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# Safety profile of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. root bark extract: Acute and sub-acute toxicity studies in Wistar rats

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**Background:** *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. has been widely prescribed in African traditional medicine system for the management of hernia, yellow fever, gastrointestinal, liver conditions and sterility, as well as for some other ethno-medicinal uses.

**Aim:** The study was to investigate the safety margins of ethanol extract of *I. gabonensis* root barks (EEIGRB) in Wistar rats.

**Setting:** This research is a toxicology investigation.

**Methods:** The acute and sub-acute toxicity studies conducted on the EEIGRB, according to the Organization for Economic Cooperation and Development (OECD) methods.

**Results:** The acute toxicity studies revealed that $LD_{50}$ was > 5000 mg/kg. In the sub-acute study, significant increase in body weights ($p < 0.05$) was observed at 200 mg/kg and 400 mg/kg in the weeks 2, 3 and 4 compared with week 0. There were no statistically significant ($p > 0.05$) changes in the haematological, hepatic and renal indices except for significant reduction ($p < 0.05$) in serum concentrations of sodium and creatinine at 400 mg/kg of EEIGRB compared with control group. Histopathological examination of the liver and kidney revealed that at 200 mg/kg, there was a slight hepatic necrosis in the liver and a slight tubular necrosis in the kidney, whereas at 400 mg/kg, there was a moderate foci necrosis in the liver and a slight glomerular distortion occurred in the kidney.

**Conclusion:** The results indicate that EEIGRB was found to be practically safe after acute administration, and there were histomorphological alterations in the liver and kidney after prolonged administration in the sub-acute dosages.

**Keywords:** *Irvingia gabonensis*; acute toxicity; sub-acute toxicity; Wistar rats; biochemical parameter.

## Introduction

The practice of traditional medicine and the use of medicinal plants in most developing countries as a normative basis for the maintenance of good health have been widely reported (Jordan, Cunningham & Marles 2010). Medicinal plants are the backbone of traditional medicine as the world population continually source plant products as their primary sources of medicine (Nunn 2002). Over 80% of Asian and African countries still rely deeply on medicinal plants for their primary health care needs (Yang et al. 2019). Many medicinal herbs are therapeutic at one dose and toxic at another, and with the growing demand for plant-based medicines, there are serious concerns for their use and safety (Saad et al. 2006; World Health Organisation 2013). Despite the increasing number of reports on the medicinal benefits of most plants, the *in vivo* toxicological implications of treatment with extracts from some of these plants are yet to be investigated (Ekor 2013; Gandhare, Kavimani & Rajkapoor 2013). Hence, there is need to carry out detailed toxicity studies to validate the safety of these herbal medicines in the short- and long-term use.

*Irvingia gabonesis* belongs to the family Irvingiaceae, and it is an indigenous fruit tree of West and Central Africa (Dienagha & Miebi 2011; Nangue et al. 2011). It is an economically important tree because of its wide use and is known by common names such as wild mango, African mango and bush mango because the tree bears mango-like fruit. The plant is called by different names in different languages of Nigeria: 'Pekpeara' in Nupe, 'Ogwi' in Bini, 'Ogbono/Ugiri' in Igbo, 'Uyo' in Efik, 'Oro' (tree) and 'Apon' (kernel) in Yoruba and 'Goron biri' in Hausa (Orwa et al. 2009). Traditionally, it has been reported to be useful in treating ailments such as hernia, yellow fever, gastrointestinal and liver conditions, sterility and

urethral discharge, and it is also considered to be an anti-poison agent (Ayuk et al. 1999; World Agroforestry Centre 2004). Bark shavings are used to relieve pain (Okolo et al. 1995) and stop diarrhoea and dysentery, and powdered chocolate prepared from the kernels is applied to burns (Irvine 1961). In an ethno-medicinal study carried out in Ilorin, Nigeria, it has been found that the root bark is also used as a fertility enhancer among males (Nuhu et al. 2018). Despite the ethno-medicinal uses of *I. gabonensis* in traditional medicine, there seems to be little or no report on the safe utilisation of the root bark. Therefore, this study was undertaken to investigate the safety margins of ethanol extract of *I. gabonensis* root bark (EEIGRB) in Wistar rats.

# Materials and methods

## Collection, identification and preparation of plant

*Irvingia gabonensis* was collected at Oke Egbo village, Ondo East local government area of Ondo State, Nigeria, in December 2017. The plant was identified by Mr Bolu Ajayi of the Herbarium Unit, Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, and a specimen voucher number (UILH/001/1364) was deposited. The root bark was peeled from the tree, cleaned and air-dried for 3 weeks, reduced to a powdery form and stored in an air-tight container for further use.

