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Toxicological Evaluation of the Leaves of *Mangifera indica* L. (Mango) on Albino Rats (*Rattus norvegicus*)

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ABSTRACT: The increasing use of plant-based medicines necessitates safety evaluations of all medicinal plants. This study evaluated the effects of mango (*Mangifera indica*) leaf extracts on albino rats (*Rattus norvegicus*). The phytochemistry of the plant's extract was evaluated, followed by a determination of its acute toxicity using 24 rats. Thereafter, the chronic toxicity of the extract was determined using another set of 24 rats, separated into four groups of six rats each. Rats in group 1 (the control) were administered distilled water, while groups 2, 3, and 4 received daily doses of 1000, 2000, and 3000 mg kg^{-1} body weight, respectively. The rats' body weights and reactions were monitored for 90 days before blood, liver, and kidney samples were collected for hematological and histopathological examinations. The phytochemistry revealed phenols, alkaloids, tannins, flavonoids, steroids, glycosides, and saponins. The acute toxicity test recorded no mortality at doses up to 5000 mg kg^{-1}, and all the rats behaved normally, except for one that was sluggish. The chronic toxicity test revealed no significant ($p>0.05$) weight difference between the control and treated rats. The packed cell volume, hemoglobin, and red and white blood cells of the rats fed 2000 and 3000 mg were significantly altered ($p<0.05$), while lymphocytes exhibited no significant alterations in any of the groups. The treated rats' livers revealed dose-dependent necrosis, whereas their kidneys showed atrophy and epithelial cell degeneration. The results obtained suggest that a single dose of the plant's extract is not harmful, but repeated high-concentration dosing for a long time may result in toxicity.

Keywords: Acute toxicity; Livers; Medicinal plants; Necrosis; Red blood cells.

تقييم السموم في أوراق مانغيفيرا إنديكال مانجو (على جرذان ألبينو) راتوس نورفيغيكوس

تاج الدين أولانريواجو يحيى ، تيتيلا فوزية ساليسو ،إستر أو. أولادي، كليمنت يارو ، نورا إليازو، عبدالرزاق إيزوافا، محمد أ. ياري

الملخص: يستلزم الاستخدام المتزايد للأدوية النباتية إجراء تقييمات للأمان في جميع النباتات الطبية .قيمت هذه الدراسة آثار مستخلصات أوراق المانجو) مانجيفيرا إنديكا (على فئران البرص) راتوس نورفيجيكوس .(وقد تم تقييم الكيمياء النباتية لاستخراج النبات، وتلت ذلك سمية حادة باستخدام 24 فأرا. وبعد ذلك ، تم تحديد السمية المزمنة للمستخلص باستخدام مجموعة أخرى من 24 فأرا، مقسمة إلى أربع مجموعات، كل منها مع ستة فئران .تم إعطاء الجرذان في المجموعة 1) المكافحة (الماء المقطر، بينما تلقت المجموعات 2 و 3 و 4 جرعات يومية من 1000 و 2000 و 3000 ملغم 1 - كغم من وزن الجسم، على التوالي .تم مراقبة أوزان جسم الجرذان وتفاعلاتها لمدة 90 يوما قبل جمع عينات من الدم والكبد والكلى لإجراء فحوصات الدم وعلم أمراض النسج .كشفت الكيمياء النباتية عن الفينولات والقلويات والتانينات والفلافونويدات والستيرويدات والجليكوزيدات والصابونات .ولم يسجل إختبار السمية الحادة أي وفاة بجرعات تصل إلى 5000 ملغم من الكغم1-، وتتصرف جميع الجرذان بشكل طبيعي، باستثناء واحدة كانت بطيئة .لم يكشف إختبار السمية المزمنة عن فرق كبير ($p>0.05$) في الوزن بين عنصر التحكم والجرذان المعالجة .فقد تم تغيير حجم الخلايا المعبأة، والهيموغلوبين، وخلايا الدم الحمراء والبيضاء للجرذان بشكل كبير($p<0.05$) ، بينما لم تظهر الخلايا اللمفاوية في جميع المجموعات أي تغييرات كبيرة .كشفت أكباد الجرذان المعالجة عن نخر يعتمد على الجرعة، في حين أظهرت الكلى ضمورا وتنكس الخلايا الظهارية .تشير النتائج التي تم الحصول عليها إلى أن جرعة واحدة من مستخلص النبات ليست ضارة، ولكن تكرار الجرعات عالية التركيز لفترة طويلة قد يؤدي إلى السمية.

