thonningii* pods on food consumption during the subchronic toxicity assessment.
Values are expressed as mean ± SEM (n = 5 per sex). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the neutral control; ^{a} $P < 0.05$, ^{b} $P < 0.01$ compared to the satellite control.

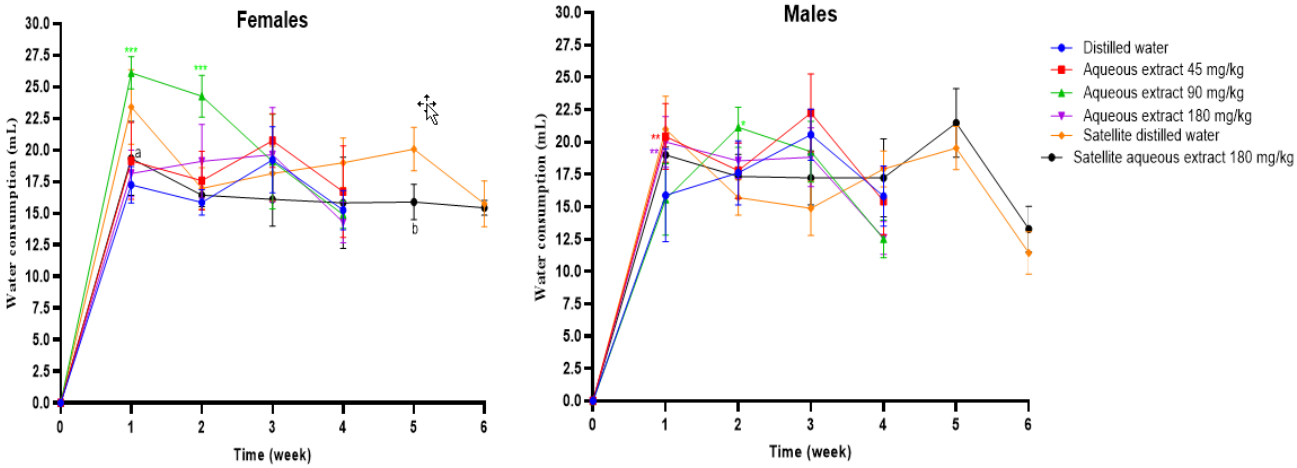

Figure 6. Overall effects of the aqueous extract of *Bauhinia thonningii* pods on water consumption in female and male rats during the subchronic toxicity assessment.
Values are expressed as mean ± SEM (n = 5 per sex). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the neutral control; ^{a} $P < 0.05$, ^{b} $P < 0.01$ compared to the satellite control.

