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# Toxicological evaluation of the leaves of *Guiera senegalensis* (J.F. Gme), *Cassia occidentalis* (Linn), and *Ziziphus mauritiana* (Lam)

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## Abstract

**Background:** There is a renewed global interest in plant-based medicine in the last few decades as a result of affordability and efficacy of the form of medicine. However, the safety of many medicinal plants and plant preparations has not been determined. To this end, this study determined the safety of the methanol extracts of the leaves of *Guiera senegalensis*, *Cassia occidentalis*, and *Ziziphus mauritiana*, which are plants commonly used in plant medicine in Africa.

**Results:** Phytochemistry of the plants' extracts showed alkaloids were the most abundant phytochemical in the extracts, followed by tannins, saponins, flavonoids, and glycosides, respectively. All the rats behaved normally at doses up to 2000 mg kg<sup>-1</sup>, while at 3000 mg kg<sup>-1</sup>, rats in *C. occidentalis* group developed breathing problems. At 5000 mg kg<sup>-1</sup>, *C. occidentalis* group showed convulsion, whereas *Z. mauritiana* and *G. senegalensis* rats displayed temporary weakness. After 60-day dosing, significant differences ( $p < 0.05$ ) were observed in the liver enzymes (ALP, AST, ALT, TP, and ALB) of most of the test rats compared with the control. Kidney function test showed the Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, urea, and creatinine of the test rats were reduced compared with the control, but only significant ( $p < 0.05$ ) in a few of the parameters. Fatty metamorphosis, lymphocytic infiltration, and karyorrhexis were observed in the liver tissues of the treated rats, but not in the control.

**Conclusion:** These results show the plants are nontoxic at moderate doses, but could be injurious at high doses or if taken repeatedly for an extended period.

**Keywords:** Alkaloids, Creatinine, Phytochemical, Toxicity, Urea, *Guiera senegalensis*

## 1 Background

Several scientific investigations and, to some extent, clinical trials have emphasized the importance of plants in the treatment of many diseases. Most developing nations, especially in Africa, rely on traditional medicines for different health needs as a result of their availability [1]. It is estimated that about 80% of the world's population, mostly in the developing countries, depend on plant medicines [2, 3]. The use of plant medicines dates back to several thousands of years [4], but the form of

medicine practically vanished with the evolution of modern medicine and synthetic drugs in the eighteenth century. However, plant medicine is currently on the rise because many medicinal plants have been discovered to contain several bioactive compounds employed in pharmaceutical industries [4]. Other reasons for the renewed interest in plant medicines are because they are cost effective and simple to prepare [5, 6]. Plant medicine has become so prominent that many drugs in current use contain bioactive substances of plant origin [7]. It is estimated that about 25% of all modern medicines are derived from plants [7, 8].

The renaissance in plant-based medicine has opened up a lot of job opportunities worldwide. Particularly, in

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Nigeria, it has lifted many people out of poverty by providing daily living to the practitioners, marketers, and other classes of workers. Plant-based medicine outfits and shops are springing up steadily in the country, engaging able-bodied men and women, thereby reducing the menace of unemployment ravaging the country. Moreover, it has helped cure some ailments which have defied conventional treatment methods. Plant-based medicine has gained so much prominence and acceptability in Nigeria that at a point the country proposed to establish a university of alternative medicine. This was conceived to boost the activities of the industry and integrate it fully into the country's health care delivery system.

The popularity of plant medicine in Nigeria can be attributed partly to the abundance of plant species with medicinal properties in the country. Of the numerous medicinal plants native to Nigeria, *Guiera senegalensis*, *Cassia occidentalis*, and *Ziziphus mauritiana* are frequently used in the northern part of the country. *G. senegalensis* is called Sabara by Hausa and other tribes in northern Nigeria. The plant contains abundant phenolic and flavonoidal compounds and is often used in the region to treat diarrhea and fever as well as increase milk production in lactating women [9, 10]. *C. occidentalis*, called Sanga-Sanga in northern Nigeria, has alkaloids, phlobatannins, tannins, flavonoids, saponins, terpenes, etc., and is used to treat leg pains, swollen feet, and hepatitis, among others [10–12]. *Z. mauritiana* is called Magarya in Hausa and is rich in alkaloids, flavonoids, tannins, steroids, and saponins [13]. Its leaves are frequently used to treat wounds, infection, fever, liver diseases, and asthma [13, 14]. However, the safety of these plants has not been established. Though some studies had been carried out in this regard, their results are inconsistent. For example, while Nuhu and Aliyu [11] reported hypoproteinemia and elevated liver enzymes in rats fed with extracts of *C. occidentalis*, Silva et al. [15] found no effects of short- and long-term administration of *C. occidentalis* in treated rats. Vashishtha et al. [16] also linked the consumption of *C. occidentalis* beans to the outbreak of the hepatomyoencephalopathy syndrome among children in western Uttar Pradesh. Therefore, a study aimed at determining the safe doses of these plants becomes imperative, so as to prevent untended fatalities. To this end, this study evaluated the toxicity of the leaves of *Guiera senegalensis*, *Cassia occidentalis*, and *Ziziphus mauritiana*.