## Extraction of *Irvingia gabonensis* root bark

Two kilograms of powdered root bark of *I. gabonensis* was extracted with 5 litres (L) of 70% ethanol in a glass jar for 3 days (72 h) at room temperature by using the maceration technique. The extract was filtered, the filtrate was further concentrated by using a rotary evaporator at 40 °C and final evaporation to dry the extract was performed by using the water bath set at a temperature of 50 °C. The percentage yield of the extraction was 10.34% and coded as ethanol extract of *I. gabonensis* root bark (EEIGRB). It was stored in a desiccator for subsequent use.

## Qualitative phytochemical screening of ethanol extract of *I. gabonensis* root bark

The EEIGRB was subjected to qualitative phytochemical tests as described by Evans (2009) to detect the presence or absence of secondary metabolites.

## Experimental animals

Wistar rats of both sexes weighting 100 – 140 grams were sourced from the Animal Facility Units of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. They were managed in well-ventilated cages at room temperature under normal day and night cycle, kept on pelletised animal feed (Vital feed®, Jos) with access to water *ad libitum*.

## Acute toxicity studies

Acute oral toxicity-limit dose method was adopted by the Organization for Economic Cooperation and Development (OECD Test Guideline 425). Six rats were used; two rats were picked, weighed and given EEIGRB orally with a dose of 2000 and 5000 mg/kg body weight. The rats were observed for 30 min, 4 h and the 24 h for signs or symptoms of toxicity, before introducing EEIGRB to the remaining four rats. The observation included: changes in skin and fur, eyes and mucous membrane and respiratory and behaviour pattern. Animals were observed for signs and symptoms of toxicity and mortality for 14 days (OECD 2001).

## Sub-acute toxicity studies

The study was carried out following OECD (2008) 407 guidelines. Twenty-four Wistar rats were randomly divided into four groups of six rats each. Group 1, which served as the control, received distilled water of 1 mL/kg. The rats in groups 2, 3 and 4 were administered orally with EEIGRB at doses of 100, 200 and 400 mg/kg body weight, respectively, for 28 consecutive days by using the orogastric cannula. Rats were maintained under standard conditions with food and water *ad libitum* for the entire period with close observation for signs and symptoms of toxicity and mortality. On the 29th day, animals were anaesthetised under diethyl ether inhalation and then euthanised. Their organs and blood samples were collected for further investigations.

## Change in the weights of body and organs

The body weights of the animals were recorded at the beginning of the experiment and repeated at a 7-day interval until the termination of the experiment. Doses of EEIGRB administered were adjusted accordingly. On the 29th day, the rats were weighed; blood was pricked from the tail for haematological evaluation, before sacrificed. The animals' anatomical sections were dissected carefully to obtain the kidney, liver, heart, spleen and lungs. All organs were weighed and observed macroscopically. The relative organ–body weight (ROW) ratio of each rat was calculated as follows:

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on the of sacrifice (g)}} \times 100 \quad \text{[Eqn 1]}$$

## Haematological indices evaluation

The blood samples for the haematological tests were collected into vacuum tubes containing ethylene diamine tetra-acetate acid (EDTA) as anticoagulant and taken to the laboratory. Blood indices including white blood cells (WBCs), red blood cells (RBCs), haemoglobin (HB), platelets, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were analysed by using an automated haematology analyser (Cell-Dyn™ Abbott, United States [US]).

### Biochemical parameters evaluation

The blood samples for biochemical analyses were collected into plain universal bottles, allowed to clot and centrifuged at 3000 revolutions per minute (rpm) for 10 min. The serums obtained were analysed to estimate the effect of EEIGRB on biochemical indices by using a photoelectric colourimeter (AC-115 Optima, Japan). The enzymes parameters estimated were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, creatinine, urea and electrolytes (chloride, sodium, potassium and bicarbonate ions) as liver and kidney function tests performed by colourimetric method by using Randox assay kits and automated biochemistry analyser (Jaijoy et al. 2011; Park, Choi & Kwak 2011).

### Histopathology studies

The kidney and liver of the sacrificed rats were harvested and cut into 3-cm-thick slices and fixed in 10% formalin solution for sectioning. The fixed specimens were sliced, processed and embedded into paraffin blocks. The blocks were cut into 5-micrometre (µm)-thick, paraffin sections by a rotary microtome. The sections were stained with haematoxylin and eosin H and E for histological observations. The stained sections were finally viewed under a light microscope for morphological changes (Rolls 2011).

