



Effect of aqueous and ethanol leaves extracts of *Justicia carnea* on selected biochemical and haematological parameters in Albino Mice

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Open Access Research Journal of Science and Technology, 2022, 06(01), 018–027

Publication history: Received on 03 June 2022; revised on 19 August 2022; accepted on 21 August 2022

Article DOI: <https://doi.org/10.53022/oarjst.2022.6.1.0048>

Abstract

This study investigated the effect of aqueous and ethanol leaves extracts of *Justicia carnea* on selected biochemical and haematological parameters in albino mice. LD50 for the ethanol extract was carried out using the guidelines proposed by organization for Economic Cooperation and Development (OECD). Twenty four mice were divided into six groups of four mice/group. Groups 1 and 2 received distil water and 3%DMSO respectively as normal control, groups 3 and 4, 5 and 6 received 500 and 1000mg/kgbw of aqueous and ethanol leaves extracts respectively for six days. Mice were sacrificed 24 hours after the sixth day of treatment and blood sample collected for biochemical and haematological investigations. Result of quantitative phytochemical constituent of the leaves revealed the presence of alkaloids (1.01×10^{-1}), terpenoids (8.55×10^{-1}), saponin (449.43), sterols (30.58), phenolic acids (38.73), glycosides (39.27), flavonoids (430.24), tannin (744.24) mg/100g, oxalate (102.11) and phytate (12.37) ppm. Aqueous leaves extract showed significant ($p \leq 0.05$) increase in PCV, Hb and platelet values, while others were non-significant while ethanol extract was non-significant ($p \geq 0.05$) in all haematological parameters when compared to control. Significant decrease ($p \leq 0.05$) was observed in AST, ALT activities and Total bilirubin concentration in all the groups for aqueous extract and non-significant differences ($p \geq 0.05$) in all liver markers for all ethanol extract treated groups when compared to control. Aqueous and ethanol extracts of *Justicia carnea* did not have any toxic effect on the experimental animals however aqueous extract boosted haemoglobin level.

Keywords: *Justicia carnea*; Acute toxicity; Phytochemicals; Flavonoids; Bilirubin

1. Introduction

Over past decades, herbal or alternative medicine has gained public interest because of their easy accessibility, effectiveness, affordability and acceptability [1, 2]. Detailed information about the ethnopharmacological properties of these plant are revealed through standard experimental methods which accounts for the use of these medicinal plant in an effective way [3, 4]. An estimation done by World Health Organization revealed that 80% of the population of developing countries use traditional medicine in primary medicinal problem. Several evidence have shown immense potential of medicinal plants for prevention, diagnosis and treatment of various diseases [5, 6]. Herbal plants are used regardless of their toxicity and pharmacological properties. The toxicity of these herbal plants is unknown and the world's population considers it safe for consumption [7] though it forms the base in drug discovery [8]. There is limited information about the pharmacology and toxicology for the commonest herbal plant used in traditional medicine. Therefore, there is need for toxicity profiling of herbal plants [9] to maximize their benefits for mankind [10]. Toxicology is an important aspect of pharmacology which deals with the adverse effect of bioactive substance on living organism before use as drug or chemical in clinical use [11] while toxicity is the science of poison. Phytochemical interactions of poison lead to injury or death of living tissues [12] *Justicia carnea* was named after a Scottish horticulturist, James Justice in the 18th century which belongs to the Acanthaceae family [13]. The flowering plant is a native of South America

