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RESEARCH ARTICLE

Safety assessment of the ethanol extract of *Caesalpinia bonduc* (L.) Roxb. Root in Wistar rats: Acute and subacute (28-day) oral toxicity studies

Mariette Sindete[1], Adam Gbankoto[1], Habib Ganfon[2], Razack Osseni[3], Achille Yemoa[4], Anatole Laleye[5]

[1]Laboratory of Experimental Physiology and Pharmacology, Faculty of Sciences and Technology, University of Abomey Calavi, Benin, [2]Laboratory of Pharmacognosy and Phytotherapy, Training and Research Unit in Pharmacy, Faculty of Health Sciences, University of Abomey Calavi, Benin, [3]Unit of Physiology, Faculty of Health Sciences, University of Abomey Calavi, Benin, [4]Laboratory of Analytical Chemistry and Drug Analysis, Training and Research Unit in Pharmacy, Faculty of Health Sciences, University of Abomey Calavi, Benin, [5]Unit of Human Biology, Faculty of Sciences Health, University of Abomey Calavi, Benin

Correspondence to: Adam Gbankoto, E-mail: qgoba@yahoo.fr

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ABSTRACT

Background: *Caesalpinia bonduc* (CB) root is used in African Pharmacopoeia for the prevention and treatment of sexual failure. **Aims and Objective:** The current study performed to evaluate the acute and subacute oral toxicity of the ethanolic extract of CB root. **Materials and Methods:** Two groups of three female Wistar rats were used for acute oral toxicity test. A single dose of 2000 mg/kg body weight (BW) of the ethanolic extract of CB root was administered to the test group; the control group received dimethyl sulfoxide. Rats were observed individually during the first 4 h and then daily until days 14. For subacute toxicity model, the male rats were divided into four groups ($n = 6$). Experimental groups received 31, 25, 125, and 500 mg/kg of BW of the ethanolic extract of CB root orally daily for 28 days. The blood hematological and biochemical parameters, as well as histopathology of liver and kidneys, were studies. **Results:** No toxicological signs were observed in rats when acutely exposed to the ethanolic extract of CB root. After the repeated administration of CB root extract, hematological and biochemical parameters were unaltered except hemoglobin and erythrocyte number in the exposed animal, but they were considered to be temporary effects and not an indication of toxic effects. No macroscopic changes and no noticeable histological changes were seen in the histopathology analysis of kidneys and liver. **Conclusion:** Ethanolic extract of CB root at single dosage level up to 2000 mg/Kg BW is nontoxic and can show protection of some vital organs when administered to 28 days.

KEY WORDS: *Caesalpinia bonduc*; Wistar Rat; Acute Toxicity; Subacute Toxicity; Histopathology

INTRODUCTION

Herbal medicine is becoming increasingly common in developing countries where the prescription of traditional medicines for the resolution of multitude health problems. Usually, a specific part of the plant (root, leaves, fruit, flowers, and seeds) is used in traditional preparations or as pure active principles formulated into a suitable preparation.[1] Many medicines commonly used today are of herbal origin. Indeed, about 25% of prescription drugs contain at least one active ingredient derived from plant material.[1,2] Plant-derived medicines are used in all civilizations and cultures and, hence, plants have always played a key role in health-care systems worldwide.[1] In most developing countries, the indigenous modes of herbal treatment are parts of the remedies, with a considerable extent of effectiveness, are

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socially accepted and economically viable and, mostly, are the only available.[3]

Male sexual problems concerning ejaculation disorders, erectile dysfunction, and inhibited sexual desire are reported around 31–52% of population.[4] However, the World Health Organization has emphasized that sexual health is a state of physical, emotional, mental, and social well-being in relation to sexuality; it is not merely the absence of disease, dysfunction, or infirmity. Sexual health requires a positive and respectful approach to sexuality and sexual relationships, as well as the possibility of having pleasurable and safe sexual experiences, free of coercion, discrimination, and violence. For sexual health to be attained and maintained, the sexual rights of all persons must be respected, protected, and fulfilled.[3] Therefore, the displeasure of sexual desire has gained much attention from the public nowadays. Since sexual behavior enhancement is believed to increase the relationship satisfaction and self-esteem in humans[5] and the search for aphrodisiac or food or drug that arouses the sexual instinct, induces sexual desire, and increases pleasure and performance has gained much attention throughout history.[5]

At present, many plants based aphrodisiacs have gained much attention. This use of plants as supplements to boost, revitalize, and ultimately improve sexual function[6,7] has been recognized worldwide as an immediate treatment[8] and continues to gain ground. The root of *Caesalpinia bonduc* (CB), a plant in a family of Caesalpiniaceae, is also reputed for aphrodisiac effect.[9] Although the aphrodisiac activity of this plant has been clearly shown, safety consumption is very much essential and requires attention.[5] However, considering that herbal medicine can be potentially toxic, further studies regarding the safety and toxicity of medicinal plants are necessary before their use.[10]

Almost any substance can be harmful at some doses but, at the same time, can be without harmful effects at some lower dose. Between these two limits, there is a range of possible effects, from subtle long-term chronic toxicity to immediate lethality.[1,11] The large array of toxic chemicals produced by plants (phytotoxins), usually referred to as secondary plant compounds, is often held to have evolved as defense mechanisms against herbivorous animals, particularly insects and mammals. Many chemicals that have been shown to be toxic are constituents of plants that form part of the human diet.[1,11] CB (L.) Roxb is a scrambling, pricky, woody liana. Leaves are of 4–7 pairs and rachis is prickly.

Leaflets are of seven to nine pairs, ovate-elliptic, pubescent below, entire, and mucronate. Flowers are yellow in axillary and terminal racemes. Pods are oblong and dehiscent. Seeds are one or two, globose or ovoid, and gray.[12] It is distributed throughout the tropical and sub-tropical zone. The seed of CB claimed a purgative, anthelmintics, antimalarial, and anti-inflammatory action.[13] The oil from seed is used in convulsion and paralysis. The roots are considered as anthelmintics and decoction of the root prescribed in fever.[13] The root has also aphrodisiac properties.[9] The leaves of CB used in the treatment of smallpox, disorder of liver. The flower is bitter cures Kapha and Vata.[13] The purpose of this study was to evaluate the acute and subacute toxicity of the ethanolic extract of CB root in Wistar rats.

