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Hematotoxicity and nephrotoxicity of long-term administration of *Guiera senegalensis* (J.F. Gme), *Cassia occidentalis* (Linn), and *Ziziphus mauritiana* (Lam) leaves obtained in Birnin Kebbi, Nigeria

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ABSTRACT

Introduction: Previously, we established the phytochemical composition and short-term administration safety of *Guiera senegalensis* (sabara), *Cassia occidentalis* (coffee senna), and *Ziziphus mauritiana* (jujube) leaves, which are common medicinal plants in Northern Nigeria. In the current study, heavy metal contents and long-term administration effects of the plants' leaf extracts on hematological parameters and the kidneys of albino rats (*Rattus norvegicus*) were investigated. The heavy metals analyzed were copper, lead, cadmium, nickel, and manganese, while the hematological parameters evaluated were packed cell volume, hemoglobin, red blood cells, white blood cells, lymphocytes, and monocytes.

Methods: Twenty-four mixed-sex rats were distributed into four groups of six rats each. Group 1 was made the control, while groups 2, 3, and 4 were administered 1000 mg kg^{-1} one of the plants extracts for 90 days. Blood and kidney samples were collected across the groups for hematological and histopathological examinations.

Results: The heavy metals were present in the extracts within the World Health Organization's acceptable limits. The treated rats were anemic compared to the control. However, on average, only the *C. occidentalis* group showed significant differences ($P<0.05$) in hematological parameters. Unlike the control, the kidneys of the rats fed with *Z. mauritiana* and *G. senegalensis* showed vacuolation of cytoplasm and tubular degeneration, while the *C. occidentalis*-fed rats had inflammation and dilated Bowman's capsules.

Conclusion: These findings reveal that constant administration of high doses of the extracts for a long time may cause health hazards. People are advised to seek an expert's advice before using the plants.

Implication for health policy/practice/research/medical education:
The results of this study showed that constant administration of *Guiera senegalensis, Cassia occidentalis,* and *Ziziphus mauritiana* extracts for a long time can cause health problems, providing guidance to professionals and consumers on how to use the plants. Researchers may find the information useful as well.

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Introduction

Plant parts have been used to treat ailments since the dawn of time (1,2). There is historical evidence of the use of plants in the treatment of various illnesses throughout all civilizations (3). Medicinal plants have a significant role in the delivery of healthcare, especially in developing countries (4). Of the 1328 new chemical substances approved as drugs between 1981 and 2016, 326 were of

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plants origin (5). Plant preparations are used for healthcare by at least four billion people, or 87.5% of the world's population, mainly in developing countries (6). In Nigeria, herbal remedies are used by almost 90% of the population (7). Plant medicines are becoming increasingly popular due to their perceived safety when compared to synthetic drugs. Approximately 8% of all hospital admissions in the United States are due to adverse reactions to synthetic drugs, with about 100 000 of these resulting in death each year (9,10). Plant remedies are also becoming more popular because they are less expensive, readily available, and simple to prepare and administer. Furthermore, some plant-based medicines have been used for millennia and have no known alternative in mainstream medicine (11). Plants have proven to be effective not only as treatments for a variety of diseases that affect humans and animals, but also as a source of bioactive molecules for drug development (12,13).

However, several experimental studies, as well as recorded incidences of toxicity after consuming some plant preparations, have made researchers to raise questions regarding the safety of plant medicines. Experts are concerned that plant medicines may not be as safe as previously believed (14). Recent improvements in scientific equipment have made it possible for the detection of the toxicity of some plants previously thought to be safe. Scientific advances have allowed for the detection of minute amounts of carcinogenic and toxic chemicals in plant preparations (15). Consequently, all medicinal plants and plant products must be evaluated for their safety before being approved for human consumption. As a result, the effects of methanol extracts of *Guiera senegalensis* (English and Hausa: Sabara), *Cassia occidentalis* (English: Coffee senna), and *Ziziphus mauritiana* (English: Jujube) leaves on hematological parameters and kidneys of albino rats (*Rattus norvegicus*) were investigated. These medicinal plants are commonly used to treat diseases in Northern Nigeria, Sub-Saharan Africa, and other parts of the world (16-18). There is, however, a scarcity of data on their potential toxicity. Blood and tissues are sensitive pathological indicators of a toxicant-exposed animal's overall health (19).

