Nigerian Journal of Pharmaceutical and Applied Science Research, 8(2): 55-61 May 2019
(ISSN 1485-8059 Available at www.nijophasr.com

Phytochemical and Anti-Anaemic Properties of Ethanol Leaf Extract of *Justicia carnea*Vahl (Acantheceae)

[1*],Patrick O. Igbinaduwa [2]Kosa Maureen Kabari and [2]Thelma C. Chikwue
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Nigeria.
[2]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Port-Harcourt, Nigeria.

ABSTRACT
Justicia carnea is a medicinal plant reported to have diverse pharmacological functions, including blood-boosting potentials. The study investigated the phytochemical, proximate parameters as well as the effects of the ethanol extract of *Justicia carnea* (JC) leaves in phenylhydrazine induced-anemia in albino rats were investigated. Quantitative phytochemical screening and proximate analyses were carried out using standard methods. The experimental animals were randomly grouped into six groups of three rats each – group 1 (non-anemic control), group 2 (anemic control), group 3 (100 mg/kg of JC extract), group 4 (200 mg/kg of JC extract), group 5 (500 mg/kg) and group 6 (1000 mg/kg). Phenylhydrazine was administered once a day at the dose of 10mg/kg to induce aneamia. After 7 days of administration, animals were sacrificed.Serum was collectedfor biochemical analysis and organs harvested for histopathological analysis. Quantitative phytochemical analysis showed the presence of secondary metabolites such as saponins (2.50 mg/100g), tannins (2.16 mg/100g), alkaloids (1.88 mg/100g). Saponins were the most abundant secondary metabolites while flavonoids (0.08%) were in minute quantity. Calcium (1930µ) and potassium (14800µ) were the most abundant minerals in the aqueous extract of the leaf while zinc (73µ) was present in low concentration and lead (1.0µ) in minute quantity. Packed cell volume (PCV), red blood cell (RBC), platelet concentration and hemoglobin (Hb) concentrations decreased significantly after 4 days of phenylhydrazine induction, but after 7 days of administering extracts of *Justicia carnea*, PCV values, RBC, platelet and Hb increased significantly. Other components of the blood such as eosinophil, basophil, monocytes, and lymphocytes also showed positive improvement. Ethanol leaf extract of *Justicia carnea* reversed phenylhydrazine induced anemia in the treated animals. This study validates the ethnomedicinal use of the plant as a traditional blood supplement in the management of anemia.

Key words: *Justicia carnea*, Anemia, Haematology, Biochemicals.

INTRODUCTION
Anemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. It occurs at all stages of the life cycle, but is more prevalent in pregnant women and young children. In 2002, iron deficiency anemia (IDA) was considered to be among the most important contributing factors to the global burden of disease (WHO, 2002). Anemia causes hypoxia due to a failure to meet tissue oxygen demand. It is a disease that results from a decrease in hemoglobin level inside erythrocytes or lack of dysfunctional RBC resulting in deficiency of RBC. This leads to reduced oxygen flow to the body organs (Lichtman *et al*, 2015).Most pregnant women and some sick individuals in the rural areas of Rivers state, Nigeria use the plant *Justicia carnea* (blood root) as a traditional blood supplement for the management of anemia. The plant has a number of secondary metabolites (Kumar *et al*, 2009) and phytochemicals (Fasuyi, 2006) that may be responsible for its observed therapeutic uses.This study seeks to validate the ethno medicinal use of the plant, *Justicia carnea* in the management of anemia and verify its toxicity to the liver and kidney.

MATERIALS AND METHODS
Plant Collection and Identification
The leaves of *J. carnea* were collected from the botanical garden of department of Pharmacognosy, faculty of Pharmaceutical sciences, University of Port Harcourt in May 2018 and its botanical identification was authenticated by Mr. Mikalu Suleiman. A voucher specimen catalogued UPH/V/1279, was deposited in the departmental herbarium for reference purpose.

