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Toxicological Assessment of *Irvingia gabonensis* Leaf Extracts in Albino Rats: A Comparative Study between Aqueous and Ethanol Extraction Methods

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Abstract

Background: This study aimed to assess the toxicity of *Irvingia gabonensis* leaf extracts in albino rats and investigate their effects on body weight, liver function parameters, and renal function parameters. The research specifically compared the outcomes of aqueous and ethanol extraction methods. Methods: Acute toxicity was evaluated by administering a single oral dose of *Irvingia gabonensis* leaf extracts to rats and monitoring them for 24 hours and during a 14-day observation period. Sub-acute toxicity was assessed through a 28-day administration of the leaf extract. Body weight changes, liver function parameters, and renal function parameters were measured and compared among treatment groups. Results: No signs of toxicity or mortality were observed in rats treated with *Irvingia gabonensis* leaf extracts obtained through either aqueous or ethanol extraction methods. The calculated lethal dose required to cause mortality in 50% of the tested animals (LD_{50}) exceeded 5000 mg/kg body weight. Oral administration of the leaf extract at doses of 400 and 800 mg/kg body weight did not induce any observable signs of toxicity or adverse effects during the 28-day study period. Male and female albino rats treated with the leaf extract showed significant weight gains compared to the control group. Higher doses (800 mg/kg) of both aqueous and ethanol extracts led to increased levels of total protein, albumin, and globulin in male albino rats, with the ethanol extract exhibiting a more pronounced effect. The administration of the ethanol extract, particularly at the lower dose (400 mg/kg), resulted in decreased levels of liver enzymes (AST, ALT, and ALP),

suggesting potential liver protective properties. Additionally, bilirubin levels, a marker of liver dysfunction, were significantly reduced in all treatment groups, with the lowest levels observed in the groups receiving higher doses of both aqueous and ethanol extracts. The administration of *Irvingia gabonensis* leaf extracts did not significantly affect renal function parameters in both male and female albino rats. Conclusion: *Irvingia gabonensis* leaf extracts obtained through aqueous and ethanol extraction methods showed no acute or sub-acute toxicity in albino rats. The extracts demonstrated potential beneficial effects on liver function parameters, particularly at higher doses. However, further research is needed to validate these findings and determine the optimal dosage for potential therapeutic applications in humans.

Keywords

Irvingia gabonensis Leaf, LD50, Acute and Sub-Acute, Toxicology, Histopathology

1. Introduction

Throughout history, the use of medicinal plants and their extracts has played a crucial role in healthcare practices. Traditional medicine, which heavily relies on the utilization of medicinal plants, is widely practiced, particularly in developing countries, with the goal of promoting and maintaining good health [1]. One of the significant advantages of medicinal plants is their natural and sustainable source of therapeutic compounds, which not only makes them environmentally friendly but also offers a holistic approach to healing [2]. Moreover, the accessibility and affordability of medicinal plants make them a valuable resource in many regions worldwide [3].

Medicinal plants are rich in a diverse array of bioactive compounds that interact with the human body, offering potential healing and wellness benefits [4]. These plants continue to play a vital role in meeting the basic medical needs of a significant portion of Asian and African countries, accounting for over 80% of their healthcare resources [5]. However, despite their increasing popularity, concerns exist regarding the safety and proper use of medicinal herbs, as they can possess both therapeutic and potentially harmful properties, depending on the dosage and specific plant species [6] [7]. Regrettably, there remains a significant lack of in-depth research on the *in vivo* toxicological effects of extracts derived from many of these medicinal plants, even though their medicinal benefits have been reported [8]. Thorough toxicity studies are crucial to assess the safety of herbal medications for both short-term and long-term usage. *Irvingia gabonensis*, a plant known for its potential therapeutic benefits in managing diabetes and obesity, as well as its analgesic, antimicrobial, antioxidant, and gastrointestinal properties, requires comprehensive evaluation. Different parts of the *Irvingia gabonensis* plant, including the bark, kernels, leaves, and roots, have been utilized in traditional medicine to address various health conditions. Additionally,

research has explored the industrial applications of African mango, yielding promising outcomes in the development of food, cosmetic, and pharmaceutical products. The seeds of the plant, utilized in the preparation of "African soup", are believed to possess antidiabetic properties, while the leaves have been traditionally employed in the treatment of diabetes. Experimental investigations focusing on *Irvingia* extracts derived from the leaves and stems have demonstrated their potential to regulate blood sugar and lipid levels [9]. In a clinical trial conducted by Adamson *et al.* [10], it was observed that *Irvingia gabonensis* seeds had the ability to lower plasma lipids and increase HDL cholesterol levels. Additionally, various studies have investigated the potential of *Irvingia gabonensis* extracts to mitigate the toxicity caused by different chemical agents [11]. The research conducted by Gbadegesin *et al.* [12] [13] has provided evidence of the effectiveness of *Irvingia gabonensis* extracts in reducing the toxicity associated with these chemical agents. Therefore, the aims of this study would be to conduct a toxicological assessment of *Irvingia gabonensis* leaf extracts in albino rats and compare the outcomes between aqueous and ethanol extraction methods. The study would focus on evaluating the potential toxicity of the extracts and assessing their effects on various parameters such as body weight, liver function, and renal function in order to provide insights into the safety and potential benefits of using *Irvingia gabonensis* leaf extracts.

2. Materials and Methods

2.1. Plant Material

The leaf sample utilized in this study was obtained from a fully grown Irvingia gabonensis tree located in Uturu, Isikwato LGA of Abia State, Nigeria. The taxonomic identification of the plant was conducted by a taxonomist affiliated with the Department of Plant Science and Biotechnology at Abia State University in Uturu.

2.2. Preparation of Aqueous Leaf Extracts of *Irvingia gabonensis*

The leaves of the plant were carefully separated from the stem and processed into a fine powder using a hammer mill. Subsequently, 200 g of this powder was dissolved in 1600 ml of water and allowed to steep for an unspecified duration. The extract was then filtered through muslin cloth and Whatman No. 1 filter paper to obtain a clear liquid. The resulting extracts were stored under unspecified conditions, presumably in a refrigerated environment, for potential reconstitution and later use. During the administration to animal the extract was desolved in water and was administered orally according to their body weight.

2.3. Preparation of Ethanol Leaf Extracts of *Irvingia gabonensis*

The plant leaves were carefully separated from the stem, ground with a hammer mill, and 200 g of the fine powder were soaked in 1200 ml of ethanol for 48 hours. The extract was then filtered using whatman no. 1 filter paper and muslin cloth. The solution obtained was dried in a water bath at 50˚C till the ethanol

solvent evaporated entirely, and the percentage yield was calculated.

2.4. Experimental Design

For the study, 100 rats: 50 male and female albino rats, weighing between 100 and 150 g each were purchased from the animal house of the biochemistry department at Abia State University in Uturu, Nigeria. Before the experiment started, the rats were given fourteen (14) days to get used to the lab environment. The rats were housed in typical environmental settings with 12 hr/12 hr light/dark cycles, humidity between 35% and 60%, and temperatures between 25˚C and 28˚C. Additionally, they were given unlimited access to water and were fed regularly with standard rat food. The study strictly complied with the ethical principles of the World Health Organization's good laboratory practice regulations from 1998 and the United States' guidelines for using experimental animals [14].

2.5. Acute Toxicity (LD_{50}) (Median Lethal Dose) Test

The acute toxicity study of *I. gabonensis* was conducted in accordance with the OECD [15] 423 guideline, with minor modifications. The study consisted of two phases. During the first phase, the animals were divided into three groups of three rats each and received oral administration of *I. gabonensis* leaf extracts in aqueous and ethanolic forms using oral gavage at doses of 10, 100, and 1000 mg/kg. In the second phase, the animals were divided into three groups of one rat each and administered higher doses (1600, 2900, and 5000 mg/kg) of aqueous and ethanolic leaf extracts of *I. gabonensis*. Following treatment, the rats were observed for a period of 24 hours and provided unrestricted access to food and water. Within this 24-hour period, any changes in behavior, toxicological symptoms, and occurrences of mortality were monitored closely.

2.6. Sub-Acute Toxicological Studies

In the sub-acute toxicological study, albino rats were subjected to oral administration of aqueous and ethanolic leaf extracts of *I. gabonensis* for a duration of 28 days using oral gavage. The concentrations of *I. gabonensis* employed in the toxicological assessment were as follows: Group I: Normal control Group II: 400 mg/kg body weight of aqueous leaf extract of *Irvingiagabonensis* Group III: 800 mg/kg body weight of aqueous leaf extracts of *Irvingia gabonensis* Group IV: 400 mg/kg body weight of ethanolic leaf extract of *Irvingia gabonensis* Group V: 800 mg/kg body weight of ethanolic leaf extract of *Irvingia gabonensis* Throughout the 28-day study period, the albino rats received oral administration of the respective aqueous and ethanolic leaf extracts of *I. gabonensis.*

2.7. Biochemical Parameters Evaluation

Following a period of twenty-eight (28) days during which the albino rats were orally administered with the aqueous and ethanolic extracts of *I. gabonensis,* the rats were subjected to further procedures. Prior to these procedures, the rats

were subjected to an overnight fasting period. To initiate the subsequent steps, the rats were anesthetized using chloroform. After anesthesia, the rats were sacrificed in accordance with ethical guidelines. Cardiac puncture was performed using a syringe and needle to draw blood samples from each animal. The blood samples were collected into dry sample bottles for clinical chemistry analysis and EDTA (Ethylllenediaminetetraacetic acid) containers for haematological analysis.

For the blood collected in the dry sample bottles, a 15-minute period was allowed for clotting before centrifuging at 12,000 rpm for 5 minutes. This centrifugation process facilitated the separation of serum from the clotted blood. The separated serum was then carefully transferred into sterile sample test tubes, ensuring aseptic conditions, to facilitate the measurement of biochemical parameters.

2.8. Liver Enzymes

Liver enzyme levels were assessed as liver function tests using the colorimetric method. The following enzymes were measured: alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Additionally, total protein, albumin, creatinine, urea, and electrolytes including chloride, sodium, potassium, and bicarbonate ions were analyzed. The analysis was conducted using Randox assay kits and an automated biochemistry analyzer, following the manufacturer's instructions [16] [17].