الكلمات المفتاحية: السمية الحادة، الكبد، النباتات الطبية، النخر، خلايا الدم الحمراء.

TOXICOLOGICAL EVALUATION OF THE LEAVES OF *MANGIFERA INDICA* L. (MANGO)

1. Introduction

There has been a global renaissance in plant-based medicines since the middle of the 19th century because they are affordable, potent, and simple to prepare. Worldwide, approximately 21,000 medicinal plants have been documented for disease treatment [1]. The renaissance is partly because plants contain many bioactive compounds used to produce pharmaceutical drugs [2]. At the minimum, 30% of pharmaceutical drugs are derived directly or indirectly from medicinal plants [3]. Plant-based medicines are used to treat ailments by at least 80% of the world's population [4].

Mango (*Mangifera indica* L.), which belongs to the family Anacardiaceae, is a cosmopolitan tree, whose parts are often used to make plant-based medicines [5]. Extracts of *M. indica* are used to treat diabetes, bronchitis, diarrhea, asthma, kidney diseases, scabies, respiratory problems, syphilis, and urinary disorders [6-8]. The plant is antimicrobial, antioxidant, anti-diabetic, and immunomodulatory, and it combats tumors [4]. *M. indica* contains abundant minerals, such as nitrogen and potassium, and also contains protein [9, 4]. The plant is also rich in phytochemicals, including mangiferin, phenolic acids, benzophenones, flavonoids, carotenoids, quercetin, isoquercetin, ascorbic acid, and tocopherols [10-12]. The oil from *M. indica* contains monoterpenes, sesquiterpenes, non-terpenoid hydrocarbons, oxygenated hydrocarbons, α-gurjunene, *trans*-caryophyllene, α-humulene, α-selinene, α-glucosidase, and camphor [10, 13].

In recent times, however, there has been a growing concern about the safety of plant-based medicines. Studies have shown that some plant-based medicines are toxic due to their constituents, while others induce toxicity due to interactions and contamination during production [2]. The toxic effects of plant-based medicines on humans depend on the chemical composition of the medicine and the extraction methods used, as well as on the state of the consumer's cell membrane, cell surface, tissues, and extracellular matrix [10, 12]. Thus, there is a need for toxicological studies of all plants used in making plant medicines [8]. Although there are several studies on the health-promoting efficacy of *M. indica*, there is a dearth of studies on the toxicity of the plant [10, 13]. Consequently, this study evaluated the toxicity of *M. indica* leaf extracts in albino rats (*Rattus norvegicus*).

2. Materials and Methods

2.1 Management of experimental animals

Twenty-four (24) male and female albino rats (*Rattus norvegicus*) with a mean weight of185±10 g and aged 50 days were used for this study. The rats were housed in well-ventilated metallic cages (six rats per cage) in a well-ventilated animal house at a room temperature of about 32 °C. Before beginning the experiment, the rats were given 14 days to acclimate to their new environment. Water and pellet feeds from Premier Feed Mills in Ibadan, Nigeria, were freely available to the rats.

2.2 Collection of plant materials and identification

Fresh leaves of *M. indica* were plucked within the Kebbi metropolis in February 2021. The leaves were identified by a botanist in the Department of Biological Sciences, Federal University Birnin Kebbi, Kebbi State, Nigeria. A sample of the authenticated materials was deposited in the department's herbarium with voucher number FUBK/a/6.

2.3 Preparation and extraction

The plant's leaves were gently washed with distilled water, dried in the shade, pulverized into powder, and sieved through a stainless-steel mesh with a diameter of 200 mm to ensure size consistency. To extract the bioactive components, 150 g of the powdered material was soaked in 900 ml of 98% methanol for 48 hours. The extract obtained was filtered using a muslin cloth, and the methanol was evaporated using a rotary evaporator at 45 °C until a constant dry weight of the extract was achieved. The dried extract was kept in a desiccator.

