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Haematological and biochemical studies on *Justicia secunda* infusion used in treatment of anaemia

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Abstract

The effect of ethanolic and infusion of *Justicia secunda* leaves in the animal organs was investigated using standard analytical protocols. A total of 40 albino rats divided into four groups of five rats each comprising one normal groups and three groups treated with 125, 250 and 500mg/kg body weight respectively. The infusion of *J. secunda* leaves was prepared using conventional methods. The hematological, biochemical and histological studies were also carried out as reported in previous literatures. The findings confirmed that *Justicia secunda* leaves are not toxic at the doses administered, there were significant concentrations of essential minerals such as sodium, potassium, calcium, iron, phosphorus, and sulfur. The hematological study conducted on the infusion of the leaves revealed several positive effects on blood parameters in experimental rats. The extract significantly $P<0.05$ elevated hemoglobin and platelet count, particularly at higher doses, compared to the control group. The liver function tests showed no significant alterations in AST, ALT, TP, and ALB levels; however, the infusion resulted in some alterations in ALP activity and total bilirubin concentration. The histopathological examination of liver tissues showed mild congestion and degenerative changes in higher dose groups, indicating potential dose-dependent hepatomegaly and stress on hepatic architecture.

Keywords: *Justicia secunda*; Acute toxicity; Hematological study; Liver function parameter; Histopathological examination

1. Introduction

Anemia is defined as a reduction in the blood's capacity to transport oxygen, which can occur due to a decrease in the total number of erythrocytes (each containing a normal amount of hemoglobin), a lower concentration of hemoglobin per erythrocyte, or a combination of both factors [1]. It is a serious global public health issue, particularly in developing countries in Africa such as Nigeria, where it is linked to increased risks of morbidity and mortality. In countries with endemic malaria, anemia is among the most common preventable causes of death in children under five years of age and in pregnant women [2], posing a significant threat to global health [3]. This condition is characterized by hemoglobin levels falling below 13 g/dl in males or 12 g/dl in females [4].

Medicinal plants have long been recognized for their beneficial properties in managing various ailments, primarily due to their bioactive components known as phytochemicals [5] and secondary metabolites, which can protect against diseases [6]. Examples of plants and fruits documented for their use in treating anemic conditions include Cocos nucifera (Benin Republic) [7], leaf extracts of Tectona grandis (Togo) [8], and *Justicia secunda Vahl* in Benin [9]. While synthetic drugs are commonly available for treating specific diseases, their high cost and associated side effects have shifted attention towards the use of medicinal plant products for the prevention and management of various ailments [10] [11]. The genus *Justicia*, named after the 18th-century Scottish botanist James Justice, belongs to the large family Acanthaceae, which consists of about 600 species of herbs and shrubs native to the tropics and subtropics [12]. *Justicia secunda* is a flowering plant widely distributed across various parts of Africa. In Nigeria, the shrubs of *J. secunda* are

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commonly grown around homesteads and used as fences, as they are easy to grow and propagate from stem cuttings by pushing the stems 1 to 2 inches into the soil [13]. A survey among the Igbo local populace in Nigeria revealed that the plant is locally called "ogwu obara," meaning blood tonic. The deep purple juice from the leaves of this plant is extracted either by soaking or boiling in water and can be consumed beverages or tea. Traditionally, several species of *Justicia* are used in the management of anemia, inflammation, gastrointestinal disorders, respiratory tract infections, fever, pain, diabetes, diarrhea, liver diseases, rheumatism, and arthritis, and are noted for their antioxidant and cardioprotective properties [12] [14] [15] [16]. However, there is no documented scientific and experimental evidence on the use of *Justicia secunda* leaves in modulating lipid profiles in experimental animals. Therefore, this study was designed to ascertain both its hematological and biochemical status in rats treated with *J. secunda* leaves.

Figure 1 *Justicia secunda* plant

Figure 1 depicts the plant studied, which is a flowering evergreen perennial herb, shrub, or subshrub with more or less woody stems. The leaves exhibit epidermal cell wall outgrowths known as cystoliths and feature a leaf stalk (petiole) with full blade margins. The terminal or axillary flowers are conspicuous, with bracts and bracteoles that are equally noticeable and arranged in an overlapping manner.

2. Material and methods

2.1. Plant materials

Fresh leaves of *J. secunda* were collected from Obinze in the Owerri west Local Government Area of Imo state and were authenticated by Mr. Francis Iwunze of the Department of Forestry and Wildlife, School of Agriculture and Agricultural Technology, Federal University of Technology Owerri (FUTO).