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precisely Brazil and widely distributed in Africa even Nigeria. It is a perennial shrub with a characteristic pink to purple or orange flower colour which blooms all through the summer. *Justicia carnea* functions mostly as ornamental plant [14]. It grows 3-4 feet wide and a height of 1.8m [15]. It is propagated by pushing 1-2 inches of the stem into the soil and kept until new leaves appear [16]. The ethnopharmacological properties of several species of *Justicia* include respiratory, gastrointestinal disorder, inflammation including application in rheumatism and arthritis, headache and fever which maybe is associated with sedative and analgesic properties, cancer, diabetes and HIV [13, 17]. Characterization based on GC-MS analysis of ethanol extract of *Justicia carnea* suggested the presence of isonicotonic acid N-oxide, phosphinodithioic acid, palmitic acid, 7H-purine, 7-benzyl-2,6-dichloro,9,12,15-octadecatrien-1-ol and 2,2,3,3,4,4,5,5,5-Nonafluoro-pentanoic acid methyl ester[18]. The haematological and lipid modulatory effect of aqueous and ethanol extract of *Justicia carnea* yielded a positive effect as described by Orjiakor[19], Onyeabo *et al.* [20]. According to Alozie *et al.* [21], aqueous extract of *Justicia carnea* demonstrated considerable antiplasmodial activity. Presently, the study determined the oral median lethal dose of ethanol leaf extract of *Justicia carnea* as well as its adverse effect on liver histopathology, haematological and biochemical parameters.

2. Material and methods

2.1. Source and identification of plant materials

Fresh leaves of *Justicia carnea* were harvested from Obinze and Obiti communities in Owerri-west and Ohaji local government areas respectively, Imo state, Nigeria. The plant sample was identified and authenticated by Dr. Ekeke Chimezie at the herbarium unit of the department of plant science and biology (PBS), University of Port Harcourt. The specimen was registered with voucher number UPH/PSB/2017/055.

2.2. Source of experimental animal

60 albino mice of both sexes weighing 20g-30g were used. They were acquired from the Animal House, Department of Biochemistry, and University of Port Harcourt. They were acclimatized for 7 days in standard cages under standard conditions under controlled temperature of 20°C and optimum humidity before use and placed on standard feed and given access to water ad libitum.

2.3. Plant extraction

Fresh leaves of *Justicia carnea* were harvested, washed with clean water and air-dried under a shade for seven days. The dried leaves were pulverized into coarse powder. The powdered leaves weighed 2.5kg each, which was macerated in distilled water and absolute ethanol (99.9%) at room temperature for 24 and 72 hours respectively. The powdered leaf extract was filtered using Whatman filter paper and the filtrate was condensed and evaporated to dryness using a rotary evaporator and water bath at 50°C. The residue weighed 75g and 50g respectively and were stored in air tight containers in a refrigerator until when needed.

2.4. Phytochemical screening

Quantitative phytochemical studies of leaf extract of *Justicia carnea* was determined using high performance liquid chromatography (HPLC) and Gas Chromatography to determine its constituent using standard procedures.

2.5. Acute oral toxicity study

The acute oral toxicity studies were determined using the guidelines proposed by Organization for Economic Cooperation and Development (OECD). The test was carried out using standard procedures as described by Ghosh.²¹ Twelve experimental animals were randomly divided into four groups of three mice per group. The extract was administered orally with single graded dose at 1250, 2500, 3750 and 5000mg/kg body weight of *Justicia carnea* using intubation canula. The treated groups were observed for toxicity, mortality for 3 hours and subsequently for the next 24 hours and no death was recorded at 5000mg/kgbw. Close attention was given to the animal for the next 6 days. The acute oral toxicity study for the aqueous leaf extract was determined using lorke's method as described by Orjiakor[19], Alozie *et al.*[22]

2.6. Experimental design

Twelve adult mice of weight range 20-30g were divided into three groups of four rats per group. Group 1 received mice feed and water only while groups 2 and 3 were treated orally with 500 and 1000mg/kg bw of aqueous leaf extract of *Justicia carnea* respectively for three days. Another set of twelve adult mice of weight range 20-30g were divided into

three groups of four rats per group. Group 1 received mice feed and water only while groups 2 and 3 were treated orally with 500 and 1000mg/kg bw of ethanol leave extract of *Justicia carnea* respectively for three days.

