African Scientist Vol. 25, No. 1 March 31, 2024
Printed in Nigeria

1595-6881/2023 $80.00 + 0.00

https://africansciientistjournal.org

AFS2024005/25103

Haematological and Histopathological Effects of *Justicia carnea* (Jehovah's Witness Plant)

Osayi Brenda Isichei-Ukah*, Omamuyovwi Blessed Igho-Daro and Onaiwu Idahosa Enabulele

Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

*Corresponding author Email: brenda.isichei@uniben.edu, Tel: +234 (0) 803 297 5574

(Received February 14, 2024; Accepted in revised form February 28, 2024)

ABSTRACT: *Justicia carnea* is a medicinal plant used widely in Nigeria as a blood-booster. The toxicity of the extracts of *J. carnea* was carried out to evaluate its haematological and histopathological effects on Wistar albino rats. The aqueous and ethanol extracts used for this study were obtained using the Soxhlet extraction method. A total of fifteen (15) rats in five treatment groups were used for this study. Different concentrations of the leaf extracts were administered to the animals for fourteen (14) days, after which they were observed for mortality and toxicity. There was no mortality recorded for the period. The results of the haematological parameters for the experimental rats showed that there was an increase in the number of red blood cells (RBC), haemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). There was a decrease in white blood cells (WBC) and lymphocytes (LYM), though not statistically significant ($p>0.05$) compared to the control. The histopathological examination of the liver and kidneys showed congestion of blood vessels, infiltration of fluids and heamorrhages. Therefore, this study has shown that *J. carnea* has blood-boosting effects but had adverse pathological effects in the organs studied.

Keywords: *Justicia carnea*, Haematological, Histopathological and Wistar albino rats

Introduction

Justicia carnea (Jacobinia or Jehovah's Witness plant in Nigeria) is a medicinal plant used widely in Nigeria and reported to have a blood-boosting potential (Onyeabo *et al.,* 2017). Several species of *Justicia* have also been reported to have diverse antimicrobial functions (Igbinaduwa *et al.,* 2020). In various parts of Africa, several species of *Justicia* are used in traditional medicine for the treatment of anaemia, inflammation, and respiratory and gastrointestinal diseases (Onyeabo *et al.,* 2017). These functions possessed by the plant are associated with its bioactive constituents comprising mainly phenols and flavonoids (Uroko *et al.,* 2017).

It has been reported that some species of *Justicia* possess anti-inflammatory activity by inhibiting the release of the hormone, prostaglandin, or other inflammatory mediators from the cell membrane by initiating the stability of the membrane (Mamta *et al*., 2013). Inflammation is the response of the body to infections by microorganisms, burns, and injuries that may endanger human health (Shingala *et a*l., 2021). The reports of Cheng *et al*., (2017), state that clinically, it can be characterized by edema, redness, pain and loss of tissue function. This process of inflammation involves changes in the flow of blood, tissue destruction, increased vascular permeability and local inflammatory mediators such as prostaglandins, leukotrienes, and cyclooxygenases (Christiakov *et al.,* 2018). Other species of *Justicia* such as, *J. flava,* and *J. adhatoda* also possess anti-inflammatory activity (Correa and Alcantara, 2012).

Following the conventional use of the plant as a blood booster, Onyeabo *et al.* (2017), in the research on the haematological and biochemical studies on *J. carnea* leaf extract in phenylhydrazine-induced anaemia in albino rats reported that the plant was able to reverse the anaemic conditions of the rats by increasing their packed cell

volume (PCV) of blood level, red blood cell, haemoglobin, and platelet count level. Orjiakor *et al*. (2019), also proved and confirmed the blood-boosting ability of the plant by showing an increase in the blood level of albino rats when the haematological parameters were analyzed. The investigations of Igbinaduwa *et al*. (2020), also confirmed the haematological and anti-anaemic properties of the plant. The reports of Akintimehin *et al*. (2021), stated and further concluded that, due to the plant's abundance in iron content, it can increase red blood cells.

