Research Article

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Biochemical and Safety Examination of Ethanol Extract of *Justicia Carnae* on PHZ -Produced Anaemia in Wistar Rats

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Abstract

The leaves of *Justicia carnae* were screened for a hematological and biochemical response using serum enzyme activities in rats. Twenty-five rats were used in this research and were grouped into 5 of 5 rats each. Group 1 was a negative control group and was treated with distilled water. Groups 2 entreated anaemic, 3, 4 and 5 were treatment groups and received 100, 200 and 400 mg/kg body weight of the *J. carnae* extract, respectively. Anaemic was induced with Phenylhydrazine (PHZ). The rats were treated for 14 days, thereafter was sacrificed and blood collected from the heart for analysis. The effect of *J. carnae* extract was checked on hematological parameters and serum enzyme activities. All results in treatment groups were compared with the untreated anaemic group 2 at statistical confidence $p<0.05$. The normal control group saved as a reference point. There was a progressive elevation of haematological parameters as the dose of the extract increased from 100, 200, to 400 mg/kg body weight compared to the untreated anaemic group. Hematological parameters, PCV, RBC, Haemoglobin increased significantly at ($p<0.05$). Total leukocyte count elevated but not significant. Differential leukocyte count indicated very mild lymphopenia neutrophilia, monocytopenia, and eosinopenia but were not significant. Clinical biochemical parameters, AST, ALT and ALP showed slight decrease but not significant. Creatinine, total protein, urea, and bilirubin mildly increased as the dose of the extract increased but within normal range and so was not statistically significant. The MCV, MCH and MCHC values suggested normocytic normochromic anaemic condition. It was concluded that the extract of *J. carnae* is safe to blood cells, liver, and kidney marker enzymes at doses < 400 mg/kg body weight.

Keywords: Anaemia, Blood cells, Enzymes, *Justicia carnae*, Phenylhydrazine Anaemia, Blood cells,

الفحص الكيميوحيوي لمستخلص الإيثانول ل *Justicia Carnae* على فقر الدم المستحدث ب PHZ في الجرذان

الخلاصة

تم فحص أوراق نبتة *Justicia carnae* من أجل دراسة الاستجابة الدمّية والكيميوحيوية باستخدام أنشطة إنزيمات المصل في الجرذان من نوع Wistar. تم استخدام عشرين جرذاً في هذا البحث وتم تجميعها في 4 من 5 جرذاً لكل منها. عُدّت المجموعة الاولى مجموعة سيطرة سلبية وعولجت بالماء المقطر فقط ، أما المجموعات 2 و 3 و 4 فقد عُدّت مجموعات معالجة زودت بـ 100 و 200 و 400 ملغم / كغم من وزن الجسم من مستخلص *J. carnae* ، على التوالي. تم استحداث فقر الدم بواسطة فينيل هيدرازين (PHZ) ، وتم علاج الجرذان لمدة 14 يومًا ، وبعد ذلك تمت قتلها وجمع الدم من القلب لإجراء الاختبارات. تم فحص تأثير مستخلص *J. carnae* على المعايير الدمّية وأنشطة انزيمات المصل. تمت مقارنة جميع النتائج في مجموعات العلاج مع مجموعة السيطرة بمستوى المعنوية الإحصائية ($P<0.05$) . تم حفظ المجموعة غير المعالجة كمجموعة سيطرة . كان هناك ارتفاع تدريجي للمعايير الدمّية بزيادة جرعة المستخلص من 100 ، 200 ، إلى 400 ملغم / كغم من وزن الجسم.