MATERIALS AND METHODS

Plant Collection and Authentication

The fresh root of CB was collected from the area in Sèhouè (6°55'54'' N - 2°16'27'' E), Atlantique Department of Southern Benin. Authentication was carried out at the National Herbarium of Benin, University of Abomey-Calavi where voucher sample was deposited (AA 6743/HNB). Root was gently washed twice under running tap water and then washed again in distilled water to remove the sand and dried at room temperature for 21 days and crushed into coarse powder with electric grinder (Flour mills Nigeria, El Motor N°1827).

Preparation of Plant Extracts

Two hundred and fifty grams of the powder of CB root was extracted in 95% ethanol (3 × 500 ml) using cold maceration for 72 h by continuous agitation with an orbital shaker VWR®, model 5000 at 180 rpm. Macerat was filtered on cotton and Whatman N°1 filter paper (Whatman International Ltd; Maidstone, England). The filtrate obtained was evaporated in a rotary evaporator (VWR® IKA, RV8) under reduced pressure and was vacuum dried at a temperature of 40°C. The dried extract obtained after oven-dried at 40°C was stored in light-resistant air-tight container, labeled and kept in a refrigerator at 4°C until needed for the experiment. The extract was reconstituted in dimethyl sulfoxide (DMSO) for use during the experiment.

Experimental Animals

Adult healthy Wistar albino rats (*Rattus norvegicus*) female (6) and male (24) of 10–12 weeks old, body weight (BW) 180–200 g were used to evaluate acute and subacute toxicity studies. Both sexes were maintained separately in standard cages with top grill, and the selected female rats were nulliparous and non-pregnant. Each animal was assigned a unique identification number. The animals were sourced and kept in the laboratory animal room of the Teaching and Research Unit in Human Biology of the Faculty of Health Sciences (FSS) of Cotonou, University of Abomey-Calavi, Benin. The rats were kept in a controlled environment at ambient temperature with 12/12 h light natural and dark cycles throughout the experiment. They were fed with standard rat pellet food (Complete Food, Group Veto Services S. A., Benin) and drinking water *ad libitum* during the experiment. The pellet diet consisted of 18%

crude protein, 1.16% calcium, 0.80% phosphorus, 0.82% lysin, 14.4% crude fiber, and 7.12% crude fat. Dried paddy husk was used as the bedding material who were cleaned every alternate day. The experiments were carried out to minimize animal suffering in accordance with the internationally accepted principles for laboratory use and care of European Parliament And The Council of The European Union.[14]

Acute Oral Toxicity Study

The oral acute toxicity study of the ethanolic extract of CB root was conducted over females rats as per the Organisation for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals section 4, n° 423 limit test, adopted in 2001.[15] This method is a step-by-step procedure that begins with the maximum dose of 2000 mg/kg BW and then depending on the mortality and/or morbidity of the animals, is lowered to 300, 50, or 5 mg/kg BW doses to allow a judgment of the test substance's acute toxicity[16] and provides information on short-time toxicity level of the test extract which helps in the selection of doses for repeated oral toxicity study.[17] The six females rats were grouped into two groups of three rats each and kept in their cage for 5 days acclimatization.[18] The first group treated with vehicle (DMSO) to establish a comparative negative control group according to the OECD guideline, while the second was considered as test group which received a single dose of 2000 mg/kg BW of the ethanolic extract of CB root. All substances were administered orally using an oropharyngeal metal cannula. All the animals were kept at overnight fasting 12 h before experiment with free excess to water.

Subacute Oral Toxicity Test

The oral acute toxicity study of the ethanolic extract of CB root was conducted over male rats as per the OECD guidelines for the testing of chemicals section 4, n° 407 limit test, adopted in 2008.[19] Twenty-four male rats were assigned into four groups of six rats each based on BW stratification. Group 1 received vehicle (DMSO) and served as control; Groups 2, 3, and 4 received doses of CB root ethanol extract at doses of graded doses of 31.25; 125 500 mg/kg BW, respectively, by gavage repeated for 28 consecutive days using an oropharyngeal metal cannula.

Clinical Observations and Survival

After dosing, the rats have further fasted for 4 h, all animals were observed individually to monitor physical and behavioral alteration providing special attention to first 30 min, 2 h, 4 h, 6 h, 10 h, and 24 h after exposure[20,21] and then twice daily for mortality and morbidity until the end of treatments. The clinical observation included changes in fur, skin, eyes, and autonomic activity such as piloerection, changes in pupil size, lacrimation, and unusual breathing pattern. Changes in gait and posture were also monitored along with stereotype activities such as excessive grooming and repetitive circling.[17,21] The period of observation was 1 week before administration of test drug until the end of treatments.

BW and Food Intake

The food consumption was recorded daily during treatment. The rats were given a certain amount of food, and the amount of remaining food was measured at the same time in the next day. The food consumption was calculated by subtracting leftover food from the total food provided. The BW of animals was recorded at the end of each week was carefully monitored before study commencement, once weekly during the study and on the day of sacrifice.

Urine Analysis

All animals from each group were housed in metabolic cages for urine collection at the end of the treatment period, and fresh urine samples were collected overnight and used for urinalysis including urine volume. Test strips (DUS™ 10, Standard Diagnostic, Korea) were dipped in urine sample, and parameters such as glucose, bilirubin, ketone bodies, leukocytes, specific gravity, occult blood, pH, protein, urobilinogen, and nitrite were examined.

Hematological and Biochemical Analyses

At the end of treatment, the animals were subjected to overnight fasting with free access to tap water before being anesthetized under diethyl ether in a saturation closed jar and when the rats became unconscious, the blood samples were obtained by retro-orbital puncture with the help of capillary tubes and were collected into non-heparinized tubes for biochemical analyses and ethylenediaminetetraacetic acid tubes for hematological analyses. In hematological studies, erythrocytes, hemoglobin (Hgb), hematocrit, mean globular volume, mean concentration Hgb, mean corpuscular Hgb concentration, leukocytes, neutrophil, lymphocyte, monocyte, eosinophil, and platelets were determined. For biochemical analysis, blood without additive was left at room temperature for 1 h and was centrifuged at 3000 rpm at 4°C for 15 min[22] to collect the serum and tests were done using fully automated biochemistry analyzer and standard diagnostic test kits. Biochemical studies were carried out to determine the toxic effect of the extract on liver function indices (Aspartate aminotransferase [AST], Alanine aminotransferase [ALT], glucose, and protein), kidney function indices (urea, and creatinine), serum electrolytes (chloride, sodium, and potassium), and lipid profile (total cholesterol).[23,24]

Organ Weight Determination and Gross Necropsy

After exsanguinations, vital organs such as liver and kidney were quickly removed and their weight (absolute and relative) was determined after noting any sign of gross lesion. They

were removed and weighted to give their absolute organ weight (g)[17] washed and placed in 10% neutral buffered formalin solution. The relative organ weight was calculated using the formula given below: Relative weight = (Weight of the organ/BW of the animal on sacrifice day) × 100.[21,25] The macroscopic examination included a study of the external surfaces, cranial, pelvic cavity, all orifices, and thorace.