Despite the therapeutic importance of medicinal plants, toxic substances are present in large numbers of plants examined. Olaniyan *et al*. (2016) reported that contamination of plants may occur due to the presence of contaminants, such as heavy metals, aflatoxin and pathogenic microorganisms from the soil or in their process of herbal preparations. These contaminated plants being consumed without determining their efficacy and safety can lead to unexpected toxic effects, which results in changes in the functions of different organs in the body, as hepatic and renal damage has been linked recently to the use of medicinal plants in the treatment of various diseases (Mapanga and Musabayane, 2010). Herbal plant usage has been on the increase due to its medicinal potential, hence the scientific investigation on the safety of *J. carnea* for use as an alternative medicine.

This study aimed to evaluate the toxicity of *J. carnea* in Wistar albino rats with a focus on hematological analysis and histopathological effects of *J. carnea* on the liver and kidneys of Wistar rats.

Materials and methods

Source of Justicia carnea: The fresh leaves of *Justicia carnea* were collected from Upper-Agbarho town, Ughelli-North Local Government Area, Delta State, Nigeria. The leaves were identified in the Department of Plant Biology and Biotechnology, University of Benin, Benin City and a voucher number, UBH-J386 was deposited.

Preparation of plant extracts: The leaves were washed with distilled water and air-dried for two weeks. They were then grounded (pulverized) into powdered form using a dry electric blender (SAISHO, Model-S-748). The powdered leaves were kept in airtight containers until use. The leaves were extracted using the Soxhlet extraction method. The solvents used were aqueous (distilled water) and ethanol (Hatbamu *et al*., 2010).

Experimental animals: A total of 15 healthy male Wistar albino rats, weighing 116g and above, obtained from the animal house of the Department of Anatomy, University of Benin, Benin City were used in this study. They were kept in plastic cages with wire screen tops at room temperature and were acclimatized for a week. They had free access to their feeds and water *ad libitum*, and fresh wood shavings used as bedding materials in the cages were replaced each day.

The animals were placed into five (5) groups of three (3) rats each. Group one (1), the control received distilled water, Group two (2) received 20 mg/kg of aqueous extracts of *Justicia carnea*, Group three (3) received 40 mg/kg of aqueous extracts of *Justicia carnea*, Group four (4) received 20 mg/kg of ethanol extracts of *Justicia carnea* and Group five (5) received 40 mg/kg of ethanol extracts of *Justicia carnea*. Both extracts of *Justicia carnea* (aqueous and ethanol) and distilled water (control) were orally administered to the rats daily for fourteen (14) days using an oral gavage. The rats were observed daily for mortality.

Haematological analysis: After the administration of the extracts on the fifteenth (15^{th}) day, the blood samples of the animals were collected for haematological analysis. They were first anaesthetized in a closed chamber containing chloroform to render them unconscious for dissection. The blood samples were collected from the heart (cardiac puncture) using sterile needles and syringes and placed in anticoagulant bottles containing ethylene diamine tetraacetic acid (EDTA). The bottles containing the blood samples were transported to the Department of Haematology, University of Benin Teaching Hospital, Benin City for analysis. The parameters analyzed were white blood cells (WBC), lymphocytes (LYM), granulocytes (GRAN), red blood cells (RBC), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT) and packed cell volume (PCV). This haematological analysis was carried out using an automated haematology analyzer (AHA) (Tefferi *et al.*, 2005).