أظهرت المعايير الدمّية : حجم الكريات المرصوصة PCV ، عدد كريات الدم الحمر RBC وخضاب الدم Hb زيادة معنوية بمستوى($P<0.05$). أظهر إجمالي عدد كريات الدم بيض ارتفاعًا تدريجيًا من خلال المستخلص على الرغم من عدم أهميته إحصائياً. أشار تعداد خلايا الدم البيضاء التفريقي إلى انخفاض طفيف غير معنوي في أعداد العدلات و اللمفيات والخلايا الوحيدة والحمضيات. أظهرت المعلمات الكيميوحيوية السريرية مستويات طبيعية من إنزيمات المصل AST و ALT و ALP على الرغم من وجود انخفاض طفيف مع زيادة جرعة مستخلص *J. carnae* . زاد الكرياتينين والبروتين الكلي واليوريا والبيليروبين بشكل طفيف مع زيادة جرعة المستخلص ولكن ضمن المعدلات الطبيعية وبالتالي لم تكن ذات دلالة إحصائية. أشارت قيم MCV و MCH و MCHC إلى حالة فقر الدم من نوع normocytic normochromic. يستنتج من الدراسة ان مستخلص *J. carnae* آمن لخلايا الدم والكبد وإنزيمات الكلى بجرعة 400 ملغم / كغم من وزن الجسم.

Introduction

Plants have beneficial properties because they contain phytochemical compounds (1) and GCMS analysis has shown that phytochemicals are made up of primary and secondary metabolites that can protect the plants, human and animals against diseases (2)(3)(4). GC-MS analysis to identify phytochemicals present in *Justicia carnae* leaves was done by(5). *Justicia* of family, Acanthacea consists of about 600 species including herbs and shrubs, and are found plenty in Africa, (6) (7). *Justicia carnae* is a flowering plant abundantly distributed in different parts of Africa. Grown around homes and act as a fence and can be propagated from stem cutting (8).

The Igbo tribe in Nigeria calls the plant '*Ogwu obara'* literally meaning drug for blood production.(9) reported its use in the control of inflammation, respiratory tract infection, gastrointestinal disorders, diabetes, diarrhoea, and liver diseases. It also possesses cardioprotective, antitumour, and antiviral activities(10), antioxidant activity (11).

Anaemia is decreased below the normal range of red blood cells (RBCs), packed red cell volume (PCV) or haemoglobin concentration (Hb), in the blood. It is a sign of a disease and not a disease. It has three major categories of which include: hypoproliferation, maturation defects, and hemolysis/blood loss. It is a hidden epidemic worldwide and can have serious consequences if left untreated. The use of Phenylhydrazine (PHZ) and compounds related to it were used as an antipyretic agent and demonstrated toxicity to RBC on red blood cells (12). This compound was, however, found to be useful in experimental models, hence an approved method of inducing haemolytic anemia for the study haematinic properties of new agents, erythropoietin regenerative response of plant materials through clinical, pathological, and morphological studies (13) (14). Phenylhydrazine administration has been shown to cause haematotoxicity which leads to haemolytic anaemia by altering iron metabolism, activating, and interferes with the binding of erythropoietin on its receptors and the formation of Heinz bodies in RBC as a side effect,(15).

AST and ALT elevation in conditions of hepatocyte damage in the inflammatory condition of the liver, hypoxic states, hepatotoxicity by toxicants, trauma, and some plant extracts, (16). Liver ALP elevation also in hepatocyte and biliary epithelial damage. They could also be ALP elevation in osteoblast, intestinal epithelial, and corticosteroid stimulation when used for treatment(17) (18).

Hyperproteinaemia is associated with dehydration occasioned by vomiting, diarrhoea, impaired renal concentration ability, excessive sweating or decreased water intake,(19). Elevated urea production is associated with intestinal haemorrhage, increased dietary urea or increased protein catabolism, (20). Elevated creatinine occure in pathological processes that cause a decrease in glomerular filtration rate which could be pre-renal, renal or post renal, (21). Hyperbilirubinaemia occur in diseases associated with haemolysis of blood as seen in babesiosis, anaplasmosis, trypanosomiasis, snake bite and some plant toxicants,(22).

We designed this research to examine the safety of *J. carnae* extract after recovering from anaemia by examining its effect on blood cells; biochemical liver enzyme markers ALT, AST, and ALP. Kidney markers urea, creatinine, bilirubin, and total protein. To investigate the haematinic and haematopoetic potential of leaf extract of *Justicia canae* in phenyl-hydrazine-induced haemolytic anemia in albino Wistar rats.

