Haematological and Biochemical Changes in aqueous leaf extract of *Justicia carnea* treated male Wistar rats exposed to lead acetate

ABSTRACT

Aim: To evaluate the effect of oral administrattion of the leaf extract of *Justicia carnea* on some haematological and biochemical parameters in rats.
Study design: It was a laboratory and experimental animal based study.
Place and Duration of Study: The study was carried out In Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Rivers State, Nigeria.
Methodology: Forty (40) male Wistar rats were obtained and randomly distributed into eight (8) groups of 5 rats each as follows: Group 1 served as negative control/received 1ml of distilled water daily; Group 2 served as positive control/treated with 10mg/kg.bw of lead acetate (Pb); Groups 3 to 5 received 400, 600 and 800mg/kg body weight of aqueous leaf extract of Justicia carnea (ALEJC) respectively daily and Groups 6 to 8 received 400, 600 and 800mg/kg bw ALEJC + 10mg/kg.bw Pb respectively daily. After all treatments, blood samples were harvested from the study animals via cardiac puncture Data were statistically analysed using the Statistical Package for Social Sciences (SPSS) version 21.0. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Post-Hoc multiple comparison test and $p< 0.05$ was considered statistically significant.
Results: The result of the present study indicated significant ($p<0.05$) increases in the levels of RBC, Hb, PCV, WBC and platelets in the 2 sets of rats when compared to those of groups 1 and 2. The amount of total/conjugated bilirubin were also seen to be markedly ($p<0.05$) raised in all ALEJC treated rats. The levels of liver enzymes were significantly ($p<0.05$) and beneficially regulated by ALEJC treatment compared to that of the group 2.
Conclusion: Therefore, the plant extract may be a biologically helpful and antianaemic candidate but the arbitrary use of the ALEHC must be checked in order to prevent undesirable effects.

Keywords: antianaemic agent, biochemical, haematologica lmarkers, Justicia carnea,

1. Introduction

Most native populations, especially of the developing world, depend on medicinal plants as an integral component of their healthcare resource for diverse ailments and diseases [1]. However, considering the need for standardization and safety, more studies are encouraged on medicinal plants [2]. There is almost an unending list of herbal plants used in the treatment or management of different disorders/diseases in our locale, but the focus of the present study is on *Justicia carnea* (of the family, Acanthanceae) plant. The genus Justicia was named after the 18th century Scottish botanist James-Justice. It is a flowering plant that is vastly grown in Tropical world. In Nigeria, *Justicia carnea,* has over the years been recognized to be a medicinal plant that serve as a quick blood tonic as well as in the treatment of others disorders/conditions [3]. Traditionally, the leaves of *Justicia carnea* are usually boiled and the decoction which is usually crimson red in colour are administered in anaemic patients and others. Tradomedicinal practitioners often administer it to mostly anaemic patients, postpartum women and post-menstruation in women, to help replenish their depleted blood level [3, 4].
Justicia carnea is usually considered as an ornamental plant and is widely distributed in different regions of Africa. In Nigeria, it is a common homestead grown plant often acting as fences [3].

Justicia carnea has been proven by several scientific studies to possess blood-boosting/tonic potentials [4, 5]. It is believed to possess phytoconstituents that enables it to effectively restore depleted blood levels to normal volume within a short treatment time [3].

Considering the importance of the use of haematological and biochemical parameters in the diagnosis and treatment of anaemia and related disoders [6], the present study set out to scientifically evaluate and validate the effect of the administration of the leaf extract of *Justicia carnea* on some haematological and biochemical parameters in male Wistar rats exposed to lead acetate.

2. Material and Methods

2.1 Research Design

The current study was an experimental/laboratory-based with the male Wistar rats as study model. The animals were obtained and accommodated in the Animal House of the Faculty of Basic Medical Sciences, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria. The weights of the animals ranged between 160g and 200g and were maintained in the 12 hour light/dark cycle under room temperature and about ambient humidity. They were acclimatized to the new handlings and accommodation for 14 days to the take-off of experimentations. The rats were allowed access to normal rat feed and tap water *ad libitum*.