2.2. Preparation of Extracts

Infusion of *Justicia secunda***:** Leaves of *Justicia secunda* were prepared by boiling 1000ml of water at 100^{o}C, the ground leaves of 100g weight of *Justicia secunda w*ere soaked in the water for 30mins and the resulting infusion were filtered to obtain the extract. Reddish brown slurry were obtained and were stored at 4^{o}C until further use for hematological, biochemical and histopathological analysis.

2.3. Sub-Acute Toxicity Study

A total of forty male Swiss albino rats were utilized for the sub-acute toxicity study. The rats were divided into four groups, each containing ten rats. The groups were administered different doses of the extract for a duration of twenty-eight days: 0 mg/kg (control), 125 mg/kg.b.wt, 250 mg/kg.b.wt, and 500 mg/kg.b.wt. After the 28-day administration period, the animals were sacrificed, and blood samples were collected for various hematological, biochemical and histopathological analyses. This study was conducted based on the modified method described by Onoja et al., 2017.

2.4. Sample Collection

On the 28th day of the study, blood samples were collected from the animals using the method described by [18], with modifications. Blood samples were collected in sterile Eppendorf tubes by inserting a heparinized capillary tube just below the eyeball of the animals. The animals were treated humanely during blood collection process, initially inducing sleep by placing them in glass jars containing cotton balls soaked with isoflurane. After 2.5 minutes, the animals were euthanized by returning them to airtight glass jars containing cotton balls soaked with isoflurane to effect death within 2 minutes. Any deceased rats were then buried in a nearby bush.

2.5. Acute toxicity study

The acute toxicity study was conducted following the method described by [19]. Twenty-one albino mice weighing between 20-35g were utilized for this study. The animals were randomly grouped based on their body weight. Prior to the study, the animals were fasted overnight but provided with access to portable drinking water ad libitum. The extract was administered orally, and the acute toxicity study was conducted in two phases. **Phase 1**: Twelve rats were subdivided into three groups, each containing three animals. These groups were administered doses of Normal, 10, 100, and 1000 mg/kg body weight of the extract, respectively. The animals were observed for 24 hours for signs such as paw licking, salivation, stretching of the entire body, weakness, respiratory distress, coma, and mortality within the first 4 hours. Subsequently, they were observed daily for 14 days. **Phase 2:** Twelve mice were randomly divided into four groups, each containing three rats. These groups were administered extract doses of 1000, 1600, 2900, and 5000 mg/kg body weight, respectively. The doses were determined based on deductions from the phase 1 study. Similar to phase 1, the animals were observed for signs of toxicity and mortality for the first critical 4 hours and then daily for 14 days. LD_{50} Calculation: After conducting the acute toxicity study, the LD_{50} value was calculated using the formula: $LD_{50} = \sqrt{}$ (Maximum tolerated dose × Minimum toxic dose). The LD_{50} represents the median lethal dose and is calculated as the square root of the product of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

2.6. Elemental Analysis using Atomic Absorption Spectrometry (AAS)

Sample digestion was determined according to [20].The mineral compositions were identified using FS240AA agilent atomic absorption spectrophotometer using the method described by [21].

2.7. Haematological Analysis:

Blood samples were subjected to haematological analysis, including measurements of hematocrit, total red blood cells (RBCs), total white blood cells (WBCs), lymphocytes, neutrophils, monocytes, eosinophils, and basophils, following the method described by [22]

2.8. Determination of Full Blood Count (FBC)

The hematology studies involved, determining the full blood count (FBC) using the Sysmex K-21n automatic multi-parameter blood cell counter. Here's how it works:

2.8.1. Principle

The Sysmex K-21n employs volumetric impedance method to measure various blood parameters. Blood cells, including white blood cells (WBC), red blood cells (RBC), and platelets, are counted using direct current (DC) detection method. Hemoglobin (HGB) concentration is measured using a non-cyanide method. Specially formulated reagents cause WBC membrane to shrink around the nucleus, allowing separation of white cells according to their volume.

2.8.2. Procedure

- The machine undergoes self-check and background check before starting the analysis.
- Once ready, the sample number is set, and the sample is introduced to the sample probe.
- The analysis is initiated by pressing the start switch.
- The machine counts the blood cells and measures hemoglobin concentration.
- Results are displayed on the liquid crystal display (LCD) screen and printed onto a ticket format.
- The machine becomes ready for the analysis of another sample.
- After completing the work, the check after analysis and shutdown procedures were executed before turning off the power.