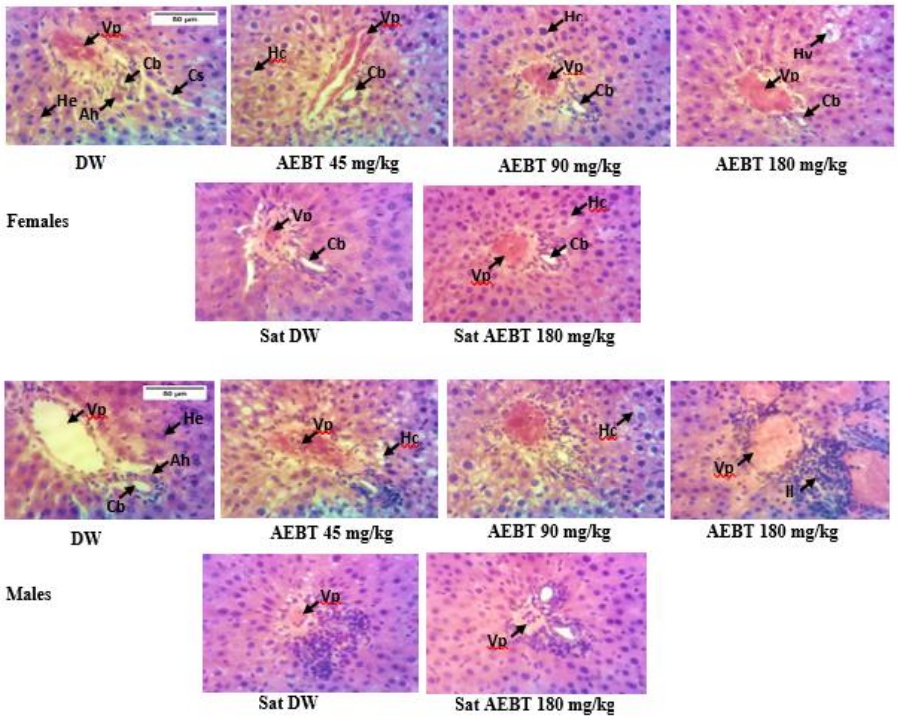

Figure 7. Representative photomicrographs of liver sections from rats treated with the aqueous extract of *Bauhinia thonningii* pods during the subchronic toxicity assessment (HE × 100).

DW = Distilled water; AEBT= Aqueous extract of *Bauhinia thonningii*; Sat DW = Satellite distilled water; Sat AEBT = Satellite aqueous extract of *Bauhinia thonningii*. Vp = Portal vein; Cb = Bile canaliculus; Ah = Hepatic artery; Cs = Sinusoidal capillaries; Hc and Hv = Cytolyzed and vacuolated hepatocytes; Il = Leukocyte infiltration.

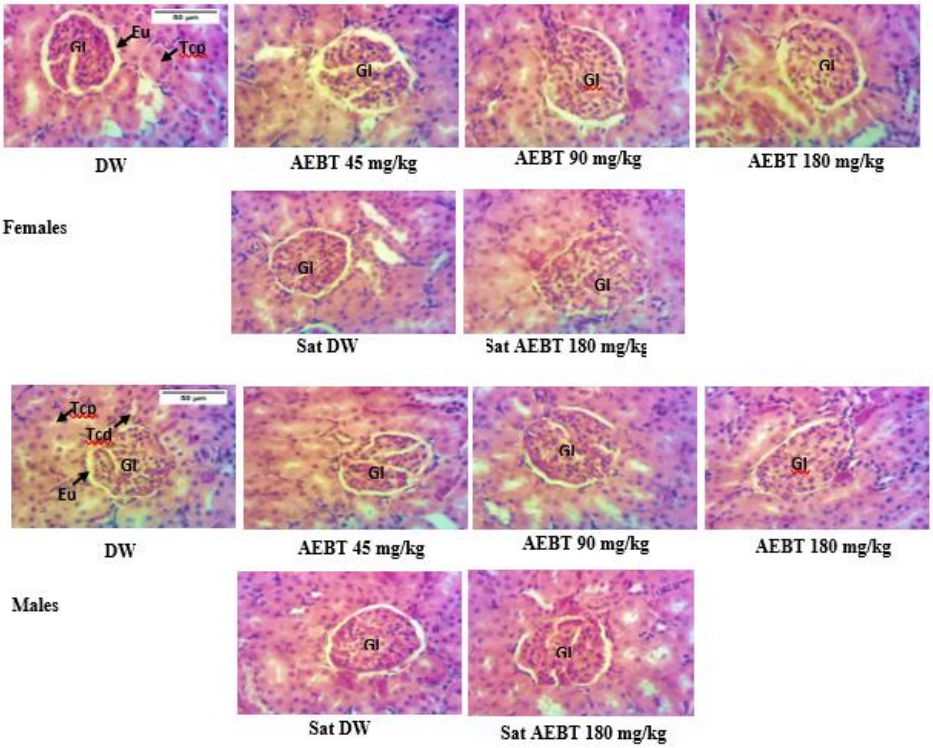

Figure 8. Representative photomicrographs of kidney sections from rats treated with the aqueous extract of *Bauhinia thonningii* pods during the subchronic toxicity assessment (HE × 100).

DW **=** Distilled water**;** AEBT= Aqueous extract of *Bauhinia thonningii***;** Sat DW = Satellite distilled water**;** Sat AEBT **=** Satellite aqueous extract of *Bauhinia thonningii*. Gl **=** Glomerulus; Eu **=** Urinary space; Tcp **=** Proximal convoluted tubule; Tcd **=** Distal convoluted tubule.

Table 1. Effect of the aqueous extract of *Bauhinia thonningii* pods on relative organ weights after the subchronic toxicity assessment.