2.4 Qualitative screening of the extracts

The qualitative screening was conducted using conventional procedures as detailed by Yahaya *et al*. [2].

Test for flavonoids

A small quantity of 10% ferric chloride solution was mixed with 0.5 g of the extract. The presence of flavonoids was indicated by the appearance of a green or blue color.

Test for tannins

Five milliliters (5 ml) of water were added to 0.5 g of the extract, followed by a few drops of 10% ferric chloride. The presence of tannins was indicated by a blue-black, green, or blue-green precipitate.

Test for alkaloids

For 2 minutes, 0.2 g of the extract was boiled with 2% H_2SO_4. A few drops of Dragendorff's reagent were added after the liquid was filtered. The presence of alkaloids was revealed by an orange-red precipitate.

Test for steroids

One milliliter (1 ml) of the extract and 5 ml of anhydrous acetic acid were mixed thoroughly. Four (4) drops of the above mixture were placed in a porcelain dish, and one drop of concentrated H_2SO_4 was added. A change in color from rose, through red, violet, and blue, to green showed the extract contained steroids.

Test for glycosides

One milliliter (1 ml) of the extract was mixed with 1 ml of glacial acetic acid containing one drop of ferric chloride solution. Exactly 1 ml of concentrated H_2SO_4 was added to the liquid, which resulted in the development of two layers. The presence of glycosides was shown by the development of a brown ring at the interface of the two layers.

Test for saponins

In a graduated cylinder, 1 ml of the extract was diluted to 20 ml with distilled water, and was shaken for 15 seconds. The presence of saponins was shown by the formation of a foam layer after 15 minutes.

Test for phenols

Exactly 0.5 g of the extract was dissolved in 2 ml of distilled water, and a small quantity of ferric chloride was added. The presence of phenols was determined by the appearance of a red, purple, or green color.

2.5 Acute toxicity test

The acute toxicity of *M. indica* extracts was determined using the "classical LD50" approach as outlined by Trevan [15]. Twenty-four (24) rats (males and females) were randomly divided into four groups, each of six rats. Group 1 was the control, while groups 2, 3, and 4 were orally administered 1000, 3000, and 5000 mg of *M. indica* leaf extract per kg of body weight of the rats, respectively. The rats were monitored for 72 hours to establish the general toxicity of the extracts based on observation of behavioral change.

2.6 Chronic toxicity test

A new batch of 24 rats was separated into four groups, each containing six rats. *M. indica* leaf extracts were administered to groups 2, 3, and 4 on a daily basis in doses of 1000, 2000, and 3000 mg kg^{-1} body weight of the rats, respectively. Group 1 was designated as the control group, with rats receiving only standard feed and water. The rats' weights were tracked and general observations recorded for 90 days before the animals were sacrificed via cervical dislocation. Hematological tests were performed on blood samples, and histological examinations were performed on the liver and kidneys.

2.7 Body weight measurement

The rats' body weights were measured at the start of the experiment using a digital weighing scale manufactured by KERN, Germany. Throughout the rest of the experiment, the measurements were taken every other day in the morning.

2.8 Hematological examination

Each rat was held firmly to a workbench using office pins. About 2.5 ml of blood was drawn from the rats' tails into ethylenediamine tetra acetic acid (EDTA) bottles using a 5 ml syringe and a 20-gauge needle. The Sysmex auto-analyzer was used to assess the amounts of blood parameters, including packed cell volume (PCV), hemoglobin (Hb), white blood cells (WBC), red blood cells (RBC), and lymphocytes (LYM).

2.9 Histopathological analysis

The rats' liver and kidney tissues were processed for histological analysis, as described by Yahaya *et al*. [16]. The rats' chests were cut open in a dorsal-ventral orientation with a surgical blade. The livers and kidneys were collected, and about 5 mm of each tissue was stored in 10% neutral buffered formalin. The tissues were dehydrated in increasing volumes of alcohol before being embedded in paraffin wax (65, 80, and 100%). With a rotary microtome (model YR421), the embedded tissues were sectioned at 5 μm, placed on glass slides, and air-dried. Hematoxylin and eosin dyes were used to stain the slides, which were then examined under a light microscope for histological abnormalities.