Phytochemical Screening and proximate Analysis
This was carried out using standard procedures as described by Harbone (1998) and AOAC (1990).
Preparation of *J.carnea* Extract. The plant material *J. carnea* was dried under shed and crushed with manual milling machine(Corona, China).

***Corresponding Author**: patrick.igbinaduwa@uniben.edu; Phone: +234(0)7063806665

Two hundred and fifty grams(250 g) of the fine powder was weighed and stored in an air tight container in a cool dry place until needed for experiment.

Extraction

Powdered plant material (250.0 g) was macerated in 2.5 L methanol for 72 hours with intermittent shaking after which it was decanted and filtered. The extract obtained was transferred and concentrated with a rotary evaporator. The crude extract obtained was stored in refrigerator until they were ready to be used.

Experimental Specimen (Wistar rat)

Wister rats in six groups of three animals each were acclimatized in the animal house of the Department of Pharmacology and Experimental Biology, University of Port Harcourt, for 14 days. Animals were fed pellet diet and water *ad-libitum*. Anemia was induced using Phenyl hydrazine HCl (groups 2 to 6). After 4 days the extract was administered to rats (groups 3 to 6) according to their body weights for 7 days. Animals were, sacrificed under anesthesia and blood samples and tissues (liver and kidney) were harvested for analysis.

Experimental protocol

Group 1(negative control): The animals of this group received distilled water

Group2 (positive control): The animals of this group were administered with phenyl hydrazine hydrochloride only.

Group3 (Test group1): The animals of this group were administered with phenyl hydrazine hydrochloride and treated with 100mg/kg extract of *Justicia carnea*.

Group 4(Test group 2): The animals of this group were administered with phenyl hydrazine hydrochloride and treated with 200mg/kg of extract of *Justicia carnea.*

Group 5(Test group 3): The animals of this group were administered with phenyl hydrazine hydrochloride and treated with 500mg/kg of extract of *Justicia carnea.*

Group 6 (Test group 4): The animals of this group were administered with phenyl hydrazine hydrochloride and treated with 1000mg/kg of extract of *Justicia carnea.*

Tissue processing: Harvesting and Fixation: The tissues (liver and kidney) were harvested and fixed in 10% formal saline for about six (6) to twelve (12) hours (Gbenou*et al*., 2006). It was post fixed in EDTA to calcify the tissues.

Dehydration: The tissues were dehydrated using a graded series of ethanol (50%, 75% 95% and absolute alcohol) for 1-2 hours while in absolute alcohol it was put twice for 1-2 hours each time.

Clearing: A suitable clearing agent (Xylene) was used to clear the tissues twice for 1-2 hours each time.

Infiltration: Molten paraffin (60°C) was used to fill up tissue pores to give it some form of semi rigidity.

Embedding: This involved the use of an embedding medium (embedding mould) and a suitable histological wax; paraffin wax to embed the tissue. The tissues were placed in molten paraffin wax at a constant temperature of 56-60°C in a paraffin bath, it was changed twice and each embedding was for 2 hours.

Sectioning: This involved the use of a precision instrument to produce thin uniform sections. A rotary microtome with an adjustable microtome blade inclined at angle of 90° was used.

Floatation: This involved floating section ribbons in a warm water bath, selected were mounted on the slide and cleared with xylene.

Staining: The Hematoxylin and Eosin (H and E) stains were used. The hematoxylinas the primary stain while the Eosin served as the counter stain.

Staining protocols: The rehydrated sections was stained in Hematoxylin solution for 30 minutes and washed in tap water for 1-3 minutes, until sections turn blue ("bluing'), 1percent acid ethanol was used to differentiate them, this removes excess dye allowing nuclear details to emerge, it was washed in running tap water until blue and stained in Eosin solution for 10 minutes and again washed in running tap water, it was dehydrated, cleared and mounted. Tap water provides the alkalinity necessary for the *"bluing" process.*

Microscopy: A light microscope to which a camera was attached was used to view slides; pictures were taken at varying magnifications.