2.9. Histopathology Studies

The female rats were sacrificed, and their kidneys and livers were removed, sliced into 3-cm-thick pieces, and fixed in 10% formalin solution for sectioning. The fixed specimens were cut into pieces, processed, and embedded in blocks of paraffin. A rotary microtome was used to slice the blocks into 5-micrometer (m) thick paraffin sections. For histological analysis, the sections were stained with hematoxylin and eosin H and E. Finally, a light microscope was used to examine the stained sections for morphological changes [18].

2.10. Statistical Analysis

The results were expressed as mean ± SD (Standard deviation) and subjected to a paired samples T Test and statistical significance obtained at $P < 0.05$. The data generated from the animal study were analyzed with a one-way analysis of variance (ANOVA) and the means compared with a Duncan multiple range comparison test using a statistical Products and Service Solutions (SPSS) version 22 and statistical significance established 95% confidence level ($P < 0.05$).

2.11. Ethical Consideration

The experimental protocol shall be presented to the research and publication department of Abia State University Uturu, Nigeria. The Animal Welfare Act of 1985 of the United States of America for research and Institutional Animal Care

and Use Committee (IACUC) protocol will be strictly followed.

3. Results

3.1. Acute Toxicity Assessment

The rats did not exhibit any signs of toxicity or mortality within 24 hours or during the 14-day observation period after a single oral dose of aqueous and ethanol leaf extract of *Irvingia gabonensis*, as determined by the limit test. The LD50 in rats was greater than 5000 mg/kg body weight.

3.2. Sub-Acute Toxicity Studies

During the 28-day study, oral administration of *Irvingia gabonensis* leaf extract at 400 and 800 mg/kg body weight in aqueous and ethanol did not cause any observable signs of toxicity, such as fatigue, weakness, convulsions, hyperactivity, dullness, diarrhea, or diuresis. All animals successfully completed the experiment.

Table 1 examined the effects of an aqueous leaf extract of I. gabonensis on male albino rats' body weight. Results showed that the control group had an average body weight of 83.52 ± 3.00 grams, while the 400 mg/kg group had a slightly higher average weight of 86.62 ± 1.05 grams.

On Day 14, the control group's average body weight increased to 130.58 ± 0.95 grams. The 400 mg/kg group had a similar weight, while the 800 mg/kg group had a lower average. The "Weight Gain" column showed a 56.33 grams weight gain, while the 400 mg/kg group had a 51.01 grams gain.

It is important to note that the values in Table 1 represent the mean ± SD (standard deviation) for a sample size (N) of 5. The mean provides the average value of body weight within each group, while the standard deviation indicates the variability or spread of the data around the mean.

Table 2 presents the effect of an aqueous leaf extract of *I. gabonensis* on the body weight of female albino rats. The study included a control group and two treatment groups receiving different doses of the extract (400 mg/kg and 800 mg/kg). Body weight measurements were taken on Day 1 and Day 28 of the experiment.

On Day 1, the control group had an average body weight of 83.52 ± 3.00 grams, while the 400 mg/kg group had a slightly higher average of 86.62 ± 1.05 grams, and the 800 mg/kg group had a lower average of 81.17 ± 3.20 grams.

On Day 28, the control group's average body weight increased to 119.30 ± 0.93 grams, while the 400 mg/kg group had a similar average of 119.81 ± 4.25 grams, and the 800 mg/kg group had a lower average of 107.33 ± 5.83 grams.

The "Weight Gain" column represents the difference between Day 28 and Day 1 body weight for each group. The control group had a weight gain of 35.78 grams, the 400 mg/kg group had a weight gain of 33.19 grams, and the 800 mg/kg group had a weight gain of 26.16 grams.

Table 1. Effect of *I. gabonensis* aqueous leaf extract on body weight of male albino rats.

	I. gabonensis Aqueous Leaf Extract		
	Control	400 mg/kg	800 mg/kg
Day 1	83.52 ± 3.00	86.62 ± 1.05	81.17 ± 3.20
Day 14	130.58 ± 0.95	130.81 ± 4.17	118.38 ± 5.80
Weight Gain	56.33	51.01	45.84

Values represent the mean ± SD for N = 5.

Table 2. Effect of *I. gabonensis* aqueous leaf extract on body weight of female albino rats.

	I. gabonensis Aqueous Leaf Extract		
	Control	400 mg/kg	800 mg/kg
Day 1	83.52 ± 3.00	86.62 ± 1.05	81.17 ± 3.20
Day 28	119.30 ± 0.93	119.81 ± 4.25	107.33 ± 5.83
Weight Gain	51.66	47.25	39.08

Values represent the mean ± SD for N = 5.

The values in the table represent the mean ± SD for a sample size of 5. The treatment groups receiving the I. gabonensis leaf extract showed lower body weight on Day 28 compared to the control group, with lower weight gain over the experimental period.

Table 3 examines the impact of an ethanolic leaf extract of *I. gabonensis* on male albino rats' body weight. The experiment involved three groups: a control group, two treatment groups receiving different doses (400 mg/kg and 800 mg/kg), and body weight measurements taken on Day 1 and Day 14. The control group had an average body weight of 78.66 ± 3.02 grams, while the 400 mg/kg group had a slightly higher average weight of 82.55 ± 3.02 grams.

On Day 14, the control group's average body weight increased to 119.30 ± 0.93 g. The 400 mg/kg group had a slightly lower average weight of 118.06 ± 4.75 g, while the 800 mg/kg group had a similar average weight of 120.68 ± 13.23 g. The "Weight Gain" column showed a weight gain of 51.66 grams, 43.01 g for the 400 mg/kg group, and 38.96g for the 800 mg/kg group.

Table 3 shows that the ethanolic leaf extract of *I. gabonensis* had varying effects on body weight compared to the control group. The 400 mg/kg dose showed a slightly lower weight gain, while the 800 mg/kg dose showed a further reduction in weight gain. The mean represents the average body weight within each group, while the standard deviation indicates variability.

Table 4 examines the effects of an ethanol leaf extract of *I. gabonensis* on female albino rats' body weight. The experiment involved three groups: a control group, two treatment groups, and two groups receiving different doses. The average body weight of the control group was 78.66 ± 3.02 grams on Day 1, while the 400 mg/kg group had a significantly higher average body weight of 144.53 ±10.50 grams. The 800 mg/kg group had even higher average body weight of

Table 3. Effect of *I. gabonensis* ethanolic leaf extract on body weight of male albino rats.

	I. gabonensis Ethanolic Leaf Extract		
	Control	400 mg/kg	800 mg/kg
Day 1	78.66 ± 3.02	82.55 ± 3.02	86.84 ± 12.10
Day 14	119.30 ± 0.93	118.06 ± 4.75	120.68 ± 13.23
Weight Gain	51.66	43.01	38.96

Values represent the mean ± SD for N = 5.

Table 4. Effect of *I. gabonensis* ethanol leaf extract on body weight of female albino rats.

	I. gabonensis Ethanol Leaf Extract		
	Control	400 mg/kg	800 mg/kg
Day 1	78.66 ± 3.02	144.53 ± 10.50	204.92 ± 9.65
Day 28	119.30 ± 0.93	156.23 ± 16.62	211.13 ± 7.30
Weight Gain	51.66	8.09	3.03

Values represent the mean ± SD for N = 5.

204.92 ± 9.65 grams. The control group's average body weight increased to 119.30 ± 0.93 grams on Day 28, while the 800 mg/kg group had the highest average body weight of 211.13 ± 7.30 grams.

The "Weight Gain" column shows the difference between body weight on Day 28 and Day 1, with the control group experiencing a 51.66 grams gain. The 400 mg/kg group showed a significantly lower gain of 8.09 grams, while the 800 mg/kg group had the lowest gain of 3.03 grams. Treatment groups receiving I. gabonensis ethanol leaf extract showed different effects on body weight.

The results presented in Table 5 and Table 6 show the effect of *I. gabonensis* aqueous and ethanolic leaf extracts on the organ weights of male and female albino rats after 28 days of administration. The organ weights measured include the kidney and liver.

In both male and female rats, the kidney weights were slightly higher in the groups treated with the leaf extracts compared to the control group, although the differences were not statistically significant. For example, in the male rats, the kidney weights ranged from 0.80 ± 0.04 to 0.88 ± 0.09 in the different treatment groups, while in the female rats, the kidney weights ranged from 0.72 ± 0.04 to 0.79 ± 0.09. These findings suggest that the administration of *I. gabonensis* leaf extracts did not significantly affect kidney weight compared to the control group.

Regarding liver weights, there were no substantial differences observed among the treatment groups compared to the control group. In both male and female rats, the liver weights remained relatively consistent across all groups. For instance, in the male rats, the liver weights ranged from 3.80 ± 0.37 to 4.24 ± 0.54, while in the female rats, the liver weights ranged from 3.79 ± 0.37 to 4.23 ± 0.54.

Table 5. Effect of *I. gabonensis* aqueous and ethanolic leaf extract on organ weight of male albino rats after 28 days administration.

Control		Aqueous Extract		Ethanolic Extract	
		400 /kg	800 mg/kg	400 mg/kg	800 mg/kg
Kidney	0.80 ± 0.04	0.88 ± 0.09	0.86 ± 0.06	0.83 ± 0.08	0.80 ± 0.02
Liver	3.80 ± 0.37	4.24 ± 0.54	4.20 ± 0.17	4.22 ± 0.21	4.20 ± 0.71

Values represent the mean ± SD for N = 5. Group I, Normal control; Group II, 400 mg/kg of *I. gabonensis* leaf extract and Group 3, 800 mg/kg of *I. gabonensis* leaf extract.

Table 6. Effect of *I. gabonensis* aqueous and ethanol leaf extract on organ weight of female albino rats.