2.10 Data analysis

All of the analyses were performed using the Statistical Package for Social Sciences (SPSS) version 20 for Windows. The analysis of variance (ANOVA) was used to compare data between the test and control groups. The statistical significance level was set at $p<0.05$.

3. Results

3.1 Phytochemicals in the extract

Table 1 below shows the phytochemicals detected in the crude extracts of *M. indica* leaves. Phenols and flavonoids were abundant, while glycosides, alkaloids, tannins, saponins, and steroids were present in moderate amounts.

Table 1. Phytochemicals detected in the crude extracts of *M. indica* leaves.

Alkaloids	Inference
Tannins	+
Saponins	+
Phenols	++
Steroids	+
Flavonoids	++
Glycoside	+

++ indicates abundantly available ; + indicates moderately available

3.2 Acute toxicity of the extracts

Table 2 shows the reactions of the rats treated with different doses of *M. indica* leaf extracts. No deaths were recorded, and all the rats behaved normally, except for one in the group fed 5000 mg kg^{-1}, which was sluggish.

Table 2. Reactions of the treated rats (n = 6 per group) to different doses of *M. indica* leaf extracts.

Group	Dosage (mg kg^{-1} body weight)	Observation period (hour)	Behavioral change	Mortality
1	Distilled water	72	None	0
2	1000	72	None	0
3	3000	72	None	0
4	5000	72	Sluggishness (n=1)	0

3.3 Effects of the extracts on body weight

The body weights of the rats treated with *M. indica* leaf extracts are shown in Table 3. Compared with the control, no significant differences ($p>0.05$) were observed.

Table 3. Change in body weights of the treated rats (n = 6 per group) to different doses of *M. indica* leaf extracts.

Dosage (mg kg^{-1} body weight)	Initial body weight (g)	Final body weight (g)
Control (Distilled water)	197.33 ± 5.32^a	210.34 ± 6.31^a
1000 ml	200.01 ± 2.31^a	205.39 ± 6.09^a
2000 ml	198.35 ± 1.31^a	212.00 ± 3.22^a
3000 ml	201.33 ± 3.34^a	206.15 ± 3.54^a

Values were expressed as mean ± SD (n = 6); values with the same subscript "a" are not significantly different from control at $p> 0.05$ (ANOVA).

3.4 Effects of the extracts on hematological parameters

Table 4 compares the hematological parameters of rats fed *M. indica* leaf extracts to those of the control rats. The packed cell volume (PCV), hemoglobin (Hb), and red blood cells (RBC) of the treated rats were reduced, and this reduction was significant ($p<0.05$) in the rats fed 2000 and 3000 mg of the extracts. The white blood cells (WBC) of the treated rats were increased, but significantly only in the group fed 3000 mg. There was no noticeable change in the lymphocytes (LYM) of the treated rats.

Table 4. Hematological parameters of rats treated with *M. indica* leaf extracts.

Parameters	Control	1000 mg	2000 mg	3000 mg
PCV (L L^{-1})	28.18±2.83[a]	26.63±3.49[a]	25.90±2.50[b]	22.67±2.7[b]
Hb (g dL^{-1})	11.63±1.83[a]	11.00±0.89[a]	0.9.37±0.58[b]	6.82±0.22[b]
WBC (mc mm^{-3})	1.28±0.87[a]	1.31±0.55[a]	1.59±0.40[a]	2.99±1.6[b]
RBC (mc mm^{-3})	5.06±0.78[a]	4.94±0.60[a]	3.91±0.18[b]	3.64±0.67[b]
LYM (c μL^{-1})	82.83±6.49[a]	82.17±1.73[a]	82.27±0.99[a]	82.63±0.95[a]

Values were expressed as mean ± SD (n = 6); mean values with different subscripts "a" and "b" along the same row are statistically different from control at $p < 0.05$ (ANOVA). PVC = packed cell volume; Hb = hemoglobin; WBC = white blood cells; LYM = lymphocytes.