Materials and Methods

Experimental animals

The approval of the Animal Ethics Committee of Federal University Birnin Kebbi, Nigeria, was sought before commencing this study. The animals were managed according to the committee's guidelines for animal care and use in research and teaching. Twenty-four albino rats of both sexes, weighing between 204 and 211 g, were used for the study. The rats were kept in iron cages at room temperature (30 ± 3°C) in the animal house of the Department of Biological Sciences, Federal University, Birnin Kebbi. The rats were provided with adequate ventilation as well as free access to water and pellet feed from the Lagos-based Vital Feed Company.

Plant materials and extraction

Fresh leaves of *G. senegalensis*, *C. occidentalis*, and *Z. mauritiana* were obtained in Birnin Kebbi, Nigeria, in May 2021. A taxonomist from the Department of Plant Science and Biotechnology at Kebbi State University of Science and Technology, Aliero, identified the plants. The herbarium section of the department retained 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. The leaves were dried in the shade to a constant dry weight, after which it was crushed into a fine powder using a TENCAN laboratory grinder (Model Number XQM-20-100). For 24 hours, about 100 g, 50 g, and 100 g of powder from *Z. mauritiana*, *C. occidentalis*, and *G. senegalensis*, respectively, were soaked in flasks containing 600 mL, 300 mL, and 600 mL of 98% methanol. The methanol was evaporated using a rotary evaporator at 45°C after the extracts were filtered using a muslin cloth. Prior to use, the dried extracts were kept in a desiccator.

Heavy metal analysis

The heavy metal contents of the plant samples were determined following Yahaya et al (20). The samples were digested by placing 5 g of each sample in a beaker, and 40 mL of concentrated HNO_3 was added. The mixture was carefully heated until it was reduced to around 20 mL. More concentrated HNO_3 was added while the heating continued until a clear, light-colored solution was observed. The contents were filtered into a 100-mL volumetric flask, cooled, and then filled to the mark with distilled water. The UNICAM atomic absorption spectrophotometer was used to determine the levels of copper (Cu), lead (Pb), cadmium (Cd), nickel (Ni), and manganese (Mn) in the plant samples.

Phytochemical analysis

The phytochemicals in the plants' extracts were determined in our previous study (21).

Experimental design

The rats were divided into four groups, each with six rats. For 90 days, the rats in groups 1, 2, and 3 were given 1000 mg kg^{-1} extracts of *G. senegalensis*, *C. occidentalis*, and *Z. mauritiana*, respectively. A control group was set up in the same way, but these rats were not given any extract. Blood and kidney samples were obtained from each group at the end of the experiment for hematological and histological tests, respectively. The dose given (as described above) was based on the results of our prior studies of acute toxicity tests (21). Although the extracts had no observable effects at doses up to 5000 mg kg^{-1}, 1000 mg kg^{-1} appeared to be

safest and health-boosting level.

Blood sample collection and analysis

The procedures of Yahay et al (22) were followed in blood collection and preparation prior to analysis. The rats' tails were sterilized by swabbing them with a 70% ethanol solution, after which surgical blades were used to pierce the tips of their tails. The flow of blood was enhanced by gently drawing the tail from the body towards the tip. After that, a 5-mL syringe and a 20-gauge needle were used to draw around 2 mL of blood into bottles containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). The Sysmex XT1800i automated blood analyzer was used to determine the levels of selected hematological parameters (packed cell volume, hemoglobin, red blood cells, white blood cells, lymphocytes, and monocytes).