Control		Aqueous Extract		Ethanolic Extract	
		400 mg/kg	800 mg/kg	400 g/kg	800 mg/kg
Kidney	0.72 ± 0.04	0.79 ± 0.09	0.76 ± 0.06	0.74 ± 0.08	0.71 ± 0.02
Liver	3.79 ± 0.37	4.23 ± 0.54	3.86 ± 0.17	3.94 ± 0.21	3.93 ± 0.71

Values represent the mean ± SD for n = 5. Group I, Normal control; Group II, 400 mg/kg of *I. gabonensis* leaf extract and Group 3, 800 mg/kg of *I. gabonensis* leaf extract.

Table 7 presents the effects of different doses of aqueous and ethanol extracts of *I. gabonensis* leaves on liver function parameters in male albino rats.

The results indicate that the higher doses of both aqueous and ethanol extracts (800 mg/kg) led to a significant increase in total protein, albumin, and globulin levels compared to the control group, with the ethanol extract having a more pronounced effect.

The levels of AST, ALT, and ALP enzymes, which are markers of liver damage, were significantly reduced with the administration of the ethanol extract, especially at the lower dose (400 mg/kg).

The levels of bilirubin, which is a marker of liver dysfunction, were also significantly reduced in all treatment groups compared to the control group, with the lowest levels observed in the groups treated with the higher doses of both aqueous and ethanol extracts.

Overall, the results suggest that *I. gabonensis* leaf extracts may have a beneficial effect on liver function in male albino rats, with the ethanol extract showing a more significant impact. However, further studies are needed to confirm these findings and determine the potential mechanisms of action.

This Table 8 shows the effect of different doses of *I. gabonensis* aqueous and ethanol leaf extracts on liver function parameters in female albino rats, compared to a control group. The results are presented as mean values with standard deviation.

Overall, the extract treatment groups showed changes in liver function parameters compared to the control group. For example, the total protein levels increased with the higher doses of aqueous and ethanol extracts (800 mg/kg) compared to the control group. Similarly, albumin levels increased with the

Table 7. Effect of *I. gabonensis* aqueous and ethanolic leaf extract on liver function parameters in male albino rats.

Treatment Groups	Control	Aqueous extract (400 mg/kg)	Aqueous extract (800 mg/kg)	Ethanol extract (400 mg/kg)	Ethanol extract (800 mg/kg)
T. protein (g/dl)	4.55 ± 0.10^{a}	4.87 ± 0.12^{b}	5.13 ± 0.07^{c}	5.30 ± 0.11^{c}	5.72 ± 0.09^{d}
Albumin (g/dl)	2.75 ± 0.03^{a}	2.90 ± 0.10^{b}	3.05 ± 0.02^{c}	3.16 ± 0.03^{d}	3.22 ± 0.03^{d}
Globulin (g/dl)	1.80 ± 0.13^{a}	1.97 ± 0.05^{b}	$2.08 \pm 0.08^{b,c}$	2.14 ± 0.10^{c}	2.50 ± 0.07^{d}
AST (U/L)	44.00 ± 3.61^{c}	33.33 ± 1.16^{b}	$41.33 \pm 1.53^{b,c}$	38.33 ± 1.16^{a}	31.00 ± 3.00^{a}
ALT (U/L)	32.00 ± 2.00^{c}	$25.00 \pm 2.00^{a,b}$	26.00 ± 2.00^{b}	$22.33 \pm 2.08^{a,b}$	21.33 ± 2.08^{a}
ALP (U/L)	76.00 ± 2.65^{b}	$73.00 \pm 3.00^{a,b}$	$73.33 \pm 3.22^{a,b}$	68.33 ± 2.89^{a}	70.00 ± 2.65^{a}
Bil. (mg/dl)	0.74 ± 0.02^{b}	0.72 ± 0.03^{b}	0.66 ± 0.01^{a}	0.65 ± 0.05^{a}	0.64 ± 0.02^{a}

Values are presented as mean ± standard deviation (n = 5); and values with different superscripts are significantly ($P < 0.05$) different from any paired mean across the row.

Table 8. Effect of *I. gabonensis* aqueous and ethanol leaf extract on liver function parameters in female albino rats.

Treatment groups	Control	Aqueous extract (400 mg/kg)	Aqueous extract (800 mg/kg)	Ethanol extract (400 mg/kg)	Ethanol extract (800 mg/kg)
Total protein (g/dl)	5.43 ± 0.16^{a}	$5.71 \pm 0.35^{a,b}$	$6.13 \pm 0.07^{b,c}$	$5.90 \pm 0.32^{b,c}$	6.24 ± 0.08^{c}
Albumin (g/dl)	3.14 ± 0.01^{a}	3.60 ± 0.20^{c}	$3.45 \pm 0.12^{b,c}$	3.40 ± 0.04^{b}	3.38 ± 0.04^{b}
Globulin (g/dl)	$2.29 \pm 0.15^{a,b}$	2.10 ± 0.30^{a}	$2.68 \pm 0.16b^{c}$	$2.51 \pm 0.30^{a,b,c}$	2.86 ± 0.12^{c}
AST (U/L)	43.00 ± 1.00^{e}	34.63 ± 0.33^{b}	38.67 ± 0.58^{d}	37.33 ± 0.56^{c}	29.34 ± 0.65^{a}
ALT (U/L)	28.00 ± 1.00^{d}	24.35 ± 0.75^{b}	26.00 ± 1.21^{c}	23.13 ± 0.50^{b}	21.00 ± 1.43^{a}
ALP (U/L)	$68.33 \pm 1.53^{b,c}$	64.32 ± 0.54^{a}	68.64 ± 0.72^{c}	$64.63 \pm 1.16^{a,b}$	62.66 ± 0.86^{a}
Bil. (mg/dl)	0.73 ± 0.01^{d}	0.68 ± 0.01^{c}	0.62 ± 0.02^{b}	$0.60 \pm 0.01^{a,b}$	0.59 ± 0.04^{a}

Values are presented as mean ± standard deviation (n = 5); and values with different superscripts are significantly ($P < 0.05$) different from any paired mean across the row.

aqueous extract doses (400 and 800 mg/kg) compared to the control group. Globulin levels were higher in the ethanol extract groups (400 and 800 mg/kg) compared to the control group.

In terms of liver enzyme levels, AST levels were lower in the highest ethanol extract group (800 mg/kg) compared to the control group. ALT levels were lower in the aqueous and ethanol extract groups (400 and 800 mg/kg) compared to the control group. ALP levels did not show any significant differences between the groups. Bilirubin levels were lower in all extract groups compared to the control group.

Overall, the results suggest that *I. gabonensis* leaf extracts may have some beneficial effects on liver function parameters in female albino rats, particularly at higher doses. However, further studies are needed to confirm these findings and determine the optimal dosage for potential therapeutic use in humans.

These tables show the effect of different doses and types of leaf extracts of *Irvingia gabonensis* on renal function parameters in male and female albino rats. The renal function parameters measured include urea, creatinine, sodium (Na^{+}),

potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-).

In Table 9, the aqueous and ethanol leaf extracts of *I. gabonensis* were given at doses of 400 mg/kg and 800 mg/kg to male rats. The results show that the administration of the aqueous extract at a dose of 800 mg/kg and the ethanol extract at a dose of 400 mg/kg did not significantly alter the renal function parameters compared to the control group. However, the administration of the aqueous extract at a dose of 400 mg/kg and 800 mg/kg and the ethanol extract at a dose of 800 mg/kg significantly increased urea levels compared to the control group. Additionally, the administration of the ethanol extract at a dose of 400 mg/kg significantly increased potassium levels compared to the control group.

In Table 10, the aqueous and ethanol leaf extracts of *I. gabonensis* were given at doses of 400 mg/kg and 800 mg/kg to female rats. The results show that the administration of the ethanol extract at a dose of 800 mg/kg significantly increased creatinine levels compared to the control group. However, the administration of the aqueous and ethanol extracts at different doses did not significantly alter the other renal function parameters compared to the control group.

It is important to note that the significance levels (a, b, c, d) indicate the level of statistical significance between groups, with a being the most significant and d being the least significant. Overall, these tables suggest that the administration of different doses and types of *I. gabonensis* leaf extracts may affect renal function parameters differently in male and female albino rats.

The tables show the effect of different doses of *I. gabonensis* aqueous and ethanol leaf extract on liver function and lipid profile parameters in male and female albino rats.

In terms of liver function parameters (Table 11), both the aqueous and ethanol leaf extracts at higher doses (800 mg/kg) increased total protein and decreased AST and ALT levels in female rats. In male rats, the ethanol extracts at 800 mg/kg significantly decreased AST levels. ALP levels were not affected by any of the treatments in male rats, while in female rats, both the aqueous and ethanol extracts at 800 mg/kg increased ALP levels. Bilirubin levels were significantly decreased by all treatments in male rats, but only the aqueous extract at 800 mg/kg reduced bilirubin levels in female rats.

In terms of lipid profile parameters (Table 12), the higher doses of the aqueous and ethanol leaf extracts significantly decreased total cholesterol and LDL-C levels in male rats. In female rats, all doses of the extracts significantly reduced total cholesterol levels, while only the higher doses significantly reduced LDL-C levels. HDL-C levels were not significantly affected by any of the treatments in either male or female rats. TAG levels were only slightly decreased by the extracts in both male and female rats, with no significant differences observed.

Overall, the effect of the extracts on liver function and lipid profile parameters was more pronounced in female rats, with more significant changes observed in total cholesterol and LDL-C levels compared to male rats. However, the specific

Table 9. Effect of *I. gabonensis* aqueous and ethanolic leaf extract on renal function parameters in male albino rats.