Histopathological analysis: After administering the extracts and distilled water for fourteen (14) days, the animals were sacrificed to harvest their selected organs; liver and kidney in their anaesthetized state for histopathological analysis. The organs were weighed and placed in different universal containers containing formalin solution to preserve them, and were transported to the Department of Histopathology, University of Benin Teaching Hospital, Benin City for analysis. The organs (tissues) were dehydrated and embedded in melted paraffin wax and mounted on a microtome to cut into thin slices. These slices were fixed on microscopic glass slides at which point the wax was removed with a solvent and then rehydrated to be ready for staining. The haematoxylin and eosin (H&E) staining method was used which involved the application of the haematoxylin dye followed by rinsing in a weak acid (HCL) solution to remove excess staining and bluing in alkaline water

(eg. Scott's tap water). Thereafter, the counterstaining with eosin dye was done. Then, the slides after drying were observed under the microscope (40X) (Kiernan, 2018).
Statistical analysis of data: The data obtained from this research were analyzed using the statistical package for social sciences (version 21), and Microsoft Excel (version 2019). The values were expressed as mean ± standard deviation at 0.05 significance levels (Ogbeibu, 2015).

Results

The toxicity test of the aqueous and ethanol extracts of *Justicia carnea* leaves on the Wistar albino rats (Table 1) showed that no deaths were recorded during the period of study.

Table 1: Toxicity test of aqueous and ethanol extracts of *Justicia carnea* on the Wistar albino rats

Treatment Groups	Dosage (mg/kg) Body Weight	Mortality
Group 1 (Control)	Distilled Water	-
Group 2	20mg/kg Aqueous extract	-
Group 3	40mg/kg Aqueous extract	-
Group 4	20mg/kg Ethanol extract	-
Group 5	40mg/kg Ethanol extract	-

- Indicates no death recorded

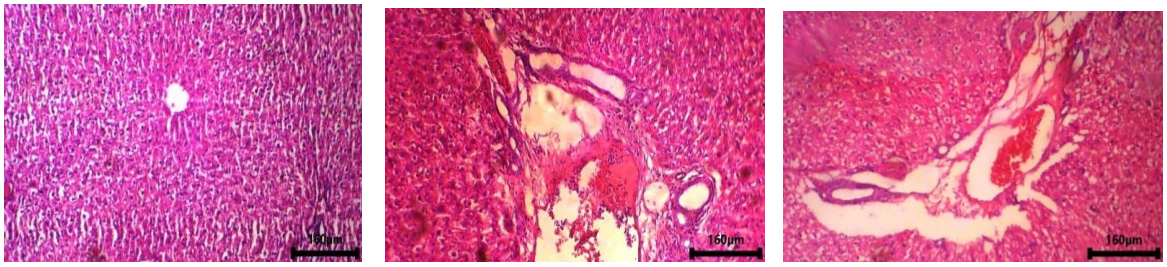
The results of the haematological parameters for the experimental rats showed that there was an increase in the number of red blood cells (RBC), haemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). Then, there was a decrease in white blood cells and lymphocytes. The increase and decrease in the parameters were not statistically significant (*p>0.05*) compared to the control.

Table 2: Haematological parameters of aqueous and ethanol extracts of *Justicia carnea*

