

Original Article

Acute Toxicity Study of Methanol Extract of Baobab (*Adansonia digitata* Linn) Fruit Shell Extract in Mice



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Article info:

Received: 05 Feb 2023

Accepted: 14 May 2023

Keywords:

Adansonia, Cerebellar cortex, Lethal dose₅₀, Liver, Kidney, Histology

ABSTRACT

Background: The natural origin of medicinal plants does not guarantee their safety, as there are no sufficient studies on the safety, efficacy, and toxicity to support their benefit claims.

Objectives: This study aimed at investigating the oral acute toxicity of *Adansonia digitata* L. (*A. digitata*) fruit shell extract in mice.

Methods: The maceration method was employed for the extraction of the *A. digitata* fruit shell using methanol. The extract was then screened for its phytochemical constituents both qualitatively and quantitatively. Lorke's method was followed for the toxicity study, and the mice were observed for clinical signs of toxicity and mortality. Further, serum was analyzed for liver and kidney function biomarkers besides the histology of the liver, kidney, and cerebellum.

Results: No single death was recorded and no sign of toxicity persisted for more than 2 hours post-administration to the extracts up to 5000 mg/kg. Therefore, the LD₅₀ of *A. digitata* fruit shell is above 5000 mg/kg. Additionally, no changes were observed in the weights as well as the relative organ weight of the mice. Further, no statistically significant changes were seen in their liver and kidney function biomarkers, besides the relatively intact histological appearance of their liver, kidney, and cerebellum.

Conclusion: The oral acute toxicity of methanol extract of *A. digitata* fruit shell is above 5000 mg/kg; hence, it is relatively safe to use it for medicinal purposes. However, a longer study duration is recommended to evaluate its toxic effects on fertility, teratogenicity, and carcinogenic potentials.

Citation: Fannami MI, Garba SH, Hamman OW, Chiroma SM. Acute Toxicity Study of Methanol Extract of Baobab (*Adansonia digitata* Linn) Fruit Shell Extract in Mice. *Pharmaceutical and Biomedical Research*. 2023; 9(3):201-210. <http://dx.doi.org/10.32598/PBR.9.3.1073.1>

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Introduction

Medicinal plants and plant extracts and products form an essential part of our daily life and are considered to be the mainstay of traditional medicine [1, 2]. They are used in the treatment and prevention of several diseases, particularly chronic and non-communicable ones, in nature [2]. The postulation that medicinal plants are natural and their preparations are harmless for the treatment of many diseases may have influenced their wide use by 70% of Asians and Africans [3, 4]. However, their origins should not assure their safety, since there are no sufficient studies on the efficacy, safety, and toxicity to support their beneficial claims [2]. Against this backdrop, the scientific community has developed three concepts. Firstly, a study must be conducted to show the safety profiles of any product/compound that are claimed to be beneficial. Secondly, the assessment of its chemical constituents, and thirdly to set guidelines to examine the proposed folkloric applications. Therefore, every medicinal plant requires verification for public acceptance and subsequently the necessity of toxicological reports [2].

Baobab (*Adansonia digitata* L.) is a part of Africa's indigenous iconic tree, which belongs to the Bombacaceae family and is widely distributed across sub-Saharan Africa and Western Madagascar [5]. The tree is fondly called "the small pharmacy tree" as all its parts, such as bark, leaves, and fruits have been reported to be an important source of traditional medicine, food, shelter, and livelihood to the communities producing them in Africa [6-8]. Recently, a dramatic increase has been seen in the global demand for baobab raw materials, especially its fruit pulp by food industries, which resulted in its increased importance and commercial value [5]. The baobab fruit pulp has been approved in Europe as a food ingredient since 2008, while in the United States, it received a Generally Recognized Safe (GRAS) status in 2009 [8].