## 2 Methods

### 2.1 Source of test animal and management

One hundred and forty (140) experimental rats (*Rattus norvegicus*) of both sexes and age 60 days with a

mean weight of  $210 \pm 10$  g were employed for this study. The rats were kept in metal cages in a well-ventilated animal house with five rats placed in each cage and maintained at room temperature ( $32 \pm 2^\circ\text{C}$ ). The rats had free access to water and rodent pellet feeds from the Vital Feeds Limited, Lagos, Nigeria.

### 2.2 Plant materials and extraction of bioactive compounds

Fresh leaves of *Guiera senegalensis*, *Cassia occidentalis*, and *Ziziphus mauritiana* were collected around Kalgo, Birnin Kebbi, Nigeria, in August 2018. The plants were identified by a taxonomist in the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero. Samples of the authenticated plants with voucher numbers V.N 48, V.N 71, and V.N 258 for *G. senegalensis*, *C. occidentalis*, and *Z. mauritiana*, respectively, were kept in the herbarium section of the department. The plant materials were washed gently to remove impurities and air-dried under shade to a constant dry weight. The dried leaves were milled into fine powder using a laboratory grinder manufactured by TENCAN (model no: XQM-(20-100). About 100, 50, and 100 g powder of *Z. mauritiana*, *C. occidentalis*, and *G. senegalensis* were soaked in flasks containing 600, 300, and 600 ml, 98% methanol, respectively, and were allowed to stand for 24 h. The extracts thus obtained were filtered with muslin cloth, and the solvent (methanol) was removed using a rotary evaporator at  $45^\circ\text{C}$ . The dried extracts were stored in a desiccator before use.

### 2.3 Qualitative screening of the extracts

The qualitative screening of the extracts was done using standard protocols as described by Sofowora [17].

#### 2.3.1 Test for glycosides

About 2.5 ml of 50%  $\text{H}_2\text{SO}_4$  was added to 5 ml of each extract in a test tube, and the mixture was heated in a boiling bath for 15 min. The mixture was allowed to cool and neutralized with 10% NaOH. About 5 ml of Fehling's solution was added, and the mixture was boiled. A brick-red precipitate was observed, which indicated the presence of glycosides.

#### 2.3.2 Test for anthraquinones

The extract (0.5 g) of each plant was shaken with 10 ml of benzene. The mixture was filtered after which 5 ml of 10% ammonia solution was added to the filtrate and shaken gently. The presence of a pink, red, or violet color in the ammoniacal (lower) phase indicated the presence of anthraquinones.

### 2.3.3 Test for saponins

About 0.5 g of each extract was dissolved in 10 ml of distilled water in a test tube. The test tube was covered tightly and shaken vigorously for 30 s, after which it was allowed to stand for 45 min. The appearance of frothing, which persists on warming, indicated the presence of saponins.

### 2.3.4 Test for phlobatanins

The presence of phlobatanins in the extract was determined using the hydrogen chloride test. The extract (0.5 g) was dissolved in distilled water and filtered after which the filtrate was boiled with 2% HCl solution. A red precipitate revealed the presence of phlobatanins.

### 2.3.5 Test for flavonoids

To a portion of the dissolved extract, a few drops of 10% ferric chloride solution were added. A green or blue color indicated the presence of flavonoids.

### 2.3.6 Test for steroids

About 0.5 g of each extract was dissolved in 2 ml acetic anhydride and placed in ice. Sulfuric acid was then carefully added, and a color change from violet to blue and finally green indicated the presence of a steroidal nucleus.

### 2.3.7 Test for tannins

About 0.5 g of the extract was dissolved in 5 ml of water, followed by a few drops of 10% ferric chloride. A blue-black, green, or blue-green precipitate indicated the presence of tannins.

### 2.3.8 Test for alkaloids

The presence of alkaloids in the extracts was tested using the Wagner-Drageendorff's test. About 0.2 g of the extracts was heated with 2% H<sub>2</sub>SO<sub>4</sub> for 2 min. The mixture was filtered, and few drops of Drageendorff's reagent were added. An orange-red precipitate showed the presence of alkaloids.

## 2.4 Quantitative screening of the extracts

The levels of the phytochemicals detected in the extracts of the plants during the qualitative screening were estimated using the method of Ajuru et al. [18].

### 2.4.1 Estimation of alkaloids

Five grams of extract of each plant was put in a 250-ml beaker, and 200 ml of 20% acetic acid in ethanol was added and made to stand for 4 h. The mixture was filtered, and the filtrate was concentrated to one quarter of the original volume using a water bath. Few drops of concentrated ammonium hydroxide

were added to the extract until the preparation was complete. The solution was allowed to settle, and the precipitate was collected by filtration and weighed. The weight of the alkaloids obtained was then expressed as a percentage of the raw extract of the plant:

$$\text{Alkaloids (\%)} = [\text{weight of alkaloids/weight of raw extract}] \times 100$$

### 2.4.2 Estimation of tannins

Five grams of each extract was weighed into a 100-ml plastic bottle containing 50 ml of distilled water and shaken for 1 h in a mechanical shaker. The filtrate obtained was put in a 50-ml volumetric flask and made up to the mark. Then, 5 ml of the filtrate was pipette into a tube and mixed with 3 ml of 0.1 M FeCl<sub>3</sub> in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120 nm wavelength within 10 min. A blank sample was prepared, and the color also developed and read at the same wavelength. A standard was prepared using tannic acid to get 100 ppm and measured. The weight was thereafter expressed as a percentage of the raw extract.