Histopathological Studies

The histological studies were performed according to Hould.[26] Each isolated organ was fixed in 10% neutral buffered formalin. The fixed tissues were dehydrated with subsequent 50%, 70%, 90%, and 100% ethanol, then, cleared in xylene. After incubation of paraffin in a 60°C incubator, they were embedded and blocked in paraffin at same temperature. Fine serial sections (5 µm thick) obtained by cutting the embedded tissue with microtome then mounted on gelatinous water coated slides and dried for 24 h at room temperature. The sections on the slides were deparaffinized with xylene, rehydrated in a descending series of alcohol and water before stained with hematoxylin and eosin dyes, dried and mounted on a light microscope for microscopic examination and histopathological analysis.

Statistical Analysis

The data were expressed as mean (± standard error of mean). The averages were analyzed using analysis of one-way analysis of variance and supplemented by Student's *t*-test. The post-test analysis was performed using Dunnett's multiple comparison tests to determine significant differences in all parameters. Values were considered significantly different when $P < 0.05$. The analysis and construction of the graphics were done using the GraphPad Prism software version 6.00 (GraphPad Prism Software, Inc., San Diego, California).

RESULTS

Clinical Observations and Survival in Acute and Subacute Oral Toxicity Tests

No mortality was observed within 4 h of continuous observation and also after 24 h in the acute toxicity studies. There was also no lethal effect observed after administration of the extract for the experimental period of 14 days, and morphological characteristics appeared normal. No salivation, diarrhea, lethargy, or unusual behaviors were observed. Respiration was normal, and no sedation was observed.

This indicates that the ethanolic extract of CB root at the dose of 2000 mg/kg BW was safe. The control group which was administered with vehicle did not produce any toxic effects or mortality within the study period. As there was no mortality recorded for this dose, the LD_{50} value was assumed to be greater than the limit test dose of 2000 mg/kg BW. Thus, the oral doses of 31.25, 125, and 500 mg/kg BW of the ethanolic extract of CB root were selected to evaluate the subacute toxicity study.

In the subacute oral toxicity studies, daily administration of CB root extract at the doses of 31.25 and 125 mg/kg BW through oral gavage for 28 days did not produce any symptoms of toxicity in rats, including the highest dose tested at 500 mg/kg BW. No mortalities were recorded in rats over a period of 28 days. None of the rats after administration showed any obvious morbidity or clinical symptoms of

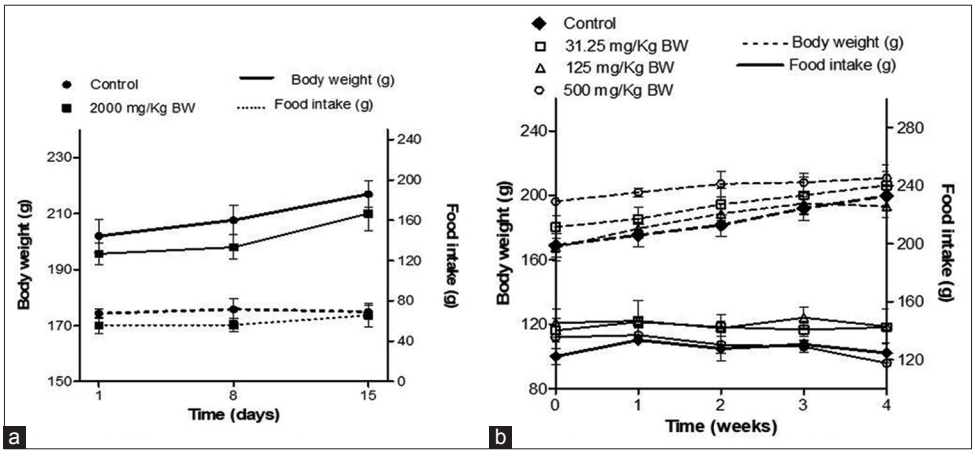

Figure 1: Effect of *Caesalpinia bondue* (CB) root extract on food intake and body weight (BW) of control and treated female Wistar rats at days 1, 8, and 15 following the acute toxicity test. The control group received dimethyl sulfoxide (DMSO): The experimental group received a single dose of 2000 mg/kg of BW of the ethanolic extract of CB. Values are means of three replicates ± standard error of mean; No significant difference was observed compared to the control (a). Effect of CB root extracts on food intake and BW of control and treated male Wistar rats at week 1; 2; 3; and 4 following the subacute toxicity test. The control group received DMSO, the three experimental groups (31.25 mg kg; 125 mg/kg; and 500 mg/kg of BW) received ethanolic extract of CB root at the dose of 31.25; 125; and 500 mg/kg of BW, respectively, orally daily for 28 days. Values are means of six replicates ± standard error of mean; No significant difference was observed compared to the control (b)

toxicity such as changes in the skin and fur, eyes, respiratory rate, salivation, perspiration, piloerection, and stereotype activities. There were no clinical signs of toxicity observed for the control group.

Effect of CB Root Extract on BW and Food Intake in Acute and Subacute Oral Toxicity Tests

The BW of Wistar rats recorded at an interval of 7 days over the treatment period of 14 days for the acute toxicity test is shown in Figure 1a. The results showed that the BW in the test extract-treated groups increased non-significantly ($P > 0.05$) when compared with the control. There were no significant ($P > 0.05$) differences in the BW changes between the control and treated rats and were noted. The BW gradually increased within normal range of BW gain. An increase in food consumption was observed during the 2 weeks for acute toxicity test; however, there were no significant differences between treated rats and the control group [Figure 1a].