Due to its high demand, baobab fruits are gathered in small or large quantities and sold to commercial companies or vendors for further processing into different products, such as baobab pulp powder, seed oil, fruit juice, and other valued products. During these operational processes, a huge quantity of baobab fruit shells (BFS) is generated as a waste product, which has less or no economic value. It is usually thrown away around the harvesting or processing areas; thus, littering the environment [6], affecting farmlands, and harboring dangerous reptiles. Despite the fact that baobab is a valuable

plant with several applications, the BFS has not yet been the subject of serious investigations. In the last two decades, only a handful of studies have been reported on the usefulness of BFS [9]. Extracts of BFS have been reported to exhibit high antioxidant capacity, probably due to the presence of bioactive compounds. Some studies reported the usefulness of BFS in the production of activated carbon for the treatment of environmental pollutants [10, 11]. More recently, Ismail et al. [6] for the first time characterized the major phenolic constituents of BFS and also demonstrated their antioxidants and inhibitory effects on α -glucosidase and α -amylase in vitro. These may serve as a novel approach to the fight against type 2 diabetes.

However, after an extensive literature search by the authors, there is a dearth of information on in vivo studies on the oral acute toxicity of BFS extract. Consequently, this study presented the first attempt to evaluate the single-dose toxicity of methanolic extract of BFS in mice. The mice were monitored for the physical sign of toxicity and mortality. Their serum biochemical parameters for kidney and liver functions were evaluated, besides the histopathology of kidneys, liver, and brain, which are vital organs in toxicology studies.

Materials and Methods

Material

Plant material

A. digitata L fruit shell was purchased from Gamboru market Maiduguri, Borno State. The taxonomic identification of the fruit shell was established by a Botanist in the Department of Biological Sciences, University of Maiduguri. The specimen is deposited at the herbarium Department of Human Anatomy, the University of Maiduguri with voucher number UM/HAH/2021/001.

Chemical substances

Ketamine injection (Swiss Parental, PVT LTD, India), methanol (Sigma Chemical Company, Saint Louis, MO, USA), and all other chemicals used in this study were of analytical grade.

Preparation of methanolic extract of *A. digitata* fruit shell

BFS were rinsed with tap water for the removal of dust and other impurities, dried under shade, pounded into powder, and sieved. Then, 1 kg of the powdered shell was

soaked in 80% methanol for three days with agitations at some intervals. The mixture was filtered using filter paper (Watchman No. 3) and concentrated to dryness using a rotary vacuum evaporator to obtain the extract. Afterward, 44.5 g of methanolic extract from BFS was obtained and kept airtight for later use. It was dissolved in distilled water before oral administration to the rats.

Phytochemical screening (qualitative and quantitative)

Qualitative screening of methanol extract of BFS

Test for alkaloids, saponin, carbohydrates, proteins and amino acids, flavonoids, tannins, cardenolide, saponin glycosides, terpenoids, cardiac glycoside, free anthraquinones, phenols, and combined anthraquinones was carried out using a standard method [12, 13].

Quantitative measurement of methanol extract of BFS

The quantities of alkaloids, phenols, saponin, tannin, and flavonoids were all measured [14-17].

Animals

Experimentations were conducted on female mice weighing between 18 to 25 g, approximately 5-6 weeks old. They were kept in the Animal House, Department of Biochemistry University Maiduguri, in accordance with the principles for the care and use of laboratory animals. The laboratory is well ventilated and the mice were exposed to 12 hours light/dark cycle and administered with standard grower mash (Vital Feed, Grand Cereal Jos, Nigeria) (proteins and amino acids, carbohydrates, fats, minerals, and vitamins) and water ad libitum.

Acute toxicity study

The study was conducted in two phases as earlier described [18], all mice were weighed before the dose administration. In phase I, four groups of mice (n=3) were orally administered with distilled water or BFS at doses of 10, 100, and 1000 mg/kg, respectively. The mice were observed for the first 4 hours and subsequently for 14 days for signs of toxicity; body weakness, hyperactivity, lack of appetite, sleep, erection of hair, brushing of the nose on the floor, salivation, stretching of the body, coma, or death. In phase II, four groups of one mouse each were orally administered with distilled water or BFS at doses of 1600, 2900, and 5000 mg/kg, respectively. They were also observed for the first 4 hours and the subsequent 14 days for any signs of toxicity and

mortality. All mice were fasted overnight and euthanized on day 15 of the experiment where blood samples were taken and organs harvested.