Genders	Treatments	Liver (g)	Kidneys (g)	Heart (g)	Spleen (g)	Lungs (g)
Females	Distilled water	2.72 ± 0.35	0.49 ± 0.03	0.31 ± 0.03	0.20 ± 0.02	0.61 ± 0.05
	AEBT 45 mg/kg	3,28 ± 0.11	0.55 ± 0.03	0.31 ± 0.01	0.26 ± 0.02	0.64 ± 0.06
	AEBT 90 mg/kg	3.13 ± 0.21	0.56 ± 0.04	0.34 ± 0.04	0.26 ± 0.03	0.68 ± 0.06
	AEBT 180 mg/kg	3.08 ± 0.16	0.63 ± 0.04	0.43 ± 0.03	0.35 ± 0.03 *	0.68 ± 0.05
	Satellite distilled water	3.19 ± 0.10	0.60 ± 0.0.1	0.40 ± 0.02	0.30 ± 0.03	0.62 ± 0.03
	Satellite AEBT 180 mg/kg	2.97 ± 0.15	0.60 ± 0.04	0.37 ± 0.02	0.28 ± 0.01	0.74 ± 0.05
Males	Distilled water	3.08 ± 0.24	0.52 ± 0.03	0.29 ± 0.03	0.28 ± 0.03	0.55 ± 0.10
	AEBT 45 mg/kg	2.80 ± 0.16	0.46 ± 0.04	0.27 ± 0.01	0.23 ± 0.03	0.60 ± 0.08
	AEBT 90 mg/kg	3.59 ± 0.10	0.57 ± 0.03	0.33 ± 0.02	0.29 ± 0.03	0.67 ± 0.03
	AEBT 180 mg/kg	2.48 ± 0.27	0.48 ± 0.02	0.33 ± 0.01	0.24 ± 0.02	0.65 ± 0.12
	satellite distilled water	3.24 ± 0.20	0.65 ± 0.04	0.40 ± 0.01	0.34 ± 0.04	0.90 ± 0.04
	Satellite AEBT 180 mg/kg	3.39 ± 0.13	0.63 ± 0.01	0.39 ± 0.01	0.31 ± 0.03	0.86 ± 0.08

Values are expressed as mean ± standard error of the mean (SEM), (n = 5 per sex). AEBT: aqueous extract of *Bauhinia thonningii* pods. *Significant difference compared with the corresponding distilled water control $P = 0.01$.

Table 2. Effect of the aqueous extract of *Bauhinia thonningii* pods on hematological parameters in female rats after the subchronic oral administration.

Parameters	Distilled water	AEBT 45 mg/kg	AEBT 90 mg/kg	AEBT 180 mg/kg	Satellite distilled water	Satellite AEBT 180 mg/kg
WBC (x 10^3 /µL)	3.54 ± 0.68	4.87 ± 0.71	5.13 ± 0.58	2.70 ± 0.31	3.75 ± 0.90	4.35 ± 0.79
Lymphocytes (%)	2.92 ± 0.54	4.53 ± 0.75	4.03 ± 0.81	2.15 ± 0.64	1.90 ± 0.25	3.56 ± 0.64
Monocytes (%)	0.22 ± 0.37	0.48 ± 0.15	0.68 ± 0.24	0.92 ± 0.35	0.49 ± 0.18	0.46 ± 0.24
Granulocytes (%)	0.22 ± 0.07	0.52 ± 0.12	1.10 ± 0.38	1.42 ± 0.40	1.32 ± 0.30	0.76 ± 0.20
Platelets (X 10^3 /µL)	929.40 ± 53.67	839.80 ± 64.68	777.00 ± 21.39	923.90 ± 14.89	771.40 ± 41.30	806.40 ± 34.99
RBC (X 10^6 /µL)	6.76 ± 0.38	4.58 ± 0.90	3.82 ± 0.92	6.48 ± 0.56	3.84 ± 0.87	3.49 ± 0.55
Hemoglobin (g/dL)	13.54 ± 0.38	13.98 ± 0.74	15.90 ± 1.12	14.42 ± 0.38	17.82 ± 0.47	17.58 ± 0.72
Hematocrit (%)	46.92 ± 2.51	38.46 ± 3.95	46.80 ± 1.69	49.64 ± 2.75	29.90 ± 4.49	28.14 ± 3.86

Values are expressed as mean ± SEM (n = 5). AEBT: aqueous extract of *Bauhinia thonningii* pods. WBC: White blood cells. RBC: Red blood cells.