### Statistical analyses

Data obtained from the study were analysed by using Statistical Package for Social Sciences (SPSS), IBM version 20. Descriptive statistics were carried out to obtain the mean ± standard error of mean (SEM). Data on ROW and haematological, hepatic and renal indices were analysed by one-way analysis of variance (ANOVA), whereas those on body weight were analysed by using repeated-measures ANOVA and Bonferroni test for comparison over time. Statistically significant differences were considered at 95% and 99% confidence intervals ($p < 0.05$ and $p < 0.01$).

### Ethical consideration

The animals were treated following the standard guidelines for the Care and Use of Laboratory Animals and with an approval of the Animal Ethics and Care Committee, Ahmadu Bello University, Nigeria (ABUCAUC/2018/096).

## Results

### Qualitative phytochemical constituents

The phytochemical analysis of EEIGRB revealed the presence of saponins, anthraquinones, cardiac glycosides, flavonoids, tannins, alkaloids, steroids and triterpenes.

### Acute toxicity evaluation

The limit test results revealed that a single oral dose of EEIGRB did not cause mortality and signs of toxicity to the rats within 24 h and during the 14 days of observation period. It showed that the $LD_{50}$ was > 5000 mg/kg body weight in rats.

### Sub-acute toxicity studies

The oral administration of EEIGRB at doses of 100, 200 and 400 mg/kg body weight produced no observable obvious signs and symptoms of toxicity such as tiredness, weakness, convulsion, hyper-activeness, dullness, diarrhoea and diuresis throughout the 28 days of study period. All the animals survived throughout the experiment.

### Effect of 28 days of oral administration of ethanol extract of *I. gabonensis* root bark on body weight

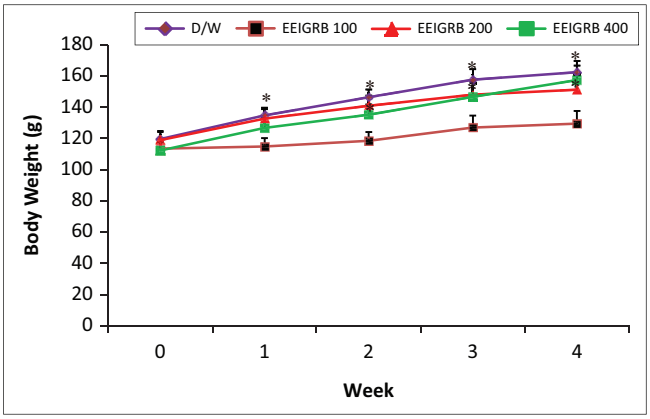
There was a progressive increase in the weight of the rats in all the groups over the 28 days. However, there was a significant increase in body weights at weeks 1, 2, 3 and 4 compared with week 0 in the control group at $p < 0.05$. Similarly, a significant increase in body weights was observed at 200 mg/kg and 400 mg/kg at weeks 2, 3 and 4 compared with week 0 ($p < 0.05$) as shown in Figure 1.

### Effect of 28 days oral administration of ethanol extract of *I. gabonensis* root bark on relative organ–body weights

The 28 days of oral administration of EEIGRB showed no significant ($p > 0.05$) change in ROWs when compared with the control group (Figure 2).

### Effect of 28 days of oral administration of ethanol extract of *I. gabonensis* root bark on haematological parameters

Oral administration of EEIGRB produced no significant ($p > 0.05$) changes in the tested haematological parameters when compared with the control group (Table 1).

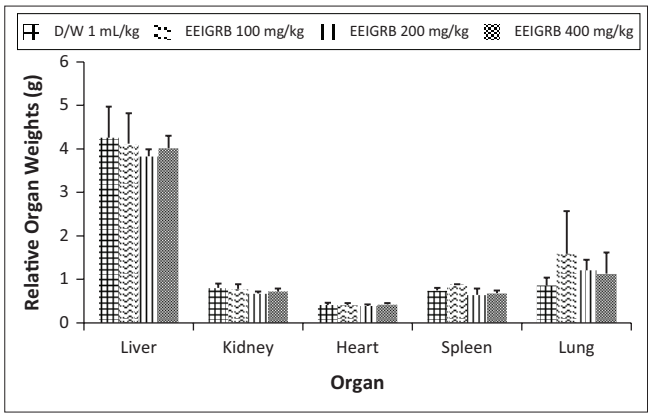



*n* = 6.

D/W, distilled water; EEIGRB, ethanol extract of *Irvingia gabonensis* root bark.

*, $p < 0.05$ compared with week 0.

**FIGURE 1:** Effect of 28 days of oral administration of ethanol extract of *I. gabonensis* root bark on body weights of rats. Values are presented as mean ± standard error of mean. Repeated measure analysis of variance followed by Bonferroni *post hoc* test.




*n* = 6.