Collection of blood samples

Blood samples were collected through cardiac puncture and kept in plain bottles. They were centrifuged at 5000 rpm for 5 minutes. The serum was transferred into another plain bottle and used for biochemical analyses.

Relative organ weight

After euthanasia on day 15, organs, like the liver, kidneys, brain, heart, lungs, and spleen were carefully harvested and weighed in grams. The relative organ weight of each mouse was then calculated as:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)} \times 100}{\text{Body weight of mouse on sacrifice day (g)}}$$

Biochemical analysis

Biochemical analyses carried out include the measurement of the activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) as well as serum total protein (TP), and albumin (ALB) to assess the liver function. While creatinine (CREA), urea, sodium (Na⁺), potassium (K⁺), chlorine (Cl⁻), and bicarbonate (HCO₃⁻) concentrations were evaluated for kidney function. All the biomarkers were processed using the available commercial kits according to the manufacturer's manual.

Histology

The liver and kidneys were harvested and fixed in 10% formalin, while the brain was fixed in 10% Bouin's fluid. After seven days, the tissues underwent routine histological preparations and were stained with Harris hematoxylin for 15 minutes and counter-stained in 1% alcoholic eosin for 30 seconds, and then mounted in DPX [19]. The prepared slides were qualitatively evaluated using a light microscope. Micrographs were snapped using a microscope (MBJX-ISCOPE, Los Angeles, USA) equipped with a digital camera (M500, X64, v. 3.7) at different magnifications.

Statistical analysis

All values obtained were expressed as Mean±SE. The one-way analysis of variance (ANOVA) was used to determine the difference between groups, followed by Tukey's post hoc test, and P<0.05 was considered statisti-

cally significant. All statistical analyses were performed using GraphPad Prism software, version 9 (San Diego, CA, USA).

Results

Types of phytochemicals present in methanol extract of BFS

Qualitative preliminary phytochemical screening of BFS revealed the existence of different phyto-constituents (Table 1).

Quantities of phytochemicals present in methanol extract of BFS

The phytochemicals found in the methanol extract of BFS were further subjected to quantitative measure-

ments to find out the quantity of each compound therein (Table 2).

Clinical signs

No mortality was recorded and no serious sign of toxicity was seen in the mice within the 14 days of observation after a single dose of BFS ranging from 10 to 5000 mg/kg. Therefore, the extract seemed to be safe at 5000 mg/kg body weight and the LD₅₀ was considered to be >5000 mg/kg.

Effect of methanol extract of BFS on anthropometric parameters in mice

Body weight

A steady increase in body weight was observed among all the groups of mice administered with single but dif-

Table 1. Phytochemical constituents of methanol extract of BFS

Phytochemically	Test/Reagents	Status
Alkaloid	Dragendrieff's	-ve
	Mayer's	+ve
	Shinoda's	+ve
Flavonoid	Ferric chloride	+ve
	Lead acetate	-ve
	Sodium hydroxide	-ve
	Keller-killiani	+ve
Cardenolide		+ve
Terpenoids		+ve
Cardiac glycoside	Salkowski's	+ve
	Lieberman Buchard's	+ve
Tannin	Ferric Chloride	+ve
	Lead Acetate	-ve
Saponing glycoside	Fehling	+ve
Carbohydrates	Molish's	+ve
	Barfoed's	-ve
	Ketoses	+ve
Phenolic compounds		+ve
Free anthraquinones		-ve
Combined anthraquinones		-ve

Table 2. Quantitative screening of methanolic extract of BFS

Phytochemical	Mean±SEM Status
Phenols	17mgGAE/g±0.02
Flavonoid	1.50mgQE/g±0.03
Tannins	12.57mgGAE/g±0.001

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ferent doses of BFS ranging from 10 mg/kg to 1000 mg/kg after 14 days (Table 3). However, the one-way ANOVA revealed no observable differences in the initial body weight [$F_{(3,8)}=1.674$, $P=0.2488$], final body weight [$F_{(3,8)}=1.097$, $P=0.4048$], and weight gain [$F_{(3,8)}=0.8018$, $P=0.5270$] among all the experimental groups of mice.