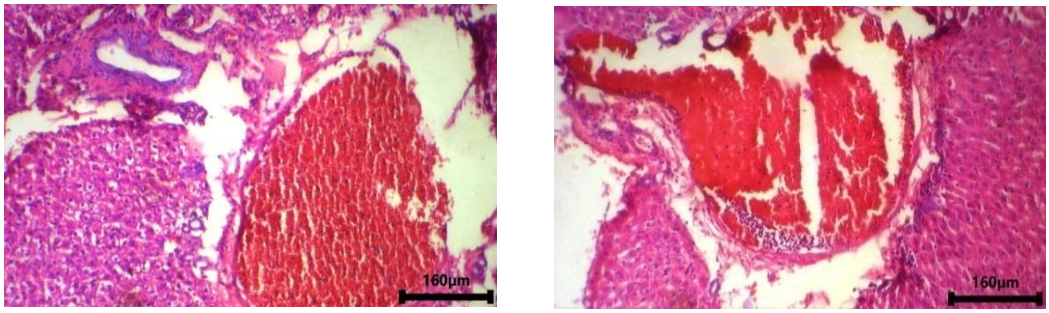
Parameter	Group 1 (Control)	Group 2	Group 3	Group 4	Group 5	*P - value*
WBC	6.40±2.14	6.10±2.17	4.15±0.35	6.33±2.71	5.47±2.65	*P > 0.05*
LYM	80.33±9.62	80.07±5.17	73.95±16.19	80.17±3.47	76.73±1.54	*P > 0.05*
GRAN	9.50±6.17	9.10±4.99	9.80±6.36	9.17±1.26	9.10±0.79	*P > 0.05*
RBC	5.49±1.29	6.08±0.52	6.38±3.39	6.25±0.47	6.34±0.26	*P > 0.05*
HGB	11.23±2.97	12.93±1.51	13.37±7.35	13.03±0.95	13.17±0.4	*P > 0.05*
MCV	64.60±3.14	67.47±0.75	67.87±0.81	66.47±4.2	67.00±1.54	*P > 0.05*
MCH	20.33±0.7	21.20±0.7	21.40±1.27	20.83±0.4	20.73±0.29	*P > 0.05*
MCHC	31.60±2.44	31.50±0.85	30.63±1.46	31.47±1.4	31.00±0.3	*P > 0.05*
PLT	195.67±58.5	269.67±12.5	335.50±282.14	315.67±137.02	334.33±30.14	*P > 0.05*
PCV	0.14±0.05	0.21±0.01	0.34±0.19	0.19±0.1	0.25±0.03	*P > 0.05*

Values are mean ± standard deviation
Key: WBC: white blood cells, LYM: lymphocytes, GRAN: granulocytes, RBC: red blood cells, HGB: hemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, PLT: platelets, PCV: packed cell volume
Group 1: control (distilled water), Group 2: aqueous 20 mg/kg, Group 3: aqueous 40 mg/kg, Group 4: ethanol 20 mg/kg, Group 5: ethanol 40mg/kg.
P>0.05-not statistically significant.

The photomicrograph of the liver sections showed that Group 1 (control) appeared normal with the central vein without congestion, interstitial spaces, and hepatocytes (cells of the liver) radiating. Groups 2 and 3 showed the dilation and congestion of blood vessels with blood and infiltrates of inflammatory cells; Groups 4 and 5 showed the dilation of the portal triad, which consists of the central vein, bile duct and artery, with the congestion of blood and the presence of inflammatory cells.
The photomicrograph of the kidney section showed Group 1 (control) appearing normal with glomerular cells and interstitial spaces without congestion. Groups 2 to 5 showed the interstitial spaces and glomerular cells being congested with blood (haemorrhage).



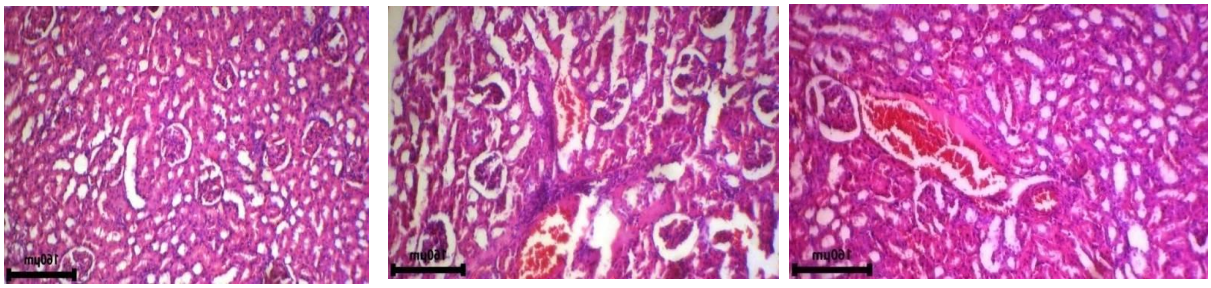
Group 1 (control) **Group 2** **Group 3**