Materials and methods

Plant Materials

Fresh leaves of *J. carnae* were collected from the University environment in

Umudike, Nigeria, and was identified by Prof. M. C. Dike at the Taxonomy section of College of Natural Resources and Environmental Management of the University.

Picture 1. *Justicia carnae* leaves and with flower

Preparation of Plant Extract

The identified leaves of *J. carnae* were dried under shade for 10 days and grinned to a coarse powder using a manual grinder (Corona-Landers C 1A SA). Extraction was done by the Soxhlet method described by (23) and 35g of coarse powdered sample was introduced into the extraction chamber using ethanol as solvent. Throughout the extraction time of 48 hrs the temperature was kept at 70^0 C in the chamber. The extract was concentrated in an oven at 30^0 C and the dried extract weighed and kept in a labelled sterile specimen bottle for the work.

Different doses of 100, 200 and 400 mg/kg body weight were prepared and administered to rats in groups 2, 3, and 4 respectively. These doses were calculated from a stock solution dissolved in distilled water.

Haematology and Biochemical Investigation

For Haematological screening, PCV and differential counts were measured by the micro-haematocrit method as described by(24). Haemoglobin concentrations was determined by cyanomethemoglobin method, Kachmar (25).

Using RBC, PCV and (Hb), the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulae (26) (27) (28) (29).

A biochemical investigation was performed using ELISA reagent kits. The measure included alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), determined by the method of (16). Using serum enzyme levels to determine liver and kidney state (17). Urea by(20) and Creatinine by (21). Total protein was determined by the Biuret method as described by (19). Samples were analyzed immediately to avoid artifactual changes (30).

Experimental Animals

Adult albino rats were purchased from University Farm. Approval was obtained from the College of Veterinary Medicine of the University in line with the guidelines for the care and use of laboratory animals provided by the National Research Council (31). The rats were acclimatized and fed *ad libitum.*

Induction of Anaemia by Phenylhydrazine (PHZ)

This was done according to the modified method described by (32). Haemolytic anaemia was induced in the rats intra-peritoneally with 2.5% phenyl hydrazine hydrochloride (Fisher Scientific Company, New Jersey, USA) at a dose of 30 mg/kg body weight. The anaemia was maintained by the administration of 15 mg/kg body weight of 2.5% phenylhydrazine

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hydrochloride at interval of 3 days, for the duration of the experiment.

Experimental design

Twenty-five rats were used for the research, they were grouped into 5 of 5 rats each. Group 1 was the normal control group and was administered distilled water orally. Groups 2 was the untreated anaemic group, 3, 4 and 5 were the treatment groups that received 100, 200 and 400 mg/kg body weight of the *J. carnae* extract respectively orally by intubation. The rats were treated for 14 days, thereafter they were sacrificed and blood collected from the heart for analysis. The effect of *J. carnae* extract was checked on haematological parameters and serum enzyme activities.

Statistical analysis

Analysis of statistical data was computed using Statistical Package for Social Sciences (SPSS) version 20. Values were expressed as mean ± Standard Error of Mean (SEM) and were further subjected to one - way analysis of variance (ANOVA) to compare doses with untreated anaemic group . Duncan post-hoc test was used to separate the mean that showed significant difference. The statistical confidence was set at $p<0.05$.

Results and discussion

The result of Fig 1 shows the values presented as means ± SEM (standard error of mean) of RBC, PCV, Hb, and TWBC (Total White Blood Cell) at significant difference $p<0.05$. Treatment was 100, 200, 400 mg/kg compared to untreated anaemic group.

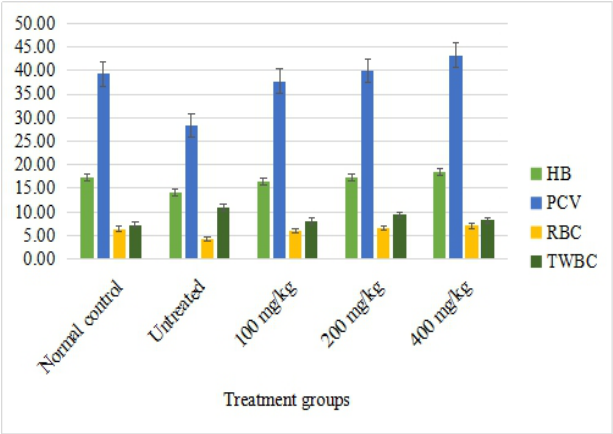

Figure 1: shown Haematology profile of Wistar rats.