Mice relative organ weight

The values for relative organ weights were indicated in Table 4. One-way ANOVA results showed no statistically significant differences ($P>0.05$) in the relative organ weights evaluated, which included brain [$F_{(3,8)}=1.427$, $P=0.3050$], heart [$F_{(3,8)}=0.3575$, $P=0.7854$], lungs [$F_{(3,8)}=0.8167$, $P=0.5199$], liver [$F_{(3,8)}=1.115$, $P=0.3985$], left kidney [$F_{(3,8)}=2.503$, $P=0.1332$], right kidney [$F_{(3,8)}=0.2554$, $P=0.8555$], and spleen [$F_{(3,8)}=2.115$, $P=0.1766$] among all the treatment groups.

Effect of methanol extract of BFS on biochemical parameters in mice

Serum levels of liver function biomarkers

The toxicological effect of methanol extract of BFS was evaluated on liver function biomarkers. One-way ANOVA results showed that there was no significant change ($P>0.05$) in the serum levels of ALT [$F_{(3,8)}=1.060$, $P=0.4182$], ALP [$F_{(3,8)}=1.436$, $P=0.3025$], AST [$F_{(3,8)}=0.8171$, $P=0.5197$], TP [$F_{(3,8)}=3.549$, $P=0.0675$], and ALB [$F_{(3,8)}=0.1561$, $P=0.9228$] at all treatment doses

of BFS in comparison to the control group of mice (Figure 1).

Serum levels of kidneys function biomarkers

The toxicological effect of methanol extract of BFS was ascertained on the kidney function parameters. One-way ANOVA showed no statistically significant differences ($P>0.05$) in the serum levels of urea [$F_{(3,8)}=1.431$, $P=0.3040$], creatinine [$F_{(3,8)}=0.7520$, $P=0.5523$], sodium [$F_{(3,8)}=1.347$, $P=0.3262$], potassium [$F_{(3,8)}=1.209$, $P=0.3672$], and chloride [$F_{(3,8)}=4.608$, $P=0.0373$] compared to the control group (Figure 2). However, one-way ANOVA revealed a statistically significant difference in the level of bicarbonate [$F_{(3,8)}=5.602$, $P=0.0229$]. Tukey's post hoc test confirmed the marked increase ($P<0.05$) in bicarbonate levels in mice receiving BSF 10 mg/kg compared to the control group.

Effect of methanol extract of BFS on the histology of kidneys, liver, and cerebellum in mice

Histology of liver

A qualitative analysis of the liver histology was conducted to ascertain the effects of methanol extract of BFS. The photomicrograph of the control mice livers showed normal sinusoids, hepatocytes, and central vein (Figure 3a). The photomicrographs of the livers of mice that received BFS ranging from 10 mg/kg to 5000 mg/kg

Table 3. Effect of single weights (g) of mice on day 14 dose of BFS on body

Treatment	Mean±SEM		
	Initial Weight (g)	Final Weight (g)	Weight Gained (g)
Control	15.4±1.51	29.23±0.44	13.83±1.93
BFS 10 (mg/kg)	14.13±1.11	29.13±3.98	18.83±1.41
BFS 100 (mg/kg)	17.37±0.86	32.17±2.35	17.77±4.07
BFS 1000 (mg/kg)	16.33±0.57	34.27±0.97	17.93±1.53

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Table 4. Effect of a single administration of different doses of BFS on relative organ weight of mice on day 14

Groups	Mean±SEM						
	Brain	Heart	Lungs	Liver	R Kidney	L Kidney	Spleen
Control	1.51±0.01	0.5±0.03	0.89±0.16	4.26±0.8	0.58±0.10	0.58±0.02	0.47±0.04
BFS 10 (mg/kg)	1.37±0.18	0.60±0.19	1.15±0.23	3.96±0.6	0.58±0.10	0.68±0.05	1.76±0.79
BFS 100 (mg/kg)	1.25±0.02	0.47±0.03	0.91±0.05	4.28±0.4	0.59±0.02	0.56±0.03	0.72±0.24
BFS 1000 (mg/kg)	1.23±0.04	0.49±0.04	0.88±0.03	3.22±0.6	0.66±0.07	0.62±0.02	0.49±0.06

PBR

body weight also showed no significant difference than the control group (Figure 3b-g).