The guideline for the care and use of experimental animals [7] were observed.

2.2 Plant Materials

The leaves of *Justicia carnea* were collected from a private garden located in Port Harcourt, Rivers State, Nigeria. A specimen of the *Justicia carnea* leaves were authenticated at the herbarium section of the Department of plant science and biotechnology, University of Port–Harcourt with herbarium number UPH/C/096.

2.3 Aqueous Extraction of Justicia carnea Leaves

Justicia carnea leaves were air dried under shade to prevent the ultraviolet decomposition of volatile and light sensitive photochemical constituents of the plant. After the leaves have dried out, they were pulverized into fine powder. The obtained dried powder of the plant was extracted using the cold maceration method. Two hundred grammes (200g) of the blended powder were dissolved in 1liter of water in a 2L conical flask. After 72 hours of periodic macerations, the mixture was filtered using the whatman number 1 filter (Whatman England) and

cotton wool to isolate plant sample parcels. The rotary evaporator and water bath at 40°C respectively were used to drive off the remaining water and the recovered. Semi-solid extract was then kept in sterile screw-capped container, labeled accordingly and refrigerated at 4°C.

2.4 Dosage Preparation of Test Samples

The dosages of 400, 600 and 800mg/kg b.w were adopted by the present study based on the report of Anthonia et al., [8], who reported the LD_{50} of the *Justicia carnea* leaves to be above 5000mg/kg.

It is noteworthy to state that the doses of 600mg/kg and 800mg/kg of the extract and 10mg/kg of the lead acetate (PbAc) were determined based on earlier reported effective doses [8], the administration of 10mg/kg of lead acetate (PbAc) in the study models via their drinking water commenced for two weeks prior to the commencement of the administration of the extracts and continued through the entire period of administration.

2.5 Study Protocol

Following the acclimatization of the 40 male Wistar rats, they were randomly distributed into eight (8) groups of five (5) rats each and were labeled as follows:

Group 1: served as negative control and received only 1ml of distilled water daily.
Group 2: served as positive control and were treated with lead acetate (Pb) + 1ml of distilled water daily for 6 weeks.
Group 3: received 400mg/kg body weight of aqueous leaf extract of *Justicia carnea* (ALEJC) for 6 weeks.
Group 4: received 600mg/kg b.w ALEJC for 6 weeks.
Group 5: received 800mg/kg b.w ALEJC for 6 weeks.
Group 6: received 400mg/kg b.w ALEJC + Pb for 6 weeks.
Group 7: received 600mg/kg b.w ALEJC + Pb for 6 weeks.
Group 8: received 800mg/kg b.w ALEJC + Pb for 6 weeks.

Nature/Source of Data

2.6 Harvesting of Samples from Study Animals

After the period of treatments, using the method of cardiac puncture, blood samples were harvested from the rats for laboratory screening after being sedated with 80% chloroform soaked in a cotton wool inside a desiccator. Exactly 5ml of blood was taken per rat and put into the

Ethylenediaminetetraacetic acid (EDTA) and lithium heparin sample bottles respectively before the automated analyses of the respective parameters.

The data obtained from the present study were subjected to statistical analyses using the Statistical Package for Social Sciences (SPSS) version 21.0. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Post-Hoc multiple comparison test and $p < 0.05$ was considered statistically significant. The values were expressed as mean ± standard error of mean (SEM).

3. Results

Table 1 shows the outcome of the effect of administration of aqueous leaf extract of *Justicia carnea* (ALEJC) on some haematological parameters in male Wistar rats.

The RBC level was found to be significantly ($p<0.05$) increased in groups 4, 5 and 8 (i.e. 600 and 800mg/kg ALEJC and 800mg/kg ALEJC + Pb treated groups) when compared to those of groups 1and 2 (i.e. untreated and lead (Pb) alone treated rats respectively). On the other hand, group 6 treated with 400mg/kg ALEJC + Pb indicated significantly ($P<0.05$) reduced RBC level when compared to that group 3, treated with 400mg/kg ALEJC.