There was a progressive increase in values of Hb, PCV, and RBC when the dose of the extract increased compared to untreated anaemic group.

Hb (g/dl) 16.5 ± 0.39, 17.3 ± 0.39 and 18.5 ± 0.39, when compared to the untreated anaemic group 14.2 ± 0.39,

PCV (%) 37.7 ± 0.29, 40.00 ±0.29 and 43. ± 0.29, when compared to the untreated anaemic group 28.25 ±0.29.

RBC (X 10^6 mm^3) 6.0 ± 0.21, 6.5 ± 0.21 and 7.00 ± 0.21, when compared to the untreated anaemic group 4.25 ± 0.21

The increase in values of Hb, PCV, and RBC was statistically significant at $p<0.05$.

TWBC (X10^3 mm^3) 10.00 ± 0.37, 9.34 ± 0.37 and 8.15 ± 0.37, when compared to the untreated anaemic group 10.95 ± 0.37,

The mild decrease in values of TWBC recorded as a dose of extract increased were not statistically significant at $p<0.05$

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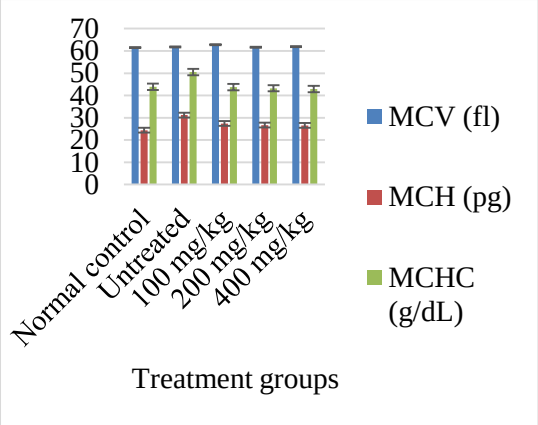

Fig 2: shown MCV, MCH, and MCHC values of Wistar rats.

Fig 2 shows the values presented as means ± SEM of MCV, MCH, and MCHC at significant difference p<0.05.Treatment was 100, 200, 400 mg/kg compared to untreated anaemic group.

MCV(fl) 62.79 ± 0.18, 61.71 ± 0.18 and 61.90 ± 0.18 when compared to the untreated anaemic group 66.50 ± 0.18,

MCH (pg) 27.42 ± 0.44, 26.72 ± 0.44 and 26,57 ± 0.44, when compared to the untreated anaemic group 31.19 ± 0.44,

MCHC (g/dl) 43.72 ± 0.73, 43.24 ± 0.73 and 42.92 ± 0.73, when compared to the untreated anaemic group 50.51 ± 0.73,

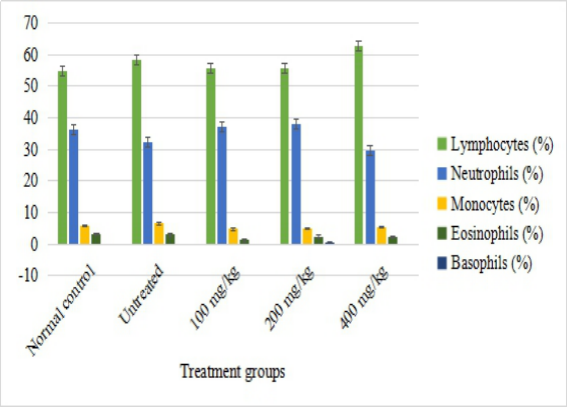

Figure 3: shown Differential leukocyte count of Wistar rats.

The graph in Fig 3 represent the values of differential blood count of leukocytes as mean ± SEM at significant difference p<0.05.Treatment was 100, 200, 400 mg/kg compared to untreated anaemic group.