D/W, distilled water; EEIGRB, ethanol extract of *Irvingia gabonensis* root bark.

**FIGURE 2:** Effect of 28 days of oral administration of ethanol extract of *Irvingia gabonensis* root bark on relative organ–body weights of rats. Values are presented as mean ± standard error of mean; there were no significant differences when compared with the control group (distilled water).

## Effect of 28 days of oral administration of ethanol extract of *I. gabonensis* root bark on hepatic indices

The 28 days of oral administration of EEIGRB did not produce significant ($p > 0.05$) changes in the ALT, AST, total proteins and albumin in treated groups, but it produced a significant increase ($p < 0.01$) in the ALP at 400 mg/kg when compared with the control group (Table 2).

## Effect of 28 days of oral administration of ethanol extract of *I. gabonensis* root bark on renal indices

A significant reduction in serum concentrations of sodium and creatinine at $p < 0.05$ was observed at 400 mg/kg group when compared with the control group (Table 3).

## Effect of 28 days oral administration of ethanol extract of *I. gabonensis* root bark on the histology of the livers and kidneys

Histological examination of the liver revealed a vascular congestion at a dose of 100 mg/kg and a slight hepatic necrosis at 200 mg/kg of EEIGRB. Moderate foci necrosis and lymphocyte hyperplasia were observed at 400 mg/kg of EEIGRB (Figure 3). Normal glomerulus and tubules were observed with the doses of 100 mg/kg. Slight tubular necrosis and slight glomerular distortion were observed at 200 and 400 mg/kg, respectively (Figure 4).

**TABLE 1:** Effect of 28 days of oral administration of ethanol extract of *Irvingia gabonensis* root bark on the haematological parameters of Wistar rats.

| Haematological indices | Treatment groups | | | |
|---|---|---|---|---|
| | D/W (1 mL/kg) | EEIGRB 100 mg/kg | EEIGRB 200 mg/kg | EEIGRB 400 mg/kg |
| WBC (×10⁹/L) | 4.83 ± 0.12 | 4.77 ± 0.49 | 4.63 ± 0.38 | 5.13 ± 0.41 |
| RBC (×10⁹L) | 6.00 ± 0.10 | 6.07 ± 0.12 | 6.00 ± 0.05 | 6.07 ± 0.12 |
| HB (g/dL) | 12.43 ± 0.23 | 13.23 ± 0.82 | 12.50 ± 0.44 | 13.80 ± 0.44 |
| PCV (%) | 37.67 ± 1.33 | 40.33 ± 2.60 | 37.00 ± 1.00 | 42.33 ± 1.45 |
| MCV (fL) | 88.40 ± 1.00 | 83.77 ± 2.53 | 88.13 ± 0.69 | 87.53 ± 0.03 |
| MCH (pg) | 29.53 ± 0.62 | 28.43 ± 1.38 | 29.87 ± 0.45 | 29.73 ± 0.55 |
| MCHC (g/dL) | 33.40 ± 0.57 | 33.47 ± 0.17 | 31.90 ± 1.15 | 31.67 ± 0.24 |
| Platelets (×10⁹L) | 226.33 ±40.99 | 191.00 ± 18.00 | 179.33 ± 5.78 | 175.67 ± 16.18 |
| LYMPH% | 60.30 ± 1.76 | 60.20 ± 3.04 | 55.23 ± 1.78 | 59.57 ± 1.37 |
| GRAN% | 35.27 ± 1.87 | 35.33 ± 1.82 | 39.70 ± 1.39 | 35.53 ± 1.83 |
| MID% | 4.73 ± 0.03 | 5.97 ± 0.49 | 5.10 ± 0.50 | 4.40 ± 0.75 |

*n = 6.*

Note: Values are expressed as mean ± standard error of mean. There were no significant differences between values for control and treatments groups (distilled water).

D/W, distilled water; EEIGRB, ethanol extract of *Irvingia gabonensis* root bark; fL, femtolitres; HB, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; Pg, picograms; RBC, red blood cells; WBC, white blood cells.

**TABLE 2:** Effect of 28 days of oral administration of ethanol extract of *Irvingia gabonensis* root bark on the hepatic indices of Wistar rats.

| Liver biomarkers | Treatment groups | | | |
|---|---|---|---|---|
| | D/W (1 mL/kg) | EEIGRB 100 mg/kg | EEIGRB 200 mg/kg | EEIGRB 400 mg/kg |
| ALT (IU/L) | 40.75 ± 1.03 | 38.00 ± 1.00 | 38.67 ± 2.72 | 47.67 ± 8.65 |
| AST(IU/L) | 242.50 ± 4.05 | 235.33 ± 7.69 | 241.00 ± 6.08 | 243.33 ± 6.77 |
| ALP (IU/L) | 35.78 ± 2.18 | 33.70 ± 4.16 | 30.80 ± 0.85 | 43.50 ± 3.36* |
| TP (g/dL) | 12.13 ± 0.54 | 10.87 ± 0.44 | 11.80 ± 1.03 | 12.50 ± 1.06 |
| Albumin (g/dL) | 2.93 ± 0.10 | 2.67 ± 0.09 | 3.00 ± 0.17 | 2.97 ± 0.17 |

*n* = 6.