Considering the changes on haemoglobin concentration (Hb), it was observed that group 2, the lead (Pb) only treated group had significantly ($P<0.05$) decreased Hb level when compared to that of group 1, normal untreated rats. All other groups indicated significantly ($P<0.05$) elevated Hb levels when compared to that of group 2. It is important to note that although Hb levels were dose-dependently increased in both the ALEJC+ Pb treated rats and the ALEJC treated rats, the increases were greatest in the ALEJC alone treated rats.

The trend of changes in the packed cell volumes (PCV) of the rats were exactly same as narrated in Hb levels.

The effects of the administrations on the WBC levels revealed significantly ($P<0.05$) higher levels of WBC in virtually all ALEJC treated groups when compared to those of groups 1 and 2; these increases were also significantly ($P<0.05$) in groups 6, 7 and 8 when compared to groups 4 and 5.

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Table 1: Effect of administration of Aqueous Leaf Extract of *Justicia carnea* (ALEJC) on some Haematological Parameters in male Wistar rats.

Groups and Treatment	Parameters				
	Red Blood Cell (RBC) Count ($X10^{12}$/L)	**Haemoglobin concentration (g/dL)**	**Packed Cell Volume (%)**	**White Blood Cell (WBC) Count ($X10^{9}$/L)**	**Platelet Count ($X10^{9}$/L)**
Group 1: Control	4. 70 ± 0.2	14.15 ± 0.70	42.50 ± 2.11	6.90 ± 0.60	176.70 ± 15.85
Group 2: Pb only treated	3.30 ± 0.43	7.67 ± 0.80 [a]	21.50 ± 2.01 [a]	6.50 ± 0.44	166.70 ± 21.33
Group 3: 400mg/kg ALEJC	6.25 ± 0.80	20.10 ± 0.94 [a, b]	60.33 ± 2.85 [a, b]	9.97 ± 1.00 [a, b]	254.70 ± 8.62 [a, b]
Group 4: 600mg/kg ALEJC	7.75 ± 0.95 [b]	22.40 ± 1.11 [a, b]	64.70 ± 4.41 [a, b]	8.13 ± 1.11 [a, b]	194.70 ± 22.31 [c]
Group 5: 800mg/kg ALEJC	9.30 ± 1.04 [a, b]	25.80 ± 2.05 [a, b, c]	79.00 ± 3.81 [a, b, c, d]	7.20 ± 0.75 [c]	182.00 ± 10.47 [c]
Group 6: 400mg/kg ALEJC +Pb	4.95 ± 0.50 [e]	13.90 0.74 [b, c, d, e]	41.70 ± 2.23 [b, c, d, e]	11.27 ± 0.63 [a, b, d, e]	307.33 ± 29.01 [a, b, d, e]
Group 7: 600mg/kg ALEJC +Pb	6.48 ± 1.12	17.70 ± 0.90 [a, b, d, e, f]	54.17 ± 2.81 [a, b, d, e, f]	11.30 ± 0.70 [a, b, d, e]	255.33 ± 13.52 [a, b, d, e]
Group 8: 800mg/kg ALEJC +Pb	9.22 ± 2.50 [a, b, f]	23.00 ± 1.70 [a, b, f, g]	69.00 ± 5.01 [a, b, e, f, g]	11.73 ± 1.05 [a, b, d, e]	263.33 ± 26.82 [a, b, d, e]

Values represent mean ± SEM, n=6; [a] Significant at p<0.05 compared to Group 1; [b] Significant at p<0.05 when compared to group 2; [c] Significant at p<0.05 when compared to group 3; [d] Significant at p<0.05 when compared to group 4; [e] Significant at p<0.05 when compared to group 5; [f] Significant at p<0.05 when compared to group 6; [g] Significant at p<0.05 when compared to group 7. Pb for lead acetate—(PbAc

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Table 2: Effects of administration of Aqueous Leaf Extract of *Justicia carnea* (ALEJC) on WBC Differentials