2.9. Biochemical analysis

For the biochemical tests, the blood samples collected were first centrifuged and the serum were used for testing as described previously in the literatures[23] [24] [25] using kits from RANDOX, Diamond Road Crumlin, Co. Antrim, United Kingdom. For the haematological and random blood glucose tests, whole blood was used without centrifugation. It was Random Blood glucose Test in the sense that the animals were feed normally in the morning and blood collection and testing were done later in the day.

2.10. Histopathological Study:

Tissue microscopy was performed immediately after blood sample collection on liver organ. Tissue sections, with a thickness of 5 microns, were prepared and fixed in 10% formalin before embedding in paraffin for histopathological analysis. The tissues were stained with hematoxylin and eosin (H & E) and examined under a light microscope (Olympus CHO_2) for any alterations compared to normal structures, following the method described by [26].

2.11. Statistical analysis

The results were expressed as Mean ±SD. Data were analyzed using one-way analysis of variance (ANOVA) using SPSS version 20.0. Differences between means were considered to be significant at ($p < 0.05$) using the post hoc test (Least Square Difference).

3. Results and discussion

3.1. Acute toxicity study of *Justicia secunda*

The acute toxicity study (LD_{50}) of the aqueous leaf extract of *Justicia secunda* leaves on mice (Table 1) showed that no deaths were recorded amongst the test animals, even at 5000 mg/kg.

Table 1 No. of animal with observed parameter/no. of animals used

Treatments								
	Phase 1				Phase 2			
Behavioral Response	**Control**	**10**	**100**	**1000**	**1000**	**1600**	**2900**	**5000**
Diarrhea	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Salivation	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Hyperactivity	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Aggression	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Lethargy	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Respiratory changes	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Tremors/convulsion	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Coma	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Death	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

LD_{50} > 5000 mg/Kg body weight

3.2. Mineral compositions of *Justicia secunda*

The mineral contents presented in Table 2 shows that the ethanolic extract of *Justicia Secunda* leaves was rich in minerals. It contained high concentration of iron (39.57±0.00) while calcium (8.67±0.00), selenium (7.68±0.00), potassium (6.79±0.00), magnesium (6.28±0.00), sodium (5.68±0.00) manganese (5.52±0.00), iodine (4.98±0.00), zinc (4.34±0.00) were moderately present, while molybdenum, cadmium, arsenic and boron were not found in the plant extract.

Table 2 Mineral composition of *Justicia secunda*

Mineral	**Composition (ppm)**
Iron	39.57±0.00
Selenium	7.68±0.00
Calcium	6.87±0.00
Potassium	6.79±0.00
Magnesium	6.28±0.00
Sodium	5.68±0.00
Manganese	5.52±0.00
Iodine	4.98±0.00
Zinc	4.34±0.00
Chromium	1.30±0.00
Copper	1.13±0.00
Arsenic	0.00±0.00
Cadmium	0.00±0.00
Boron	0.00±0.00
Lead	0.00±0.00
Molybdenum	0.00 ±0.00

Table 3 Effect of *Justicia secunda* infusion on hematological parameters

Groups	**Control**	**125mg/kg**	**250mg/kg**	**500mg/kg**
WBC(x10*3/ul)	6.66±0.80	10.17±1.59*	10.74±1.81*	11.88±1.52*
LYM (%)	86.46±4.63	81.39±15.83	80.36±16.53	88.09±5.65
NEU (%)	12.82±2.40	8.78±0.64	9.35±2.23	8.66±1.01
MON (%)	0.54±0.26	2.12±0.82	1.94±0.60	2.10±0.47
BAS (%)	1.13±0.90	0.60±0.7	0.75±0.13	0.79±0.12
RBC(10^6/µl)	6.01±0.36	6.43±0.41	6.65±0.64	6.66±0.66
HGB (g/dl)	11.72±0.54	12.66±0.42	12.76±1.11	13.46±1.58
HCT (%)	35.36±1.76	35.16±1.84	38.58±2.38*	40.50±2.12*
PLT(10^3/µl)	538.00±11.06	688.20±56.65*	704.20±51.90*	720.20±28.49*
MCV(µmˆ3)	59.08±1.39	54.78±2.78	58.16±2.32	55.68±3.00
MCH (pg)	19.58±0.54	19.12±0.33	19.22±0.42	19.18±0.85
MCHC (g/dl)	33.18±0.48	33.48±2.70	33.08±1.07	33.54±0.37
RDW (µmˆ3)	15.48±0.51	14.34±1.46	17.06±1.10	16.06±0.69
PDW(%)	15.70±0.25	15.38±0.16	15.40±0.10	15.40±0.32
PCT (%)	0.44±0.09	0.51± 0.08	0.55 ±0.07	0.55± 0.03