Histology of kidneys

Qualitative analysis of the mice’s kidney histology was carried out to ascertain the effects of methanol extract of BFS. Photomicrographs of the control mice kidneys showed normal convoluted tubules and glomeruli (Figure 4a). The photomicrographs of the kidneys of mice

that received a single dose of BFS ranging from 10 mg/kg to 5000 mg/kg body weight also showed a normal appearance of convoluted tubules and glomeruli (Figure 4b-g).

Histology of the cerebellum

Qualitative analysis of the histology of the mice cerebellar cortex was performed to check the effects of single-dose exposure to methanol extract of BFS. Photo-

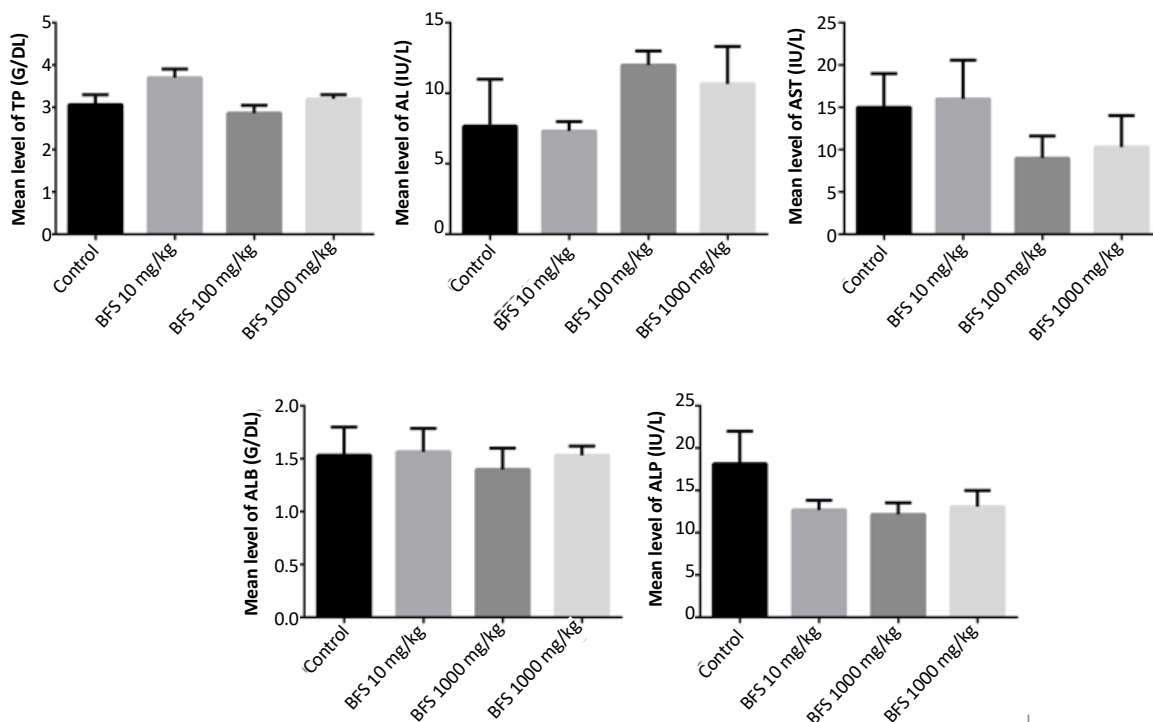
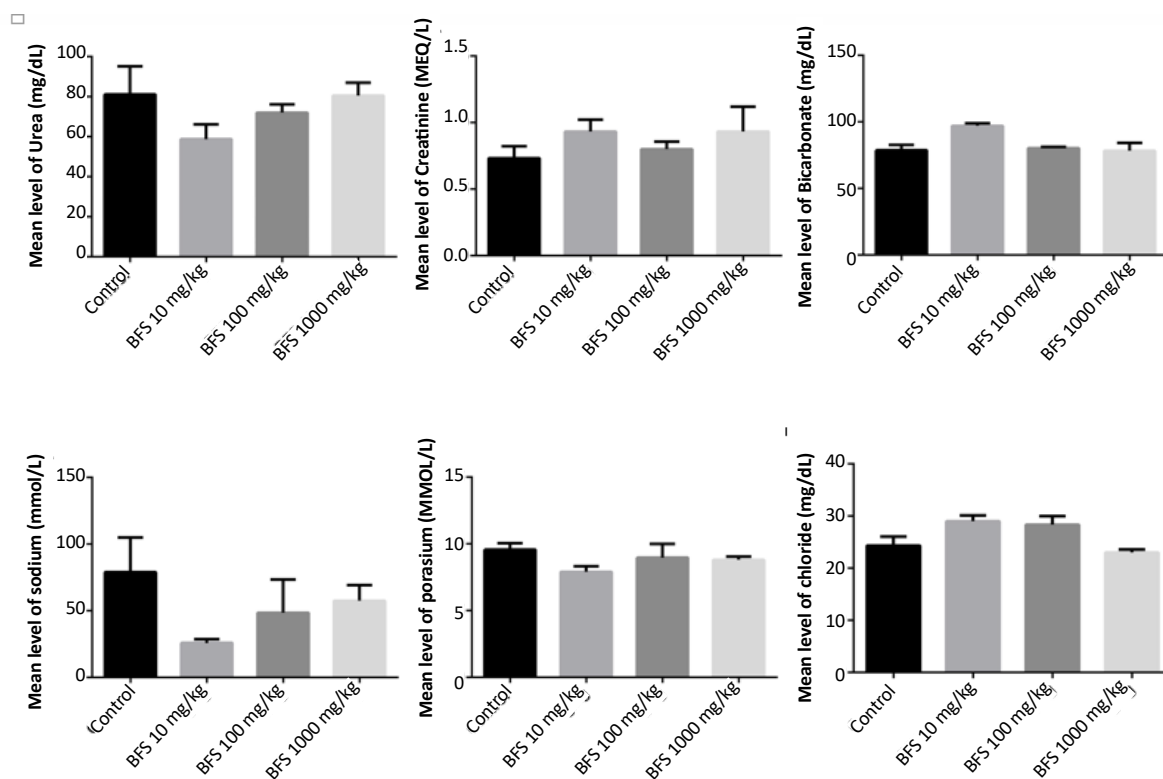