Group and Treatment	Neutrophil (%)	Lymphocyte (%)	Eosinophil (%)	Basophil (%)	Monocyte (%)
Group 1: Control	26.00 ± 2.55	69.50 ± 2.20	1.67 ± 0.76	0.50 ± 0.34	2.33 ± 1.33
Group 2: Pb only treated	20.33 ± 1.41	73.50 ± 2.40	1.83 ± 0.70	0.33 ± 0.21	3.83 ± 1.17
Group 3: 400mg/kg ALEJC	31.50 ± 4.00 [b]	55.50 ± 4.28 [a, b]	4.50 ± 1.36 [a, b]	0.67 ± 0.50	6.50 ± 1.00 [a]
Group 4: 600mg/kg ALEJC	35. 33 ± 3.00 [a, b]	57. 70 ± 3.24 [a, b]	2.17 ± 0.70	0.20 ± 0.17	4.17 ± 0.90
Group 5: 800mg/kg ALEJC	30.83 ± 4.01 [b]	58.33 ± 3.56 [a, b]	4.67 ± 0.76 [a, b, d]	0. 20 ± 0.16	6.00 ± 1.12 [a]
Group 6: 400mg/kg ALEJC +Pb	23. 70 ± 3.06 [d]	70.33 ± 3.76 [c, d, e]	2.17 ± 0.40 [e]	0. 20 ± 0.17	4.67 ± 1.00
Group 7: 600mg/kg ALEJC +Pb	26.70 ± 1.12 [d]	65. 70 ± 1.96 [c]	2.33 ± 0.01	0. 20 ± 0.17	5.17 ± 0.90
Group 8: 800mg/kg ALEJC +Pb	36.00 ± 1.11 [a, b, f, g]	59.00 ± 3.20 [a, b, f]	2.17 ± 0.80 [e]	0.00 ± 0.00	2.83 ± 0.75 [c, e]

Values represent mean ± SEM, n=6; [a] Significant at p<0.05 compared to Group 1; [b] Significant at p<0.05 when compared to group 2; [c] Significant at p<0.05 when compared to group 3; [d] Significant at p<0.05 when compared to group 4; [e] Significant at p<0.05 when compared to group 5; [f] Significant at p<0.05 when compared to group 6; [g] Significant at p<0.05 when compared to group 7. Pb for lead acetate—(PbAc

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Table 3: Effects of administration of Aqueous Leaf Extract of *Justicia carnea* (ALEJC) on some biochemical parameters

Group and Treatment	Total Protein (g/L)	Albumin (g/L)	Total Bilirubin (µmol/L)	Conjugated Bilirubin (µmol/L)	AST (iu/L)	ALT (iu/L)	ALP (iu/L)
Group 1: Control	30.00 ± 7.17	48.00 ± 1.32	19.40 ± 2.58	9.67 ± 2.30	12.00 ± 2.04	9.00 ± 2.00	13.50 ± 3.41
Group 2: Pb only treated	9.50 ± 1.26 [a]	17.50 ± 1.82 [a]	9.10 ± 2.03	0.67 ± 0.33 [a]	19.67 ± 3.20 [a]	36.33 ± 4.80 [a]	34.50 ± 4.67 [a]
Group 3: 400mg/kg ALEJC	37.03 ± 5.65 [b]	48.03 ± 2.03 [b]	39.15 ± 5.04 [a]	5.83 ± 1.01 [b]	11.50 ± 1.30 [b]	7.00 ± 1.24 [b]	11.83 ± 2.76 [b]
Group 4: 600mg/kg ALEJC	27.00 ± 5.55 [b]	45.17 ± 4.33 [b]	26.18 ± 4.17 [c]	5.17 ± 1.01	10.00 ± 1.90 [b]	11.20 ± 2.70 [b]	10.50 ± 1.50 [b]
Group 5: 800mg/kg ALEJC	36.00 ± 10.61 [b]	50.70 ± 5.70 [b]	20.52 ± 4.00 [c]	9.67 ± 1.33 [b]	14.70 ± 5.34	16.67 ± 4.60 [b, c]	4.83 ± 1.08 [a, b]
Group 6: 400mg/kg ALEJC +Pb	35.67 ± 4.39 [b]	48.70 ± 1.41 [b]	20.30 ± 3.61 [c]	7.00 ± 1.05 [b]	11.00 ± 1.50 [b]	8.00 ± 1.95 [b, e]	7.33 ± 1.02 [b]
Group 7: 600mg/kg ALEJC +Pb	49.50 ± 6.01 [b]	46.50 ± 3.02 [b]	49.37 ± 3.21 [a, d, e, f]	24.30 ± 3.01 [a, b, c, d, e, f]	9.33 1.43 [b]	9.50 ± 2.10 [b]	5.70 ± 1.33 [b]
Group 8: 800mg/kg ALEJC +Pb	39.67 ± 9.34 [b]	49.33 ± 2.40 [b]	55.45 ± 4.45 [a, c, d, e, f]	16.02 ± 2.00 [a, b, c, d, e, f, g]	10.00 ± 1.55 [b]	8.83 ± 2.04 [b]	11.00 ± 4.00 [b]