PLCC	78.80 ±13.83	81.80 ±6.37	87.00± 3.08	91.40 ±7.09**
PLCR(%)	12.69± 0.92	12.80 ±1.90	14.00± 2.79*	15.40± 1.74*

Data are presented as mean ± SEM in n = 3 determinations. Values with asterisk (*) between groups indicate significant difference ($p < 0.05$) compared to control. WBC white blood cell count: LYM lymphocyte count: GRAN granulocyte Count: RBC red blood cell count: HGB hemoglobin: PLT platelet count:PCV packed cell volume: MCV mean corpuscular volume: MCH mean corpuscular haemoglobin and MCHC mean corpuscular haemoglobin concentration

From table 3, there was a significant difference ($p < 0.005$) in WBC, HGB, and PLT levels in the rats treated with 125 mg/kg, 250 mg/kg, and 500 mg/kg (10.17%, 12.66%, 538.88%; 10.74%, 12.76%, 704.20%; and 11.88%, 13.46%, 720.20% respectively) when compared to the control groups (6.66%, 11.72%, and 538.00%). There was also a significant difference ($p > 0.005$) in RBC levels in the treated groups with 125 mg/kg (6.43%), 250 mg/kg (6.65%), and 500 mg/kg (6.66%) when compared to the control group (6.01%). There were no variations in LYM, NEU, MON, MCV, and MCHC levels in the treated rats at doses of 125 mg/kg, 250 mg/kg, and 500 mg/kg when compared to the control groups.