The food consumption of control was decreased for the 4th week. The results of food consumption show increasing

Table 1: Effect of *Caesalpinia bonduc* root extract on urinalysis in acute and subacute oral toxicity tests

Parameters	Observation	Acute oral toxicity		Subacute oral toxicity			
		Single dose (mg/kg)		Repeated dose (mg/kg)			
		Control	2000	Control	31.25	125	500
Number of animals		3	3	6	6	6	6
Leukocytes (WBC/μL)	Negative	3	3	6	6	6	6
	trace	0	0	0	0	0	0
	+70	0	0	0	0	0	0
Nitrite (±)	Negative	3	3	6	6	6	6
	Trace	0	0	0	0	0	0
	Positive	0	0	0	0	0	0
Urobilinogen (mg/dL)	0.1	3	3	6	6	6	6
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
Protein (mg/dL)	Negative	0	0	0	0	0	0
	+30	3	0	1	4	1	0
	++100	0	1	5	2	3	6
	+++300	0	2	0	0	2	0
Occult blood (Red blood cell/μL)	Negative	3	3	6	6	6	6
	Trace	0	0	0	0	0	0
	+25	0	0	0	0	0	0
Ketone body (mg/dL)	Negative	3	3	6	6	6	6
	±5	0	0	0	0	0	0
	+15	0	0	0	0	0	0
Bilirubin	Negative	3	3	6	6	6	6
	+	0	0	0	0	0	0
	++	0	0	0	0	0	0
Glucose (mg/dL)	Negative	3	3	6	6	6	6
	±100	0	0	0	0	0	0
Specific gravity	≤ 1.010	2	0	2	3	0	1
	1.015	1	0	4	0	4	2
	1.020	0	1	0	3	0	3
	1.025	0	2	0	0	2	0
	≥1.030	0	0	0	0	0	0
pH	>6	0	0	0	0	0	0
	6–8	3	0	6	5	4	3
	<8	0	3	0	1	2	3
Volume (ml/12 h)		5.53±0.75	5.00±0.72	3.27±0.55	3.4±0.45	4.2±0.47	4.3±0.42

Volume values are mean±standard error of mean, no significant difference

BW of rats associated with increased food consumption. The BW of Wistar rats recorded at an interval of 7 days over the treatment period of 28 days in subacute oral toxicity test is shown in Figure 1b. The results showed that the BW in the test extract-treated groups increased non-significantly ($P > 0.05$) when compared with the control, but after 4 weeks, the BW returned to normal for animals treated with height dose.

The increase in BW for all groups was mostly dose-dependent as a greater increase in BW was observed in high dose group. Daily administration of CB extract at different doses for 28 days did not result in any significant change in the mean food intake of treated groups compared to the control; however, decrease in food consumption was observed at the last week of treatment for the rats treated with the high dose of extract [Figure 1b].

Effect of CB Root Extract on Urinalysis in Acute and Subacute Oral Toxicity Tests

The details of urine analysis of Wistar rats are given in Table 1. The volume of urine in the rats of the treated lots did not increase significantly compared to the control group with the vehicle for both acute and subacute toxicity. No significant difference was observed in the qualitative analysis of urine parameters, including glucose, bilirubin, ketone bodies, specific gravity, occult blood, pH, protein, urobilinogen, and nitrite in the urine of rats collected at the end of treatment [Table 1].

Table 2: Effect of *Caesalpinia bonduc* root extract on hematological parameters in acute and subacute oral toxicity tests

Parameters	Acute oral toxicity		Subacute oral toxicity			
	Single dose (mg/Kg)		Repeated dose (mg/Kg)			
	Control	2000	Control	31.25	125	500
HGB (g/dL)	14.03±0.55	14.03±0.25	12.10±0.73	14.62±1.34*	14.37±0.21*	13.88±0.52
Hematocrit (%)	48.67±7.50	45.67±5.13	41.50±1.87	50.17±3.06*	48.00±3.68*	46.17±1.72
ERT (T/L)	7.67±0.60	7.23±0.44	6.10±0.42	8.01±0.28*	7.61±0.22*	6.91±0.13
MGV (fL)	63.33±5.68	63.00±7.21	69.50±1.87	60.17±1.47*	65.33±2.42	67.83±1.72
MCH (pg)	18.33±1.15	19.33±0.57	21.00±1.67	18.17±2.92	18.50±1.38	20.83±3.76
MCHC (%)	29.00±3.46	31.00±2.64	30.50±2.74	28.83±1.72	28.83±2.71	29.00±3.34
Leukocytes (G/L)	6.90±0.70	5.73±1.18	6.10±0.48	7.70±1.41	5.85±0.72	5.83±0.88
Neutrophils (G/L)	17.00±2.08	17.00±1.73	2.23±0.11	2.52±0.25	1.69±0.66	1.89±0.14
Eosinophils (G/L)	1.33±0.88	1.66±0.33	00±00	00±00	0.12±0.02	0.06±0.03
Monocytes (G/L)	6.66±0.88	5.66±2.72	0.36±0.08	0.80±0.32*	0.60±0.13	0.61±0.14
LPC (G/L)	78.33±2.03	83.00±2.88	3.52±0.83	4.29±0.86	3.31±0.09	2.58±0.97
Platelets (103/µL)	755.00±18.33	822.70±68.96	978.20±11.81	658.70±51.58*	777.20±26.30*	884±18.26

Values are mean±standard error of mean (n=3 of acute toxicity test and six of subacute toxicity test). Hgb: Hemoglobin, ERT: Erythrocytes, LPC: Lymphocytes, MGV: Mean globular volume, MCH: Mean Concentration Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration. *P<0.05 significantly different from the control group

Table 3: Effect of *Caesalpinia bonduc* root extract on biochemical parameters in acute and subacute oral toxicity tests

Parameters	Acute oral toxicity		Subacute oral toxicity			
	Single dose (mg/kg)		Repeated dose (mg/kg)			
	Control	2000	Control	31.25	125	500
Glucose (G/L)	0.73±0.10	0.45±0.06*	0.61±0.15	0.53±0.28	0.45±0.04	0.22±0.04*
Urea (G/L)	0.62±0.08	0.75±0.06	0.77±0.08	0.67±0.07	0.68±0.05	0.75±0.04
Creatinine (mg/L)	6.72±0.27	4.32±0.42*	5.33±0.18	5.56±0.37	5.61±0.53	6.22±0.14
TC (G/L)	0.65±0.08	0.62±0.07	0.69±0.08	0.74±0.05	0.59±0.03	0.59±0.07
Proteins (G/L)	60.00±1.00	66.33±1.66	72.26±4.11	74.44±2.46	76.00±1.14	74.94±8.08
ALT (UI/L)	62.67±1.85	46.33±1.2*	67.50±6.89	77.83±7.16	68.00±5.65	85.33±5.85
AST (UI/L)	158.70±9.33	186.70±1.76*	138.70±15.11	168.80±11.30	193.70±21.23*	236.00±11.03*
Na^+ (mEq/L)	140.30±1.45	140.20±1.90	136.50±4.71	143.80±8.11	136.60±6.12	139.00±3.19
Cl^-(mEq/L)	104.20±2.48	106.50±0.86	103.10±3.19	106.30±1.40	110.10±3.09	105.10±1.86
K^+ (mEq/L)	4.41±0.68	3.71±0.45	3.72±0.39	3.55±0.40	5.39±0.77	3.74±0.17

Values are mean ± standard error of mean (n=3 of acute toxicity test and six of subacute toxicity test). AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, TC: Total cholesterol, K^+: Potassium ion, Na^+: Sodium ion, Cl^-: Chloride ion. *P<0.05 significantly different from the control group

Effect of CB Root Extract on Hematological Parameters in Acute and Subacute Oral Toxicity Tests

The hematological profile of control and treated groups of acute and subacute oral toxicity tests are tabulated in Table 2. The results concluded that all hematological parameters such as total white blood cell count and Hgb are within the normal range in both control and treated groups in acute oral toxicity tests. There is no difference between the groups. Some significant changes were observed in the parameters of rats when compared with the control group in subacute oral toxicity test [Table 2].