2.7. Blood sample collection

Experimental animals were sacrificed 24 hours after the third day of treatment through cervical dislocation. The thoracic region was opened up revealing the heart and blood was collected by cardiac puncture. Blood samples were collected in ethylene diamine tetraacetic acid (EDTA) bottles for haematological studies and heparinized bottles for biochemical studies.

2.8. Hematological investigation

Component of haematological parameter such packed cell volume (PCV) was determined using haematocrit method, white blood cell count (WBC) was estimated using haemocytometer. Differential white cell count was determined using the method described by Osim *et al.* [23] The erythrocyte sedimentation rate was estimated.

2.9. Biochemical assays

Components of biochemical assay such as total bilirubin was estimated using colometric method as described by Jendassik and Grof [24] the activities of aspartate transaminase, alanine transaminase and alkaline phosphate were determined using standard commercial kits from Randox as described by Reitman and Frankel [25]. Albumin concentration was estimated using bromocresol green method while total protein concentration was determined using biuret method.

2.10. Histological techniques

Histological investigation was determined on the organ (liver) which was harvested from the mice, immediately after sacrifice and fixed in 10% formolsaline. The organs were placed in ascending concentration of alcohol for dehydration to occur; the organs were embedded in molten paraffin wax, then mounted on a rotary microtome to obtain thin section of 5micron and 15micron. Staining was done using aqueous dyes (Haematoloxylin and eosin) and viewed under a microscope.

2.11. Statistical analysis

Data were analysed using SPSS for windows version 16 (USA). Data were presented in mean \pm standard error of mean ($M \pm SEM$). Descriptive statistics was done using Analysis of variance (ANOVA). Multiple comparison was done by post Hoc Turkey at $p \leq 0.05$.

3. Results

Table 1 Quantitative phytochemical compositions of leave of *Justicia carnea*

COMPONENTS	CONCENTRATION (mg/100g)
Alkaloids	1.01×10^{-1}
Terpenoids	8.55×10^{-1}
Saponin	449.43
Sterols	30.58
Phenolic acids	38.73
Glycoside	39.27
Flavonoids	430.24
Tannin	744.24
Oxalate	102.11ppm
Phytate	12.369ppm

Table 2 LD₅₀ determination of ethanol leaves extract of *Justicia carnea* in mice

Group	Dose (mg/kgbw)	No of animal/grp (n=3)	No of dead animal
1.	1250	3	0/3
2.	2500	3	0/3
3.	3750	3	0/3
4.	5000	3	0/3

Table 3 Effect of aqueous leaves extract of *Justicia carnea* on some haematological parameters in mice

Group	PCV (%)	Hb (g/dl)	RBC (X 10 ¹² /L)	ESR (mm/hr)	Platelet (X 10 ⁹ /L)
Normal Control (1ml distilled water)	36.25 ± 1.31	12.10 ± 0.43	5.33 ± 0.17	2.25 ± 0.25	240.00 ± 14.72
500mg/kgb.wt. Aqueous Extract only	31.00 ± 0.41	10.18 ± 0.18	4.55 ± 0.10	2.75 ± 0.25	200.00 ± 0.00
1000mg/kgb.wt. Aqueous Extract only	34.00 ± 2.00	11.35 ± 0.65	5.05 ± 0.32	1.75 ± 0.25	367.50 ± 12.50 ^a

Data are reported as mean ± standard error of mean (M ± SEM), n = 4; Values with superscript^{"a"} indicates a statistical significant difference (p ≤ 0.05) when compared to the control while values without similar superscript are not statistically significant (p ≥ 0.05); PCV = Packed Cell Volume, Hb = Haemoglobin, RBC = Red Blood Cells, ESR = Erythrocytes Sedimentary Rate.