### 2.4.3 Estimation of flavonoids

One hundred grams of each plant extract was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered using Whatman filter paper no. 42 (125 mm). The filtrate was later transferred into a crucible, evaporated to dryness over a water bath, and weighed and expressed as a percentage of the raw extract.

### 2.4.4 Estimation of saponins

The extracts were ground, and 20 g was transferred into 200 ml of 20% ethanol. The suspension produced was heated over a hot water bath for 4 h with continuous stirring at about 55 °C. The mixture was filtered, and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90 °C. The concentrate was transferred into a 250-ml separator funnel, and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated, and 60 ml of *n*-butanol was added. The combined *n*-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight and estimated as a percentage of the raw extract.

#### 2.4.5 Estimation of glycosides

One gram of the extract was macerated in 50 ml of distilled water and filtered. Then, 4 ml of the alkaline picrate solution was added to 1 ml of the filtrate. The mixture was boiled for 5 min, allowed to cool, and weighed and expressed as a percentage of the raw extract.

#### 2.5 Experimental design

The toxicity test of methanol extracts of the three plants was determined in two phases. In the first phase, the "Classical LD<sub>50</sub>" method described by Trevan [19] was used to establish the range of doses capable of producing toxic effects. Ninety rats were randomly divided into 3 groups containing 30 rats each with a group assigned 1 of the extracts and labeled *G. senegalensis*, *C. occidentalis*, and *Z. mauritiana* group, respectively. The thirty rats in each group were subdivided into 5 containing 6 rats each and administered 500, 1000, 2000, 3000, and 5000 mg kg<sup>-1</sup> of the extract, respectively. A control group was equally set up, but rats in this group were not given any extract. The rats were monitored for 72 h, and general toxicities of the extracts were evaluated according to a set of signs determined by Almeida [20].

In the second phase, a new set of 36 rats were distributed equally into 4 groups. Rats in groups 1, 2, and 3 were administered 1000 mg kg<sup>-1</sup> extracts of *G. senegalensis*, *C. occidentalis*, and *Z. mauritiana*, respectively, for 60 days. The fourth group was made the control and were not fed any extract. At the end of the treatments, the rats were sacrificed by cervical dislocation after sedating them with chloroform in a tightly covered bell-shaped glass jar. Blood samples were then collected for liver function tests (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), and albumin) and kidney function tests (creatinine, urea, Cl<sup>-</sup>, Na<sup>+</sup>, and K<sup>+</sup>). The livers of the rats were also harvested for histological examination.

#### 2.6 Blood sample collection procedures

Blood samples were obtained from the rats based on the method of Hugo and Russel [21]. The rats were sedated with chloroform in a bell-shaped glass jar in the laboratory. Total death was prevented to allow continuous flow of blood for proper blood collection. Each rat was pegged down on a work bench and held firmly with office pins. A surgical blade was used to cut through the chest region of the rats in the dorsal-ventral direction. The blood was then collected from a beating heart using a heparinized capillary tube through capillary action into EDTA bottle. EDTA serves as an anticoagulant.

#### 2.7 Liver and kidney function tests

The biochemical parameters, including the ALT, AST, and ALP, were estimated by ultraviolet, colorimetric, and spectrophotometric methods, respectively, as described by Bergneyer and Bernt [22]. The albumin and protein content were calculated by the biuret method as described by Parasuraman et al. [23].

For the determination of kidney functions, creatinine and urea levels were analyzed using the methods of Tietz [24]. The serum ions, including Cl<sup>-</sup>, Na<sup>+</sup>, and K<sup>+</sup>, levels were determined using the flame photometry method as described by Akhigbe et al. [25].

#### 2.8 Histopathological examination

The liver tissues of the rats were prepared for histopathological examination using the method of Taylor et al. [26]. The tissues were excised and processed using routine hematoxylin and eosin staining techniques. Analysis of the tissues was done using qualitative methods with emphasis on the morphology, architectures, and cytological structures of the tissues.

#### 2.9 Data analysis

Data were expressed as mean ± standard error of mean (SEM). The statistical difference between the control and test groups was analyzed using Student's *t* test. The value of *p* ≤ 0.05 was considered significant.

### 3 Results

#### 3.1 Qualitative screening of the extracts

Table 1 shows the results of qualitative analysis of extracts of the three plants. Of all the phytochemicals screened, only alkaloids, tannins, saponins, flavonoids, and glycosides were detected in the extracts.

#### 3.2 Quantitative screening of the extracts

The results of quantitative analysis of the extracts of the three plants are presented in Table 2. *Z. mauritiana* has the highest concentration of alkaloids with 7.8%, followed by *C. occidentalis* with 6.4%, and the least was

**Table 1** Phytochemicals detected in the methanol extracts of *Z. mauritiana*, *C. occidentalis*, and *G. senegalensis*

Phytochemical	<i>Z. mauritiana</i>	<i>C. occidentalis</i>	<i>G. senegalensis</i>
Alkaloids	++	++	++
Tanins	++	++	+
Saponins	+	+	+
Flavonoids	+	+	+
Glycosides	+	+	+
Anthraquinones	-	-	-
Phlobatanins	-	-	-
Steroids	-	-	-

"++," present in abundance; "+," sparingly present; "-", not detected

**Table 2** Quantities of major phytochemicals detected in the methanol extracts of *Z. mauritiana*, *C. occidentalis*, and *G. senegalensis*