The extract of CB caused an increase in Hgb, erythrocytes, and hematocrit was observed, which is mainly significant ($P < 0.05$) and non-dose-dependent in rats treated with 31.25 and 125 mg/kg BW. The decrease in platelets was also observed at these doses. The high dose revealed no significant difference from the control. All the other parameters showed non-significant and non-dose-dependent changes when compared with the control.

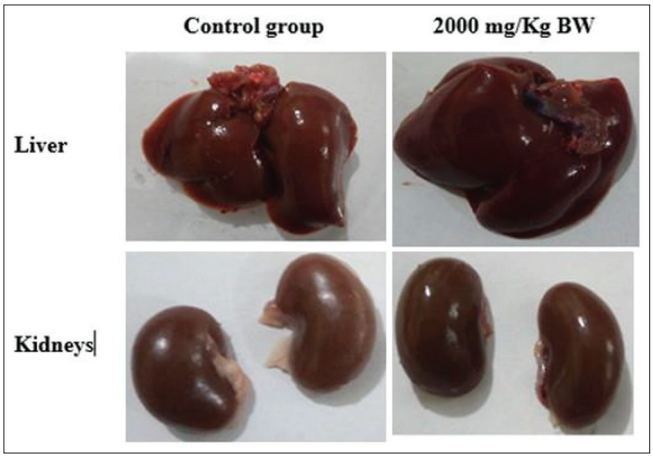

Figure 2: Effect of *Caesalpinia bonduc* (CB) root extract on gross necropsy study of liver and kidneys of control and treated rats in acute oral toxicity test. The control group received dimethyl sulfoxide; the experimental groups received a single dose of 2000 mg/kg of body weight of the ethanolic extract of CB root

Effect of CB Root Extract on Biochemical Parameters in Acute and Subacute Oral Toxicity Tests

Table 3 shows the result of biochemical parameters of acute and subacute oral toxicity for groups of rats treated with CB root extract. In acute oral toxicity test, study of biochemical parameters showed that the CB root extract decreases significant ($P < 0.05$) glucose, creatinine, and ALT level; however, significant increase ($P < 0.05$) was observed for AST level. No significant alterations were observed for the serum concentrations of lipid profile markers and electrolytes of extract-treated animals.

The result in rats at the end of subacute toxicity test showed a dose-dependent decrease in glucose level which was significant in high dose, however, a dose-dependent increase in AST level for three doses of CB root extract was observed which was significant ($P < 0.05$) for the doses of 125 and 500 mg/Kg of BW of extract. A non-significant increase in creatinine levels was also observed. The rest of the parameters showed minor fluctuations mostly non-significantly when compared with the control.

Effect of CB Root Extract on Gross Necropsy in Acute and Subacute Oral Toxicity Tests

Liver and kidney are the primary organs affected by metabolic reactions caused by any toxicant. The macroscopic observations of these vital organs from female rats in acute toxicity test [Figure 2] did not produce any macroscopic abnormal changes in color, size, shape, texture, and atrophy or hypertrophy compared with the control or developed any lesions after administration of CB root extract at a single dose. No changes were also observed on gross observation of these organs in both control and treated groups in subacute oral toxicity test.

Effect of CB Root Extract on Absolute and Relative Organ Weight in Acute and Subacute Oral Toxicity Tests

The acute and subacute effect of CB root extract on absolute and relative organ weights in rats is presented in Figures 3

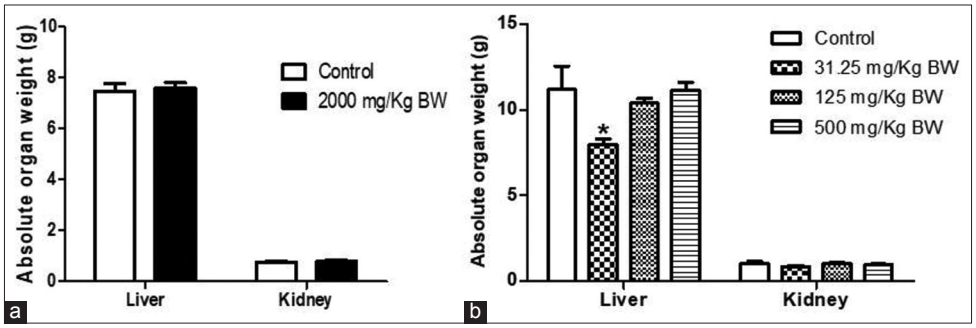

Figure 3: Effect of *Caesalpinia bondue* (CB) root extract on the absolute weight of the liver and kidney of female rats in acute toxicity test of 14 days (a) and male rats in subacute oral toxicity test 28 days (b) at the end of the treatment. The control group received dimethyl sulfoxide, the experimental groups received a single dose of 2000 mg/kg of body weight (BW) (a) and repeated dose of 31.25 mg/kg; 125 mg/kg; and 500 mg/kg of BW (b) for 28 days of the ethanolic extracts of CB root. Values are means of three replicates ± standard error of mean (SEM) (a) and six replicates ± SEM (b). *$P < 0.05$ significantly different from the control group

and 4. Absolute liver and kidney weight in the rats treated with a single dose of 2000 mg/kg BW of the extract of CB root [Figure 3a] was not significantly altered in these rats, whereas absolute liver weight significantly increased ($P < 0.05$) in the rats treated with 31.25 mg/kg BW of extract of CB root [Figure 3b].

The relative weight of the liver and kidney in the rats treated with 2000 mg/kg BW of extract had also no modification [Figure 4a]. The relative weight of the liver in the rats treated with 31.25 mg/kg BW of the extract of CB root [Figure 4b] significantly increased ($P < 0.05$). No other significant differences in absolute or relative organ weights were observed between the control and treated groups.