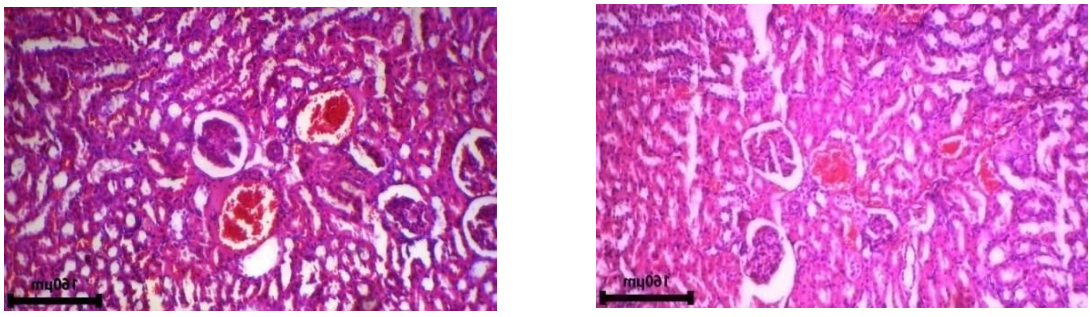
Group 4 **Group 5**

Plate 1: Microscopic view of the liver of Wistar albino rats (160 µm) administered with the aqueous and ethanol extracts of *Justicia carnea.*

Key: Group 1 (Control): Distilled water, Group 2: 20 mg/kg of aqueous extract of *Justicia carnea*, Group 3: 40 mg/kg of aqueous extract of *Justicia carnea*, Group 4: 20 mg/kg of ethanol extract of *Justicia carnea,* Group 5: 40 mg/kg of ethanol extract of *Justicia carnea*



Group 1 (control) **Group 2** **Group 3**



Group 4 **Group 5**

Plate 2: Microscopic view of the kidney of Wistar albino rats (160 µm) administered with the aqueous and ethanol extracts of *Justicia carnea.*

Key: Group 1 (Control): Distilled water, Group 2: 20 mg/kg of aqueous extract of *Justicia carnea*, Group 3: 40 mg/kg of aqueous extract of *Justicia carnea*, Group 4: 20 mg/kg of ethanol extract of *Justicia carnea*, Group 5: 40 mg/kg of ethanol extract of *Justicia carnea*

Discussion

Justicia carnea is an unconventional medicine, traditionally used for managing a myriad of diseases (Onyeabo *et al*., 2017; Akintimehin *et al*., 2021). The assumption that herbal preparations/remedies are safe and effective has influenced the indiscriminate use of such remedies, most especially among rural communities, where these remedies can be administered for a long time without considering the dose or concentration that will bring about toxic side effects (Ben-Arye *et al*., 2016).

White blood cells play a major role in immunity by providing the body with the ability to protect or defend itself against infection and diseases and it usually increases in the presence of foreign agents (Eyong *et al*., 2004). The decrease in the WBC in this study is similar to the reports of Kemp and Franco (2002), who also reported a decrease in the white blood cell count when investigating the activity of a plant that was known to be a treatment for upper respiratory tract infections and further concluded that this decrease could be due to the prolonged usage of the plant for treatment. This result also agrees with Akintimehin *et al*. (2021), who reported a decrease in the level of WBC and granulocytes, when also investigating *J. carnea.*

The increase in platelet count shows that the extract can function in preventing the loss of blood through blood clotting. Platelets also play a role in immunity by capturing and engulfing microorganisms and by the formation of clots. They prevent the spread of bacteria, which is a protective mechanism (Morrell *et al*., 2014). The increase in the PCV, RBC and HGB agrees with *J. carnea* having a blood-boosting potential. According to Orjiakor *et al*. (2019), the blood-stimulating effect of this plant could be due to the presence of biologically active constituents in it, facilitating the action of haematopoeitic cells and stabilizing the circulation of blood. The increase in RBC, PCV and PLT also agrees with the previous work of Onyeabo *et al*. (2017) on the reversed effect of *J. carnea* leaf extract on anaemia-induced experimental rats and Orjiakor *et al*. (2019) on its effect on the haematological indices of anaemia-induced rats.