Printing and Interpretation of micrographs:

The micrographs served as results and provided the basis for comparative studies and analysis (Tchogou*et al*, 2016)

Blood sample analysis: The blood samples were analyzed using an automated machine in which the parameters were gotten at end of the process. The

parameters include hemoglobin level (Hb level), neutrophil, basophil, eosinophil, white blood cell, platelets, packed cell volume (PCV), monocytes, and lymphocytes.

Statistical analysis

Quantitative data obtained from this study was expressed as mean ± SEM. Analytical basis of comparison was strictly based on values obtained. Statistical analysis was done using the Kruskal Wallis test and posthoc was done with Dunn's multiple comparison tests.

RESULTS

Phytochemical screening showed that the plant is mainly composed of saponins and tannins. Alkaloids, phenols and flavonoids were also present (Table 1).

PROXIMATE ANALYSIS

Proximate analysis showed that *Justicia carnea* has high carbohydrate and protein contents. Ash, moisture and crude fibre were moderate while fats and moisture contents were low.

Mineral and Metal Determination

Mineral contents of the aqueous leaf extract of *Justicia carnea* is shown in table 3. The plant has high concentration of calcium, potassium and iron while zinc was present in low concentration and lead in minute quantity

Table 1: Secondary Metabolites in *Justicia carnea*

Chemical constituents	Quantity
Carbohydrate	30.7%
Protein	24.31%
Crude Fibre	18.76%
Ash content	10.49%
Crude fat	8.19%
Moisture content	7.55%

Table 2: Proximate Composition of *Justicia carnea*.

Secondary metabolites	Quantity
Saponins	2.50mg/100g
Tannins	2.16mg/100g
Alkaloids	1.88mg/100g
Phenol	1.12mg/100g
Flavonoids	0.08mg/100g

Table 3: Mineral Contents of Aqueous Leaf Extract of *Justiciacarnea*

Mineral/metal	Quantity (ppm)
Potassium	14800.00
Iron	218.00
Calcium	1930.00
Zinc	73.00
Lead	1.00

The result of the hematological analysis of the blood samples of the Wistar rats is shown in Table 1. The plant extract showed an improvement in the hematological indices that were examined at the lowest dose of 100 mg/kg.

Table 4: Heamatological indices of the Wistar Rats

	HB	**WBC**	**PCV**	**PLT**	**ESP**	**BSP**	**MNC**	**NUP**	**LMP**
Negative control	15.20 ± 0.50	11.17 ± 3.70	45.67 ± 1.52	150.00 ± 30.82	1.67 ± 0.27	2.00 ± 0.47	1.67 ± 0.27	55.33 ± 2.13	39.33 ± 1.44
Positive control	10.20 ± 1.46*	8.20 ± 0.47	30.67 ± 4.37*	149.33 ± 15.76	2.00 ± 0.58	1.33 ± 0.67	1.33 ± 0.88	1.15 ± 0.67	1.15 ± 0.67
Extract 100mg/kg	15.73 ± 0.72*	15.60 ± 0.36**	47.33 ± 2.19*	174.67 ± 21.76	1.33 ± 0.33	2.33 ± 0.33	1.67 ± 0.33	41.00 ± 6.66	52.33 ± 6.64
Extract 200mg/kg	14.63 ± 0.38*	8.00 ± 0.85	44.00 ± 1.15*	173.00 ± 30.66	1.33 ± 0.67	1.00 ± 0.58	1.33 ± 0.33	56.67 ± 6.06	40.00 ± 5.77
Extract 500mg/kg	15.10 ± 1.10*	10.8 ± 0.93**	45.33 ± 3.33*	181.67 ± 9.53	1.67 ± 0.33	0.00 ± 0.00	1.00 ± 0.58	63.00 ± 2.08	35.33 ± 2.91
Extract 1000mg/kg	14.30 ± 1.35*	11.90 ± 1.75	42.33 ± 3.71	191.67 ± 11.67*	0.33 ± 0.33*	1.67 ± 0.33	0.67 ± 0.33	58.00 ± 4.00	41.00 ± 4.51