Extract	Alkaloids (%)	Tannins (%)	Flavonoids (%)	Saponins (%)	Glycosides (%)
<i>Z. mauritiana</i>	7.80	2.85	0.66	1.90	2.90
<i>C. occidentalis</i>	6.40	3.74	3.60	3.00	0.37
<i>G. senegalensis</i>	6.00	0.6	0.90	3.00	0.30

*G. senegalensis* with 6.0%. The highest level of tannins was detected in *C. occidentalis* with 3.74%, while 2.84% and 0.6% were recorded in *Z. mauritiana* and *G. senegalensis*, respectively. The concentration of saponins in *Z. mauritiana* was 1.9%, while 3.0% was found in both *C. occidentalis* and *G. senegalensis*. Glycoside content in *Z. mauritiana* was 2.9%, while 0.37% and 0.3% were detected in *C. occidentalis* and *G. senegalensis*, respectively. The levels of flavonoids found in *Z. mauritiana*, *C. occidentalis*, and *G. senegalensis* are 0.66%, 3.6%, and 0.9%, respectively.

### 3.3 Acute toxicities of the extracts

Table 3 shows the reactions of the treated rats to the varied doses of the extracts of the three plants. Up to 2000 mg kg<sup>-1</sup>, the rats across the groups did not react negatively, while at 3000 mg kg<sup>-1</sup>, rats in the *C. occidentalis* group developed fatigue and breathing problem. At 5000 mg kg<sup>-1</sup>, the rats treated with *C. occidentalis* showed convulsion, while the rats administered with *Z. mauritiana* and *G. senegalensis* extracts showed weakness. There were no cases of death, indicating the LD<sub>50</sub> of the extracts of the three plants are above 5000 mg kg<sup>-1</sup>.

### 3.4 Effects of the extracts on liver enzymes

Table 4 shows the effects of the plant extracts on the liver enzymes of the rats after 60-day dosing. Compared with the control (Fig. 1), significant differences ( $p < 0.05$ ) were not observed in the levels of TP and ALB (g dl<sup>-1</sup>) of the rats administered *Z. mauritiana*, *G. senegalensis*, and *C. occidentalis*. However, the treated rats showed significant ( $p < 0.05$ ) elevated levels of the ALP, AST, and ALT (U l<sup>-1</sup>).

### 3.5 Effects of the extracts on the kidney function parameters

The effects of 60-day dosing on the kidney functions of the rats are revealed in Table 5. The treated and control rats showed no significant difference ( $p < 0.05$ ) in the levels of Na<sup>+</sup> (mEq l<sup>-1</sup>), K<sup>+</sup> (mmol l<sup>-1</sup>), and Cl<sup>-</sup> (mEq l<sup>-1</sup>). However, significant differences exist between the treated and control rats in the levels of urea (mg dl<sup>-1</sup>) and creatinine (ml min<sup>-1</sup>).

### 3.6 Histopathological effects of the extracts

Figures 2, 3, and 4 describe the effects of the extracts on the liver tissues of the treated rats. Figures 2 and 3 are the liver sections of the rats administered *G. senegalensis* and *Z. mauritiana*, showing karyorrhesis, mild congestion, and lymphocytic infiltration. Fatty metamorphosis, inflammation, pyknotic-looking hepatocyte nuclei, and lymphocytic infiltration were observed in the liver sections of the rats treated with *C. occidentalis* (Fig. 4). No abnormalities were observed in the liver tissues of control rats (Fig. 1).

## 4 Discussion

The toxicity of the leaves of three medicinal plants, namely *Z. mauritiana*, *C. occidentalis*, and *G. senegalensis*, was established in this study. The study was initiated to prevent unintended fatalities among users of the medicinal plants in Nigeria and other parts of the world. Some phytochemicals, including alkaloids, tannins, glycosides, saponins, and flavonoids, were detected in the extracts of the plants (Tables 1 and 2), which indicate the plants could confer some health benefits. These bioactive substances are well known and reported in the literature for their health-boosting activities. The nonlethal property of the extracts across the groups at doses up to 5000 mg kg<sup>-1</sup> (Table 3) shows the LD<sub>50</sub> values of the

**Table 3** Reactions of the rats to different doses of *Z. mauritiana*, *C. occidentalis*, and *G. senegalensis*

Extract	Doses (mg kg <sup>-1</sup> ) administered for 3 days				
	500	1000	2000	3000	5000
<i>Z. mauritiana</i>	Nil	Nil	Nil	Nil	Weakness
<i>C. occidentalis</i>	Nil	Nil	Nil	Weakness and gasping for breath	Convulsion
<i>G. senegalensis</i>	Nil	Nil	Nil	Nil	Weakness
Control	Nil	Nil	Nil	Nil	Nil