Histopathological examinations

The rats were sacrificed by cervical dislocation, and the kidneys were collected and processed for histopathological examination as conducted by (23). About 3-5 mm thick samples were cut from the kidney tissues and processed for histological demonstration. The tissues were fixed for 5–10 hours in 10% formal saline, after which they were immersed in various grades of alcohol (97%–100%) to eliminate water from the fixed tissue. After that, the tissues were immersed in molten wax and allowed to harden. Varying concentrations of xylene were used to remove any remaining alcohol and paraffin wax from the dehydrated tissues. The embedded tissues were mounted on a wooden block, and the sections and ribbons were floated out with a 20% alcohol solution. The sectioned tissues were floated out in warm water (around 35°C) to ensure proper tissue adhesion to microscopic slides. The prepared slides were stained using the hematoxylin and eosin techniques. The slides were placed under a light microscope for histological examination.

Quality control and assurance

All of the reagents used in this study were of high grade. Each reagent container was washed in a detergent solution and thoroughly rinsed with water and the reagent. To ensure the accuracy of the heavy metal analysis, the background contamination of the samples was examined. After five samples, the blank samples were tested, and all results were reproduced three times. The values were confirmed to be reproducible at a 95% confidence level. As a result, the mean value of each heavy metal was employed for further analysis. The precision and accuracy of the analyzed heavy metals were checked against standard reference materials for every heavy metal.

Data management and analysis

The data on the hematological parameters were expressed as Mean ± Standard deviation (SD). The statistical difference between the control and test groups was analyzed using the student's *t* test. The value of $P \leq 0.05$ was considered significant.

Results

Concentrations of heavy metals in the medicinal plants

Table 1 shows the concentrations of Cd, Mn, Pb, Cu, and Ni in the samples of *Z. mauritiana, G. senegalensis,* and *C. occidentalis* obtained in Birnin Kebbi, Kebbi State. All the heavy metals were present in the plants within the World Health Organization's permissible limits. On average, Mn had the highest concentrations, followed by Ni, Cu, Cd, and Pb, in that order.

Effects of the extracts on the hematological parameters of the rats

The effects of the plants' extracts on the rats' hematological parameters are presented in Table 2. When compared to the control, the test groups had a mild reduction in packed cell volume (PCV), hemoglobin (Hb), red blood cells (RBCs), lymphocytes (LMC), and monocytes (MNC), while white blood cells (WBCs) increased. These alterations were significant ($P \leq 0.05$) in the rats fed with *C. occidentalis* and *G. senegalensis* (LMC only).

Histopathological effects of the extracts

Figures 1-4 show the histology of the kidney tissues of the rats dosed with the plants' extracts. The control rats showed normal kidney tissues (Figure 1), while tubular degeneration and vacuolation of cytoplasm were observed in the kidneys of rats administered *Z. mauritiana* (Figure 2). The kidneys of the *G. senegalensis* group revealed interstitial congestion and tubular degeneration of the proximal convoluted tubule (Figure 3). Figure 4 shows that the rats treated with *C. occidentalis* had interstitial

Table 1. Levels of heavy metals in *Ziziphus mauritiana, Guiera senegalensis,* and *Casia occidentalis* obtained in Birnin Kebbi, Kebbi State, Nigeria

Plant	Cd	Mn	Ni	Cu	Pb
Z. mauritiana	0.009 ± 0.001	1.0 ± 0.07	0.58 ± 0.09	0.52 ± 0.04	BDL
G. senegalensis	0.007 ± 0.001	1.6 ± 0.05	0.47 ± 0.02	0.57 ± 0.03	BDL
C. occidentalis	0.008 ± 0.001	1.0 ± 0.03	0.71 ± 0.08	0.51 ± 0.02	BDL
WHO Limits (24)	0.02	2.0	5.0	10.0	0.3

WHO, World health organization; BDL, Below detection level.
Values are expressed as mean ± SD and mg/kg.