Values represent mean ± SEM, n=6; [a] Significant at p<0.05 compared to Group 1; [b] Significant at p<0.05 when compared to group 2; [c] Significant at p<0.05 when compared to group 3; [d] Significant at p<0.05 when compared to group 4; [e] Significant at p<0.05 when compared to group 5; [f] Significant at p<0.05 when compared to group 6; [g] Significant at p<0.05 when compared to group 7. Pb for lead acetate—(PbAc.

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The result on the changes in platelet levels indicated that, the levels significantly (P <0.05) increased in virtually all treated groups when compared to those of groups 1 and 2; and these increases were more in the ALEJC + Pb treated groups.

The data on Table 2 represent the outcome of the effects of administration of aqueous leaf extract of *Justicia carnea* (ALEJC) on WBC differentials.

A look at the neutrophil levels reveals that 3, 4, 5, and 8 had significantly raised levels when compared to group 2. Groups 3 and 4 showed significantly (P <0.05) decreased levels when compared to that of group 4.

The percentage of lymphocytes was found to significantly (p<0.05) decreased in groups 3, 4, and 8 compared when respectively compared to those of groups 1 and 2.

The percentage of eosinophil was seen to significantly (P <0.05) increase in groups 3 and 5 when compared to those of groups 1 and 2.

The variances of the percentage levels of basophil did not show any significant (p>0.05) changes across all the treated groups.

On the other hand, percentage of monocytes only significantly increased in groups 3 and 5 when compared to that of group 1; but group 8 had significantly (p<0.05) reduced levels compared to those of groups 3 and 5.

Table 3 displays the effects of administration of aqueous leaf extract of *Justicia carnea* (ALEJC) on some biochemical parameters which include total protein, albumin, bilirubin and some liver enzymes levels.

The total protein level was only significantly (P <0.05) reduced in group 2 when compared to that of group 1. However, the levels of total protein in all treated groups were significantly (P <0.05) raised compared to that of group 2.

The changes in albumin levels across all respective groups followed exactly the same trend as in total protein narrated above.

The result of the changes in total and conjugated bilirubin indicated drastically (P <0.05) reduced levels in group 2 compared to group 1; but that their levels significantly (P <0.05) increased in groups 7 and 8 when compared those of other treated groups.

Considering the changes in the liver enzymes (AST, ALT and ALP) levels, it was found that group 2 had significantly (P <0.05) elevated levels compared to both control (group 1) and all other treated groups (i.e. from 3 to 8).

4. Discussion

The continual consumption of medicinal plants in our locality is often linked to the notion that they possess diverse beneficial and therapeutic potentials [9]. *Justicia carnea*, a medicinal plant, known for its local ethnobothanical application as blood tonic plant, has been the foci with several interests [5]. More often than not, some of these investigations are not exhaustive as to properly understand the fundamental effects of such medicinal plants [10]. The present study, thus, evaluated the possible effects of the aqueous leaf extract of *Justicia carnea* (ALEJC) on

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some haematological and biochemical parameters in multi-organ lead acetate-induced toxicity in male Wistar rats. The key findings of the investigation are as discussed in the following paragraphs.