Note: Values are expressed as mean ± standard error of mean.

ALB, albumin; ALP; alkaline phosphatase; ALT, alanine aminotransferase; AST; aspartate aminotransferase; D/W, distilled water; EEIGRB, ethanol extract of *Irvingia gabonensis* root bark; TP, total protein.

*, The mean difference is statistically significant ($p \leq 0.01$) compared with the control group (distilled water).

**TABLE 3:** Effect of 28 days of oral administration of ethanol extract of *Irvingia gabonensis* root bark on the renal indices of Wistar rats.

| Kidney biomarkers | Treatment groups | | | |
|---|---|---|---|---|
| | D/W (1 mL/kg) | EEIGRB 100 mg/kg | EEIGRB 200 mg/kg | EEIGRB 400 mg/kg |
| Urea (μmol/L) | 31.25 ± 2.99 | 34.43 ± 2.41 | 29.47 ± 1.47 | 37.77 ± 3.99 |
| Creatinine (μmol/L) | 1.03 ± 0.08 | 0.79 ± 0.06 | 0.73 ± 0.12 | 0.67 ± 0.03* |
| $Na^+$ (mmol/L) | 169.13 ± 3.57 | 171.47 ± 1.73 | 165.57 ± 3.83 | 145.30 ± 3.00* |
| $K^+$ (mmol/L) | 21.88 ± 1.46 | 20.00 ± 4.39 | 17.43 ± 1.71 | 20.37 ± 1.39 |
| $Cl^-$ (mmol/L) | 108.25 ± 6.80 | 105.00 ± 5.77 | 91.00 ± 2.00 | 91.33 ± 1.86 |
| $HCO^{3-}$ (mmol/L) | 24.00 ± 3.67 | 31.00 ± 1.15 | 30.00 ± 5.51 | 25.00 ± 1.73 |

*n* = 6.

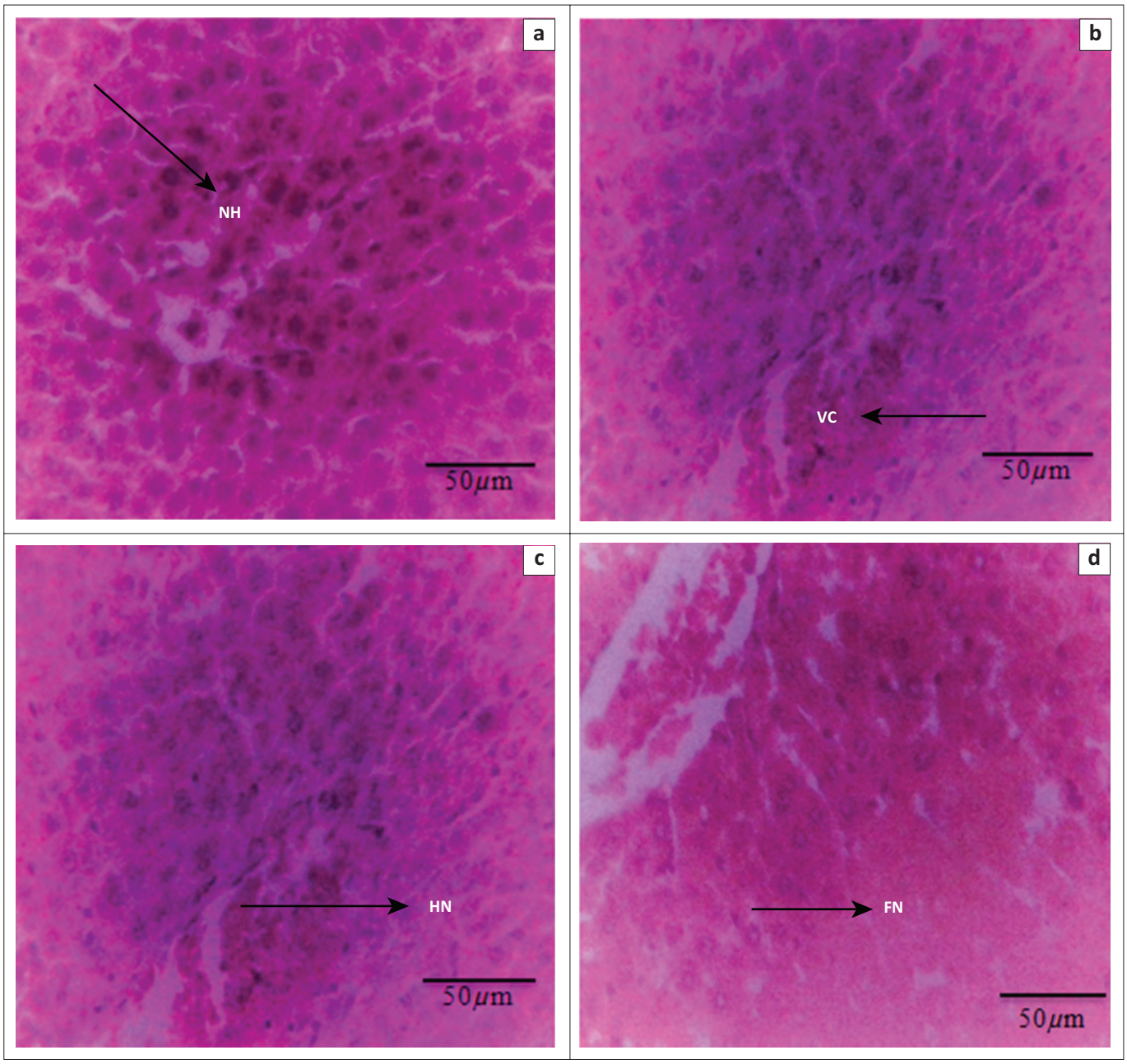
Note: Values are expressed as mean ± standard error of mean.