Effect of CB Root Extract on Histopathological Changes in Acute and Subacute Oral Toxicity Tests

Histopathological analysis of the organ samples of liver, kidney from rats in acute (A) and subacute (B) oral toxicity test are tabulated in Figure 5. Multiple sections of the liver of rat showed normal hepatocytes sinusoidal spaces and centrilobular vein in extract-treated group (A). Sections of rat liver from the extract-treated Group 4 (B) showed almost normal cellular architecture. Liver sections in acute and subacute oral toxicity tests showed normal liver architecture. No histopathological changes of kidney were noted both in the treated and in the untreated group. All sections from the kidney exhibited normal glomerular and unremarkable typical tubule interstitial parenchyma, with no hyaline changes or vascular necrosis.

DISCUSSION

Plants provide a wide variety of phytochimiques components useful to humankind. These substances can be extracted and used for the preparation of drugs, or the plant itself can be used directly as a medication.[24] Plants contain some toxic substances, and it is better to evaluate them according to standard procedures. Toxicity tests are carried out effectively on rodents (rats or mice) due to their availability and low cost and the wealth of toxicology data in literature already available for these species.[24,27] Considering the numerous reported therapeutic potentials of CB root as an alternative medicine effective for various diseases, whose sexual motivation.[9] A safety profile was established through acute and subchronic toxicity study, as a guide for the management of its application and usage in herbal preparations. This study will serve to prevent exposing humans to potential toxicity-related health risks while using CB root. Acute toxicity test assesses the adverse effects that occur within a short time after administration of a single dose of a test substance. This testing is usually done in the development of a new chemical or product to provide information on its potential toxicity.[1,27] Acute toxicity study showed that the lethal dose was more than 2000 mg/kg BW. This dose also failed to show the toxicity signs throughout the experimental period; no significant modification in the general behavior and no mortality were observed in the treated group. BW change is an important index for the assessment of toxicity[1] as well as food consumption. They have been used as an indicator of adverse effects of drugs and chemicals.[18] In the present study, there was a gradual normal increase in the mean BW of the treated group such as control group as well as the mean BW gain for rats. However, the difference was statistically insignificant. In addition, there was no significant difference in the absolute weight of liver and kidneys of treated rats compared to control group.

The extract had so no effect on the normal growth of rats. Analysis of blood parameters is relevant for toxicity risk evaluation, as any changes in the hematological and biochemical systems have a higher predictive value for human toxicity when data are translated from animal studies.[18] The hematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal.[18] Hematological analysis easily detects the abnormalities in body metabolic processes and reveals very important information about the response of the body to injury, deprivation, and/or stress.[20]

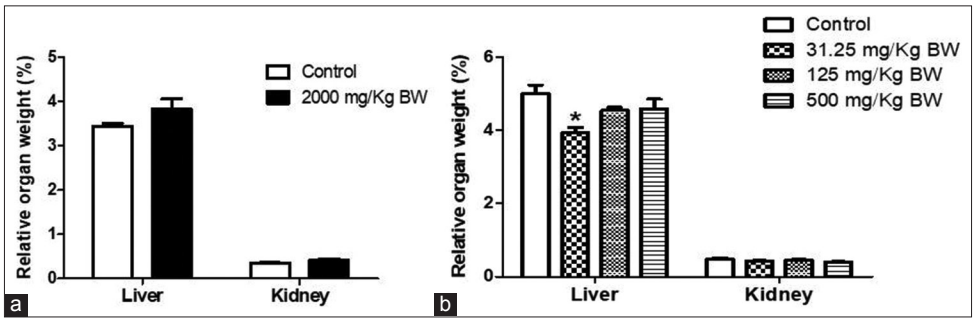

Figure 4: Effect of *Caesalpinia bonduc* (CB) root extract on the relative weight of the liver and kidney of female rats in acute toxicity test of 14 days (a) and male rats in subacute oral toxicity test 28 days (b) at twhe end of treatment. The control group received dimethyl sulfoxide; the experimental groups received a single dose of 2000 mg/kg of body weight (BW) (a) and repeated dose of 31.25 mg/kg; 125 mg/kg; and 500 mg/kg of BW (b) 28 days of the ethanolic extract of CB. Values are means of three replicates ± standard error of mean (b). *$P < 0.05$ significantly different from the control group