3.5 Histopathological effects of the extracts

Figures 1a–d show the liver tissues of the control and treated rats. Normal hepatocytes were observed in the liver of the control rats, while dose-dependent necrosis was observed in the treated rats. The kidney tissues of the control and treated rats are shown in Figures 2a–2d. Normal hepatocytes were observed in the control group, while the rats fed 1000, 2000, and 3000 mg kg^{-1} of the extracts revealed mild atrophic glomerulus, tubular atrophy, and epithelial cell degeneration.

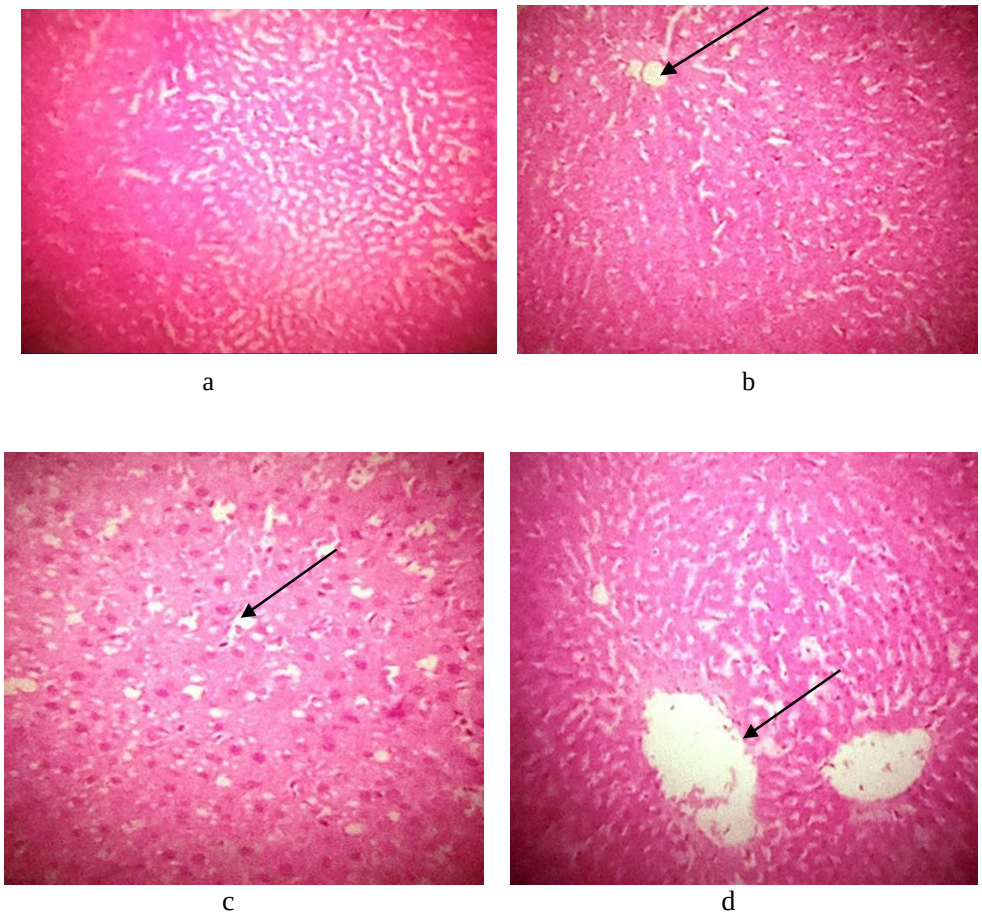

Figure 1. Photomicrography of the liver tissues of the control and treated rats.
a = control rats showing normal hepatocytes.
b = rats treated with 1000 mg kg^{-1} *M. indica* extract showing mild necrosis.
c = rats treated with 2000 mg kg^{-1} *M. indica* extract showing moderate necrosis
d = rats treated with 3000 mg kg^{-1} *M. indica* extract showing moderate necrosis.