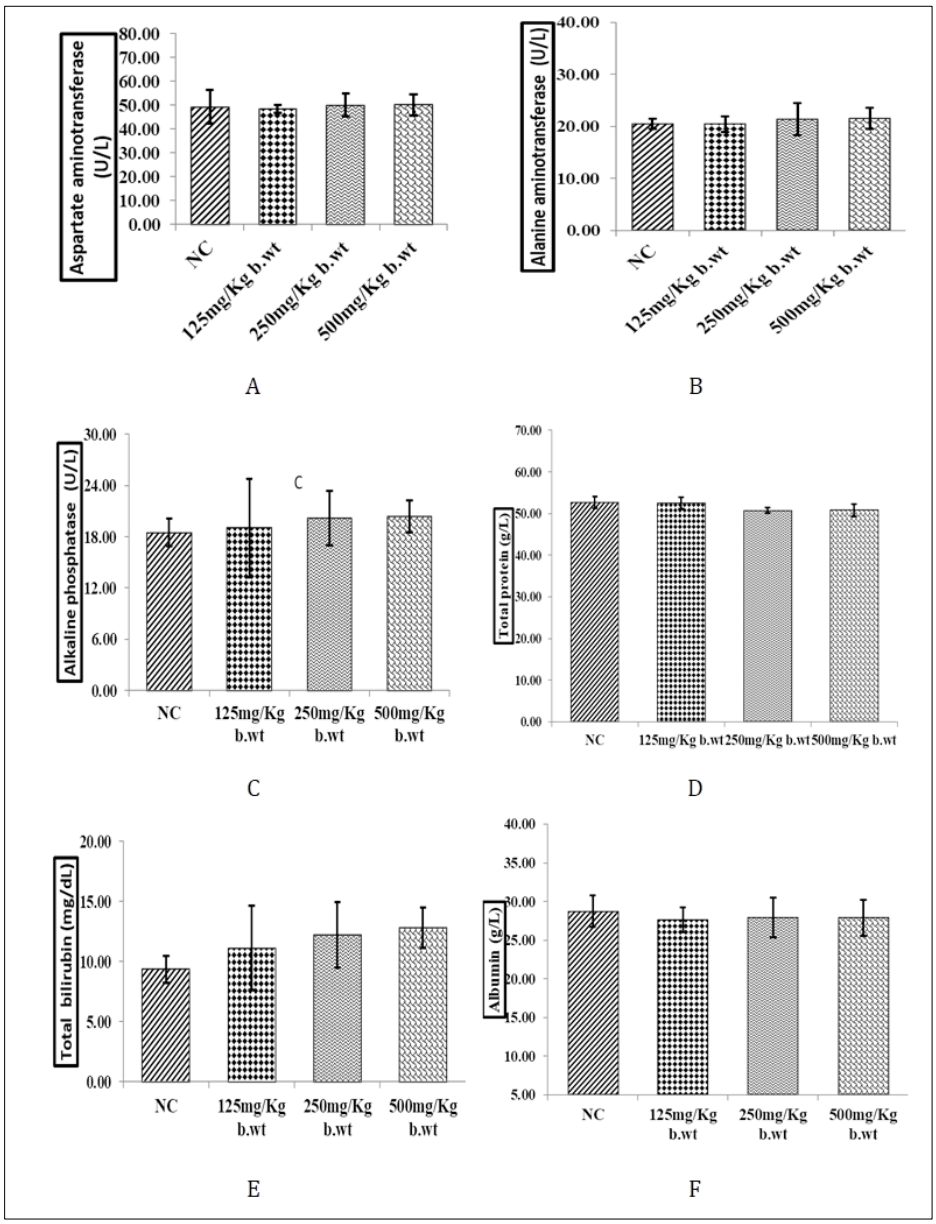

Figure 1 Liver Marker Enzymes and function parameters

Liver function test (liver enzymes assay). (A), Aspartate amino transferase (B) Alanine amino transaminase, (C). Alkaline phosphatase, (D) Total Protein, (E) Total Bilirubin, (F) Albumin

The plant extracts did not significantly increase (P value > 0.05) the levels of liver enzymes Aspartate amino transferase (AST) and alanine transaminase (ALT) in the treated rats. However, there was a significant increase in alkaline phosphatase (ALP). The results of the liver function tests indicate that the plant extract has no adverse effect on the treated rats when compared with the control groups. The plant extracts a significant increase (P value > 0.05) in total bilirubin in the treated rats, no variation in protein and albumin when compared to the control groups.