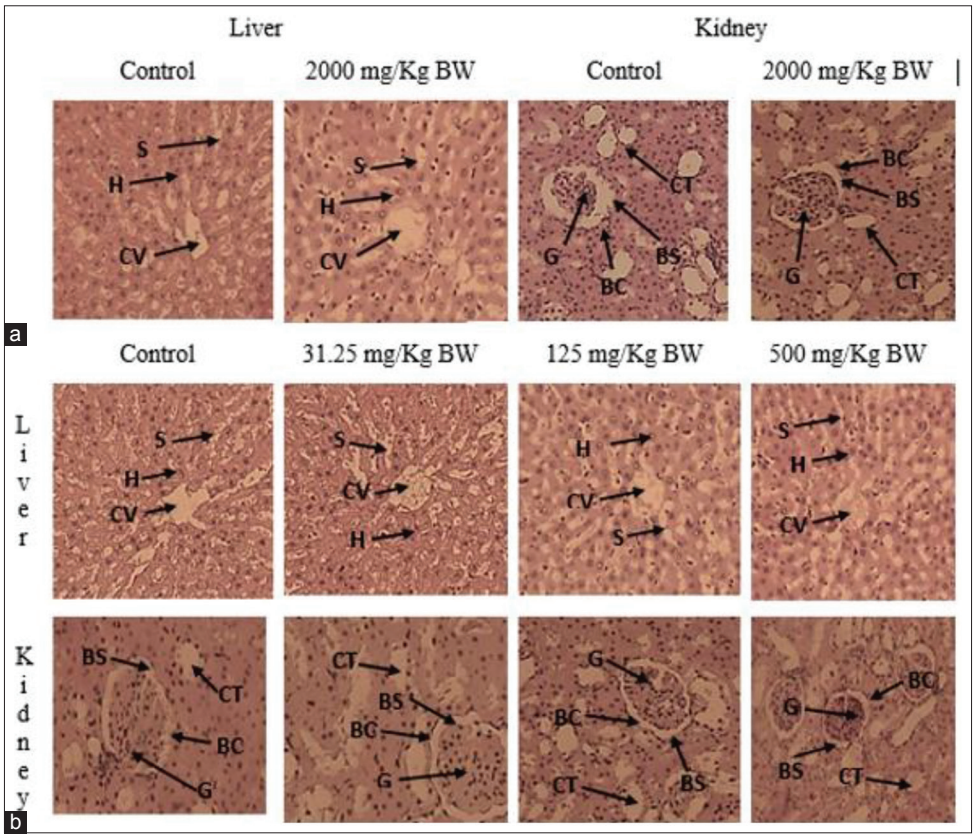

Figure 5: Photomicrographs (H and E. magnification × 400) of histoarchitecture of kidney and liver of rats. Effect of *Caesalpinia bonduc* (CB) root extracts histopathological changes of the liver and kidney of female rats in acute toxicity test of 14 days (a) and male rats in subacute oral toxicity test 28 days (b). The control group received dimethyl sulfoxide, the experimental groups received a single dose of 2000 mg/kg of body weight (BW) (a) and repeated dose of 31.25 mg/kg; 125 mg/kg; and 500 mg/kg of BW (b) for 28 days of the ethanolic extract of CB root. Liver: Control (a) group liver section showed the normal appearance of hepatocytes (H). Centrilobular vein (CV) and sinusoids (S) are preserved and ordered. No morphological difference was observed for 2000 mg/kg BW (a) when compared with the control. Control (b) group liver section showed the normal appearance of hepatocytes (H). CV and sinusoids (S) are preserved and ordered only that the sinusoids were less developed at the level of the groups treated with 125 and 500 mg/kg BW compared to the controls. Liver of 31.25 mg/kg BW shows no morphological difference when compared with the control. Kidneys: Renal Glomeruli (G) and convoluted tube (CT) without alterations are visible in control and extract treated groups. Control (a) and treated groups. Control (a) and treated group (a) showing no difference in morphology glomeruli (G), tubules (CT), Bowman's capsule, and Bowman's space

However, in the present study, all changes of hematological parameters were with in normal limits. No toxicity to the white cell, red blood cells, and Hgb experimental rats compared to controls. The numerous physiological mechanisms reflected by these hematological parameters (respiration, immunology, etc.) therefore remain normal in these treated rats although the applied dose was very much higher.[28] In toxicological evaluation, biochemical parameters have significant roles as a marker because of their response to clinical signs and symptoms produced by toxicants.[1] The biochemical investigation showed that significant modification of assessed AST level occurred in rats treated with 2000 mg/kg BW. The levels of other enzymes in the liver and kidneys of group of rats stayed with in normal ranges, which demonstrated that the test extract had no membrane stabilizing effect on these organs.[20]

Histopathological slides provide a more in-depth study of any toxic effects or diseases by investigating its effects on tissues as the preparation process preserves the tissue architecture.[17] In histopathological studies, no major alteration in vital organs liver and kidney were found. Although the method used in this study is not intended for determining precise LD_{50} value, it helps to identify the dose level at which animals are expected to survive. The extract has low acute toxicity and may be included in category five with LD_{50} estimated at 2000–5000 mg/kg.[19]

Subacute toxicity study examines toxicity caused by repeated dosing over an extended period of 28 days of oral administration in rodents. This test provides information on target organs and on the potential of the test chemical to accumulate in the organism and then is used as the basis for the determination of the no observed effect level.[1] In the present subacute toxicity study, the rats that were treated with the ethanol extract of CB root at doses 31.25, 125, and 500 mg/kg BW showed no particular clinical sign, no signs of morbidity and mortality. During the experimental period, no death or no apparent behavioral changes were observed compared with

the control group. The administration of CB root extract did not cause any significant changes in BW of the experimental rats compared to the witnesses. No significant changes were observed for food consumption. Analysis of organ weight in toxicology studies is an important endpoint for identification of potentially harmful effects of chemicals. The main requirement in toxicological experiments is the ability to assess the effects extract on specific organs.[20] In the present study, no changes were observed in the gross examination of the organs of treated animals when compared to the control group. The relative organ weight of the liver and kidneys recorded in the treatment groups showed a significant difference compared to control group for 31.25 and 500 mg/kg BW doses. This may imply that the secretory capacity of the organ has been affected or the cell constriction or inflammation of the organs, which would have led to a decrease in their weight.[20]

The hematological values such as hematocrit, erythrocytes, and Hgb are higher than those of control rats at 31.25 and 125 mg/kg BW. Those variations are not considered as pathologically significant which can induce blood cell breakdown; those variations bring a beneficial effect for blood cells.[29,30] Evaluation of hepatic and renal function is of prime importance to assess the toxic properties of extracts and drugs. Relevant renal function biomarkers including creatinine and urea levels did not change in male rats administered lowest doses of CB root used in this study. An elevated renal biomarker is an indication that poor excretion may occur; this is equally relevant to the hepatic function enzymes since both the liver and kidneys are the major sites for substance elimination in the body.[31] An increase in transaminases was observed at dose of 125 and 500 mg/kg BW. Transaminases are enzymes with significant metabolic activity within cells. Their increase reflects a cellular lesion, especially in the liver.[28] There was no alteration in body electrolytes in all the treated rats throughout the experiment. An increase in serum electrolytes is an indication of decreased reabsorption suggestive of renal tubular dysfunction. This may result in disturbances in cellular homeostasis.[29] In histopathological studies, no major alteration in vital organs liver and kidney were found. These potential adverse effects of this extract may be attributable to the presence of alkaloids, tannins, and saponins in the extracts, some of which have been reported to be toxic to the liver and kidney.[29]

CONCLUSION

The results from this study provide important data on toxicological properties of CB root which is traditionally used as an aphrodisiac plant. In the evaluation of acute toxicity, oral administration of CB root extract at a dose of 2000 mg/kg BW did not produce any major toxicological effects. In the subacute study, no severe treatment-related toxicity was observed during the study after the rats were administered with CB root extract up to a dose of 500 mg/kg BW for 28 days. Almost all the parameters were normal without any major changes; however, minor alterations in few parameters were observed, which may or may not be treatment related, thus carrying little or no toxicological importance. However, further study is required to investigate and confirms its safety in humans.