From the histopathological results of the organs of the experimental animals, as compared with the control, the dilation and congestion of blood vessels with blood, the dilation of the portal triad which consists of the central vein, bile duct and artery, and the presence of infiltrates of inflammatory cells, as observed in the liver are abnormalities that can lead to a failure in the function of the liver and death, if left untreated (Hellsten *et al*., 2012). The dilation of the blood vessels, a condition called vasodilation, is a process whereby the blood vessels widen, or expand, as a result of the relaxation of the muscular wall of the vessels to allow for the increased flow of blood, thereby lowering blood pressure, while the dilation of the portal triad (central vein, bile duct, and artery) is a form of expansion that can cause the narrowing of other vessels in the liver thereby restricting the supply of blood to them, as reported by Hellsten *et al*. (2012). However, congestion of blood is the excess accumulation of blood in vessels and it could be due to an increased flow of blood (Elmore, 2006).

The congestion of blood and haemorrhage observed in the glomerular cells and interstitial spaces of the kidney across the groups administered compared with the control can lead to a failure in the function of the kidney and possibly death if not treated (Russell and Cullen, 2013). As compared with the control, the intake of the extracts by the experimental animals led to a condition of haemorrhage in the kidney, which is a process of active bleeding, by which blood escapes from the blood vessels, either into the organs or tissues in the body (internal bleeding), or outside the body (external bleeding) as a result of injury. Roth (2011) reported that this escape of blood into the organs of the body can be due to the pressure of blood flowing through the blood vessels leading to a tear or a burst of the vessels causing haemorrhage.

From these histopathological results, the administration of the *J. carnea* plant extracts without any form of morbidity or mortality of the experimental animals but with slight abnormalities in their organs (liver and kidney) could be due to the blood-boosting ability of the plant and its daily dosage for two (2) weeks. This result is comparable with Akintimehin *et al*. (2021), who reported a liver and kidney structure with slight abnormalities of mild fat congestion and haemorrhage.

Conclusion

The findings from this study have confirmed that *J. carnea* functions as a blood-booster due to the increase in red blood cells and packed cell volume. However, the histology of the liver and kidneys showed pathological effects of infiltration of fluids and hemorrhage. Therefore, more research into the adequate dosage and duration of treatment is required.

References

Akintimehin E, Karigidi K, Omogunwa T, Adetuyi F: Safety assessment of oral administration of ethanol extract of *Justicia carnea* leaf in healthy Wistar rats: haematology, antioxidative and histology studies. Clin Phytosci, 7:2. 2012.

Ben-Arye E, Samuels N, Goldstein LH, Mutafoglu K, Omran S, Schiff E: Potential risks associated with traditional herbal medicine use in cancer care: a study of Middle Eastern oncology healthcare professionals. Cancer, 122: 598-610. 2016.

Cheng L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L: Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, 9(6): 7204-7218. 2017.

Christiakov D, Melnichenko A, Grechko A, Myasoedova V, Orekhov A: Potential of anti-inflammatory agents for treatment of atherosclerosis. Exp Mol Pathol, 104(2): 114-124. 2018.

Correa G, Alcantara A: Chemical constituents and biological activities of species of *Justicia* - a review. Braz J Pharmacogn, 22(1): 220-238. 2012.

Elmore S: Histopathology of the lymph nodes. Toxicol Pathol, 34: 425-454. 2006.

Eyong E, Umoh I, Ebong P, Eteng M, Antai A, Akpa A: Haematoxic effects following ingestion of Nigerian crude oil and crude oil polluted shellfish by rats. Nigerian J Physiol Sci, 19(1-2): 1-6. 2004.