Treatment groups	Control	Aqueous extract (400 mg/kg)	Aqueous extract (800 mg/kg)	Ethanol extract (400 mg/kg)	Ethanol extract (800 mg/kg)
Urea (mg/dl)	18.38 ± 0.87^{a}	23.39 ± 0.66^{c}	20.73 ± 0.91^{b}	17.17 ± 1.10^{a}	18.68 ± 0.73^{a}
Creatinine (mg/dl)	$0.82 \pm 0.07^{a,b}$	$0.75 \pm 0.05^{a,b}$	0.74 ± 0.06^{a}	0.84 ± 0.02^{b}	$0.79 \pm 0.03^{a,b}$
Na^{+} (mEq/L)	121.00 ± 0.80^{a}	124.21 ± 0.67^{b}	124.37 ± 1.07^{b}	$123.72 \pm 0.85^{a,b}$	$122.25 \pm 2.81^{a,b}$
K^{+} (mEq/L)	4.38 ± 0.07^{b}	4.13 ± 0.05^{a}	4.25 ± 0.07^{a}	4.16 ± 0.06^{a}	4.16 ± 0.10^{a}
Cl^{-} (mEq/L)	80.68 ± 0.89^{a}	81.98 ± 0.69^{a}	85.51 ± 0.87^{b}	$83.41 \pm 2.88^{a,b}$	85.06 ± 0.62^{b}
HCO_3^- (mEq/L)	20.30 ± 0.56^{a}	20.17 ± 0.35^{a}	20.57 ± 0.35^{a}	19.90 ± 0.46^{a}	20.27 ± 0.21^{a}

Values are presented as mean ± standard deviation (n = 5); and values with different superscripts are significantly ($P < 0.05$) different from any paired mean across the row.

Table 10. Effect of *I. gabonensis* aqueous and ethanol leaf extract on renal function parameters in female albino rats.

Treatment groups	Control	Aqueous extract (400 mg/kg)	Aqueous extract (800 mg/kg)	Ethanol extract (400 mg/kg)	Ethanol extract (800 mg/kg)
Urea (mg/dl)	$16.56 \pm 0.33^{a,b}$	20.49 ± 0.55^{d}	18.30 ± 0.13^{c}	15.87 ± 0.42^{a}	17.7 ± 0.56^{b}
Creatinine (mg/dl)	0.79 ± 0.02^{c}	0.75 ± 0.01^{b}	0.72 ± 0.01^{a}	0.86 ± 0.01^{d}	0.82 ± 0.02^{c}
Na^{+} (mEq/L)	120.05 ± 2.33^{a}	122.18 ± 0.72^{a}	121.68 ± 1.73^{a}	122.33 ± 0.67^{a}	122.54 ± 1.06^{a}
K^{+} (mEq/L)	4.29 ± 0.09^{b}	4.09 ± 0.11^{a}	$4.14 \pm 0.08^{a,b}$	$4.22 \pm 0.01^{a,b}$	$4.17 \pm 0.05^{a,b}$
Cl^{-} (mEq/L)	81.86 ± 0.59^{a}	82.07 ± 1.72^{a}	85.02 ± 0.61^{b}	$83.59 \pm 2.61^{a,b}$	$84.25 \pm 0.62^{a,b}$
HCO_3^- (mEq/L)	19.63 ± 0.21^{b}	$21.10 \pm 0.44^{a,b}$	18.93 ± 0.67^{c}	18.10 ± 0.70^{a}	19.20 ± 0.10^{b}

Values are presented as mean ± standard deviation (n = 5); and values with different superscripts are significantly ($P < 0.05$) different from any paired mean across the row.

Table 11. Effect of *I. gabonensis* aqueous and ethanolic leaf extract on lipid profile parameters in male albino rats.

Treatment groups	Control	Aqueous extract (400 mg/kg)	Aqueous extract (800 mg/kg)	Ethanol extract (400 mg/kg)	Ethanol extract (800 mg/kg)
Total cholesterol (mg/dl)	88.84 ± 1.20^{c}	$86.48 \pm 1.28^{b,c}$	$85.00 \pm 0.62^{a,b}$	82.34 ± 2.12^{a}	$84.46 \pm 0.70^{a,b}$
HDL-C (mg/dl)	52.24 ± 1.57^{a}	$55.76 \pm 0.34^{b,c}$	56.40 ± 0.80^{c}	$54.06 \pm 1.26^{a,b}$	56.33 ± 0.54^{c}
TAG (mg/dl)	124.93 ± 0.75^{b}	$121.30 \pm .1.30^{a}$	120.30 ± 1.49^{a}	121.27 ± 2.04^{a}	121.03 ± 0.97^{a}
LDL-C (mg/dl)	11.62 ± 0.67^{b}	6.47 ± 1.15^{a}	3.74 ± 0.94^{a}	4.02 ± 3.37^{a}	3.92 ± 1.17^{a}
VLDL-C (mg/dl)	24.99 ± 0.15^{b}	24.26 ± 0.26^{a}	24.06 ± 0.30^{a}	24.25 ± 0.41^{a}	24.21 ± 0.19^{a}

Values are presented as mean ± standard deviation (n = 5); and values with different superscripts are significantly ($P < 0.05$) different from any paired mean across the row.

Table 12. Effect of *I. gabonensis* aqueous and ethanol leaf extract on lipid profile parameters in female albino rats.

Treatment groups	Control	Aqueous extract (400 mg/kg)	Aqueous extract (800 mg/kg)	Ethanol extract (400 mg/kg)	Ethanol extract (800 mg/kg)
Total cholesterol (mg/dl)	112.12 ± 1.62^{b}	107.17 ± 1.03^{a}	105.66 ± 0.67^{a}	105.22 ± 0.71^{a}	107.09 ± 0.46^{a}
HDL-C (mg/dl)	60.19 ± 1.67^{a}	58.71 ± 1.46^{a}	59.67 ± 0.95^{a}	57.05 ± 1.65^{a}	58.55 ± 0.91^{a}

Continued

TAG (mg/dl)	130.07 ± 1.60^{c}	126.37 ± 1.01^{b}	123.50 ± 0.95^{a}	125.47 ± 1.07^{a,b}	126.17 ± 0.75^{b}
LDL-C (mg/dl)	25.92 ± 0.73^{b}	23.19 ± 2.43^{a,b}	21.29 ± 2.51^{a}	23.41 ± 2.48^{a,b}	23.31 ± 0.66^{a,b}
VLDL-C (mg/dl)	26.01 ± 0.32^{c}	25.27 ± 0.20^{b}	24.70 ± 0.19^{a}	25.09 ± 0.21^{a,b}	25.23 ± 0.15^{b}

Values are presented as mean ± standard deviation (n = 5); and values with different superscripts are significantly (P<0.05) different from any paired mean across the row.

effects of the extracts on different parameters varied depending on the dose and type of extract used.

The tables show the effect of different doses of aqueous and ethanol leaf extracts of *I. gabonensis* on various hematological indices of male and female albino rats. The hematological indices evaluated include red blood cell count (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), total white blood cell count (TWBC), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

In Table 13, it can be observed that the administration of *I. gabonensis* leaf extracts at different doses (400 and 800 mg/kg) resulted in a dose-dependent increase in RBC count, PCV, and Hb concentration of male albino rats. The highest dose of ethanol extract (800 mg/kg) showed the most significant increase in all the three parameters. However, there was no significant difference in TWBC and PLT counts among the treatment groups.

Table 14 shows that the administration of *I. gabonensis* leaf extracts at different doses (400 and 800 mg/kg) also resulted in a dose-dependent increase in RBC count, PCV, Hb concentration, and PLT count of female albino rats. The highest dose of ethanol extract (800 mg/kg) showed the most significant increase in all the four parameters. However, there was no significant difference in TWBC count among the treatment groups.

It is also noteworthy that the MCV, MCH, and MCHC values of the rats were not consistent among the treatment groups and were sometimes lower than the control values. These values suggest that the size, hemoglobin content, and concentration of hemoglobin in the red blood cells of the rats were affected differently by the leaf extracts.

Table 15 shows the effect of different doses of aqueous and ethanol extracts of *Irvingia gabonensis* leaves on the differential white blood cell count of male albino rats. The results are expressed as percentages of neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Overall, the results suggest that the extracts have a minimal effect on the white blood cell differential count. There were slight changes in the percentage of neutrophils and lymphocytes, with the highest dose of the aqueous extract (800 mg/kg) and the ethanol extract (800 mg/kg) showing a slight increase in neutrophils and a decrease in lymphocytes. There were also minor changes in the percentage of monocytes and eosinophils, with the ethanol extract (400 mg/kg) showing a slight increase in monocytes and eosinophils.

Table 13. Effect of *I. gabonensis* aqueous and ethanolic leaf extract on haematological indices of male albino rats.

Treatment groups	Control	Aqueous extract (400 mg/kg)	Aqueous extract (800 mg/kg)	Ethanol extract (400 mg/kg)	Ethanol extract (800 mg/kg)
RBC ($\times 10^6$/mm^3)	5.91 ± 0.22^a	6.05 ± 0.13^a	6.08 ± 0.21^{a,b}	6.30 ± 0.14^b	6.77 ± 0.13^c
PCV (%)	40.00 ± 1.23^a	42.00 ± 1.23^b	42.60 ± 1.82^b	44.40 ± 0.89^c	45.40 ± 0.55^c
Hb (g/dl)	14.96 ± 0.15^a	15.40 ± 0.34^{a,b}	15.42 ± 0.25^{a,b}	15.86 ± 0.36^{b,c}	16.26 ± 0.47^c
TWBC ($\times 10^3$/mm^3)	8.66 ± 0.34^a	9.59 ± 0.75^b	9.40 ± 0.21^b	9.70 ± 0.57^b	9.40 ± 0.12^b
PLT ($\times 10^3$/mm^3)	230.80 ± 6.69^a	232.00 ± 7.38^a	230.80 ± 7.26^a	232.40 ± 5.23^a	233.20 ± 3.96^a
MCV (fl)	67.70 ± 1.26^a	69.46 ± 0.99^b	70.08 ± 1.15^b	70.49 ± 1.06^b	67.09 ± 1.12^a
MCH (pg)	25.33 ± 0.73^b	25.47 ± 0.24^b	25.39 ± 0.59^b	25.18 ± 0.31^b	24.03 ± 0.59^a
MCHC (g/dl)	37.42 ± 0.78^b	36.68 ± 0.60^{a,b}	36.23 ± 1.08^{a,b}	35.73 ± 0.75^a	35.82 ± 1.21^a

Values are presented as mean ± standard deviation (n = 5); and values with different superscripts are significantly (P < 0.05) different from any paired mean across the row. RBC, Red Blood Cells; PCV, Packed Cell Volume; HB, Haemoglobin; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration; WBC, White Blood Cell.

Table 14. Effect of *I. gabonensis* aqueous and ethanol leaf extract on haematological indices in female albino rats.