4.1 Effects of Administration of Aqueous Leaf Extract of *Justicia carnea* (ALEJC) on Haematological Parameters in the Study Animals

A. Effects of ALEJC on RBC, Hb and PCV levels

The outcome of the effect of administration of aqueous leaf extract of *Justicia carnea* (ALEJC) on some haematological parameters in male Wistar rats reveals a remarkable boosting effect on RBC, Hb and PCV levels in the animals not exposed to lead and a corroboratory dose dependent ameliorations in the levels of same parameters in the lead poisoned animals. In fact the PCV values of the non-lead exposed rats indicated increasing dose-dependent polycythaemic levels. As seen in Table 1, these findings were not only similar to the earlier reports of Nji *et al.,* [11] and Akintimehin *et al.,* [5], who also recorded markedly raised RBC, Hb and PCV levels following treatments of laboratory animals with extracts of *Justicia carnea*, but showed a much more raised levels of these parameters.

Considering this property of the *J. carnea* extract, different schools of thought [4, 9] have attributed it to the phytochemical and proximate compositions of the plant. Validating this opinion, Akuodor et al., [12] and Olufunke, [9] asserted that plants under the genius Justicia share this property. More so, this category of plants have been noted for their effective potencies in the management of inflammation, gastrointestinal disorders, respiratory tract infection, fever, pain, diabetes, diarrhoea, liver diseases, rheumatism and arthritis as well as anti-inflammatory, anti-tumoral, antiviral and analgesic activities [5, 8, 13]. Thus, the present study validates the fact that the ALEJC may be a potent candidate for blood tonic or anti-anaemic agent in a mammalian model. Further, advance studies on the finger print identification of the actual active ingredients possibly responsible for the erythrocyte boosting effect in the ALEJC as well as their likely isolation may be very beneficial in fully tapping its therapeutic potency.

B. Effects of ALEJC on WBC Count and Differentials

Generally the result of the ALEJC treatment on the WBC levels revealed comparatively significant leucocytosis in virtually all ALEJC treated groups with respect to groups 1 and 2; these increases were even markedly higher in groups 6, 7 and 8 (i.e. groups co-treated with different doses of ALEJC and lead (this may be attributed to the possible additional toxic effect of lead). The result of the WBC differentials followed a similar trend with that of foregoing as could be seen in the percentages of neutrophils and eosinophils. Nonetheless this was observed to be different with the changes in percentages of lymphocytes, which appreciably decreased in the extract only treated rats and increased in the extract plus lead treated rats; but the reverse was almost the case with monocytes. The basophil had no significant alterations in the treated rats as against the untreated.

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Conditions like toxicity, infections, inflammation, allergic reaction, immunostimulatory therapeutic agents, etc., may directly or indirectly result in leucocytosis [14], thus, the multi-organ toxicity effect in the lead exposed rats may be responsible for the markedly raised WBC levels in the ALEJC treated lead exposed rats of the present study. This finding is consistent with the submission of Farkhondeh et al., [15], which recorded significantly raised total and differential WBC level in lead exposed experimental guinea pigs. This finding validates the earlier submissions of Shah et al., [16] and Vijayakumar et al., [17] which emphasized that exposure to toxicants, radiation and some chemotherapies as well as viral, bacterial, parasitic, and fungal infections may lead to lymphocytopenia.

On the other hand, the significant leukocytosis observed in the ALEJC treated non-lead exposed rats in the present study appears to be an immunostimulatory effect and this corroborates with the finding of Auwal et al., [18], which noted that the varying combination of different phytochemicals (including tannins, saponins and steroids) of some medicinal plants may result in significant leucocytosis in Wistar rats. Therefore, it is important to state here that, such attribute of the ALEJC may be beneficial in lecopaenic conditions but may be unhelpful otherwise. It indicates that caution should be taken in the arbitrary consumption of the plant extract in the study models and the likes. The foregoing finding of the present study reveals the need for proper characterization and standardization of the beneficial phytochemicals of the *Justicia carnea* plant as to eliminate or reduce to the barest minimum the associated unwanted constituents of the plant.