$Cl^-$, chloride ion; D/W, distilled water; EEIGRB, ethanol extract of *Irvingia gabonensis* root bark; $HCO^{3-}$, bicarbonate ion; $K^+$, potassium ion; $Na^+$, sodium ion.

*, The mean difference is statistically significant ($p \leq 0.05$) compared with the control group (distilled water).

# Discussion

Preliminary phytochemical screening gave a brief idea about the qualitative nature of active phytochemical constituents present in plant extracts, which will help the investigators in future regarding the selection of the particular extract for further investigation or isolating the active constituent(s) (Mishra et al. 2011). Different phytochemical agents can trigger the common mechanisms of toxicity at the level of organism, organ, tissue and cell (Rubino 2015). In this study, phytochemical screening revealed the presence of some secondary metabolites that are known to possess numerous pharmacological properties and may be responsible for various activities of EEIGRB. This result is in agreement with the findings of Ojo et al. (2014) who reported the presence of alkaloids, tannin, phlobatannins, saponin, flavonoids, anthraquinones, phenol and cardiac glycosides on the stem bark of *I. gabonensis*. The acute toxicity studies are important for determining the safe dose of drugs that can be used clinically or experimentally in animals



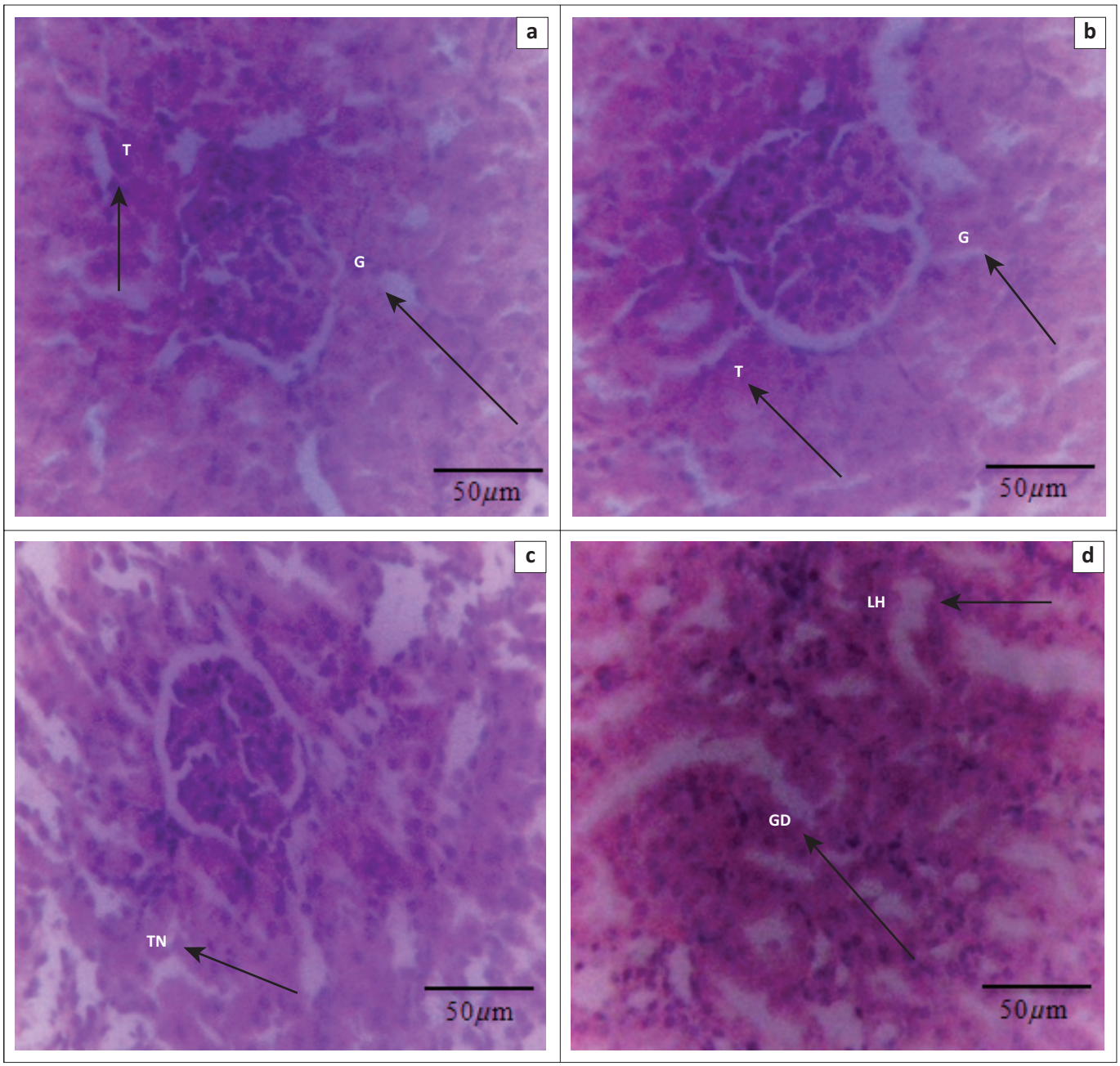

FN, foci necrosis; HN, hepatic necrosis; NH, normal hepatocytes; VC, vascular congestion.

**FIGURE 3:** Photomicrograph of the liver section of (a) control group rats (1 mL/kg distilled water) showing normal hepatocytes; (b) after a dose of 100 mg/kg of ethanol extract of *Irvingia gabonensis* root bark showing vascular congestion; (c) 200 mg/kg of ethanol extract of *Irvingia gabonensis* root bark showing a slight hepatic necrosis; (d) 400 mg/kg of ethanol extract of *Irvingia gabonensis* root bark showing a moderate foci necrosis (haematoxylin and eosin stain; original magnification ×400).