Table 3. Effect of the aqueous extract of *Bauhinia thonningii* pods on hematological parameters in male rats after the subchronic oral administration.

Parameters	Distilled water	AEBT 45 mg/kg	AEBT 90 mg/kg	AEBT 180 mg/kg	Satellite distilled water	Satellite AEBT 180 mg/kg
WBC (x 10^3 /µL)	2.62 ± 0.99	3.20 ± 0.48	3.96 ± 0.91	6.26 ± 1.30	4.42 ± 0.76	3.46 ± 0.60
Lymphocytes (%)	4.18 ± 0.50	4.40 ± 1.23	5.28 ± 1.20	7.86 ± 0.78	7.60 ± 0.73	2.98 ± 0.44 [a]
Monocytes (%)	0.20 ± 0.13	0.90 ± 0.54	1.48 ± 0.73	0.50 ± 0.18	0.28 ± 0.06	0.20 ± 0.07
Granulocytes (%)	0.44 ± 0.16	0.46 ± 0.08	0.64 ± 0.17	0.50 ± 0.16	0.52 ± 0.12	0.32 ± 0.10
Platelets (X 10^3/µL)	856.20 ± 13.55	704.60 ± 61.82	1097.00 ± 54.23	863.20 ± 31.99	798.60 ± 64.51	656.20 ± 79.34
RBC (x 10^6 /µL)	5.92 ± 0.63	3.52 ± 0.71 *	2.62 ± 0.29 **	3.55 ± 0.16 *	2.82 ± 0.40	5.15 ± 0.57 [a]
Hemoglobin (g/dL)	12.28 ± 0.85	14.76 ± 0.60 *	14.58 ± 0.45	15.76 ± 0.67 **	17.26 ± 0.07	15.70 ± 0.10
Hematocrit (%)	40.94 ± 3.17	34.18 ± 3.55	31.94 ± 1.89	30.74 ± 1.85	24.58 ± 2.15	38.16 ± 2.47 [a]

Values are expressed as mean ± SEM (n = 5). AEBT: aqueous extract of *Bauhinia thonningii* pods. WBC: White blood cells. RBC: Red blood cells. * $P < 0.05$, ** $P < 0.01$ compared to the neutral control. [a] $P < 0.05$ compared with the satellite control.

Table 4. Effect of the aqueous extract of *Bauhinia thonningii* pods on serum transaminases and alkaline phosphatase activities.

Genders	Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)
Females	Distilled water	25.96 ± 2.91	91.96 ± 4.40	469.50 ± 22.40
	Aqueous extract 45 mg/kg	10.84 ± 1.69 ***	52.38 ± 4.66 ***	575.70 ± 16.37
	Aqueous extract 90 mg/kg	16.27 ± 1.41 *	61.93 ± 5.63 **	563.80 ± 17.73
	Aqueous extract 180 mg/kg	13.53 ± 1.10 **	66.06 ± 5.40 *	512.70 ± 35.63
	Satellite distilled water	8.82 ± 2.38	77.35 ± 6.43	501.10 ± 32.22
	Satellite aqueous extract 180 mg/kg	14.87 ± 2.81	63.61 ± 6.23	417.70 ± 15.34
Males	Distilled water	21.55 ± 2.38	71.99 ± 6.49	912.00 ± 37.24
	Aqueous extract 45 mg/kg	13.42 ± 1.96 *	65.94 ± 7.08	695.60 ± 38.07
	Aqueous extract 90 mg/kg	17.72 ± 1.57	84.57 ± 6.20	823.40 ± 77.22
	Aqueous extract 180 mg/kg	9.69 ± 1.93 **	50.98 ± 2.22	641.70 ± 62.56 *
	Satellite distilled water	12.73 ± 1.58	81.89 ± 5.76	647.50 ± 33.18
	Satellite aqueous extract 180 mg/kg	11.75 ± 1.58	59.20 ± 7.59	394.10 ± 50.43 [a]

Values are expressed as mean ± SEM (n = 5 per sex). ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to the neutral control. [a] $P < 0.05$ compared with the satellite control.