It is important to note here that, the above emphasis on the effect of the ALEJC on WBC count and its differentials is unique and interesting; as several other studies [4, 8, 11] just emphasized the effects of the plant on just RBC, HB and PCV changes, thus, neglecting the plant's actual effects on WBC and platelet counts and the risks associated with their changes.

C. Effects of ALEJC on Platelets Count

It was observed from this study that platelet count was remarkably raised in virtually all the ALEJC treated rats. Although this finding is similar to those of some previous studies [5, 11], it is another pointer to the fact that the safety levels of most of these widely utilized beneficial herbs must be ascertained before promoting their use and commercialization. It is well known that having considerably elevated platelet counts, or thrombocytosis, can lead to a number of dangerous illnesses, including stroke (caused by thromboembolism) and other problems of the cardiovascular system [19]. According to Schafer's examination of the current study's ALEJC-treated mice, [19], this apparent secondary thrombocytic state may be caused by increased inflammatory activities. It is also informative to say that, in addition to exercising caution when consuming ALEJC, a screening for any underlying disorders that may worsen thrombocytosis should be conducted before to its administration.

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4.2 Effects of Administration of ALEJC on some Biochemical Parameters in the Study Animals.

A. Effects of ALEJC on Plasma Protein Level

The outcome of the present study on some biochemical parameters revealed that the total protein level was only significantly reduced in the lead-only-treated animals but were significantly improved in all the ALEJC treated animals. Similarly, changes in albumin levels across all respective groups followed exactly the same trend as seen with the total protein. It is important to state here that blood contains three main types of proteins namely albumin, globulins and fibrinogen and these proteins help the body regulate transport of substances and possibly produce and control substances (such as hormones, enzymes and antibodies) that the body needs to function normally [20, 21]. It is understood that a normal and stable plasma protein level is a reliable sign of health [22]. The fact that total protein and plasma albumin levels were positively and beneficially regulated in the ALEJC-treated animals in this study suggests that the ALEJC may be a safe and beneficial biological or therapeutic agent when used carefully and may possibly be useful in hypoproteinemic conditions.

B. Effects of ALEJC on Total and Conjugated Bilirubin

The result of the changes in total and conjugated bilirubin indicated remarkably reduced levels in the lead-only-treated animals when compared to the control animals. However, their levels are significantly increased in the groups treated with 600mg/kg ALEJC +Pb and 800mg/kg ALEJC +Pb when compared to the treated groups. It is known that the screening of total or conjugated bilirubin as one of the liver function test, reveals the health status of the liver as it is the site of bilirubin metabolism. A small level of bilirubin in the blood is presumed normal, but a raised level may be a sign of liver toxicity or disease [23]. From the above finding, it is therefore important to state that a chronic consumption of high doses of ALEJC may result in liver toxicity and may be thus unsafe. The foregoing finding is contrary to the report of Ukpabi-Ugo et al., [24], which argued that the treatment of rats with methanol extract of *Justicia carnea* leaves exerted ameliorative effect on carbon tetrachloride (CCl_4) elevated total and conjugated bilirubin. Considering these divergent outcomes on the effect of *Justicia carnea* leaves extract on total and conjugated bilirubin in same mammalian model, it is thus suggested that further investigation be done to unravel the actual effect of the plant on these liver makers.

C. Effects of ALEJC on Liver Enzymes

Considering the changes in the liver enzymes (AST, ALT and ALP) levels in the current study, it was found that only the lead (Pb) alone treated animals (group 2) indicated significantly raised levels when compared to both the normal untreated animals (control/group 1) and all other test/treated groups (i.e. from 3 to 8).