Table 2. Hematological parameters of the rats treated with methanol extracts of *Ziziphus mauritiana, Guiera senegalensis, and Cassia occidentalis* obtained in Birnin Kebbi, Kebbi State, Nigeria

Parameter	Control	*Z. mauritiana*	*G. senegalensis*	*C. occidentalis*
PCV (L L^{-1})	0.40 ± 0.12[a]	0.39 ± 0.12[a]	0.37 ± 0.11[a]	0.36 ± 0.13[b]
Hb (g dL^{-1})	15.20 ± 1.20[a]	14.70 ± 1.01[a]	14.6 ± 1.61[a]	13.01 ± 0.90[b]
RBC (mc mm^{-3})	4.20 ± 0.11[a]	4.11 ± 0.30[a]	3.91 ± 0.21[a]	3.80 ± 0.40[b]
WBC (c mm^{-3})	4, 500 ±100[a]	4, 631 ± 120[a]	4,900 ± 101[b]	4950 ± 150[b]
LMC (c μL^{-1})	2, 510 ± 50[a]	2, 460 ± 70[a]	2, 235± 60[b]	2, 229 ± 55[b]
MNC (c μL^{-1})	301 ± 20[a]	295 ± 22[a]	296 ± 15[a]	294.20[a]

PCV: Packed cell volume; Hb: Hemoglobin; RBC Red blood cells; WBC: White blood cells; LMC: Lymphocytes; MNC: Monocytes.
Values are expressed as mean ± SD; values along the row with different superscripts from the control are statistically different from the control at $P \leq 0.05$.

congestion with interstitial inflammatory cells and destruction of the epithelial lining of the tubules.

Discussion

The safety status of *Z. mauritiana, G. senegalensis,* and *C. occidentalis* was determined in this study. The plants contained permissible levels of the evaluated heavy metals (Table 1), suggesting that the plants may not induce heavy metal-related toxicities in the treated rats. This result is consistent with that of Mohammed et al (25), who reported permissible levels of evaluated heavy metals in *Moringa oleifera* obtained in the Northeast of Nigeria. The result is also in line with the report of Baba and Mohammed (26), who detected permissible levels of Zn, Cu, K, Mn, Fe, and Ni in selected medicinal plants in Kano, Northwestern Nigeria. However, the result is inconsistent with that of Zauro et al (27), who reported non-permissible levels of some heavy metals in *C. occidentalis* obtained from some villages in Kebbi State, Northwestern Nigeria. Uba and Baburo (28) also reported high concentrations of manganese in *Cassia singueana* and *Combretum micranthum* leaves obtained in Sokoto, Northwestern Nigeria. Anthropogenic activities and soil geology vary widely throughout the world, and so do the chemicals accumulated in the soils and plants. This could explain the inconsistencies in the results of various studies cited. However, the plants' extracts altered the hematological parameters of the rats, although the alteration was insignificant except in the rats fed with *C. occidentalis* (Table 2). This finding is consistent with our earlier study (21), in which short-term administration of the plant extracts produced nontoxic effects, but prolonged use induced toxic effects. This could be attributed to bioaccumulation of certain substances in the extracts to toxic levels in the blood of the rats. Our previous study showed that the plants contained alkaloids, saponins, flavonoids, and tannins, among other phytochemicals, indicating that the plants may have hemopoietic stimulating properties (29). However, these phytochemicals can be injurious to the body when constantly taken for a long time. Constantly

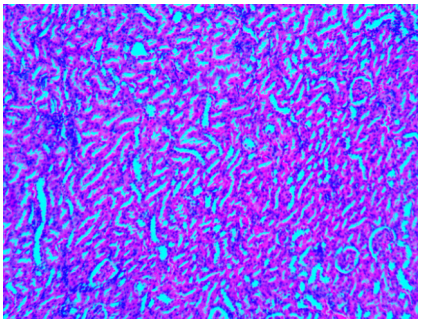

Figure 1. Photomicrograph of a kidney section of the control rats showing normal renal glomerulus, proximal convoluted tubule and distal convoluted tubule (x40).