Figure 1. TP, ALB, ALP, AST, and ALT after one-time administration of different doses of BFS extract. Data were presented as Mean±SE and analyzed using one-way ANOVA, n=3 mice for each group.

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Figure 2. Kidney function parameters after a one-time administration of different doses of BFS extract
Data were presented as Mean±SE and analyzed using one-way ANOVA, n=3 mice for each group, P<0.05.

micrograph of the cerebellar cortex of the control group of mice showed normal cell layers, which included the Molecular Layer (ML), middle Purkinje cells layer (PCL), and inner granular layer (GL) with aggregation of granular cells (Figure 5a). The photomicrographs of the cerebellum of mice that received a single dose of BFS ranging from 10 mg/kg to 5000 mg/kg (Figure 5b-g) also showed a similar pattern as there were no obvious histological aberrations.

Discussions

In this study, qualitative analysis of a methanolic extract of *A. digitata* fruit shell indicated the presence of cardenolide, terpenoids, cardiac glycoside, saponin glycoside alkaloid, and phenolic compounds. Similar results were reported after the phytochemical screening of the aqueous extract of *A. digitata* fruit pulp [20], which revealed the presence of tannins, saponin, phenol, glycosides, flavonoids, and alkaloids. In the present study, quantitative estimation of phytochemicals showed total flavonoid content to be 1.50 mgQE/g, which showed the presence of Quercetin. Quercetin is among the most commonly studied flavonoids, which possesses many health-promoting potentials, including anti-inflammato-