ALT with a longer half-life (of 42 days in serum) is more abundant in the liver than in other organs, thus, the reason for its consideration as a more liver specific enzyme marker. It is also established that hepatocellular injury is characterised by leakage of ALT into plasma [25, 26]. Therefore, the markedly elevated levels of ALT, AST and ALP in just the lead alone treated rats

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with the concomitant normal levels of these enzymes in all the ALEJC treated rats may be indicative of the fact that the kupffer cells may have being more highly traumatized by the lead poisoning. This submission is strengthened by the observation (in the present study) of the fact that increasing concentrations of ALEJC treatment in the rats did not only significantly alter the ALT, AST and ALP but did raise the levels of both total and conjugated bilirubin concentrations. This line of thought is further validated by the fact that Kupffer cells are known to possibly play a role in clearing several serum enzymes, including ALT and AST [27] and that bilirubin production involves a two-stage sequential catalytic degradation reaction that primarily takes place in the cells of the reticuloendothelial system, which also include the Kupffer cells of the liver [28, 29]. No doubt, the hepatocytes are the most of the liver cells (about 80%) and they as well secrete bile [30] evaluating their toxicity alone in liver disorder may not be an absolute approach. The present study therefore submits that the treatments with ALEJC may not show any hepatotoxic tendency but perhaps only toxic effects on kupffer cells. So, liver enzyme level evaluations should be concomitantly done with bilirubin level assessment in order to properly assess the actual impact of a substance on the liver.

D. Effects of ALEJC on Serum Electrolytes Levels

On the outcome of the effects of the administration of aqueous leaf extract of *Justicia carnea* (ALEJC) on some electrolytes, urea and creatinine levels in rats, the present study observed a marked elevation in the lead alone treated animals (i.e. group 2) when compared to both normal/control (group 1) and all other test groups of animals (i.e. from 3 to 8 except 7). On the changes in potassium ion (K^+) level, only groups 7 and 8 indicated significant elevations when compared to all other groups. Only a non-uniform changes occur in HCO^-_3 levels, where groups 3 and 5 had significantly raised levels. For Cl^- groups 4, 7 and 8 showed significantly ($p<00.05$) decreased levels compared to that of group 3. Except for higher dosages (which tended towards toxic levels), the ALEJC treatment did not indicate any adverse effects on urea, creatinine and uric acid levels.

It is known that relatively stable serum levels of electrolytes, urea, creatinine and uric acid are some of the several indicators of good health. Imbalance in electrolytes level may indicate a heart, lung or kidney related problems; and aside from the traumatic impacts on the functions of these visceral organs, dehydration is one common cause of a significant shift in electrolyte equilibrium [31, 32]. More so, electrolytes play a key physiological role, as virtually all cells, especially those of the nerve, heart, and muscle, depend on them to regulate voltages across their plasma membranes and to generate/conduct electrical impulses (nerve impulses, muscle contractions) across themselves and to other cells [33]. Plants are a wide reservoir of nephroprotective substances and possibly provide beneficial effects in different physiological media [34]. Depending on the levels of different phytochemicals in plants and their yet unknown mechanism of actions in the body, some plants are seen to elicit cytotoxic effects [34]. Therefore, considering the above finding of the present study, the ALEJC may not have significant

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nephrotoxic effects except in higher concentration. Thus, the need to be cautious with the dose of administrations or indiscriminate consumption of the crude extracts of the *J. carnea.*

5. Conclusion

This study, thus submits that the ALEJC extract may be a potent blood tonic or anti-anaemic agent in mammalian models. It was also found that the treatment with the different doses of ALEJC showed beneficial effects on most of the biochemical parameters in the study models. However, caution should be taken in the consumption of increasing doses of ALEJC in the study model, as the composition of various dissimilar active ingredients may result in the amplification or stimulation of WBC functions.

Ethical Approval

Ethical covering for the study was sought and obtained on the 20th of December, 2022 from the Central Ethics Committee for Research Management and Development, University of Port Harcourt, Nigeria with reference number: UPH/CEREMAD/REC/MM86/022.

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