Key: HB= Heamoglobin, WBC = White Blood Cells, PCV –Packed Cell Volume, PLT = Platelets, ESP – Esinophil, BSP = Basophil, MNC = Monocytes, NUP =Neutrophil, LYP = Lymphocytes

Significance = *P<0.05, **P<0.01
When the positive was compared with the negative, HB and PCV was significant at *P<0.05

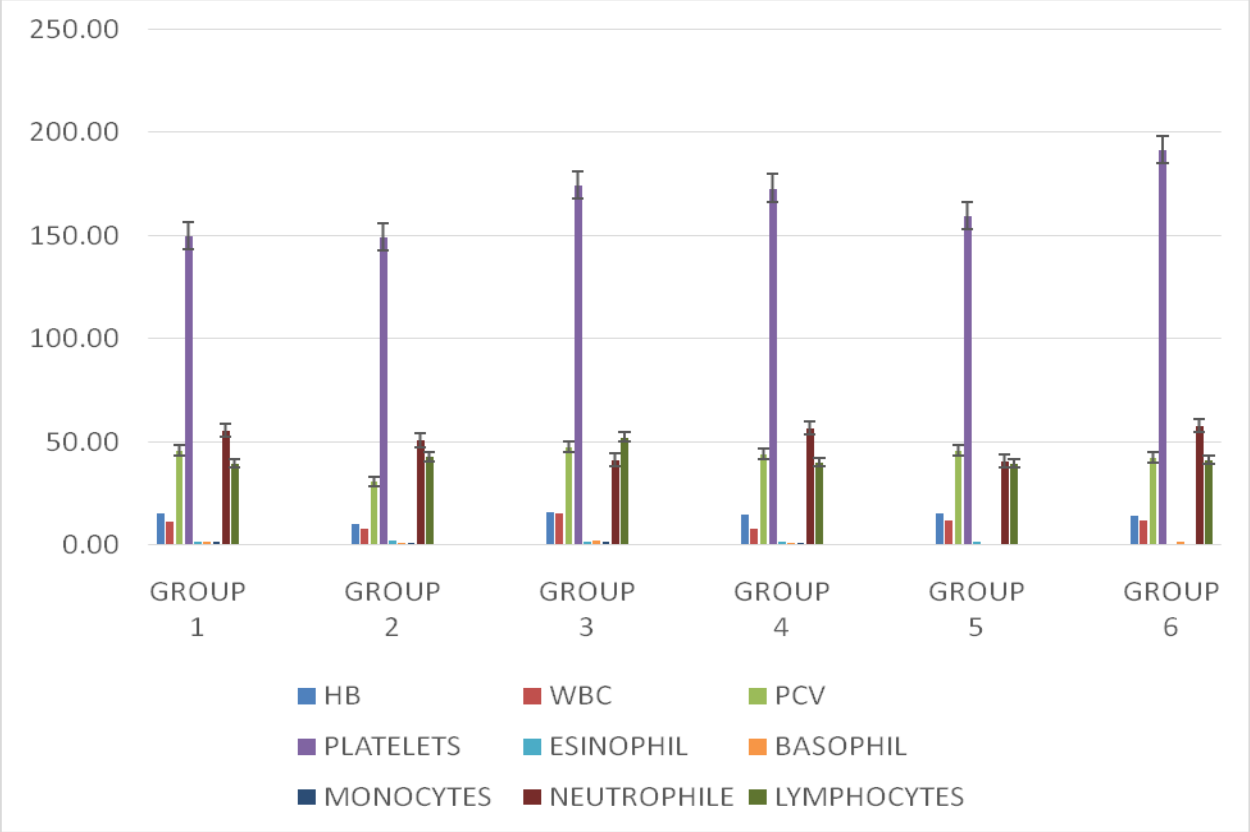

Figure 1: Representation of the biochemical assay of effect of ethanol extract of *Justicia carnea* on the hematopoeitic components in phenylhydrazine induced anemic rats.