Nil no reaction; number of rats per group (n) = 6

**Table 4** Liver enzymes of the rats dosed with *Z. mauritiana*, *C. occidentalis*, and *G. senegalensis*

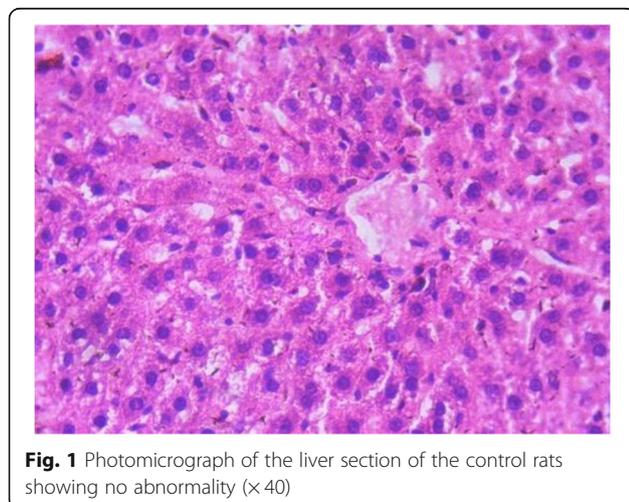
Parameter	Control	<i>Z. mauritiana</i>	<i>G. senegalensis</i>	<i>C. occidentalis</i>
TP (g dl <sup>-1</sup> )	07.23 ± 0.3 <sup>a</sup>	06.93 ± 0.4 <sup>a</sup>	7.13 ± 0.1 <sup>a</sup>	06.90 ± 0.2 <sup>a</sup>
ALB (g dl <sup>-1</sup> )	04.11 ± 0.2 <sup>a</sup>	04.05 ± 0.1 <sup>a</sup>	4.10 ± 0.6 <sup>a</sup>	04.03 ± 0.6 <sup>a</sup>
ALP (U l <sup>-1</sup> )	24.00 ± 2.6 <sup>a</sup>	13.00 ± 1.7 <sup>b</sup>	11.33 ± 1.5 <sup>b</sup>	13.01 ± 2.9 <sup>b</sup>
AST (U l <sup>-1</sup> )	86.67 ± 10 <sup>a</sup>	160.33 ± 15.60 <sup>b</sup>	194.33 ± 16.60 <sup>b</sup>	191.67 ± 5.61 <sup>b</sup>
ALT (U l <sup>-1</sup> )	53.33 ± 3.5 <sup>a</sup>	41.67 ± 3.40 <sup>b</sup>	100.66 ± 9.26 <sup>b</sup>	67.33 ± 1.76 <sup>a</sup>

Values were expressed as mean ± SE; number of rats per group (n) = 6; statistical test = Student's *t* test; values along the row with different superscript letters as the control are statistically different from control at *p* < 0.05

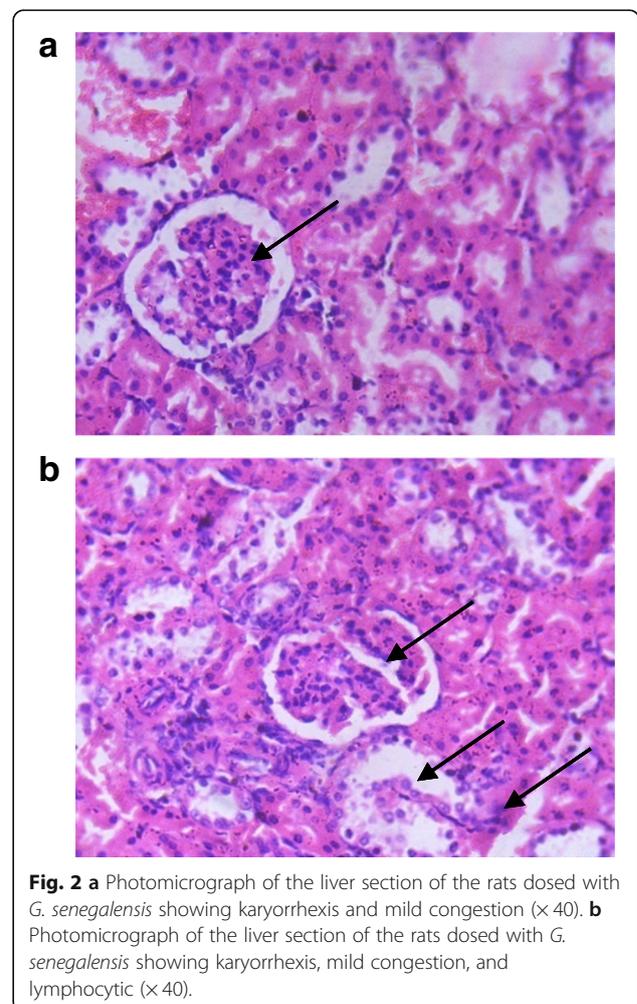
methanol extracts of the plants were within the standard range of 500–5000 mg kg<sup>-1</sup> body weight proposed by Lorke [27]. Hence, a single oral administration of the plants less than or equal 5000 mg kg<sup>-1</sup> is not expected to cause any fatality. However, as noticed in the reactions of the rats, a single oral dose of 3000 mg kg<sup>-1</sup> for *Z. mauritiana* and *G. senegalensis* as well as 2000 mg kg for *C. occidentalis* is advisable. The fatigue and convulsion displayed by some of the rats between 3000 and 5000 mg kg<sup>-1</sup> could be the effects of one or more of the phytochemicals in the extracts, particularly alkaloids, tannins, and saponins.