Hatbamu Y, Eguale T, Wubete A, Sori T: *In vitro* antimicrobial activity of some selected Ethiopian medicinal plants against some bacteria of veterinary importance. Afr J Microbiol Res, 4: 1230 – 1234. 2010.

Hellsten Y, Nyberg M, Jensen L, Mortensen S: Vasodilator interactions in skeletal muscle blood flow regulation. J Physiol. 590(24): 6297-6305. 2012.

Igbinaduwa P, Kabari K, Chikwue T: Phytochemical and anti-anaemic properties of ethanol leaf extract of *Justicia carnea* Vahl (Acanthaceae). Nigerian J Pharm Appl Sci Res, 8(2): 55-61. 2020.

Kemp D, Franco K: Possible leukopenia associated with long-term use of *Echinaceae*. J Am Board Fam Pract, 15(5): 417-419. 2002.

Kiernan J: Does progressive nuclear staining with hemalum (alum hematoxylin) involve deoxyribonucleic acid (DNA), and what is the nature of the dye – chromatin complex? Biotechnol Histochem, 93(2): 133-148. 2018.

Mamta S, Jyoti S, Rajeev N, Dharmendra S, Abhishek G: Phytochemistry of medicinal plants. J Pharmacogn Phytochem, 1: 168-182. 2013.

Mapanga R, Musabayane C: The renal effects of blood glucose-lowering plant-derived extracts in diabetes mellitus- an overview. Ren Fail, 32(1): 132-138. 2010.

Morrell C, Aggrey A, Chapman L, Modjeski K: Emerging roles for platelets as immune and inflammatory cells. Blood, 123: 2759-2767. 2014.

Ogbeibu AE: Biostatistics: A Practical Approach to Research and Data Handling. 2nd Edition. Mindex Publishing Co. Ltd, Benin City. 285 p. 2015.

Olaniyan J, Hadiza L, Hussain A, Musa B, Abubakar S: Acute and sub-acute toxicity studies of aqueous and methanol extracts of *Nelsonia campestris* in rats. J Acute Dis, 5(1): 62-70. 2016.

Onyeabo C, Achi N, Ekeleme–Egedigwe C, Ebere C, Okoro C: Haematological and biochemical studies on *Justicia carnea* leave extract in phenylhydrazine induced-anaemia in albino rats. Acta Sci Pol Technol Aliment, 16(2): 217-230. 2017.

Orjiakor C, Uroko R, Njoku O, Ezeanyika L: Nutritive properties of aqueous extracts of *Justicia carnea* leaves and its effect on haematological and some biochemical indices of anaemia-induced male Wistar albino rats. Biomed Res, 30(4): 645-654. 2019.

Roth E: Haemorrhage. *Encyclopedia of Clinical Neuropsychology*. New York, N.Y: Springer New York. pp. 1234-1235. 2011.

Russell C, Cullen M: Systems toxicologic pathology. Haschek and Rousseaux's Handbook of Toxicologic Pathology (Third Edition) 3:1509-1566. 2013.

Shingala Z, Chauhan B, Baraiya J: A review on medicinal plants as a source of anti-inflammatory agents. J Pharmacogn Phytochem, 10(6): 364-371. 2021.

Tefferi A, Hanson C, Inwards D: How to interpret and pursue an abnormal complete blood cell count in adults. Mayo Clin Proc, 80(7): 923-936. 2005.

Udedi S, Ani O, Asogwa K, Maduji F, Okafor C: *In-vitro* and *in-vivo* antioxidant activity of ethanol leaf extract of *Justicia carnea*. Int. J. Biochem. Res Rev, 29(4): 48-60. 2020.

Uroko RI, Egba SI, Achi NK, Uchenna ON, Agbafor A, Ngwu OR, Nweje-Anyalowu PC, Ogbonna CE: Effects of aqueous extracts of palm fruits (*Elaeis guineensis*) on liver function indices of male Wistar albino rats. Res J Med Plant, 11: 148-159. 2017.