Treatment groups	Control	Aqueous extract (400 mg/kg)	Aqueous extract (800 mg/kg)	Ethanol extract (400 mg/kg)	Ethanol extract (800 mg/kg)
RBC ($\times 10^6$/mm^3)	5.22 ± 0.14^a	5.71 ± 0.46^b	5.84 ± 0.38^b	6.36 ± 0.14^c	6.57 ± 0.15^c
PCV (%)	38.00 ± 1.58^a	40.60 ± 2.30^a	41.20 ± 1.30^{a,b}	40.80 ± 3.83^a	44.20 ± 2.39^b
Hb (g/dl)	13.84 ± 0.41^a	14.44 ± 0.17^b	14.90 ± 0.27^c	15.20 ± 35^c	15.82 ± 0.22^d
TWBC ($\times 10^3$/mm^3)	7.96 ± 0.18^a	8.92 ± 0.50^b	9.03 ± 0.22^b	9.62 ± 0.19^c	9.01 ± 0.50^b
PLT ($\times 10^3$/mm^3)	228.60 ± 3.78^a	232.60 ± 2.07^{b,c}	231.20 ± 2.39^{a,b,c}	229.20 ± 0.84^{a,b}	234.00 ± 3.39^c
MCV (fl)	72.85 ± 4.52^b	71.38 ± 6.18^{a,b}	70.75 ± 5.28^{a,b}	64.16 ± 6.50^a	67.33 ± 3.59^{a,b}
MCH (pg)	26.52 ± 1.13^b	25.41 ± 1.98^{a,b}	25.58 ± 1.75^{a,b}	23.89 ± 0.36^a	24.11 ± 0.85^a
MCHC (g/dl)	36.46 ± 1.52^a	35.66 ± 2.17^a	36.20 ± 1.59^a	37.60 ± 4.58^a	35.89 ± 2.21^a

Values are presented as mean ± standard deviation (n = 5); and values with different superscripts are significantly (P < 0.05) different from any paired mean across the row. RBC, Red Blood Cells; PCV, Packed Cell Volume; HB, Haemoglobin; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration; WBC, White Blood Cell.

Table 15. Effect of *I. gabonensis* aqueous and ethanolic leaf extract on white blood cell differential of male albino rats.

Treatment groups	Control	Aqueous extract (400 mg/kg)	Aqueous extract (800 mg/kg)	Ethanol extract (400 mg/kg)	Ethanol extract (800 mg/kg)
Neutrophils (%)	51.60 ± 1.52^a	54.00 ± 2.92^{a,b}	55.20 ± 2.28^b	54.20 ± 2.39^{a,b}	56.80 ± 1.92^b
Lymphocytes (%)	40.00 ± 1.87^b	38.20 ± 2.68^{a,b}	37.00 ± 2.24^{a,b}	37.40 ± 2.70^{a,b}	35.80 ± 1.64^a
Monocytes (%)	5.20 ± 0.45^{b,c}	5.40 ± 0.55^c	4.60 ± 0.55^{a,b}	4.80 ± 0.45^{a,b,c}	4.20 ± 0.45^a
Eosinophils (%)	2.80 ± 0.45^a	2.40 ± 0.55^a	2.60 ± 0.55^a	3.00 ± 1.00^a	3.00 ± 0.71^a
Basophils (%)	0.40 ± 0.06^a	0.00 ± 0.00^a	0.60 ± 0.55^a	0.60 ± 0.55^a	0.20 ± 0.45^a

Values are presented as mean ± standard deviation (n = 5); and values with different superscripts are significantly (P < 0.05) different from any paired mean across the row.

However, it is important to note that the differences observed were generally small and may not be clinically significant. Further studies are needed to confirm the effects of *Irvingia gabonensis* extracts on white blood cell counts and to determine their potential therapeutic value.

Table 16 shows the results of an experiment in which different treatment groups were administered various doses of aqueous and ethanol extracts of a plant, and their effects on different types of white blood cells (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) were measured and compared to a control group.

The results show that the percentage of neutrophils in the control group was 52.20% ± 2.17%, while the groups treated with the aqueous extract at doses of 400 mg/kg and 800 mg/kg showed a slight increase to 52.60% ± 1.67% and 54.80% ± 1.64%, respectively, with the latter being significantly different from the control group (indicated by different letters a, b, or c). The group treated with the ethanol extract at a dose of 800 mg/kg showed the highest increase in neutrophil percentage to 55.80% ± 1.48%, which was also significantly different from the control group. This suggests that both the aqueous and ethanol extracts may have a mild stimulatory effect on neutrophil production.

For lymphocytes, the percentage in the control group was 38.80% ± 2.49%, and none of the treatment groups showed a significant change in this percentage, with values ranging from 37.40% ± 0.55% to 39.80% ± 1.92%. This suggests that neither the aqueous nor the ethanol extracts had a significant effect on lymphocyte production.

The percentage of monocytes in the control group was 5.20% ± 0.84%, and while the aqueous extracts at both doses did not show a significant effect, the ethanol extract at a dose of 5.40% ± 0.89% showed a slight increase in the percentage of monocytes, which was significantly different from the control group. This suggests that the ethanol extract may have a mild stimulatory effect on monocyte production.

For eosinophils, the percentage in the control group was 3.40% ± 0.55%, and the groups treated with the aqueous and ethanol extracts at both doses showed a slight decrease in percentage, with values ranging from 2.40% ± 0.48% to 2.80% ± 0.84%. However, these changes were not significant, indicating that the extracts did not have a significant effect on eosinophil production.

Finally, for basophils, the percentage in the control group was 0.40% ± 0.05%, and only the aqueous extract at a dose of 400 mg/kg showed a slight increase in percentage to 0.40% ± 0.08%, while all other groups had a percentage of 0.00%. However, these changes were not significant, indicating that the extracts did not have a significant effect on basophil production.

Overall, the results suggest that the aqueous and ethanol extracts of the plant may have a mild stimulatory effect on neutrophil and monocyte production, but did not have a significant effect on lymphocyte, eosinophil, or basophil production. However, further studies would be needed to determine the potential benefits and risks of using these extracts for medical purposes.

Table 16. Effect of *I. gabonensis* aqueous and ethanol leaf extract on white blood differential in female albino rats.

Treatment groups	Control	Aqueous extract (400 mg/kg)	Aqueous extract (800 mg/kg)	Ethanol extract (400 mg/kg)	Ethanol extract (800 mg/kg)
Neutrophils (%)	52.20 ± 2.17^{a}	$52.60 \pm 1.67^{a,b}$	$54.80 \pm 1.64^{b,c}$	$52.60 \pm 1.82^{a,b}$	55.80 ± 1.48^{c}
Lymphocytes (%)	38.80 ± 2.49^{a}	39.80 ± 1.92^{a}	38.00 ± 1.00^{a}	39.40 ± 2.41^{a}	37.40 ± 0.55^{a}
Monocytes (%)	$5.20 \pm 0.84^{a,b}$	$4.80 \pm 1.10^{a,b}$	4.20 ± 0.45^{a}	5.40 ± 0.89^{c}	$4.80 \pm 0.45^{a,b}$
Eosinophils (%)	3.40 ± 0.55^{b}	$2.80 \pm 0.84^{a,b}$	$2.60 \pm 0.52^{a,b}$	$2.60 \pm 0.55^{a,b}$	2.40 ± 0.48^{a}
Basophils (%)	0.40 ± 0.05^{a}	0.00 ± 0.00^{a}	0.40 ± 0.08^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values are presented as mean ± standard deviation (n = 5); and values with different superscripts are significantly ($P < 0.05$) different from any paired mean across the row.

Hepatic micrographs of the male and female rats treated with aqueous and ethanolic leaf extracts of *I. gabonensis*.

Male

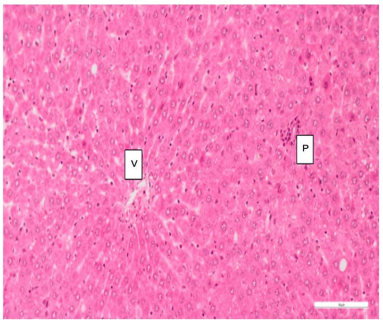


Plate 1. Normal control.

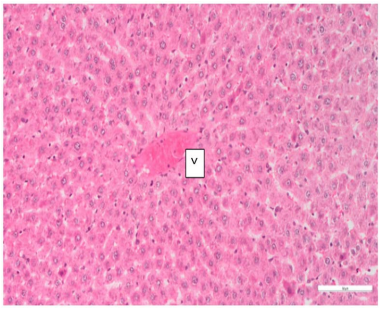


Plate 2. 400 mg/kg aqueous.

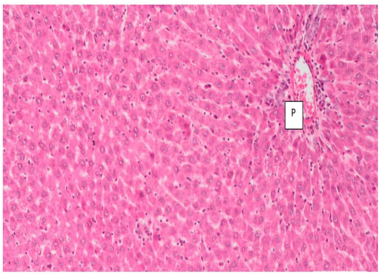


Plate 3. 800 mg/kg aqueous.

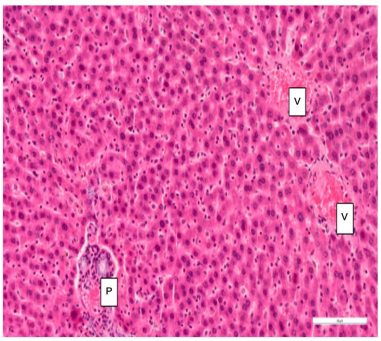


Plate 4. 400 mg/kg ethanol.

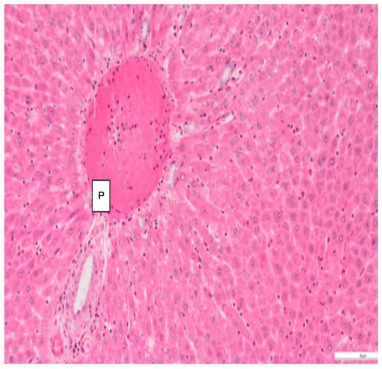


Plate 5. 800 mg/kg ethanol.