3.3. Histopathological examination of liver

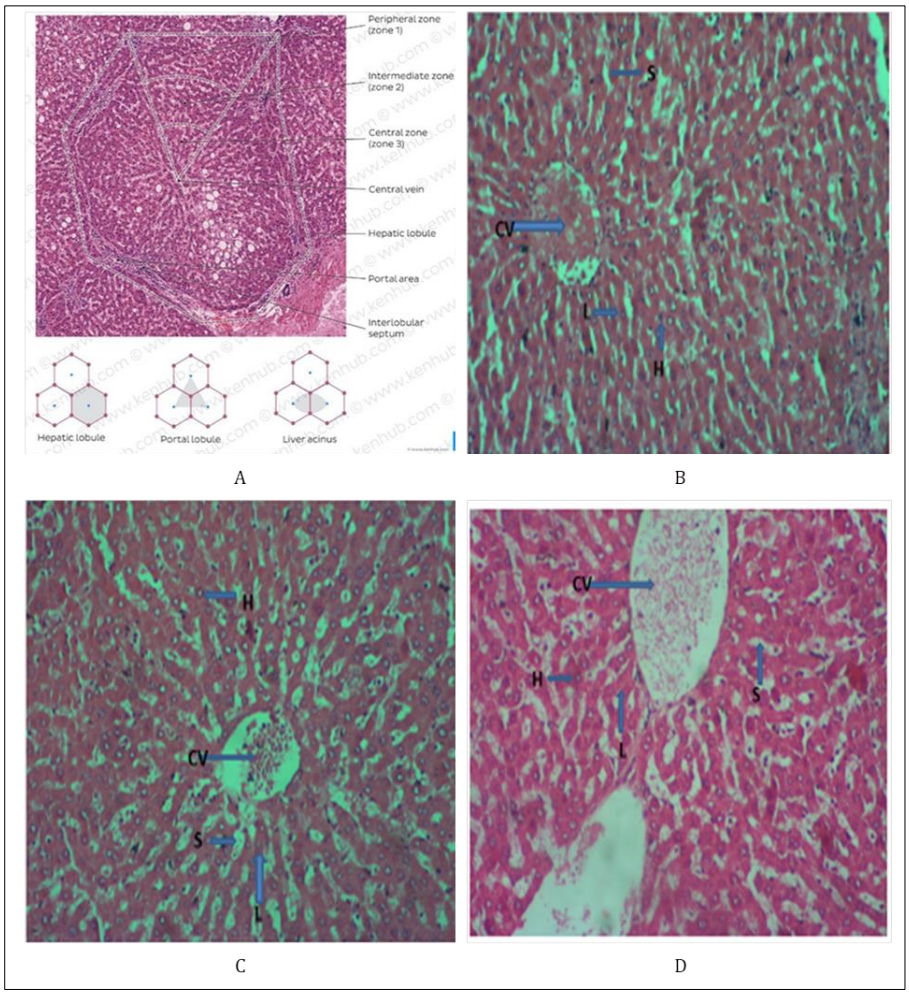

Figure 2 A-D Histopathology Plates of Liver in Control and Experimental Rats

3.4. Plate A: Transverse Section of Liver (Control) H&E Stain ×400

Plate A (normal) depicts a normal central vein (CV), lamella (L), sinusoids (S), and hepatocytes (H). No pathological changes are observed at ×400 magnification. Stain: H&E. Plates B, C, and D: Histological Sections of Liver in Rats Administered 125, 250, and 500 mg/kg bw. of infusion of *Justicia secunda*: These plates show the liver sections of rats treated with 125 mg/kg (Plate B), 250 mg/kg (Plate C), and 500mg/kg (Plate D) body weight of infusion of *Justicia secunda*. They revealed an enlarged central vein (CV) containing blood clot, congestion with aggregates of red blood cells, and slightly distorted stromal arrangement. The lamella (L), sinusoids (S), and hepatocytes (H) are well identified at ×400 magnification. Stain: H&E.

4. Discussions

The pictorial representation of *Justicia secunda* leaves used in this study is depicted in Figure 1. The acute toxicity study conducted on *Justicia secunda* leaves revealed no observed deaths or significant adverse effects in the animals treated

with the infusion. Throughout the 14-day duration of the study, there were no observable differences in body changes between the treated and control animals, indicating a lack of toxicity. Additionally, macroscopic observations showed no apparent changes in appearance between the treated and control groups. The LD_{50} (lethal dose for 50% of the test population) was not calculated because no deaths were recorded, but it was estimated to be greater than 5g/kg. LD_{50} values greater than 5,000 mg/kg are generally considered of no practical toxicological interest, indicating that *Justicia secunda* leaves were non-toxic at the doses administered. The goals of toxicity studies included determining doses that caused major adverse effects, identifying possible target organs of toxicity, and elucidating important clinical signs attributable to exposure to high doses of the test substance. Macroscopic observation of the animals treated with the infusion showed no apparent change in appearance (colour and size) of the animals when compared with the control group. This indicates that the leaves of *Justicia Secunda* were not toxic to the animals at the doses administered. This findings confirmed that the *Justicia secunda* leaves used were not toxic at the doses administered, providing reassurance regarding their safety for consumption or use in various applications. The elemental analysis of *Justicia secunda* leaves shown in table 2 revealed significant levels of beneficial minerals, including iron, calcium, and selenium. These minerals play essential roles in various physiological functions and contribute to overall health and well-being. Iron is a crucial mineral involved in the formation of hemoglobin, which carries oxygen in the blood. The high iron content in *Justicia secunda* leaves in this study made it a valuable dietary source for individuals at risk of iron deficiency or anemia. Additionally, the presence of iron in *Justicia secunda* leaves may justify its traditional use as a haematinic plant, particularly among populations where iron deficiency is prevalent. The value obtained for iron in this study was higher than iron content of *Justicia gendarussa* (0.15ppm), *Centella asiatica* (0.21ppm), *Strobilantes crispa* (0.14ppm), and *Murraya koenigii* (0.15ppm) as reported by [27]. Most of iron in the body is housed within hemoglobin and the distinctive red color of blood is attributable to the presence of Fe in the hemoglobin. Iron is as well an important component of myoglobin. Fe concentration in the body is roughly 3 to 4 grammes, which is virtually equivalent to a concentration of 40 to 50 milligrammes of iron per kilogramme of body weight [28]. From this work the iron content of the leaf was higher than the iron present on *Justicia secunda* (1.99±0.08ppm) and *Jatropha tanjorensis* (1.67±0.06ppm) as