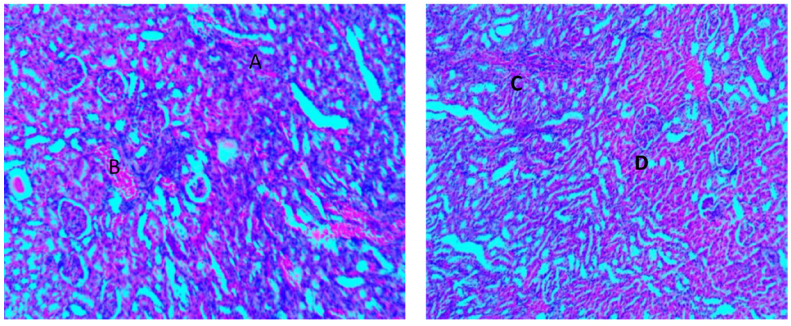

Figure 2. Photomicrographs of the kidney sections of the rats dosed with *Ziziphus mauritiana* (x40) showing: A = vacuolation of cytoplasm of the epithelial lining; B = tubular degeneration of the proximal convoluted tubule; C = mild tubular degeneration; and D = vacuolation of cytoplasm of the epithelial lining.

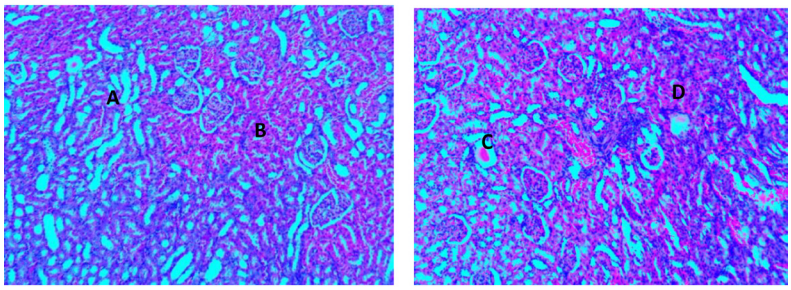

Figure 3. Photomicrographs of the kidney sections of the rats dosed with *Guiera senegalensis* (x40) showing: A = interstitial congestion; B = tubular degeneration of the proximal convoluted tubule; C = vacuolation of cytoplasm of the epithelial lining; and D = mild tubular degeneration.

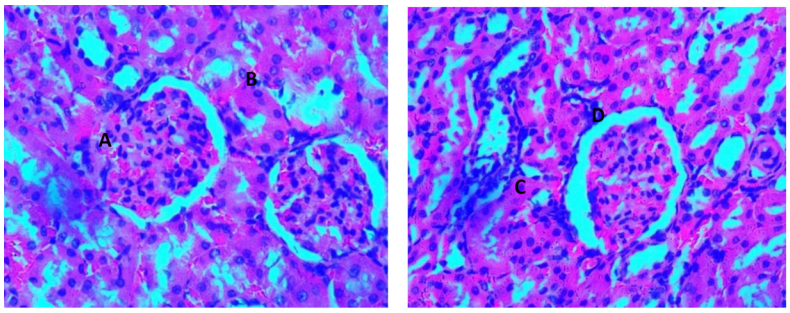

Figure 4. Photomicrographs of the kidney sections of the rats dosed with *Cassia occidentalis* (x40) showing: A = interstitial congestion with interstitial inflammatory cells; B = destruction of epithelial lining of the tubules; C = slightly dilated Bowman's capsule (spaces in between); and D = interstitial congestion with inflammatory cells.