ry, anti-diabetic, and anti-cancer properties, and also reduces the risk of cardiovascular diseases [21, 22]. These potentials are associated with its potent antioxidant capacity by strengthening endogenous defense against free radicals, while the anti-cancer activity of quercetin is due to its ability to bind cellular receptors and proteins, which controls mechanisms of cell signaling and also inhibits enzymes responsible for the carcinogens activation [23]. Alkaloids are chemical compounds mostly containing basic nitrogen atoms and are used as a remedy for gout with analgesic, antihypertensive, vasodilator, muscle relaxant, antitumor, and anti-malarial activity [20, 24]. The presence of these classes of compounds, especially flavonoids and alkaloids in the aqueous extract of the fruit pulp of *A. digitata* could be responsible for its various medicinal and anti-oxidative uses [25]. Flavonoid is considered to have good antioxidant, antiviral, anti-inflammatory, anti-allergic, and anticancer properties and are appropriate therapeutic agents [26].

The present acute toxicity study showed that oral administration of methanol extract of BSF up to the dose of 5000 mg/kg body weight did not cause any persistent clinical signs nor mortality in mice. Consequently, this study proved that BSF was non-toxic up to 5000 mg/

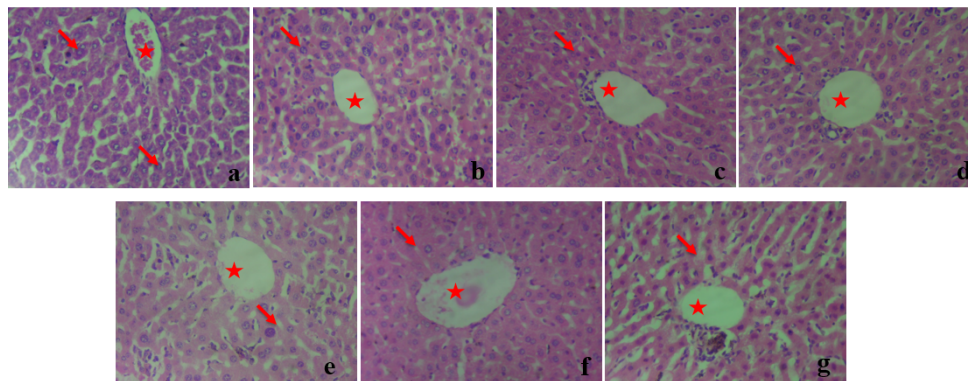


Figure 3. Photomicrographs of transverse sections of the liver of the control (distilled water) (a) showing normal histological structure of the liver with the central vein (red star) and Kuffers cells (red arrow), BFS 10 mg/kg (b), BFS 100 mg/kg (c), BFS 1000 mg/kg (d), BFS 1600 mg/kg (e), BFS 2900 mg/kg (f), and BFS 5000 mg/kg (g)
No serious histological aberrations were noticed.

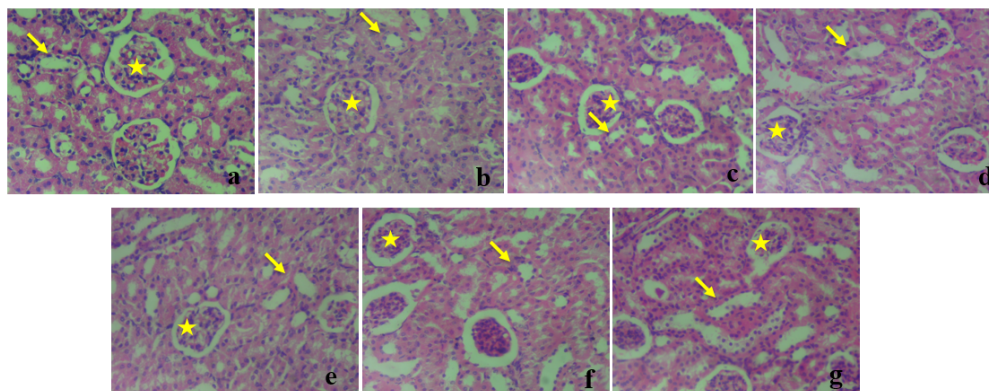


Figure 4. Photomicrographs of transverse sections kidneys of control (distilled water) (a) showing normal histological structure of the kidneys with glomerulus (yellow star) and tubules (yellow arrow), BFS 10 mg/kg (b), BFS 100 mg/kg (c), BFS 1000 mg/kg (d), BFS 1600 mg/kg (e), BFS 2900 mg/kg (f) and BFS 5000 mg/kg (g)
No serious histological aberrations were noticed.