Female

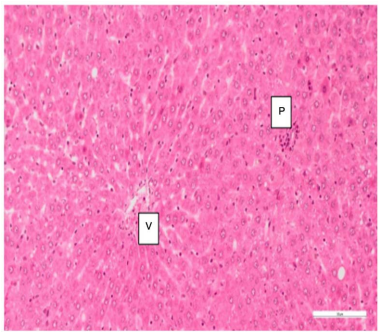


Plate 6. Normal control.

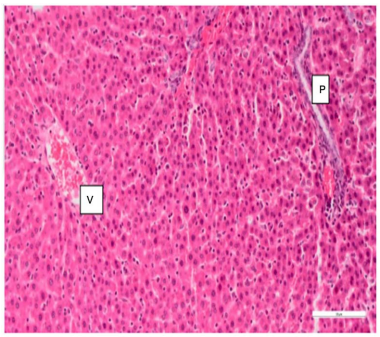


Plate 7. 400 mg/kg aqueous.

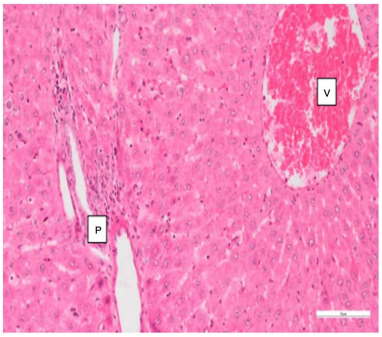


Plate 8. 800 mg/kg aqueous.

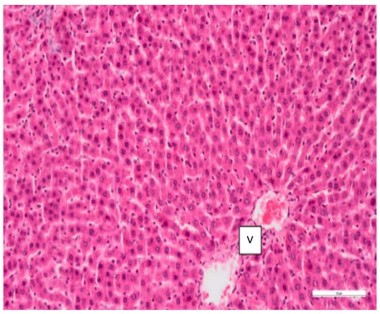


Plate 9. 400 mg/kg ethanol.

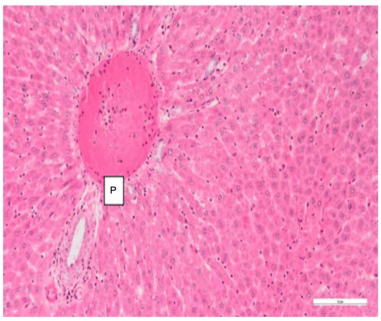


Plate 10. 800 mg/kg ethanol.

Plates 1-10 shows the hepatic micrographs of the male and female rats treated with aqueous and ethanolic leaf extracts of *I. gabonensis*. Sections of the liver presented in all the groups showed the normal hepatic histo-architecture or histomorphology. Normal hepatocytes arranged in interconnecting cords in a radial manner around the central veins (V) were observed. Normal structures of the portal areas (P) were also observed.

Renal micrograph of the male and female rats treated with aqueous and ethanolic leaf extracts of *I. gabonensis*.

Plates 11-20 shows the renal micrograph of the male and female rats treated with aqueous and ethanolic leaf extracts of *I. gabonensis*. Sections of the kidney presented in this group showed the normal renal histo-architecture or histomorphology. Normal glomeruli (G) surrounded by normal renal tubules (arrows were observed).

Male

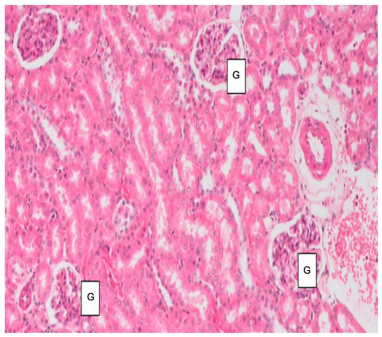


Plate 11. Normal control.

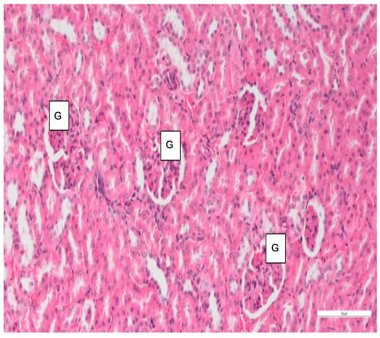


Plate 12. 400 mg/kg aqueous.

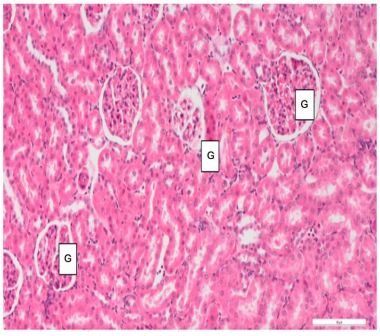


Plate 13. 800 mg/kg aqueous.

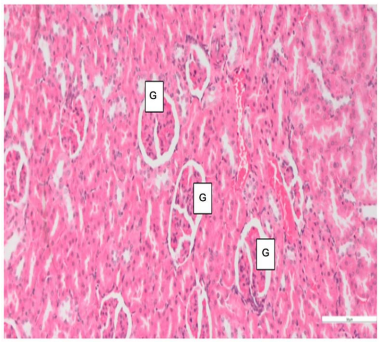


Plate 14. 400 mg/kg ethanol.

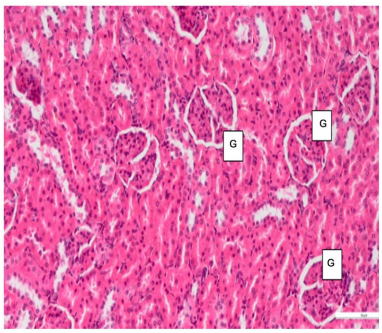


Plate 15. 800 mg/kg ethanol.

Female

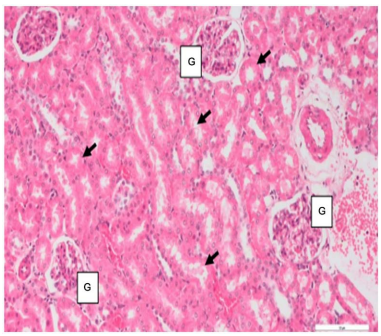


Plate 16. Normal control

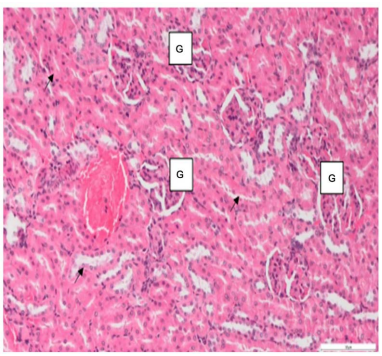


Plate 17. 400 mg/kg aqueous.

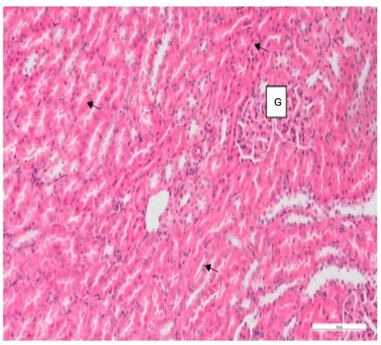
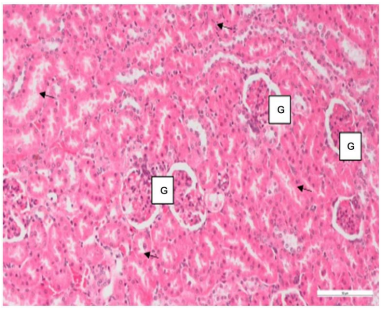


Plate 18. 800 mg/kg aqueous.



Plat 19. 400 mg/kg ethanol.

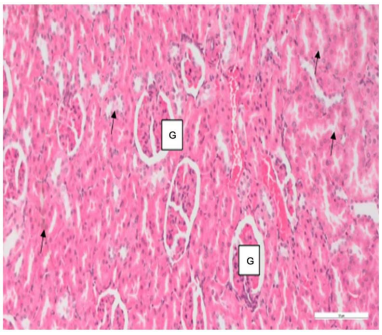


Plate 20. 800 mg/kg ethanol.

4. Discussion

Determining the safe drug dose for use in clinical or experimental settings in animals requires conducting acute toxicity studies [19]. It is well known that the extract's toxicity varies with the LD50 value; the higher the LD50 value, the lower the extract's toxicity [20]. The results of the male and female rats' acute oral toxicity tests at the grade doses of 10, 100, 1000, 1600, 2900, and 5000 mg/kg revealed no evidence of acute toxicity, behavioral changes, or mortality. This suggests that the rat LD50 was higher than 5000 mg/kg body weight. As a result, it is considered to be largely safe and non-toxic [21]. Variations in body weight are an accurate indicator of an animal's general health. Changes in body weight have been used to anticipate harmful effects of drugs and chemicals [22]. As shown in **Tables 1-4**, after 28 days of oral administration of the aqueous and ethanol leaf extracts of *I. gabonensis* at doses of 400 and 800 mg/kg body weight, it can be observed in that the treatment groups receiving the ethanol leaf extract of *I. gabonensis* displayed different effects on body weight compared to the control group. The group receiving a dose of 400 mg/kg showed a lower weight gain compared to the control group, while the group receiving a dose of 800 mg/kg exhibited the lowest weight gain.

These findings suggest that the ethanol leaf extract may have a potential impact on body weight regulation in male and female albino rats, with higher doses potentially leading to reduced weight gain. Further research and analysis would be required to understand the significance and underlying mechanisms behind these observed effects.

Organ weight is a vital marker of an animal's physiological and pathological condition. Relative organ weight is a more useful and sensitive indicator of toxicity than absolute organ weight because it connects the animals' overall health to each of their organs [23]. The results as shown in **Table 5** and **Table 6**, revealed that the relative organ weights of the male and female rats did not differ significantly from the control rats after 28 days of oral administration of the aqueous and ethanol leaf extract of *I. gabonensis* at doses of 400 and 800 mg/kg body weight. Given that weight loss is a sensitive indicator of toxicity, this suggests that the extract had no impact on the organs' functionality and could be regarded as non-toxic [24]. The results of Efosa, [22] who found no significant difference ($p > 0.05$) in the percentage change in weight and relative liver weights among all experimental groups, suggest that the extract is not toxic. This finding is consistent with their findings.