consuming high doses of tannic acid and saponins for a long time can cause eryptosis of erythrocytes (apoptosis of the erythrocytes), which is characterized by cell shrinkage, iron deficiency, abnormal blood count, and renal insufficiency (30-32). Repeatedly eating a single green plant alone for an extended period may be injurious to the body. Some phytochemicals, such as alkaloids and tannins, may accumulate gradually to toxic levels in the blood after an extended period and cause health issues (33). The reduction in the blood parameters showed that certain substances in the plants induced oxidative stress (34). The increased WBC of the treated rats is an indicator of a self-defense mechanism against exposure to xenobiotics. The results of the current study are consistent with those of Chhapola et al (35), who observed mild anemia, leukopenia, and thrombocytopenia in three children in India who were hospitalized due to *C. occidentalis* poisoning. Extracts of *C. occidentalis* were also demonstrated to produce significant decreases in PCV, HB, and RBC (36). Alawode et al (37) showed that *Z. mauritiana* decreases platelet counts in test rats compared with the controls. In mice fed with *G. senegalensis*, hematological and serological alterations were evident (38).

Furthermore, the extracts of the plants induced histological damage in the kidney tissues of the rats (Figure 2). This result confirmed our earlier study in which long-term administration of the plants' extracts induced liver damage. As suggested earlier, the histopathological effects observed in the current study could be due to the accumulation of phytochemicals in the rats' blood at toxic levels. Chronic exposure (even at low levels) to alkaloids (particularly pyrrolizidine) can induce histopathological effects and cancer through oxidative DNA damage (39,40). Tannins have been implicated in liver and kidney damage (41,42). Prolonged exposure to high saponin levels can cause small intestine, liver, and kidney damage via necrosis (43). The results of the current study are consistent with those of Muyibi et al (36), who observed dose-dependent lesions, including fatty changes and acute tubular necrosis, in the livers and kidneys of rats administered with *C. occidentalis*. The results are also in line with Silva et al (44), who found biochemical, hematological, and morphological parameters altered in rats fed with 2.5 g/kg/day extract of *C. occidentalis* for 30 days. Similarly, daily treatment of rats with *G. senegalensis* has been shown to cause endotheliotoxicity, hepatonephropathy, and pancreatic hyperplasia (38). Usman and Aminatu (45) also reported histopathological changes in the livers, kidneys, spleens, and lungs of rats treated with acute and sub-acute doses of *G. senegalensis* extracts. However, the results contradict Tanimu and Wudil (46), who found no histological changes in rats dosed with 300–900 mg/kg extracts of *C. occidentalis* for 2 weeks. The results also contradict Dahiru et al (47), who reported non-toxicity in rats fed with ≤400 mg/kg of extract of *G senegalensis* for

8 days. Furthermore, in mice fed 400 mg/kg for 42 days, an aqueous extract of *Z. mauritiana* leaf induced no toxic effects and even improved fatty liver and atherosclerosis (48). These inconsistencies could be due to varied doses administered in the studies as well as varied lengths of administration. On average, studies in which lower doses and shorter durations were used produced mild or nontoxic effects, while studies with higher doses and longer durations, like the current study, produced toxic effects.

Conclusion

Considering the results of this study, it can be concluded that constant administering high doses of *G. senegalensis, Z. mauritiana*, and *C. occidentalis* for a long time may elicit health problems. Repeated consumption of high doses of the extracts can cause anemia as well as inflammation, congestion, and tubular damage in the kidneys, with *C. occidentalis* producing more effects. People are advised to seek an expert's advice before using the plants. We recommend further studies to verify these claims.

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Authors' contributions

YTO and EOO conceptualized and drafted the manuscript, while BMDA proofread it. KN collected the data, whereas AYB did the data analysis. IA and BJD performed the experiments.

Conflict of interests

Authors declare no conflict of interests.

Ethical considerations

This study was approved by the Animal Ethics Committee of the Federal University Birnin Kebbi, Nigeria. Text plagiarism, data fabrication, and redundant publication have been carefully observed by the authors.

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