Table 4 Effect of aqueous leaves extract of *Justicia carnea* on some hematological parameters in mice

Group	WBC (X 10 ⁹ /L)	N	L	E	M
Positive Control (1ml distilled water)	5.20 ± 0.63	20.75 ± 1.65	77.75 ± 1.03	1.50 ± 1.00	0.50 ± 0.58
500mg/kg Aqueous Extract only	8.00 ± 0.82 ^a	21.75 ± 1.43	65.75 ± 1.03 ^a	1.50 ± 0.50	0.00 ± 0.00
1000mg/kg Aqueous Extract only	7.38 ± 0.59 ^a	21.50 ± 2.63	67.50 ± 1.44 ^a	1.50 ± 0.87	0.50 ± 0.50

Data are reported as mean ± standard error of mean (M ± SEM), n = 4; Values with superscript^{"a"} indicates a statistical significant difference (p ≤ 0.05) when compared to the control while values without similar superscript are not statistically significant (p ≥ 0.05). WBC = White Blood Cells, N = Neutrophils, L = Leucocytes, E = Eosinophils, M = Monocytes

Table 5 Effect of ethanol leaves extract of *Justicia carnea* on some haematological parameters in mice

Group	PCV (%)	Hb (g/dl)	RBC (X 10 ¹² /L)	ESR (mm/hr)	Platelet (X 10 ⁹ /L)
Positive control (1ml DMSO)	32.50 ± 0.87	10.85 ± 0.30	4.73 ± 0.15	2.00 ± 0.41	310.00 ± 30.82
500mg/kg Ethanolic Extract only	27.00 ± 1.78	9.00 ± 0.00	3.83 ± 0.24	1.75 ± 0.25	263.43 ± 0.53 ^a
1000mg/kg Ethanolic Extract only	29.00 ± 3.03	9.68 ± 0.99	4.05 ± 0.42	1.75 ± 0.48	269.88 ± 0.43 ^a

Data are reported as mean ± standard error of mean (M ± SEM), n = 4; Values with superscript^{"a"} indicates a statistical significant difference (p ≤ 0.05) when compared to the control while values without similar superscript are not statistically significant (p ≥ 0.05). PCV = Packed Cell Volume, Hb = Haemoglobin, RBC = Red Blood Cells, ESR = Erythrocytes Sedimentary Rate.

Table 6 Effect of ethanol leaves extract of *Justicia carnea* on some haematological parameters in mice

Group	WBC 10 ⁹ /L)	(X N	L	E	M
Positive control (1ml DMSO)	9.03 ± 0.68	25.50 ± 1.66	71.25 ± 1.25	2.00 ± 0.71	1.25 ± 0.48
500mg/kg Ethanolic Extract only	22.50 ± 1.02 ^a	38.75 ± 0.48 ^a	60.75 ± 0.48 ^a	1.00 ± 0.58	0.00 ± 0.00
1000mg/kg Ethanolic Extract only	19.00 ± 1.41 ^a	31.75 ± 1.18 ^a	67.00 ± 1.73 ^a	1.25 ± 0.75	0.00 ± 0.00

Data are reported as mean ± standard error of mean (M ± SEM), n = 4; Values with superscript^{"a"} indicates a statistical significant difference (p ≤ 0.05) when compared to the control while values with similar superscript are not statistically significant (p ≥ 0.05). WBC = White Blood Cells, N = Neutrophils, L = Leucocytes, E = Eosinophils, M = Monocytes

Table 7 Effect of aqueous leaf extract of *Justicia carnea* on liver markers in mice

Group	AST (μ/L)	ALT (μ/L)	ALP (μ/L)	Total Protein (g/L)	Albumin (g/L)	Total Bilirubin (mmol/L)
Positive Control (1ml distilled water)	75.75 ± 13.41	39.75 ± 19.21	19.33 ± 3.12	47.00 ± 4.97	29.00 ± 1.08	11.80 ± 8.36
500mg/kg Aqueous Extract only	55.75 ± 0.00 ^a	29.00 ± 0.00 ^a	15.00 ± 0.00	42.00 ± 0.00	52.00 ± 0.00 ^a	9.30 ± 0.00
1000mg/kg Aqueous Extract only	46.50 ± 16.21 ^a	25.75 ± 1.18 ^a	11.75 ± 1.75	31.00 ± 6.16	41.50 ± 5.63 ^a	9.73 ± 0.88

Data are reported as mean ± standard error of mean (M ± SEM), n = 4; Values with superscript^{"a"} indicates a statistical significant difference (p ≤ 0.05) when compared to the control while values with similar superscript are not statistically significant (p ≥ 0.05).; AST = Aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase

Table 8 Effect of ethanol leaves extract of *Justicia carnea* on liver markers in mice.