(Gandhare et al. 2013). The result for acute oral toxicity by using OECD guideline 425 limit tests for EEIGRB in rats showed no signs or symptoms of acute toxicity and mortality. This suggests that the $LD_{50}$ of EEIGRB was greater than 5000 mg/kg body weight in rats. Hence, EEIGRB is considered to be relatively safe and practically non-toxic (Adesegun, Celestina & Coker 2016). This result is in line with the finding of Efosa, Emmanuel and Usunomena (2016), who reported the acute toxicity of ethanol leaf extract of *I. gabonensis* to be above 5000 mg/kg body weight.

The body weight changes serve as a sensitive indicator of the general health status of animals. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Nandy & Datta 2012). In this research, there was a progressive increase in the body weight of the rats in all the groups over the 28 days and suggesting that the administration of EEIGRB did not exert any deteriorative effect on their normal metabolic processes relating to growth and development.

Organ weight is also an important index of the physiological and pathological status in animals. The relative organ weight is a more viable and sensitive index of toxicity than absolute organ weight as it relates the overall well-being of the animals to each of their organs (Joth et al. 2009). The 28 days of oral administration of EEIGRB showed non-significant changes in relative organ weight when compared with the control rats, which indicate that the extract did not alter the organs of




G, glomerulus; GD, glomerular distortion; LH, lymphocyte hyperplasia; T, tubules; TN, tubular necrosis.

**FIGURE 4:** Photomicrograph of the kidney section of (a) control group rats (1 mL/kg distilled water) showing the normal glomerulus and tubules; (b) after a dose of 100 mg/kg of ethanol extract of *Irvingia gabonensis* root bark showing normal glomerulus and tubules; (c) 200 mg/kg of ethanol extract of *Irvingia gabonensis* root bark showing slight tubular necrosis; (d) 400 mg/kg of ethanol extract of *Irvingia gabonensis* root bark showing lymphocyte hyperplasia and a slight glomerular distortion (haematoxylin and eosin stain; magnification ×400).

functionality and could be considered non-toxic because decreases in the weights are sensitive markers of toxicity (Waller & Sampson 2018).