reported by [29]. Calcium is essential for bone formation, blood coagulation, muscle function, and nerve impulse transmission, and vitamin B_{12} absorption [30]. The presence of calcium in *Justicia secunda* leaves indicated its potential role in supporting bone health, muscle function, and overall mineral balance in the body. Adequate intake of calcium is essential for preventing bone disorders such as osteoporosis and osteomalacia, as well as maintaining overall health and well-being. Selenium is an important component of antioxidant enzymes such as glutathione peroxidase and thioredoxin reductase. Its presence in *Justicia secunda* leaves suggested its potential role in antioxidant defense mechanisms and protecting cells from oxidative damage [31]. Selenium's antioxidant properties may contribute to the overall health benefits associated with consuming *Justicia secunda* leaves. Sodium (Na) is crucial for maintaining acid-base balance and regulating osmotic pressure in body fluids. Its presence in *Justicia secunda* leaves may contribute to overall electrolyte balance and help prevent conditions such as dehydration and stunted growth. In this study, sodium present was lower 6.48 ± 0.10ppm and 6.99 ± 0.12ppm as it was reported by [29] on *Justicia secunda* and *Jatropha tanjorensis* respectively. Potassium (K) is essential for carbohydrate metabolism, muscle and nerve function, and maintaining proper acid-base balance. Its presence in *Justicia secunda* leaves may support these physiological processes and help prevent weakness and paralysis. Magnesium plays a critical role in enzyme activation and is involved in numerous biochemical processes in the body, including protein synthesis, muscle and nerve function, bone formation, and blood pressure regulation [32]. Its presence in *Justicia secunda* leaves may support these functions and help prevent symptoms of magnesium deficiency, such as lethargy. The recommended dietary allowance (RDA) for magnesium is 400 and 320 mg/day for healthy adult males and females respectively [33]. Zinc is necessary for haemoglobin synthesis and erythroid differentiation, making it essential for healthy red blood cell production. Its presence in *Justicia secunda* leaves may support these processes and help prevent conditions such as anaemia. Manganese is important for the synthesis of mucopolysaccharides and plays a role in bone formation, sex hormone production, connective tissue health, and blood clotting [34]. Its presence in *Justicia secunda* leaves may support these functions and help prevent symptoms of manganese deficiency, such as stunted growth and leg deformities [35]. Table 3 summarizes the hematological analysis of *Justicia secunda* infusion, revealing elevated hemoglobin and platelet counts, with a significant increase observed at higher extract doses compared to the control. These results was in line with previous studies demonstrating the potential anti-anemic properties of *Justicia secunda* leaf extract in anaemia-induced experimental rats [36]. Similar blood-stimulating effects have been reported for other medicinal plants such as *Xylopia aethiopica* [37], *Tectona grandis* [38], and extracts *of M. indica, A. hybridus*, and *T. occidentalis* [39], attributed to bioactive constituents that stimulate hematopoietic cell activity and stabilize blood circulation [36]. White blood cell (WBC) counts typically increase in response to pathogens, enhancing the body's defense mechanisms [40]. WBC differentials, including granulocytes (neutrophils, basophils, eosinophils) and non-granulocytes (lymphocytes, monocytes), provide specific insights into immune system activity. Our study demonstrated a significant increase in WBC differentials, especially in the test groups, with notable differences in basophil and neutrophil counts compared to the control which is contrary to [41] report. Increased WBC and lymphocyte counts, particularly at the 500 mg/kg body weight concentration, suggest potential leucopoietic and immunomodulatory effects of the *Justicia secunda* leaf extract, enhancing WBC and