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REFERENCES

1. Loha MA, Abay SM, Ergete W, Geleta B. Acute and subacute toxicity of methanol extract of syzygium leaves on the histology of the liver and kidney and biochemical compositions of blood in rats. Evid Based Complement Altern Med 2019;2019:1-15.
2. Taylor JLS, Rabe T, McGaw LJ, Jäger AK, Staden JV. Towards the scientific validation of traditional medicinal plants. Plant Growth Regul 2001;34:23-37.
3. World Health Organization. Traditional Medicine, African Health Monitor. Vol. 4. Geneva: Amagazine of the World Health Organization Regional Office for Africa; 2013. p. 1-43.
4. Kotta S, Ansari SH, Ali J. Exploring scientifically proven herbal aphrodisiacs. Pharmacogn Rev 2013;7:1-0.
5. Wattanathorn J, Wannanon P, Muchimapura S, Thukham-Mee W, Tong-Un T, Polyiam P, *et al*. Toxicity evaluation of *Anacardium occidentale*, the potential aphrodisiac herb. Biomed Res Int 2019;2019:1459141.
6. Sahoo HB, Nandy S, Senapati AK, Sarangi SP, Sahoo SK. Aphrodisiac activity of polyherbal formulation in experimental models on male rats. Pharmacognosy Res 2014;6:120-6.
7. Jung MA, Oh K, Choi EJ, Kim YJ, Bae D, Oh D, *et al*. Effect of aqueous extract of *Dendropanax morbifera* leaf on sexual behavior in male rats. J Food Nutr Res 2017;5:518-21.
8. Adimoelja A. Phytochemicals and the breakthrough of traditional herbs in the management of sexual dysfunctions. Int J Androl 2000;23 Suppl 2:82-4.
9. Gbankoto A, Anago E, Houndjo PA, Adjahouinou DC, Gbaguidi F. Effect of aqueous and ethanolic extracts of *Caesalpinia bonduc* root on sexual behaviour of male Wistar rats. IJMCR 2015;3:1137-41.
10. Moreira SD, Tamashiro LK, Jorge BC, Balin PD, Heredia-Vieira SC, de Almeida GL, *et al*. Toxicological safety evaluation in acute and 28-day of aqueous extract from *Serjania marginata.* (Sapindaceae) leaves in rats. J Ethnopharmacol 2018;2018:1-33.
11. Chambers FL. A Textbook ofmodern toxicology. Trends Pharmacol Sci 1987;8:408.
12. Prasad GP, Trimurtulu G, Reddy KN, Naidu ML. Analytical study of kuberaksha/Kantaki karanja patra churna [*Caesalpinia bonduc* (L.) roxb. Leaf powder]. Ayu 2010;31:251-4.
13. Sonvane SM, Deshpande AN, Shaikh AR, Gadgul AB, Choutmahal SA, Bhosale PV. Evaluation of *in vitro*

antimycobacterial activity of *Caesalpinia bonduc* seed coat extracts. Intern J Pharm Res Rev 2016;5:7-11.
14. Directive 2010/63/EU. The European Parliament and the Council of the European Union. Vol. 276. Croatian: Official Journal of the European Union; 2000. p. 33-79.
15. Organization of Economic Cooperation and Development. Guidelines for the Testing of Chemicals. Vol. 4. New-York: Acute Oral Toxicity-Limit; 2001. p. 423, 14.
16. Rojas-Armas J, Arroyo-Acevedo J, Ortiz-Sánchez M, Palomino-Pacheco M, Castro-Luna A, Ramos-Cevallos N, *et al*. Acute and repeated 28-day oral dose toxicity studies of *Thymus* L. Essential oil in rats. Toxicol Res 2019;35:225-32.
17. Ghosh D, Mondal S, Ramakrishna K. Acute and sub-acute (30-day) toxicity of *Aegialitis rotundifolia* Roxb., leaves extract in Wistar rats: Safety of a rare mangrove traditionally as pain antidote. Clin Phytosci 2019;5:1-16.
18. Osseni R, Akoha S, Adjagba M, Azonbakin S, Lagnika L, Awede B, *et al*. *In vivo* toxicological assessment of the aqueous extracts of the leaves of *Carissa edulis* (*Apocynaceae*) in Wistar rats. Eur J Med Plants 2016;15:1-10.
19. Organization of Economic Cooperation and Development. Guidelines for the Testing of Chemicals. Vol. 4. New-York: Sub-Acute Oral Toxicity-Limit; 2008. p. 407, 14.
20. Al-Afifi NA, Alabsi AM, Bakri MM, Ramanathan A. Acute and sub-acute oral toxicity of *Dracaena cinnabari* resin methanol extract in rats. BMC Complement Altern Med 2018;18:50.
21. Shendge PN, Belemkar S. Acute and 28-day oral toxicity studies of methanolic extract of *Lagenaria siceraria* (*Cucurbitaceae*) fruit in rats. Drug Chem Toxicol 2019;31:1-9.
22. Abid R, Mahmood R. Acute and sub-acute oral toxicity of ethanol extract of *Cassia fistula* fruit in male rats. Avicenna J Phytomed 2019;9:117-25.
23. Tabasum S, Khari S, Kirti J. Subchronic toxicity assessment of orally methanol (70%) seed extract of *Abrus precatorius* L. in Wistar albino rats. Turk J Pharm Sci 2019;16:88-95.
24. Nkono BL, Selestin DS, Dzeufiet DP, Longo F, Kamtchouing P. Subchronic toxicity of aqueous extract of *Alstonia Boonei* de wild. (*Apocynaceae*) stem bark in normal rats. Int J Pharmacol Toxicol 2015;3:5-10.
25. Hould R. Histopathology and Cytopathology Techniques. Montreal: Maloine; 1984. p. 225-7.
26. Ezenyi IC, Mbamalu ON, Balogun L, Omorogbe L, Ameh FS, Salawu OA. Antidiabetic potentials of *Syzygium guineense* methanol leaf extract. J Phytopharmacol 2016;5:150-6.
27. Baba AF, Merzouk H, Bouanane S, Benkalfat NB, Merzouk SA, Mulengi JK, *et al*. Évaluation de la toxicité aiguë de la 2-hydroxy-méthyl-1(N-phtaloyltryptophyl) aziridine chez le rat wistar. Anl Toxicol Analy 2010;22:115-21.
28. Salau AK, Yakubu MT, Oladiji AT. Function indices of liver and kidney and haematological parameters of male Wistar rats after oral administration of aqueous extract of Terminalia avicennioides root barks. Comp Clin Pathol 2018;28:305-10.
29. Osseni R, Awede B, Adjagba M, Kpadonou C, Fall M, Laleye A, *et al*. Acute and subchronic toxicity of *Gmelina arborea* Roxb, (*Verbenaceae*) in Wistar rat. IJPR 2015;7:116-22.
30. Kale OE, Awodele O, Akindele AJ. Subacute and subchronic oral toxicity assessments of *Acridocarpus* (DC.) Guill. and Perr. Root in Wistar Rats. Toxicol Rep 2019;6:161-75.
31. Musa NH, Zain HH, Mohammed MT, Husni I. Effect of lyophilized aqueous leaf extract of *Aquilaria Subintegra* on aphrodisiac in mice. Asian Pac J Reprod 2019;8:167-73.

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