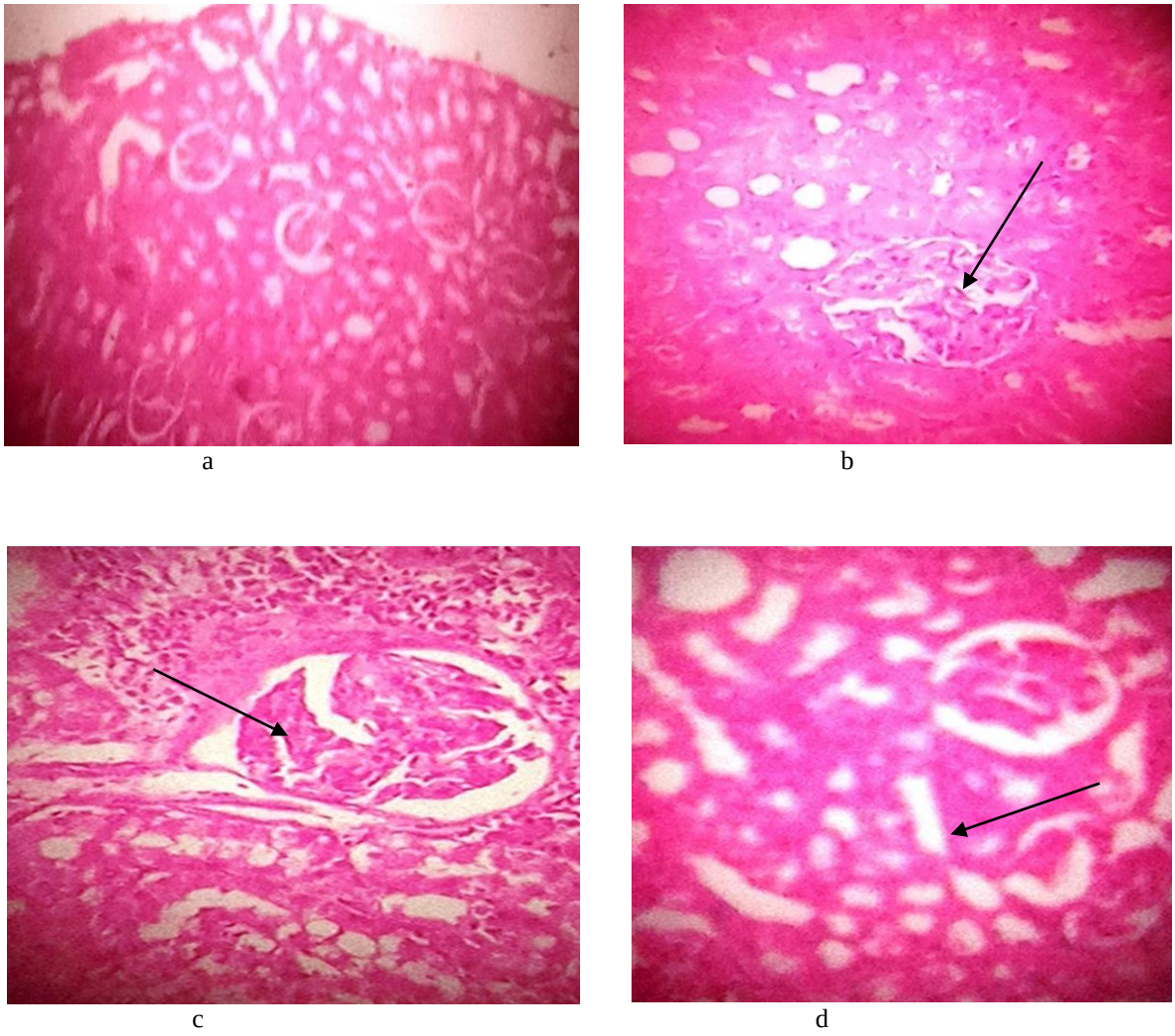

Figure 2. Photomicrography of the kidney tissues of the control and treated rats.
a = control rats showing normal glomerulus
b = rats treated with 1000 mg kg^{-1} *M. indica* extract showing mild atrophic glomerulus
c = rats treated with 2000 mg kg^{-1} *M. indica* extract showing tubular atrophy
d = rats treated with 3000 mg kg^{-1} *M. indica* extract showing epithelia cell degeneration (outerlayer)