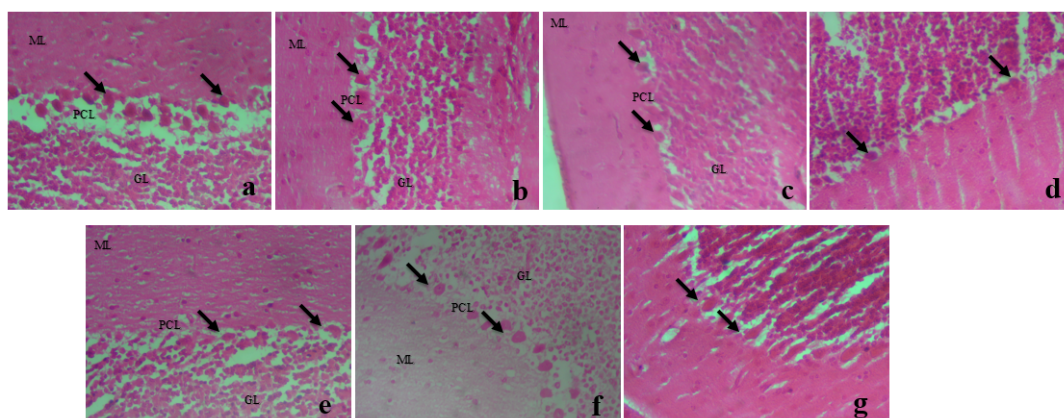


Figure 5. Photomicrographs of transverse sections of cerebellum of the control (distilled water) (a) showing normal histological cell layers of cerebellar cortex with with (ML) molecular layer, (PCL) middle Purkinje cells layer with large pyriform shape (black arrow) and inner granular layer (GL) with aggregation of granular cells, BFS 10 mg/kg (b), BFS 100 mg/kg (c), BFS 1000 mg/kg (d), BFS 1600 mg/kg (e), BFS 2900 mg/kg (f), and BFS 5000 mg/kg (g)
No serious histological aberrations were noticed.

kg, and relatively safe to use. Substances that are higher than 5000 mg/kg via the oral route of administration are considered non-toxic [27]. Weight modulation is a very important characteristic of numerous medicinal agents [28, 29]. The present study explored body weight as well as relative organ weights in order to ascertain the toxicity of BFS extract. However, no changes in the body weights nor the relative organ weight of their liver, kidneys, brain, heart, lungs, and spleen were observed. This is suggestive of the safe nature of the extract at the doses tested. Further, no biochemical derangement was observed in both liver and kidney function biomarkers analyzed, while the histology of vital organs of metabolism, including the liver and kidney was intact besides the normal appearance of the mice brain's cerebellum.

Conclusion

In conclusion, the acute oral dose of BFS methanolic extract up to 5000 mg/kg showed no evidence of toxicity or treatment-related mortality in mice. No changes were observed in the body and relative organs weights, liver and kidney function biomarkers, and also in the histology of their liver, kidneys, and cerebellum. Hence, the LD₅₀ of BFS is more than 5000 mg/kg in mice and can be said to have a high margin of safety. However, a longer study duration is recommended to evaluate its toxic effects on fertility, teratogenicity, and carcinogenic potentials.

Ethical Considerations

Compliance with ethical guidelines

The study protocol was approved by the Postgraduate Board of Studies, Faculty of Basic Medical Sciences, the University of Maiduguri, which is in accordance with the ARRIVE guidelines for the Care and Use of Animals with code PGA/18/01/01/08807.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

All authors appreciated the valuable contributions made by NI Dibal, Ephraim Ayuba, and Sunday of the Human Anatomy Department University of Maiduguri.

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