A liver function test measures the serum levels of bilirubin, albumin, ALT, AST, and ALP. The integrity of the liver is evaluated using biomarkers called liver enzymes. Significantly elevated serum levels of ALP, ALT, and AST as well as total proteins and albumin are associated with hepatic injury [25]. Aside from the liver enzyme biomarkers, low levels of albumin and total protein may indicate liver damage or disease. According to **Table 8**, there was a significant ($p > 0.05$) decrease in liver enzymes in the treated female rats when compared with the control. In this study, the liver was unaffected by the 28-day administration of *I. gabonensis* aqueous leaf extract at doses of 400 and 800 mg/kg body weight of the male and female rats. Although ALP in female rats slightly increased at a dose of 800 mg/kg body weight of the aqueous extract, these changes were still within the accepted normal laboratory reference range [26]. Granados-Echegoyen *et al.*, [27] suggesting that the extract of *I. gabonensis* may have hepatoprotective properties. However, after receiving 400 mg/kg and 800 mg/kg of aqueous and ethanol leaf extract, male and female rats showed a significant increase in total protein, albumin, and globulin as well as a significant decrease in bilirubin, showing that the plant extract not only improves serum protein concentrations but also has beneficial effects on liver conditions. Additionally, no lesions or pathological changes were seen in the liver's histopathology in the groups of treated males and females, indicating that the liver was not compromised (**Plate 1**).

Creatinine and urea are regarded as critical prognostic indicators of renal dysfunction and kidney failure for any toxic compound [28]. One of the many vital functions of the kidney is the maintenance of electrolyte balance. The electrolyte balance in human bodies is crucial for cells and organs to function normally [29]. The renal/kidney function can be assessed by measuring the concentrations of urea, creatinine, and electrolytes (sodium, chloride, and potassium) [30]. The loss of functionality and integrity of the renal system can be identified by a change in the serum concentration of urea, creatinine, and electrolytes [31]. Following administration of the aqueous and ethanol extracts to the male and female rats, the renal function as shown in **Table 9** and **Table 10**, shows a significant increase in (urea, creatinine, and chloride ion) when compared with the

control group, although still within the laboratory reference range, but no significant difference in sodium ion and bicarbonate ion. It's interesting to note that the potassium ion in the female rats slightly decreased when compared to the control. The decline was, nevertheless, within the reference and physiologically normal range [32]. Additionally, no lesions or pathological changes were found in the kidney's histopathology in any of the groups of treated males and females, indicating that the extract did not affect the kidney's physiological function at the tested doses (**Plate 2**).

A collection of clinical chemical tests called a lipid profile is used as the first step in a general medical screening for disorders of lipid metabolism. Total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), and triglyceride (TAG) imbalances are thought to be important in the lipid-related metabolic diseases as well as the risk of developing cardiac conditions like atherosclerosis, which can cause coronary heart disease, hypoxia, infarction, and necrosis [33]. Bad cholesterol, or low-density lipoprotein, is better for the heart in lower concentrations but can obstruct arteries in higher concentrations. HDL cholesterol, on the other hand, is good cholesterol that must remain within a range to safeguard the health of the heart. Additionally, it stops harmful cholesterol from accumulating on artery walls. Diabetes is frequently linked to high triglyceride levels, which indicate coronary heart disease [34]. As a result, high triglycerides will also result in higher levels of LDL and total cholesterol [34]. The results of the study on the lipid profile of male and female albino rats are shown in **Table 11** and **Table 12**. The results showed that the administration of *Irvingia gabonensis* leaf extract at the tested doses and times led to a dose-dependent decrease in the values obtained from TC, TAG, LDL-C, and VLDL-C, but intriguingly, the levels of HDL-C in the female rats decreased non-significantly. Aqueous and ethanol leaf extract from the African bush mango may have anti-atherogenic properties because it has higher levels of HDL-C and lower levels of TC, TAG, LDL-C, and VLDL-C. These results are consistent with those of Efosa [22], who demonstrated that African bush mango ethanol leaf extract has mitigating and anti-atherogenic effects in cadmium-induced hypolipidaemia in wistar albino rats.

The haematopoietic system is a vulnerable target for toxic substances, especially in the bone marrow, where RBC production occurs, and a critical indicator of physiological and pathological status in both humans and animals [35]. Haematological indices are frequently used as indicators of toxicity due to the interaction between a toxin and its potential metabolites on cellular components [36]. Free radical oxidative attack and invasion by foreign compounds regularly compromise these indices [37]. A rise in white blood cells (WBC) indicates the presence of oxidative stress, toxins, and free radicals. The administration of *Irvingia gabonensis* aqueous and ethanol leaf extract as shown in **Table 13** and **Table 14**, significantly elevated RBC, PCV, Hb, TWBC, and PLT levels in both male and female rats in this study when compared to the control rats. This suggests that the phytochemicals in the leaf extract encourage bone marrow's pro-

duction of blood cells. The significant decrease in MCV, MCH, and MCHC in the female treated rats may be a sign of hypochromic and microcytic anemia, in which the red blood cells are smaller than normal. According to research, the MCV, MCHC, and MCH can be in the low, normal, or high ranges even if the red blood cell count is normal. This might be caused by a lack of iron brought on by taking plant extracts.

The tables present the results of a study that investigated the effect of Irvingia gabonensis aqueous and ethanol leaf extracts on the haematological indices of male and female albino rats. The haematological indices measured include red blood cell (RBC) count, packed cell volume (PCV), haemoglobin (Hb) concentration, total white blood cell (TWBC) count, platelet (PLT) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

In Table 13, the results show that the aqueous and ethanol extracts of Irvingia gabonensis at various doses did not significantly affect the RBC count, TWBC count, and PLT count in male albino rats. However, the PCV, Hb concentration, MCV, and MCHC values increased with increasing dose of the extract. These results suggest that Irvingia gabonensis leaf extracts may have a positive effect on the erythrocyte and haemoglobin levels in male albino rats.

Similarly, Table 14 shows that the aqueous and ethanol extracts of *Irvingia gabonensis* did not significantly affect the RBC count and PLT count in female albino rats. However, the PCV, Hb concentration, MCV, and MCHC values increased with increasing dose of the extract. Notably, the MCV values were significantly higher in the control group compared to the treatment groups, which suggests that the extract may have a protective effect against anaemia in female albino rats.

The results suggest that *Irvingia gabonensis* leaf extracts may have a positive effect on the erythrocyte and haemoglobin levels in male and female albino rats.

Table 15 and Table 16 provide the white blood cell (WBC) differential counts in female and male rats treated with an extract. The tables indicate the relative proportions of different types of white blood cells, including lymphocytes, monocytes, basophils, neutrophils, and eosinophils. According to the results, there were no significant differences observed in lymphocytes, monocytes, or basophils between the treatment groups and the control group in both female and male rats. This suggests that the extract did not have a substantial impact on the levels of these particular white blood cells. However, there were significant findings in the differential counts of neutrophils and eosinophils. In female rats treated with the extract, there was a significant increase in neutrophils and a decrease in eosinophils compared to the control group. This indicates that the extract may have influenced the levels of these two types of white blood cells in female rats. In male rats, the results showed no significant difference in basophils and eosinophils compared to the control group. However, there were significant differences observed in lymphocytes and monocytes, as well as in the group treated with the higher concentration (800 mg/kg) of both the aqueous

and ethanol extract. This suggests that the extract may have had an impact on the levels of lymphocytes and monocytes in male rats, particularly at the higher dosage. The significance of these findings lies in the potential immunomodulatory effects of the extract. White blood cells play crucial roles in the immune system, and alterations in their proportions can reflect changes in immune response and overall health. The observed increase in neutrophils and decrease in eosinophils in female rats treated with the extract may indicate an enhanced inflammatory response or immune activation. Similarly, the significant differences in lymphocytes and monocytes in male rats suggest that the extract might have influenced the immune cell composition in this group.

The results obtained highlight the need for further investigation to identify the precise processes through which the extract affects white blood cell differentials. To clarify the extract's mechanism of action and investigate its potential therapeutic uses in the management of anemia and related disorders, more research is necessary. Furthermore, in light of the extract's potential as a therapeutic agent, it is critical to evaluate its immunomodulatory capabilities. The effects of the extract on the immune system should be better understood by conducting thorough assessments of its safety, efficacy, and wider implications. To learn more about how the extract affects immunological responses and immune system function generally, it would be beneficial to look into the functional implications of the observed changes in white blood cell differentials.

5. Conclusion

In conclusion, the evaluation of the aqueous and ethanolic leaf extract of *Irvingia gabonensis* on male and female albino rats showed no statistically significant changes ($p < 0.05$) in the body weight, organ weight, haematological, hepatic and renal indices. Additionally, the examination of liver and kidney histopathology revealed normal structure and function in both male and female treated groups. As such, the administration of the aqueous and ethanol leaf extract of *Irvingia gabonensis* at the tested doses appears to be safe for both male and female groups during acute and sub-acute treatment.

Conflicts of Interest

There is no conflict of interest declared.

References

[1] Jordan, S.A., Cunningham, D.G. and Marles, R.J. (2010) Assessment of Herbal Medicinal Products: Challenges and Opportunities to Increase the Knowledge Base for Safety Assessment. *Toxicology and Applied Pharmacology*, **243**, 198-216. https://doi.org/10.1016/j.taap.2009.12.005

[2] Benzie, I.F.F. and Wachtel-Galor, S. (2011) Herbal Medicine: Biomolecular and Clinical Aspects. 2nd Edition, CRC Press, Boca Raton.

[3] World Health Organization (2008) Traditional Medicine Fact Sheet. https://www.who.int/news-room/fact-sheets/detail/traditional-medicine

[4] Cragg, G.M. and Newman, D.J. (2013) Natural Products: A Continuing Source of Novel Drug Leads. *Biochimica et Biophysica Acta* (*BBA*)—*General Subjects*, **1830**, 3670-3695. https://doi.org/10.1016/j.bbagen.2013.02.008

[5] Yang, M., Zihuan, W., Yudan, W., Guoyin, K., Guy, S.S.N. and Shengbao, C. (2019) Acute and Sub-Acute Toxicity Evaluation of Ethanol Extract from Aerial Parts of *Epigynum auritum* in Mice. *Food and Chemical Toxicology*, **131**, Article ID: 10534. https://doi.org/10.1016/j.fct.2019.05.042

[6] Saad, B., Azaizeh, H., Abu-Hijleh, G. and Said, O. (2006) Safety of Traditional Arab herbal Medicine. *Evidence-Based Complementary and Alternative Medicine*, **3**, Article ID: 762642. https://doi.org/10.1093/ecam/nel058

[7] World Health Organization (2013) Traditional Medicine Strategy 2014-2023. P. 76.