glomerular matrix PCT = Proximal convoluted tubule, DCT= Distal convoluted tubule

The photomicrograph of the processed kidney tissues are shown in figures 2-7.
Figures 2 and 3showed glomerular capillaries (Red), mesangial cells (blue), mesangial matrix (gray), proximal convoluted tubules, distal convoluted tubules which were normal. The glomerular capsular space was patent (black). These show that histologically, the kidneys were normal.
Figure 4 showed lobulation of glomerular tuft and collapsed tubules which indicates a distorted kidney. In Figure 5, there was infiltration of interstitial tissue with inflammatory cells which is suggestive of a distorted kidney. Figure 6 and 7 presented with normal glomeruli and renal tubules which suggest a normal kidney.
The photomicrographs of the liver tissues are shown in Figures 8-13. In Figure 8, the hepatocytes, hepatic plates, sinusoidal endothelial cells, sinusoids, bile ducts and connective tissue were present. This is indicative of a normal liver.
In figures 9 and 10, the portal vein were congested with blood cells, micro vesicular steartosis and cytoplasm were replaced with fatty cells while the nuclei were centrally placed. Council mem's bodies were found without nucleus representing features of a distorted liver.
Figures 11 shows that the central vein was congested. Micro vascular steartosis were present and the nuclei were fused together, suggestive of histologic section of liver with evidence of regeneration.
In figures 12 and 13, the portal vein were patent, hepatocytes and hepatic sinusoids were normal indicative of histologically normal liver section

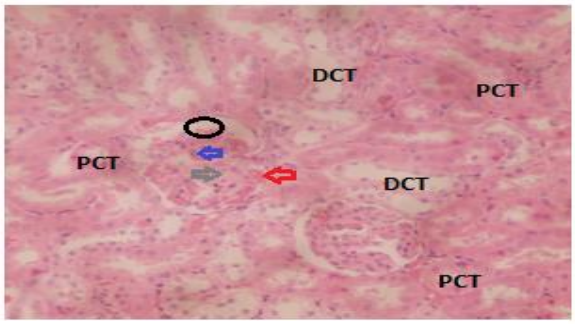

Figure 2: Photo micrograph of Kidney of animals administered Distilled water (Negative Control)

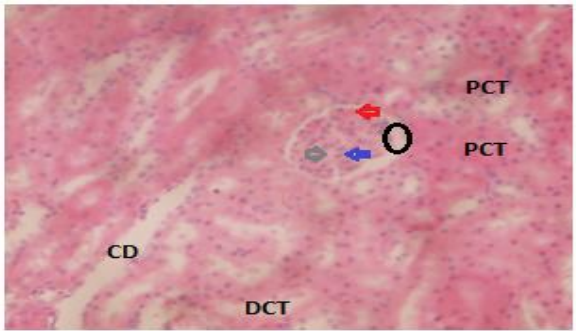

Figure 3: Photo micrograph of Kidney of animals administered Phenylhydrazine (Positve control)