The reduction in the metabolic enzymes (TP, ALB) as well as the increase in other liver enzymes (ALP, AST, ALT) of the rats after 60-day dosing (Table 4) is symptomatic of the presence of cytotoxic agents. The changes were particularly more pronounced in the rats dosed with *Z. mauritiana* and *C. occidentalis*, both of which contain relatively high tannins and alkaloids. Similar patterns were also observed in the reduction of kidney function parameters (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, urea, creatinine) of the rats at the end of the dosing period (Table 5). The changes in these health indices could result from the gradual accumulation of certain substances in

the extracts to toxic levels in the blood of the treated rats. Though any bioactive substance, both tested and non-tested in the studied extracts, could have caused these changes, the primary suspects in this study are tannins and alkaloids detected in moderate abundance in the extracts. Several studies have reported toxicities of alkaloids and tannins in animals following a prolonged treatment. For instance, alkaloids derived from



**Fig. 1** Photomicrograph of the liver section of the control rats showing no abnormality (x 40)



**Fig. 2 a** Photomicrograph of the liver section of the rats dosed with *G. senegalensis* showing karyorrhexis and mild congestion (x 40). **b** Photomicrograph of the liver section of the rats dosed with *G. senegalensis* showing karyorrhexis, mild congestion, and lymphocytic (x 40).

**Table 5** Kidney parameters of the rats treated with *Z. mauritiana*, *C. occidentalis*, and *G. senegalensis*

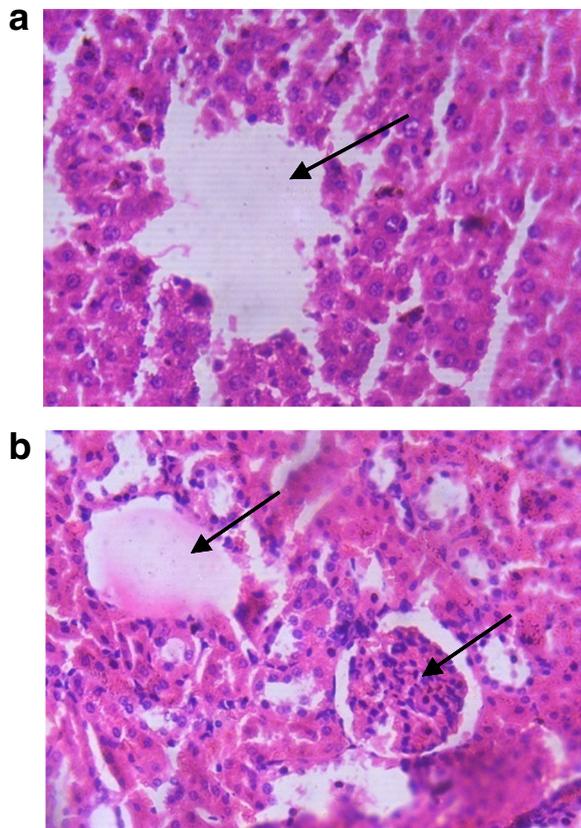
Parameter	Control	<i>Z. mauritiana</i>	<i>G. senegalensis</i>	<i>C. occidentalis</i>
Na <sup>+</sup> (mEq l <sup>-1</sup> )	139.67 ± 10.3 <sup>a</sup>	139.00 ± 10.5 <sup>a</sup>	139.67 ± 10.3 <sup>a</sup>	140.33 ± 11.8 <sup>a</sup>
K <sup>+</sup> (mmol l <sup>-1</sup> )	4.90 ± 0.5 <sup>a</sup>	4.33 ± 0.9 <sup>a</sup>	4.43 ± 0.2 <sup>a</sup>	4.13 ± 0.2 <sup>a</sup>
Cl <sup>-</sup> (mEq l <sup>-1</sup> )	107.00 ± 5.8 <sup>a</sup>	101.00 ± 6.8 <sup>a</sup>	102.67 ± 4.9 <sup>a</sup>	102.67 ± 5.1 <sup>a</sup>
Urea (mg dl <sup>-1</sup> )	4.87 ± 0.3 <sup>a</sup>	4.07 ± 0.9 <sup>b</sup>	4.38 ± 0.3 <sup>b</sup>	4.00 ± 0.6 <sup>b</sup>
Creatinine (ml min <sup>-1</sup> )	68.00 ± 3.8 <sup>a</sup>	54.68 ± 3.7 <sup>b</sup>	54.33 ± 5.8 <sup>b</sup>	47.00 ± 4.5 <sup>b</sup>

Values were expressed as mean ± SE; number of rats per group (n) = 6; statistical test = Student's *t* test; value along the row with different superscript letters as the control are statistically different from control at *p* < 0.05