Group	AST (μ/L)	ALT (μ/L)	ALP (μ/L)	Total Protein (g/L)	Albumin (g/L)	Total Bilirubin (mmol/L)
Normal control (1ml DMSO)	83.00 ± 8.04	34.00 ± 3.58	10.75 ± 5.66	52.00 ± 3.92	30.00 ± 4.45	12.40 ± 0.47
500mg/kg Ethanolic Extract only	72.25 ± 24.57	48.00 ± 5.18	30.50 ± 6.71 ^a	57.00 ± 4.18	42.75 ± 3.97	14.43 ± 3.26
1000mg/kg Ethanolic Extract only	65.25 ± 16.16	43.75 ± 6.54	24.70 ± 3.09 ^a	47.25 ± 1.03	43.00 ± 1.08	9.90 ± 1.55

Data are reported as mean ± standard error of mean (M ± SEM), n = 4; Values with superscript^{"a"} indicates a statistical significant difference (p ≤ 0.05) when compared to the control while values with similar superscript are not statistically significant (p ≥ 0.05). AST = Aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase

3.1. Histopathological result

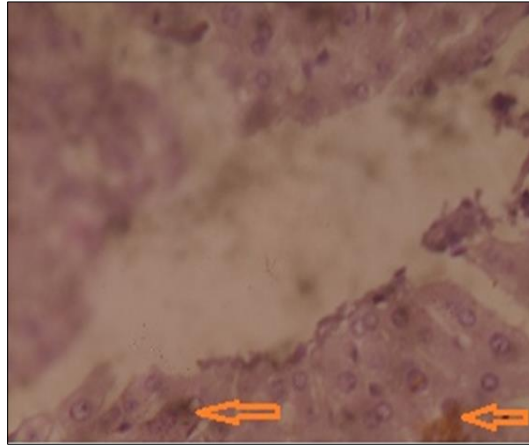


Figure 1 Photomicrograph of liver tissues of mice that received distilled water and feed for three days. (Haematoxylin and eosin) X 1000 Showing normal central vein, cords of hepatocytes with no pathology

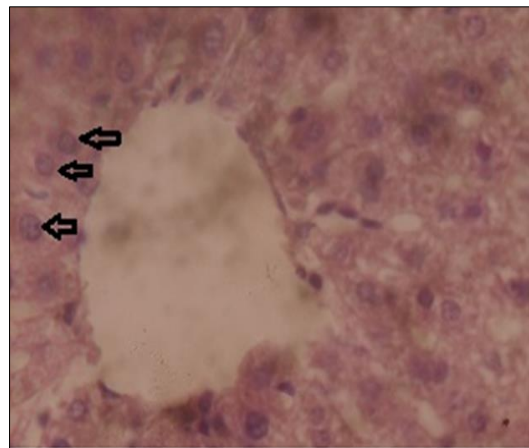


Figure 2 Photomicrograph of liver tissues of mice treated 1000mg/kg body weight of aqueous extract of *J. carnea* for three days. (Haematoxylin and eosin) X 1000 showing normal central vein, hepatic cords, with fewer normal kuffer cells lowered hepatocellular necrosis and inflammation.