The haematopoietic system is a susceptible target for toxic compounds and an important index of physiological and pathological status in man and animals, especially in the bone marrow where the production of RBCs occurs (Kifayatullah et al. 2015). Haematological indices are usually used as markers of toxicity because of the interaction between a toxin and its potential metabolites on cellular components (Arika et al. 2016). The non-significant changes in the haematological indices of the EEIGRB-administered groups relative to the control group in 28 days of administration suggest that it may be nontoxic to the haematopoietic system. However, there was a slight increase in the level of WBCs at 400 mg/kg, which suggest that the extract may contain a biologically active compound(s) that may have activated the immune system (WHO 2004). According to Webb et al. (2003) WBCs help to defend the body against infectious disease and foreign materials as part of the immune system.

Liver function tests involve evaluating serum ALT, AST, ALP, bilirubin and albumin levels. The most commonly used indicators of liver injury are the ALT and AST in the blood stream or plasma, which usually suggests chronic hepatitis or

biliary obstructions (Ramaiah 2011). In this study, 28 days of administration of graded doses of EEIGRB did not produce significant effects on the serum levels of liver enzymes, bilirubin and proteins in the experimental animals when compared with control. However, there was a significant increase in the activity of ALP at 400 mg/kg when compared with control. This increase suggests a hepatocellular injury. According to Hassan et al. (2015), increased ALP is a marker for injury to the liver, bone, leucocytes, kidney and intestine.

Creatinine and urea are considered as important prognostic markers of renal dysfunction and kidney failure for any toxic compound (Sood et al. 2015). Amongst the many important functions of the kidney is the maintenance of electrolyte balance. The balance of the electrolytes in human bodies is vital for the normal function of cells and organs (Alhassan et al. 2017). Electrolytes such as sodium and potassium are considered as the key extracellular and intracellular cations, respectively, in the body system (Hassanzadeh-Taheri et al. 2018). In this study, there was a significant ($p < 0.05$) decrease in the serum creatinine and sodium level after a dose of 400 mg/kg body weight of extract compared with the control group. The altered renal indices may result in impairment of renal tubular reabsorption and glomerular filtration, or excessive secretion of aldosterone hormones, leading to inability to absorb various ions and severe tissue oedema (Yang et al. 2019). This may be because of the phytochemical content in the EEIGRB such as alkaloids, tannins and saponins, which have been reported to be toxic to the liver and kidney (Yakubu & Musa 2012). This suggests that the extract could cause adverse effects on the functions of the kidney if given at high doses and for a long period, and renal function may have to be monitored on long-term administration of the extract. A significant decrease in serum creatinine level usually precedes a renal impairment and muscle wasting (Salawu et al. 2009).

Histopathological examination is one of the gold standards for evaluating treatment-related pathological changes in tissues and organs (OECD 2008). In this study, two vital organs (liver and kidney) were used to assess EEIGRB for toxicity. The liver and kidneys are crucial organs that perform a significant role in detoxification (Hariza et al. 2010). Histopathological examination of the liver revealed a slight hepatic necrosis at the medial dose (200 mg/kg) and a moderate foci necrosis at a high dose (400 mg/kg), which corroborates the finding from the hepatic function indices (Table 2). The histopathological examination of the kidney revealed a slight tubular necrosis at the medial dose (200 mg/kg) and lymphocyte hyperplasia and a slight glomerular distortion at a high dose (400 mg/kg), which corroborates the finding from renal function indices (Table 3) that showed a significant decrease ($p < 0.05$) in the activities of serum creatinine and sodium.

# Conclusion

The EEIGRB was found to be relatively safe after acute administration and showed mild toxicity after 28 days of repeated oral administration. However, EEIGRB was found to have hepatic toxicity and nephrotoxicity with associated histomorphological alterations in the liver and kidneys at an oral dose of 400 mg/kg for 28 days; therefore, prolonged oral administration of EEIGRB should be avoided because of the toxicity risk.

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## Competing interests

The authors have declared that no competing interests exist.

## Authors' contributions

A.N. designed and conducted the experiment and drafted the manuscript. E.M.A., U.H.D. and M.U.K. designed the experiment, gave directions and proof-read the manuscript. A.M.Z. and A.E.A. participated in the data analysis, edited and proof-read the manuscript..

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## Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

## Disclaimer

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