Table 5. Effect of the aqueous extract of *Bauhinia thonningii* pods on serum and urinary creatinine and urea levels during the subchronic toxicity assessment.

Genders	Treatments	Serum creatinine (mg/dL)	Urinary creatinine (mg/dL)	Serum urea (mg/dL)	Urinary urea (mg/dL)
Females	Distilled water	11.60 ± 1.83	85.32 ± 5.93	246.10 ± 42.18	343.20 ± 44.99
	Aqueous extract 45 mg/kg	6.20 ± 0.76	87.76 ± 5.75	346.80 ± 42.63	473.10 ± 59.56
	Aqueous extract 90 mg/kg	5.32 ± 1.16	88.68 ± 7.55	388.80 ± 45.52	227.40 ± 69.37
	Aqueous extract 180 mg/kg	4.80 ± 0.68	17.68 ± 3.50	114.30 ± 22.59	314.60 ± 76.24
	Satellite distilled water	7.32 ± 1.92	86.08 ± 3.08	334.50 ± 18.92	247.40 ± 55.93
	Satellite aqueous extract 180 mg/kg	7.72 ± 2.32	111.10 ± 7.34	211.20 ± 32.76	188.80 ± 52.28
Males	Distilled water	4.87 ± 0.77	67.36 ± 9.16	187.80 ± 6.89	460.40 ± 34.90
	Aqueous extract 45 mg/kg	2.76 ± 0.86	47.32 ± 8.50	173.20 ± 3.87	351.80 ± 37.52
	Aqueous extract 90 mg/kg	3.75 ± 0.13	40.96 ± 6.32	165.50 ± 6.01	389.20 ± 23.65
	Aqueous extract 180 mg/kg	2.60 ± 0.19	40.40 ± 4.59 ***	134.8 ± 7.45 *	236.00 ± 22.50 *
	Satellite distilled water	3.60 ± 0.28	24.48 ± 3.83	111.70 ± 5.87	363.70 ± 38.14
	Satellite aqueous extract 180 mg/kg	3.10 ± 0.57	25.88 ± 5.87	182.3 ± 2.87 [a]	231.30 ± 35.38

Values are expressed as mean ± SEM (n = 5 per sex). * $P < 0.05$, *** $P < 0.001$ compared with the neutral control; [a] $P < 0.05$ compared with the satellite control.

Table 6. Effect of the aqueous extract of *Bauhinia thonningii* pods on lipid profile parameters after the subchronic administration.

Genders	**Treatments**	**Triglycerides (mg/dL)**	**Total Cholesterol (mg/dL)**	**HDL Cholesterol (mg/dL)**
Females	Distilled water	0.98 ± 0.19	2.81 ± 0.16	1.01 ± 0.12
	Aqueous extract 45 mg/kg	0.81 ± 0.11	2.72 ± 0.13	0.74 ± 0.16
	Aqueous extract 90 mg/kg	0.71 ± 0.07	2.42 ± 0.07	0.95 ± 0.03
	Aqueous extract 180 mg/kg	0.81 ± 0.06	2.63 ± 0.20	0.88 ± 0.10
	Satellite distilled water	1.27 ± 0.20	2.83 ± 0.24	0.99 ± 0.07
	Satellite aqueous extract 180 mg/kg	1.14 ± 0.15	2.63 ± 0.05	0.70 ± 0.06
Males	Distilled water	0.81 ± 0.14	3.24 ± 0.12	0.94 ± 0.09
	Aqueous extract 45 mg/kg	1.05 ± 0.17	2.99 ± 0.17	0.79 ± 0.05
	Aqueous extract 90 mg/kg	0.80 ± 0.10	3.05 ± 0.16	1.01 ± 0.05
	Aqueous extract 180 mg/kg	0.99 ± 0.12	2.53 ± 0.06	0.85 ± 0.12
	Satellite distilled water	1.05 ± 1.16	2.78 ± 0.23	0.86 ± 0.04
	Satellite aqueous extract 180 mg/kg	1.73 ± 0.24	3.29 ± 0.44	0.71 ± 0.04

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

Table 7. Effect of the aqueous extract of *Bauhinia thonningii* pods on bilirubin, serum total protein and urinary albumin levels.