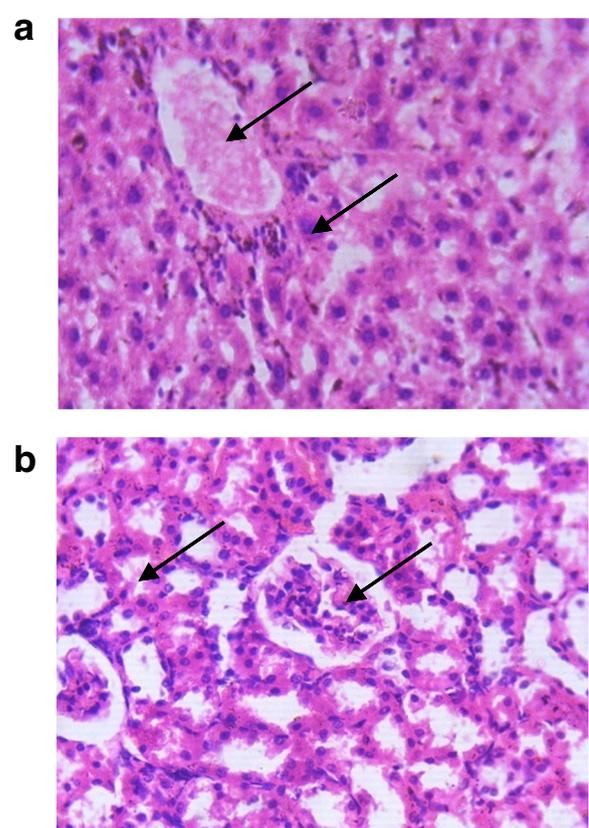
the bark of Yohimbe tree have been reported to alter liver and kidney function parameters of rats administered 14 mg kg<sup>-1</sup> dose for 15 days [28]. Tannins extracted from a plant had also been reported to induce changes in the liver enzymes of some rats administered 2.5 mg ml<sup>-1</sup> doses [29].

Additionally, the changes observed in the liver and kidney function parameters are indicative of liver toxicity or injury [30]. According to Herrine [31], some medicinal herbs contain substances that can damage

the liver. The liver is the primary target of toxins because it processes all substances ingested. The deteriorating state of the liver tissues of the treated rats is evident in their histological sections, which showed karyorrhexis, mild congestion, lymphocytic infiltration, fatty metamorphosis, inflammation, and pyknotic-looking hepatocyte nuclei. These effects were more pronounced in *C. occidentalis* and *Z. mauritiana* groups—the same pattern observed in the kidney and liver function tests.



**Fig. 3 a** Photomicrograph of the liver section of the rats dosed with *Z. mauritiana* showing karyolysis and mild congestion within the central vein (× 40). **b** Photomicrograph of the liver section of the rats dosed with *Z. mauritiana* showing karyolysis and lymphocytic infiltration (× 40)



**Fig. 4 a** Photomicrograph of the liver section of the rats dosed with *C. occidentalis* showing fatty metamorphosis (× 40). **b** Photomicrograph of the liver section of the rats dosed with *C. occidentalis* showing inflammation, lymphocytic infiltration, and pyknotic-looking hepatocyte nuclei (× 40)

Generally, many medicinal plants are safe, but eating a large amount of one type of green plant, over time, could lead to health issues. Phytochemical poisoning, such as alkaloid and tannin poisoning, can occur from consumption of any specific type of green vegetable over an extended period [32].

## 5 Conclusion

The findings of this study show the plants are rich in health-boosting bioactive substances that are pharmacologically safe in moderate doses. However, repeatedly consuming the plants for an extended period could be harmful to health. Some phytochemicals detected in the plants, particularly alkaloids and tannins, could bioaccumulate in the blood to toxic levels and cause health issues, including liver and kidney problems.

## Abbreviations

ALB: Albumin; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Cl<sup>-</sup>: Chloride ion; K<sup>+</sup>: Potassium ion; Na<sup>+</sup>: Sodium ion; TP: Total protein

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## Authors' contributions

TY conceptualized, designed, and drafted the manuscript; did the correspondence; and coordinated all the experiments. KS provided mentorship and carried out the qualitative and quantitative analysis of the extracts, while HN collected the plant materials, provided the rats, and conducted the kidney function test. EO carried out the histopathological studies and revised the manuscript, while US did the liver function test and participated in drafting the article. All authors read and approved the final manuscript.

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## Availability of data and materials

All data generated or analyzed during this study are included in this published article.

## Ethics approval and consent to participate

This study was conducted with the approval of the Animal Ethics Committee of Federal University Birnin Kebbi, Nigeria. The Guide of Care and Use of Animals in Research and Teaching as outlined by the committee was used to manage the animals.

## Consent for publication

Not applicable

## Competing interests

The authors declare that they have no competing interests.