lymphocyte production[42][43]. These findings indicate that the extract does not induce toxicity or sub-acute inflammation, supporting overall immune defense and immunity in experimental rats. Moreover, the elevated levels of hematocrit (HCT) and platelet counts by the extract suggest enhanced macrophage formation and pathogenic scavenging roles. The significant increase in platelet counts further implies potential benefits in preventing excessive blood loss through improved blood clotting mechanisms and resistance to capillary membrane leakage [44]. Notably, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in the treated groups showed no significant alterations compared to the control, consistent with previous findings [41], indicating a lack of macrocytic or hypochromic anemia induced by the extract. The analysis of the effects of *Justicia secunda* on AST, ALT, ALP activities, total protein, albumin, globulin, and bilirubin concentration provides valuable insights into the impact of the plant infusion on liver function as shown in figure 2. There were no significant difference observed in AST and ALT activities between the treatment groups 125mg/kg (48.37±1.57IU/L), 250mg/kg (50.08±4.90 IU/L), 500mg/kg (50.18±4.45 IU/L) and the control group (49.32±7.00 IU/L). This implies that the plant extract is not likely to cause liver injury, which is in agreement with [45] [46]. AST and ALT are cytosolic enzymes, and elevated levels in serum can indicate cellular leakage and loss of cell membrane integrity in the liver [47]. However, in this study, the levels of AST and ALT did not show any indication of liver injury. The result of this study shows a significant increase in ALP activity in the treated groups: 125mg/kg (19.05 ±5.74 IU/L), 250 mg/kg (20.45±3.20 IU/L) and 500mg/kg (20.40±1.87 IU/L) compared to the control group (18.52±1.64 IU/L). ALP is a membrane-bound enzyme and a marker of liver function [48]. Increased ALP activity can indicate structural damage to hepatic cells. The elevated ALP levels in the treatment groups suggest some level of cellular damage. This study also reported a significant increase in total bilirubin concentration in the treated rats 125mg/kg (11.14±3.49 mg/dL), 250mg/kg (12.27±2.72 mg/dL), and 500mg/kg (12.85±1.67 mg/dL) compared to the control group (9.37±1.13 mg/dL). Bilirubin is a metabolic breakdown product of blood and is excreted by the liver. Increased levels of bilirubin can indicate liver dysfunction or blockage of the biliary tract. The elevated bilirubin levels observed in the treated rats suggest potential impairment in liver function relative to hemoglobin breakdown [49]. This study found no significant effect on serum total protein in the doses of 125mg/kg, 250mg/kg, 500mg/kg (52.48±1.43 g/L), (50.77±0.66 g/L), (50.78±1.52 g/L) and albumin (27.62±1.62 g/L), (27.95±2.58 g/L), (27.89±2.30 g/L) levels in the treatment groups compared to the control group (52.54±1.41 g/L) (28.58±2.05 g/L) respectively. Total protein is used to assess liver function and disease progression, while albumin, primarily produced in the liver, is a major carrier protein in the bloodstream. The unchanged levels of total protein and albumin suggest no significant impact on liver function or disease progression in the treated rats. However, since total protein levels remained unchanged and albumin levels were unaffected. The findings of this work show that there were no significant decreases in liver marker enzyme activities, indicating that the infusion of *Justicia secunda* leaves has relatively no hepatoxic effects on liver functions. The high levels of phenols and flavonoids found in this ethanol extract could protect liver cells from the adverse effects of reactive oxygen species/free radicals generated from leakages in the electron transport chain and other exogenous sources [50]. The histological examination of liver samples in this study revealed significant findings across different groups. In the normal, untreated rats, the liver structure appeared typical with a normal central vein, lamella (L), sinusoids (S), and hepatocytes, and no pathological changes were observed. However, in the groups treated with *Justicia secunda* leaf infusion at concentrations of 125 mg/kg, 250 mg/kg, and 500 mg/kg, several changes were noted. There was an enlarged central vein containing blood clots and congestion of aggregated red blood cells at concentration of 125 mg/kg Group. The stromal arrangement was slightly distorted. Despite these changes, the lamella, sinusoids, and hepatocytes were still identifiable. Similar to the lower dose group of 250 mg/kg, this group showed an enlarged central vein with blood clots and slightly distorted stromal arrangement. Additionally, the lamella, sinusoids, and hepatocytes began to show signs of degenerative changes. The changes were more pronounced at 500 mg/kg Group, with further enlargement of the central vein, increased blood clot presence, and more evident degenerative changes in the lamella, sinusoids, and hepatocytes. These findings suggest that the administration of *Justicia secunda* leaf extract led to hepatomegaly (enlargement of the liver) in a dose-dependent manner. This is in line with the report by Ajayi, *et al.,* 2023), indicating potential underlying liver conditions when the extract is used repeatedly. Hepatomegaly can be indicative of various liver issues, including infections (such as hepatitis), tumors, abnormal accumulations of fats, iron, copper, and proteins, occlusion of bile ducts, and fluid-filled pockets in the liver [52]. The slight distortion in the stromal arrangement and the degenerative changes in the liver's cellular components suggest metabolic disruptions. Given the liver's critical role in numerous metabolic processes, the introduction of substances with unknown concentrations can potentially lead to these observed changes [53].

5. Conclusion

The result of these findings confirmed that *Justicia secunda* leaves were not toxic at the doses administered, providing reassurance regarding their safety for consumption or use in various applications. It has beneficial minerals which highlights its nutritional value and potential health benefits. Incorporating *Justicia secunda* into the diet can help provide essential minerals needed for optimal health and well-being. It also suggested a relatively low risk of hepatotoxicity

associated with the use of the extract, however, there was an increase level of the liver enzyme assay (ALP and TB) which could be as a result of the liver being responsible for most of the metabolic activities that occur in the body, and the use of substances with unknown concentrations is increasing. The histopathological analysis demonstrates that *Justicia secunda* leaf infusion can induce structural changes in the liver, indicating possible metabolic disturbances and highlighting the need for caution in its use and further investigation into its long-term effects.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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