[8] Ekor, M. (2013) The Growing Use of Herbal Medicines: Issues Relating to Adverse Reactions and Challenges in Monitoring Safety. *Frontiers Pharmacology*, **4**, Article 177. https://doi.org/10.3389/fphar.2013.00177

[9] Gandhare, B., Kavimani, S. and Rajkapoor, B. (2013) Acute and Subacute Toxicity Study of Methanolic Extract of *Ceiba pentandra* (L.) Gaertn. on Rats. *Journal of Scientific Research*, **5**, 315-324. https://doi.org/10.3329/jsr.v5i2.11800

[10] Sulaimon, A.O., Auta, T. and Hassan, A.T. (2015) Evaluation of Antidiabetic Activity of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Leaf and Bark in Alloxan Induced Diabetic Rats. *Biosciences Research in Today's World*, **1**, 84-89.

[11] Adamson, I., Okafor, C. and Abu-Bakare, A. (1990) A Supplement of Dikanut (Irvingia Gabonesis) Improves Treatment of Type II Diabetics. *West African Journal of Medicine*, **9**, 108-115.

[12] Gbadegesin, M.A., Adegoke, A.M., Ewere, E.G. and Odunola, O.A. (2014) Hepatoprotective and Anticlastogenic Effects of Ethanol Extract of Irvingia Gabonensis (IG) Leaves in Sodium Arsenite-Induced Toxicity in Male Wistar Rats. *Nigerian Journal of Physiological Sciences*, **29**, 29-36.

[13] Olorundare, O.E., Adeneye, A.A., Akinsola, O.A., Sanni, D.A., Koketsu, M. and Mukthar, H. (2020) *Clerodendrum volubile* Ethanol Leaf Extract: A Potential Antidote to Doxorubicin-Induced Cardiotoxicity in Rats. *Journal of Toxicology*, **2020**, Article ID: 8859716. https://doi.org/10.1155/2020/8859716

[14] Gannon, W.L., Sikes, R.S. and the Animal Care and Use Committee of the American Society of Mammalogists (2007) Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research. *Journal of Mammalogy*, **88**, 809-823. https://doi.org/10.1644/06-MAMM-F-185R1.1

[15] The Organization of Economic Co-Operation and Development (OECD) (2021) The OECD Guideline for Testing of Chemicals: 420-Acute Oral Toxicity. Paris.

[16] Jaijoy, K., Soonthornchareonnon, N., Lertprasertsuke, N., Panthong, A. and Sireeratawong, S. (2011) Acute and Chronic Oral Toxicity of Standardized Water Extract from the Fruit of *Phyllanthus emblica* Linn. *International Journal of Applied Research and Natural Products*, **3**, 48-58.

[17] Park, J.H., Choi, K.H. and Kwak, H.S. (2011) Single and 14-Day Repeat-Dose Toxicityof Cross-Linked β-Cyclodextrin in Rats. *International Journal of Toxicology*, **30**, 700-706. https://doi.org/10.1177/1091581811419678

[18] Rolls, G. (2011) Microtomy and Paratin Section Preparation. Leica Biosystem, Wetzlar.

[19] Gandhare, B., Kavimani, S. and Rajkapoor, B. (2013) Acute and Subacute Toxicity Study of Methanolic Extract of *Ceiba pentandra* (Linn.) Gaertn. on Rats.

Journal of Scientific Research, **5**, 315-324. https://doi.org/10.3329/jsr.v5i2.11800

[20] Gadanya, A.M., Sule, M.S. and Atiku, M.K. (2011) Acute Toxicity Study of "Gadagi" Tea on Rats. *Bayero Journal of Pure and Applied Sciences*, **4**, 147-149. https://doi.org/10.4314/bajopas.v4i2.29

[21] Adesegun, S.A., Celestina, I.O. and Coker, H.A. (2016) Analgesic and Antioxidant Activities of Stem Bark Extract and Fractions of *Petersianthus macrocarpus*. *Pharmacognosy Research*, **8**, 181-185. https://doi.org/10.4103/0974-8490.182912

[22] Efosa, G.E., Emmanuel, U. and Usunomena, U. (2016) Phytochemical Composition, *in-vitro* Antioxidant Activity and Acute Toxicity of *Irvingia gabonensis* (O'Rorke) Baill Ethanolic Leaf Extract. *International Journal of Biological Research*, **4**, 36-41. https://doi.org/10.14419/ijbr.v4i1.5939

[23] Nandy, S. and Datta, R. (2012) Acute and Sub-Acute Toxicity Studies of Methanol Leaves of *Pterospermium acerifolium* L. Willd in Rodents. *International Journal of Pharmacy and Life Sciences*, **3**, 1519-1529.

[24] Joth, S.L., Zakaria, Z., Chen, Y., Lau, Y.L., Ltha, L.Y. and Sasidharan, S. (2009) Acute Oral Toxicity of Methanolic Seed Extract of *Cassia fistula* in Mice. *Molecules*, **16**, 5268-5282. https://doi.org/10.3390/molecules16065268

[25] Waller, D.G. and Sampson, A.P. (2018) Medical Pharmacology and Therapeutics. 5th Edition, Elsevier, Edinburgh.

[26] Sabiu, S., Ajani, E.O., Abubakar, A.A., Nuran, O.I., Irondi, A.E., Quadri, D.F. and Abubakar, A.Y. (2015) Toxicological Evaluations of *Stigma maydis* (Corn Silk) Aqueous Extract on Haematological and Lipid Parameters in Wistar Rats. *Toxicology Reports*, **2**, 638-644. https://doi.org/10.1016/j.toxrep.2015.04.001

[27] Granados-Echegoyen, C., Perez-Pacheco, R., Alexander-Aguilera, A., Lagunez-Rivera, L., Alonso-Hernandez, N. and Chairez-Martinez, E.J. (2015) Effects of Aqueous and Ethanol Extract of Dried Leaves of *Pseudocalymma alliaceum* (Bignonaceae) on Haematological and Biochemical Parameters of Wistar Rats. *Asian Pacific Journal of Reproduction*, **4**, 129-134. https://doi.org/10.1016/S2305-0500(15)30009-9

[28] Sood, M.M., Saeed, M., Lim, V., Cordova, F., Komenda, P. and Malik, A. (2015) The Urea-to-Creatinine Ratio Is Predictive of Worsening Kidney Function in Ambulatory Heart Failure Patients. *Journal of Cardiac Failure*, **21**, 412-418. https://doi.org/10.1016/j.cardfail.2015.02.003

[29] Alhassan, A.J., Imam, A.A., Atiku, M.K., Ezema, M.D., Muhammad, I.U. and Idi, A. (2017) Acute and Sub-Chronic Toxicity Studies of Aqueous, Methanol and Chloroform Extracts of *Alstonia boonei* Stem Bark on Albino Mice. *Saudi Journal of Medicine*, **2**, 126-132.

[30] Rhiouani, H., El-Hilaly, J., Israili, Z.H. and Lyoussi, B. (2008) Acute and Sub-Chronic Toxicity of an Aqueous Extract of the Leaves of *Herniaria glabra* in Rodents. *Journal of Ethnopharmacology*, **118**, 378-386. https://doi.org/10.1016/j.jep.2008.05.009

[31] Oloyede, H. and Sunmonu, T. (2009) Potassium Bromate Content of Selected Bread Samples in Ilorin, Central Nigeria and Its Effect on Some Enzymes of Rat Liver and Kidney. *Food and Chemical Toxicology*, **47**, 2067-2070. https://doi.org/10.1016/j.fct.2009.05.026

[32] Isanga, J. and Zhang, G.N. (2007) Biologically Active Components and Nutraceuticals in Peanuts and Related Products. *Food Reviews International*, **23**, 123-140. https://doi.org/10.1080/87559120701224956

[33] Akara, E.U., Okezie, E., Ude, V.C., Uchenna-ikonne, C., Eke, G. and Ugbogu, A.E. (2021) *Ocimum gratissimum* Leaf Extract Ameliorate Phenylhydrazine-Induced

Anemia and Toxicity in Wister Rats. *Drug Metabolism and Personalized Therapy*, **36**, 311-320. https://doi.org/10.1515/dmdi-2020-0185

[34] Nigam, P. (2011) Serum Lipid Profile: Fasting or Non-Fasting? *Indian Journal of Clinical Biochemistry*, **26**, 96-97. https://doi.org/10.1007/s12291-010-0095-x

[35] Kifayatullah, M., Mustafa, M.S., Sengupta, P., Sarker, M.M.R., Das, A. and Das, S.K. (2015) Evaluation of the Acute and Sub-Acute Toxicity of the Ethanolic Extract of *Pericampylus glaucus* (Lam.) Merr. in BALB/c Mice. *Journal of Acute Disease*, **4**, 309-315. https://doi.org/10.1016/j.joad.2015.06.010

[36] Arika, W.M., Nyamai, D.W., Musila, M.N., Ngugi, M.P. and Njagi, E.N. (2016) Haematological Markers of *in Vivo* Toxicity. *Journal of Hematology and Thromboembolic Diseases*, **4**, Article ID: 1000236.

[37] Maduka, H.C.C., Okoyeb, Z.S.C. and Ejea, A. (2003) The Influence of *Sacoglottis gabonensis* Stem Bark Extract and Its Isolate Bergenin, Nigerian Alcoholic Beverage Additives, on the Metabolic and Haematological Side Effects of 2, 4-Dinitrophenyl Hydrazine-Induced Tissue Damage. *Vascular Pharmacology*, **39**, 317-324. https://doi.org/10.1016/S1537-1891(03)00042-9