Genders	**Treatments**	**Total bilirubin (µmol /L)**	**Direct bilirubin (µmol/L)**	**Serum protein (mg/mL)**	**Urinary albumin (g/dL)**
Females	Distilled water	18.56 ± 2.33	11.90 ± 0.59	0.58 ± 0.01	0.27 ± 0.02
	Aqueous extract 45 mg/kg	12.87 ± 0.88	8.55 ± 1,8	0.58 ± 0.03	0.13 ± 0.02
	Aqueous extract 90 mg/kg	15.16 ± 3.47	13.95 ± 0.9	0.49 ± 0.02	0.16 ± 0.01
	Aqueous extract 180 mg/kg	14.18 ± 1.89	8.22 ± 1.06	0.65 ± 0.01	0.25 ± 0.08
	Satellite distilled water	18.25 ± 3.96	9.98 ± 01.54	0.60 ± 0.03	0.18 ± 0.01
	Satellite aqueous extract 180 mg/kg	18.41 ± 3.28	6.20 ± 1.07	0.57 ± 0.01	0.18 ± 0.01
Males	Distilled water	11.72 ± 2.64	6.53 ± 1.68	0.57 ± 0.02	0.25 ± 0.05
	Aqueous extract 45 mg/kg	22.73 ± 5.32	12.38 ± 1.77	0.57 ± 0.03	0.21 ± 0.05
	Aqueous extract 90 mg/kg	14.78 ± 1.51	7.47 ± 1.07	0.59 ± 0.04	0.16 ± 0.03
	Aqueous extract 180 mg/kg	13.50 ± 1.82	7.72 ± 0.96	0.63 ± 0.02	0.22 ± 0.05
	Satellite distilled water	21.51 ± 4.65	4.66 ± 1.56	0.64 ± 0.01	0.20 ± 0.02
	Satellite aqueous extract 180 mg/kg	23.10 ± 5.19	6.05 ± 0.76	0.61 ± 0.02	0.17 ± 0.01

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

Conclusion

This study investigated the toxicological profile of the aqueous extract of *B. thonningii* pods through acute and subchronic toxicity assessments in rats. Oral administration of the extract at doses of 2000 and 5000 mg/kg did not induce mortality, significant alterations in stool consistency, food intake, or water consumption, indicating that the median lethal dose (LD_{50}) is greater than 5000 mg/kg body weight. Subchronic oral exposure to the extract at doses up to 180 mg/kg did not result in marked adverse effects on body weight, hematological parameters, or key biochemical markers of liver and kidney function in both male and female rats. The absence of significant alterations in serum transaminases, bilirubin, lipid profile, total protein, and renal biomarkers, together with the normal histoarchitecture of the liver and kidneys, suggests that the aqueous extract does not induce overt systemic, hepatic, or renal toxicity under the experimental conditions. However, mild and adaptive variations observed in certain physiological and biochemical parameters indicate that prolonged administration at higher doses should be approached with caution. Overall, the aqueous extract of *B. thonningii* pods appears relatively safe following acute and subchronic oral exposure, supporting its traditional use. Further long-term toxicity and mechanistic studies are warranted to fully establish its safety profile.

Abbreviations

AEBT: Aqueous extract of *Bauhinia thonningii*
ALP: Alkaline phosphatase
ALT: Alanine aminotransferase
ANOVA: Analysis of variance
AST: Aspartate aminotransferase
EDTA: Ethylenediaminetetraacetic acid

HDL: High-density lipoprotein
LD_{50}: Median lethal dose
NaCl: Sodium chloride
OECD: Organization for Economic Co-operation and Development
rpm: Rotations per minute
SEM: Standard error of the mean

Authors' Contribution

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RPTB, MLN, SLKP and SLNW conceived and designed the study. RPTB, MLN, SLPK, LMTT, MTKW, RCDD and SLNW collected and analyzed the data. SLNW, MLN, SLKP, and RPTB drafted the manuscript and critically revised it for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Conflict of interest

The authors declare no conflict of interest

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