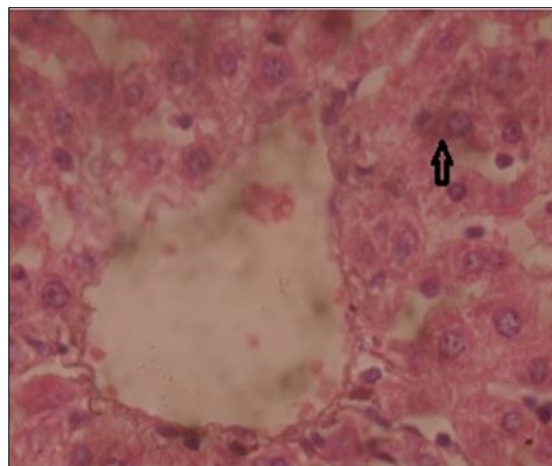


Figure 3 Photomicrograph of liver tissues of mice orally administered 3% DMSO only without treatment showing normal hepatic cords, inflamed kufer cell with higher level of hepatocellular necrosis

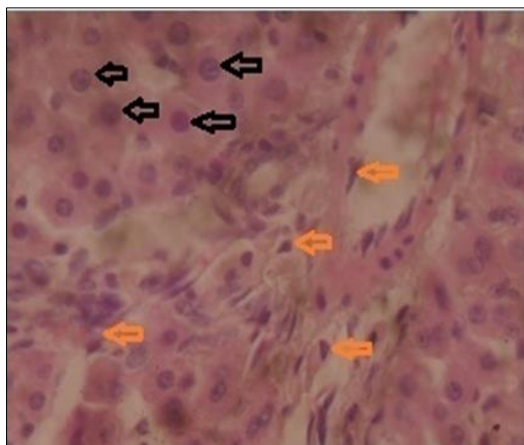


Figure 4 Photomicrograph of liver tissues of mice orally treated 1000mg/kg body weight of ethanol extract of *J. carnea*. Central vein showing lower level of inflammation, normal hepatic cords and normal kuffer cells

4. Discussion

Table 3.1 revealed the presence of alkaloids (1.01×10^{-1}), terpenoids (8.55×10^{-1}), saponin (449.43), sterols (30.58), phenolic acids (38.73), glycosides (39.27), flavonoids (430.24), tannin (744.24) mg/100g, oxalate (102.11) and phytate (12.37) ppm phytochemicals in the leaves of *Justicia carnea*. This result is consistent with studies by Orjiakor[19], Onyeabo *et al.*[20] and Patrick *et al.*[26] that reported the presence of saponins, alkaloids, flavonoids, tannins and phenols in the phytochemical screening of *J. carnea* leaves, while Patrick *et al.*[26] reported the absence of terpenoids, Orjiakor[19] and Onyeabo *et al.*[20] reported its presence. Onyeabo *et al.* [20] discovered the presence of saponin while Orjiakor [19] reported its absence. A great diversity of chemical classes is found in the *Justicia* family of plant, mainly alkaloids, lignans, flavonoids, and terpenoids [13, 27]. Several species of the *Justicia* family are used traditionally in the management of various ailments such as inflammation, gastrointestinal disorders, respiratory tract infection, fever, pain, diabetes, diarrhea, liver diseases, rheumatism and arthritis [13, 17]. The ethno pharmacological potentials of *J. species* lay on the bioactive compounds that exhibit physiological action on the body.

Result of LD₅₀ determination showed that the ethanol extract of the leaves of *Justicia carnea* was safe at 5000mg/kg bw as no death was recorded at this dose (Table 2). This finding is supported by research work on ethanol and aqueous leaves extract of *J. carnea* [19-20, 28]. Hence, it show that the ethanol effect extract of *Justicia carnea* are safe and have no acute toxicity up to the dose of 5000mg/kg body weight orally which is considered high. This result is in agreement with Onyeabo *et al.*[20] which suggest that acute toxicity test of ethanol leaf extract of *Justicia carnea* in mice recorded no mortality even up to the highest dose. The non-toxicity of aqueous leave extract of *Justicia carnea* in experimental animals at the highest dose 5000mg/kg body weight was reported by Orjiakor[19]; Alozie *et al.*[22].