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## References

- Silva O, Gomes ET (2003) Guieranone A, a naphthylbutenone from the leaves of *Guiera senegalensis* with antifungal activity. *J Nat Prod* 66:447–449. <https://doi.org/10.1021/np0204904>
- Silva O, Serrano R, Gomes ET (2008) Botanical characterization of *Guiera senegalensis* leaves. *Microsc Microanal* 14:398–404. <https://doi.org/10.1017/S1431927608080860>
- Oyebo O, Kandala N-B, Chilton PJ, Lilford RJ (2016) Use of traditional medicine in middle income countries: a WHO-SAGE study. *Health Policy Plan* 31:984–991. <https://doi.org/10.1093/heapol/czw022>
- Beyene B, Belachew B (2016) Habitamu D (2016). Review on application and management of medicinal plants for the livelihood of the local community. *J Resour Dev Manag* 22:33–39
- Houghton PJ (1995) The role of plants in traditional medicine and current therapy. *Journal of Alternative and Complementary Medicine* 1(2):131–143. <https://doi.org/10.1089/acm.1995.1.131>
- Mahomoodally MF (2013) Traditional medicines in africa: an appraisal of ten potent african medicinal plants. *Evid Based Complement Alternat Med*: e617459. <https://doi.org/10.1155/2013/617459>
- Fiot J, Sanon S, Azas N, Mahiou V, Jansen O, Angenot L, Balansard G, Ollivier E (2006) Phytochemical and pharmacological study of roots and leaves of *Guiera senegalensis* J.F. Gmel (Combretaceae). *Journal of ethnopharmacology* 106:173–178. <https://doi.org/10.1016/j.jep.2005.12.030>
- Shellar DB, Shirote PJ (2011) Natural product in drug discovery: back to future. *Biomed Pharmacol J* 4:141–146
- Kankara SS, Ibrahim MH, Mustafa M, Go R (2018) Ethnobotanical survey of medicinal plants used for traditional maternal healthcare in Katsina state, Nigeria. *South African J Bot* 97:165–175. <https://doi.org/10.1016/j.sajb.2015.01.007>
- Ene AC, Arawodi SE (2012) Ethnomedicinal survey of plants used by the Kanuris of Northeastern Nigeria. *Indian J Tradit Knowl* 4:640–645
- Nuhu AA, Aliyu R (2008) Effects of *Cassia occidentalis* aqueous leaf extract on biochemical markers of tissue damage in rats. *Trop J Pharm Res* 7(4): 1137–1142
- Sadiq IS, Shuaibu M, Bello AB, Tureta SG, Isah A, Izuagie T, Nasiru S, Kamaru MB (2012) Phytochemistry and antimicrobial activities of *Cassia occidentalis* used for herbal remedies. *J Chem Eng* 1(1):38–41
- Najafi S (2013) Phytochemical screening and antibacterial activity of leaf extract of *Ziziphys mauritiana* Lam. *Int Res J Appl Basic Sci* 4(10):3274–3276
- Dahiru D, Sini JM, John-Africa L (2006) Antidiarrhoeal activity of *Ziziphys maritiana* root extract in rodent. *Afr J Biotechnol* 5(10):941–945
- Silva MG, Aragão TP, Vasconcelos CF, Ferreira PA, Andrade BA, Costa IM, Costa-Silva JH, Wanderley AG, Lafayette SS (2011) Acute and subacute toxicity of *Cassia occidentalis* L. stem and leaf in Wistar rats. *J Ethnopharmacol* 136(2):341–346. <https://doi.org/10.1016/j.jep.2011.04.070>
- Vashishtha VM, Kumar AT, John J, Nayak NC (2007) *Cassia occidentalis* poisoning as the probable cause of hepatomyoencephalopathy in children in western Uttar Pradesh. *Indian J Med Res* 125:756–762
- Sofowora A (1993) Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp 191–289
- Ajuru MG, Williams LF, Ajuru G (2017) Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger Delta Region of Nigeria. *J Food Nutr Sci* 5(5):198–205. <https://doi.org/10.11648/jfjns.20170505.16>
- Trevarn JW (1927) The error of determination of toxicity. *Proc Royal Soc London B* 101(712):480–514. <https://doi.org/10.1098/rspb.1927.0030>
- Almeida RN (2006) Psicofarmacologia: fundamentos práticos. Guanabara Koogan, Rio de Janeiro, Brasil
- Hugo SM, Russel AO (1984) Antimicrobial activities of some African medicinal plant. *J Chem Soc Nigeria* 15:351–360
- Bergmeyer HU, Bernt E (1974). In: method of enzymatic analysis; Bergmeyer, H. U. 2nd ed., Academic press, New York, Pp 574-579.
- Parasuraman S, Raveendran R, Kesavan R (2010) Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother* 1:87–93. <https://doi.org/10.4103/0976-500X.72350>
- Tietz NW (1994) Textbook of clinical chemistry. 2<sup>nd</sup> ed. Saunders Company, Philadelphia: W.B, p 1919. [https://doi.org/10.1016/0307-4412\(86\)90182-2](https://doi.org/10.1016/0307-4412(86)90182-2)

25. Akhigbe RE, Ige SF, Afolabi AO, Oyeyipo PI, Ajao FO, Ajayi FA (2008) Water balance and serum levels of some electrolytes in oral contraceptive-treated female wistar rats. *J Med Sci* 8:591–594. <https://doi.org/10.3923/jms.2008.591.594>
26. Taylor DJ, Green NP, Stout GW (2003) Microscope techniques. Biological Science, 3rd edn. Cambridge University Press, U. K., pp 163–164
27. Lorke D (1983) A new approach to practical acute toxicity testing. *Arch Toxicol* 54:275–287. <https://doi.org/10.1007/BF01234480>
28. Yakubu MT, Bilbis LS, Lawal M, Akanji MA (2003) Evaluation of selected parameters of rat liver and kidney function following repeated administration of yohimbine. *Biochemistry* 15:50–56
29. Vejayan J, Jamunaa A, Halijah I (2016) Adverse effects of tannin contained in *Mimosa pudica* root extract. *J Appl Sci* 16:477–483. <https://doi.org/10.3923/jas.2016.477.483>
30. Reuben A (2004) Hy's law. *Hepatology* 39:574–578. <https://doi.org/10.1002/hep.20081>
31. Herrine SK (2018). Medicinal herbs and the liver. Available online at <https://www.merckmanuals.com/home/liver-and-gallbladder-disorders/drugs-and-the-liver/liver-injury-caused-by-drugs>. (Accessed 1 Nov 2018).
32. Sara O (2018). Alkaloid poisoning: symptoms & treatment. Available online at <https://study.com/academy/lesson/alkaloid-poisoning-symptoms-treatment.html> (Accessed 28 Dec 2018)

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