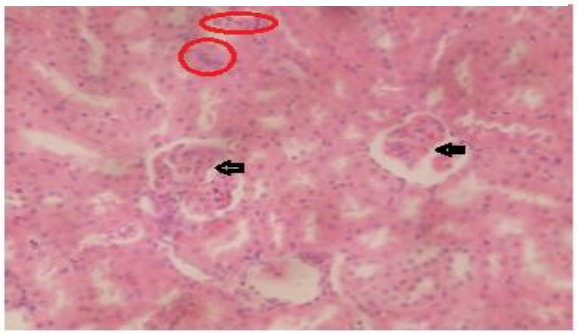

Figure 4: Photo micrograph of Kidney of animals treated with 100mg/kg of extract

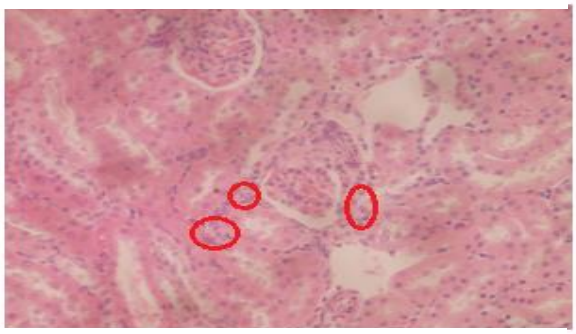

Figure 5: Photo micrograph of Kidney of animals treated with 200mg/kg of extract

Figure 6: Photo micrograph of Kidney of animals treated with 500mg/kg of extract

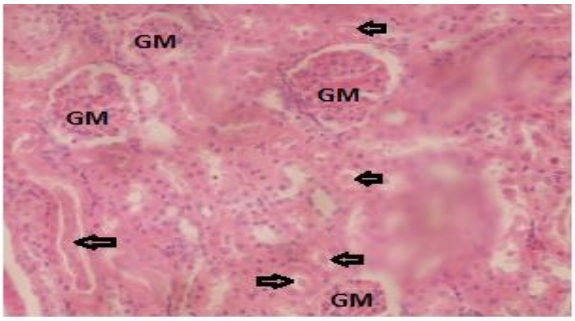

Figure 7: Photo micrograph of Kidney of animals treated with 1000mg/kg of extract

GM = glomerular matrix PCT = Proximal convoluted tubule, DCT= Distal convoluted tubule

DISCUSSION

With the increasing stress associated with modern life conditions, different types of diseases are having a free day and people are inclining towards the use of herbal products to keep various diseases atbay and boost their health (Dahiya, 2013). Regular consumption of plant foods are associated with numerous health benefits rooted in their various physiological effects as a result of their phytochemical and nutritional constituents (Hussein *et al*; 2011). Green leafy vegetables are particularly important in promoting health because of their nutritive contents (Goupy*et al*, 1995).

Phenylhydrazine induced anemia resulted indecreased hemoglobin level, RBC count, PCV and impaired erythrocyte deformability (Berger, 2007). The ethanol leaf extract of *J. Carnea* reversed the phenylhydrazine induced anemia in the animals. Oxidative damage to red blood cells resulting in the production of reactive oxygen species in the presence of phenylhydrazine has been reported by researchers (Clemens *et al.*, 1984). Therefore, the increase in the hematological indices after administration of the extract could be attributed to the phytochemicals (saponins, flavonoids and alkaloids) and antioxidants in the plant extract. These results agree with reports on extracts of *Tectonagrandis* (Diallo *et al.*, 2008),*Mangifera indica*, *Amaranthu shybridus* and *Terifairia. occidentalis* (Ogbe *et al.*, 2010), which increased the concentration of haemoglobin and red blood cells after induction with phenylhydrazine (Diallo *et al.*, 2008). The extract was able to reverse