Mean values of all haematological indices in all the extract treated groups showed non-significant decrease except mean platelet concentration in mice treated with 1000mg/kg bw aqueous extract which significantly increased when compared to control as revealed in Table 3. The rise in platelets count seen may suggest that the extracts have a stimulatory effect on thrombopoietin [29]. They, therefore, can be used in management of hemophilia. The cause of increased platelet count (thrombocytosis) in mice models may be associated with inflammation and presence of a blood disease such as abnormal bleeding induced by toxic phytochemical substances such as tannins in the extract.

Table 4 showed that mean WBC and lymphocyte counts in mice treated with aqueous leave extract of *J. carnea* in all the groups were significantly reduced and increased respectively while that of neutrophils, eosinophils, and monocytes were non-significant when compared to control. A low number of WBCs may be due to bone marrow deficiency or failure (for example, due to infection, tumour or abnormal scarring), disease of the liver or spleen, radiation therapy or exposure[30-32].

Rats treated with 500 and 1000mg/kg bw ethanolic extract showed non-significant differences in mean PCV, Hb, RBC, ESR values and significantly increased mean platelet count when compared to control while mean WBC and Neutrophils counts significantly increased and Lymphocytes significantly decreased in result from Tables 5 and 6.

The neutrophils defend the body against invasion by microorganisms, especially the bacteria.[33]. Clinically, an increase in neutrophils in the blood (i.e neutrophil 'leucocytosis' or 'neutrophilia') is usually as a result of an infection and tissue injury. The mechanism responsible for leukocytosis is the stimulation of activities of leptin and the leptin receptor that are parts of a pathway that stimulates hemopoiesis[34] The other pathological causes of neutrophilia include, Bacterial infections; Inflammation or necrosis; Metabolic disorders e.g. diabetic ketoacidosis, uremia, and eclampsia; Steroid therapy; Acute hemorrhage or hemolysis.

Significant reduction in mean AST and ALT activities in mice in all the aqueous extract treated groups and increase in albumin concentration when compared control was observed

Mean ALP activity in mice treated with 500 and 1000mg/kg Ethanolic Extract showed significant increase when compared to control group.

ALT and AST are sensitive indicators of hepatocellular injury but they lack specificity as they are also present in muscle (cardiac and skeletal), kidney, and RBCs. In hepatocyte cytoplasm AST is more abundant than ALT. Declining AST and ALT may indicate either recovery of poor prognosis in fulminant hepatic failure [35]

Alkaline phosphatase has many sources including liver, bone, placenta and the kidney. In the liver, its present in the lining of the biliary tree adjacent to the hepatocyte membrane hence any increase of its values due to liver origin indicates biliary obstruction and cholestasis [36]. Highest levels of alkaline phosphatase occur in cholestatic disorders. Elevations occur as a result of both intrahepatic and extrahepatic obstruction to bile flow and the degree of elevation does not help to distinguish between the two [37].

The histological result showed no damage even up to 1000mg/kg body weight of the ethanol extract which is in agreement with the report of Onyeabo *et al.* [20], which stated that there was no significant ($p < 0.05$) difference in the relative heart, liver and kidney weight for all groups relative to the non-anaemic and anaemic control when treated with the ethanol extract of *Justicia carnea*.

5. Conclusion

Oral administration of both aqueous and ethanol extracts of *Justicia carnea* is safe at the doses of 500 and 1000mg/kgb.wt. The extracts stimulated hemopoiesis and thrombopoiesis hence could be used in management of hemophilia.

Compliance with ethical standards

Acknowledgments

The authors wish to express their gratitude to the staff of the Department Of Biochemistry, University of Port-Harcourt, Nigeria for providing the necessary facilities needed for this research.

Disclosure of conflict of interest

We declare that are no conflict of interest.

Statement of ethical approval

All experimental protocols were in compliance with the Department of Biochemistry Research Ethics Committee (UPH/BCHREC/2022/005) on research in animals as well as internationally accepted principles for laboratory animal use and care.

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