4. Discussion

The toxicity of *Mangifera indica* leaves was evaluated in this study. The study was conceived to determine the safe doses of the plant extract to prevent or minimize its health hazards among users worldwide, particularly in Nigeria. Table 1 shows that the plant contains bioactive substances, including phenolic acids, alkaloids, tannins, flavonoids, steroids, glycosides, and saponins. These substances are well known for their health-boosting activities, but some of them could induce toxicity at certain doses. In the current study, the acute toxicity test of the plant's extract revealed no mortality nor any abnormality at doses up to 5000 mg kg^{-1} (Table 2), which is the upper limit at which a single dose of an extract is considered non-toxic [2, 17]. Table 3 further demonstrates the plant's non-toxicity, as there was no significant difference in body weight between the treated and control rats. These results are consistent with those of Zhang *et al*. [18], Ahomadegbe *et al*. [19], EASL [20], and Reddeman *et al*. [21], all of whom reported the non-toxicity of the plant.

Table 4, however, shows that long-term dosing (90 days) at 2000 and 3000 mg kg^{-1} body weight significantly altered the hematological parameters of the treated rats. The packed cell volume (PCV), hemoglobin (Hb), and red blood cells (RBC) were reduced, while the white blood cells(WBC) were increased. This result contradicts that of Ogbe *et al*. [22] and Abidakun *et al*. [23], who observed an increase in PCV, Hb, and RBC in experimental animals treated with mango extract. The inconsistency of the current study with the previous ones might be due to the longer duration and higher concentrations used in this study. In contrast to the current study's 90-day treatment with 1000, 2000, and 3000 mg kg^{-1} body weight, Ogbe *et al*. [22] and Abidakun *et al*. [23] gave experimental animals 20 mg for 14 days and 1 mg for 40 days, respectively. The plant contains alkaloids and saponins, which, at high concentrations and duration, can accumulate to toxic levels and

destroy blood cells [24, 25]. The plant also contains tannins, which have been implicated in iron deficiency, resulting in anemia [26].

Figures 1a-d and 2a-d further show that *M. indica* extracts can be toxic at high concentrations and over long periods of time. The treated rats' livers developed dose-dependent necrosis, while their kidneys developed atrophic glomerulus, tubular atrophy, and epithelial cell degeneration. Atiba *et al*. [27] reported abnormal liver morphology, such as an enlarged central vein and reduced sinusoids, in rats treated with *M. indica* leaf extracts for a long time. Zhang *et al*. [18] also reported enlargement of liver and kidney tissues in rats dosed with *M. indica* for 90 days. Amien *et al*. [28] and Nadella and Kumar [29], on the other hand, found that *M. indica* has chemo-protective effects on the kidneys and livers. However, the studies that reported chemoprotective effects used lower concentrations and shorter durations. For instance, Amien *et al*. [28] dosed experimental animals with 300 mg kg^{-1} for 14 days, while Nadella and Kumar [29] dosed them with 150 mg kg^{-1} for 10 days. The reported histopathological effects could be due to potentially toxic phytochemicals in the extracts, primarily alkaloids, saponins, and tannins. High doses and prolonged use of an alkaloid-containing substance can result in hepatocyte necrosis and renal failure [30, 31]. Tannins have also been linked to liver necrosis and kidney problems [32]. Additionally, liver and kidney damage due to necrosis of liver cells and renal tubules has been reported among mice treated with high concentrations of saponins [33, 34]. Toxicants primarily target the kidneys and liver because these organs process all foreign substances in the body [2, 35].

5. Conclusion

The results showed that *M. indica* leaves contain pharmacologically important phytochemicals such as mangiferin, phenolic acids, flavonoids, carotenoids, alkaloids, tannins, quercetin, isoquercetin, ascorbic acid, and tocopherols. However, some of these compounds, especially tannins, saponins, and alkaloids, could be toxic at certain doses. A single dose of the plant's extract is not expected to be toxic, as it is non-toxic at doses up to 5000 mg kg^{-1}, which is the maximum dose at which an extract can be considered non-toxic. However, repeated high-concentration and long-duration dosing may induce toxicity, as evidenced by the alteration of the hematological parameters and tissues of the treated rats.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

Not applicable

Ethical statement

This study was conducted in accordance with the ethical standards of the European and German Animal Welfare legislation, declaration principles set out by Helsinki and the National Institutes of Health guidelines for care and use of animals in research. All protocols were approved by the local ethics committee of the Federal University Birnin Kebbi, Nigeria (regulation CEE 86/609).

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