the phenylhydrazine induced anemia with an increase in the hemoglobin level even at a dose of 100 mg/kg. There was also a significant increase in the WBC countsuggestive of the fact that the entire hemopoietic pathway may have been stimulated by the plant extract (Okonkwo *et al*., 2015). WBC counts increase rapidly following a foreign attack on the system by pathogens and the normal physiologic response of the system will be to boost the body's defense mechanisms (Eyong *et al*., 2004). An increased number of monocytes in the blood (monocytosis) occur in response to chronic infections, in autoimmune disorders, in blood disorders, and in certain cancer (Radhika *et al;* 2013). A reduction in monocyte level was observed at the concentrations of 500mg/kg and 1000mg/kg which may indicative of the absence of anemia. A definite decrease in neutrophil count shows presence of diseases such aneamiaetc. (Berns, 2009) as was seen in the positive control but after the administration of the extract, the neutrophil count increased tremendously as was seen in the different concentrations. The lymphocytes decrease with presence of diseases (while it increases in the presence of a remedy (such as *J. carnea* extract. The increase in hemoglobin, PCV and WBC after administration of extract of *Justicia carnea* corresponds to the experiment carried out by (Chimaraoke, *et al*., 2017). The toxicity profile of *J. carnea* on the liver and kidney showed different findings at different concentration of the extract.Liver diseases remain serious health problems and are caused, among others, by drugs, chemicals etc. Many xenobiotic are capable of causing some degree of liver injury (Bass, *et al*., 1996). The liver is prone to xenobiotic-induced injury because of its central role in xenobiotic metabolism, its portal location within the circulation, and its anatomic and physiologic structure (Jones, *et al*. 1996). The kidney is the major organ that functions in the filtration, reabsorption, secretion and excretion of substances and a damage to this organ will lead to accumulation of substances that may be poisonous to the body, it is important that any product taken should have a safety profile to the kidney (Walter, 2004). Histopathological assessment of liver damage was carried out by studying hematoxylin and eosin stained slides of liver tissue. The extract was able to reverse both liver and kidney damage associated with phenylhydrazine administration. The extract at concentrations 100 mg/kg and 200 mg/kg were effective in improving the hematological properties after induction of anemia to a reasonable count but was not able to take proper care of the liver and kidney which implies that production of volatile hydrocarbons. *Biochemistry* the both concentration should be used for a long period before it will balance the organs in the body to their normal functioning state while the extract at concentrations of 500 mg/kg and 1000 mg/kg were found to considerably improve the hematological components and also restored liver and kidney damage associated with phenylhydrazine induced anemia showing that the concentrations are effective for acute treatment of anemia.

CONCLUSION

This study shows that *J. carnea* extracts possess antianemic potential, which satisfies to the use of these plant extracts in folk medicine for the management of anemia. The observations from this study revealed that leaves of *J. carnea* not only possess antianemic properties as reportedly used by traditional healers, but poses no toxicity to the vital organs such as the liver and the kidney at high concentration. Further studies are needed to determine the bioactive component present in *J. carnea* leaves that could be responsible for anti-anemic properties.

REFERENCES

AOAC.(1990). Official Methods of Analysis. 15th Edition, Washington, D.C.; Association of Official Analytical Chemist: 69-88.

Bass N.M, Ockner B.A.(1996).Drug-induced liver disease. In: Zakin D, Boyer T.D, editors. Hepatology: a textbook of liver disease. 3rd eds. Philadelphia: W.B Saunders: 962–1017

BernsJ.S. (2009) Anemia of chronic kidney disease: Target hemoglobin/hematocrit for patients treated with erythropoietic agents. http://www.uptodate.com/home. Accessed Dec. 8, 2015.

Berger, J. (2007). Review: Phenylhydrazine haematotoxicity. *J. Applied. Biomed*. 5: 125–130.

Chimaraoke O, Ngozi K. Achi, Chima A. Ekeleme-Egedigwe, Chidinma U. Ebere, Chidinma K. Okoro (2017), haematological and biochemical studies on justiciacarnea leaves extract in phenylhydrazine induced-anemia in albino rats:*ActaScientiarumPolorionumTechnology Aliment*. 16(2):217–230

Clemens, M. R., Remmer, H., Waller, H. D. (1984). Phenylhydrazine-induced lipid peroxidation of red blood cells in vitro and in vivo: Monitoring by the

and Pharmacology, 33:715– 1718.

Dahiya, K. (2013). Nutraceuticals and their impact on human health.*Journal of Plant. Biochemistry Physiology*, 1(4): 1-3.

Diallo, A., Gbeassor, M., Vovor, A., Eklu-Gadegbeku, K., Aklikokou, K. Agbonon, A. (2008). Effect of Tectonagrandis on phenylhydrazine induced aneamia in rats: *Fitoterapia*, 79: 332–336.

Eyong, E. U., Umoh, I. B., Ebong, P. E., Eteng, M. U. Antai, A. B., Akoa, A. O. (2004). Heamotoxic effects following ingestion of Nigerian crude oil and crude oil polluted shellfish by rats. *Nigerian Journal of Physiological Science***19**(1–2):1–6.

Fasuyi, A. O. (2006). Nutritional potentials of some tropical vegetable leaf meals.Chemical characterization and functional properties. African Journal of Biotechnology, 5: 49–53.

Gbenou, J. D., Tossou, R., Dansou, P., Fossou, M., Moudachirou, M. (2006).Etude des proprieties antianémiques de JusticiasecundaVahl (Acanthaceae) chez les rats de souchewistar.*Pharmaceutical Medicine of Traditional Africa*, 14: 45–54

Goupy, P., Amiot, M.J., Richard-Forget, F., Duprat, F., Aubert, S. and Nicolas, J. (1995). Enzymatic browning of model solutions and apple phenolic extracts by apple polyphenol oxidase. *Journal of Food Science*, 60: 497-501

Harborne, J.B. (1998). Phytochemical Methods.A Guide to Modern Technology of Plant analysis, 3rd Edition. Chapman and Hall, New York: 88-185.

Hussein, S. Z., Yusoff, K.M., Makpol, S. and Yusof, Y. A. M. (2011). Antioxidant capacities and total phenolic contents increase with gamma irradiation in two types of Malaysian honey. *Molecules* . *16(8),6378-6395.*

Jones A.L. Anatomy of the normal liver. In: Zakin D, Boyer T.D, editors. Hepatology: a textbook of liver disease. 3rd edition Philadelphia: WB Saunders; 1996.3–32.

Kumar, A., Iavarasan, R. I., Jayachandran, T., Decaraman, M., Aravindhan, P., Padmanabhan, N., Krishnan, M. R. V. (2009).Investigation on a tropical plant *Syzygium cumini* from Kattuppalayam, Erode District, Tamil Nadu, South India.*Pakistan Journal of Nutrition*, 8: 83–85.

LichtmanM.A(2010) Structure of the marrow and the hematopoietic microenvironment. In: Williams Hematology. 8th edition New York, N.Y.: The McGraw-Hill Companies;

Ogbe, R. J., Adoga, G. I., Abu, A. H. (2010*).* Antianaemic potentials of some plant extracts on phenyl hydrazineinducedaneamia in rabbits. *Journal of Medicinal Plants Research*, 4(8): 680–684.

Okonkwo, C. C., Agu, C. V., Njoku, O. U., Abonyi, U., Apeh, V., Anaduaka, E.G., Odo, C. E. (2015). Hypoglycaemic and haematinic properties of ethanol leaf extract of Artocarpusheterophyllus in alloxan induced diabetic rats. *African Journal of TraditionalComplementary Alternative Medicine*, 12(2):144–148.

Radhika, J., Sathya, S., Jothi, G and Japasheba, JL. (2013).Cardioprotective role of JusticiatraquebareinsisLinn.leaf extract in isoproterenol induced myocardial infarction in albino rats. *Journal of Applied Pharmaceutical Science*, 3(4):124–128.

Tchogou, AP., Sènou, M., Dougnon, TV.,Agossadou, A., Assogba, F., Kinsiclounon, EG, Loko, F. (2016). The aqueous extracts of Cocosnucifera L. (Arecaceae) effectively treat induced aneamia. Experimental study on wistar rats. International Journal of Biology, 8(3): 1–10.

Walter F. Boron (2004). Medical Physiology: A Cellular and Molecular Approach. Elsevier.
World Health Organization Report (2002): Reducing risks, promoting healthy life. Geneva, World Health Organization, 2002.www.nhlbi.nih.gov/health-topics/sickle-cell-disease