Lymphocytes (%) 55.50 ± 1.00, 55.50 ± 1.00 and 58.75 ± 0.76, when compared to the untreated anaemic group 58.75 ± 1.00,

Neutrophils (%) 37.25 ± 1.17, 38.00 ± 1.17 and 29.50 ± 1.17, when compared to untreated anaemic group 32.25 ± 1.17,

Monocytes (%) 4.75 ± 0.27, 5.00 ± 0.27 and 5.25 ± 0.27, when compared untreated anaemic group 6.50 ± 0.27,
Eosinophils (%) 2.25 ± 0.25, 2.25 ± 0.25 and 2.25 ± 0.25, when compared to untreated anaemic group 3.00 ± 0.25,
Basophlls (%) 00.0 ± 0.00, 0.25 ±0.05 and 00.0 ± 0.00, when compared to untreared anaemic group 0.0 ± 0.00
No statistically significant difference at p<0.05 and all values of leukocytes fall within the normal reference range.

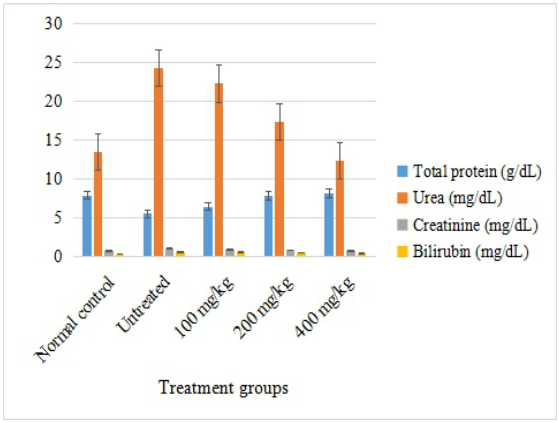

Figure 4: shown Total protein, urea, creatinine, and bilirubin of Wistar rats.

The graph in Fig 4 represents the value of serum biochemistry of total protein, urea, creatinine, and bilirubin. The value is represented as mean ± SEM at p<0.05. Treatment was 100, 200, 400 mg/kg compared to untreated anaemic group.

Total protein: 6.48 ± 0.24, 7.8539 ±1.2 and

8.19 ± 0.24, when compared to the untreated anaemic group 5 ± 0.24, *(Ref range 4.0-8.0)* Radostits *et al.*,(2000)
Urea: 22.27 ± 1.2 , 17.39 ± 1.2and 12.37 ± 1.2, when compared to the untreated anaemic group 24.28 ± 1.2, *(Ref range 10-30)*
Creatinine: 0.97 ± 0.03 , 0.90 ± 0.03 and 0.79 ± 0.03, when compared to the untreated anaemic group 1.08 ± 0.03, *(Ref range 0.6-1.6)*
Bilirubin: 0.61 ± 0.03, 0.54 ± 0.03 and 0.50 ± 0.03, when compared to the untreated anaemic group 0.67 ± 0.03, *(Ref range 0-10)*
No statistically significant difference at $p<0.05$ and all values fall within the normal reference range.

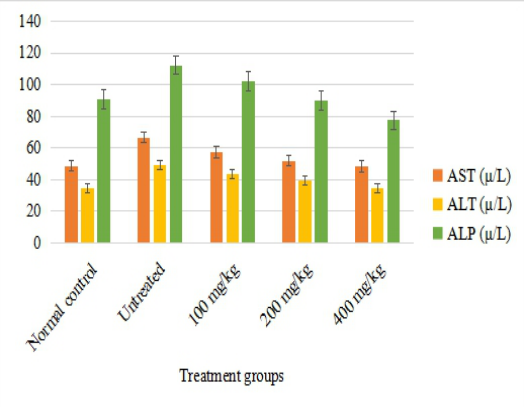

Figure 5: shown AST, ALT, and ALP in Wistar rats.

The graph in Fig 5 represents the value of serum biochemistry of AST, ALT, and ALP and represented as mean ± SEM at $p<0.05$. Treatment was 100, 200, 400 mg/kg compared to untreated anaemic group.

AST (µ/L) 57.29 ± 1.68, 52.03 ± 1.68 and 48.31 ± 1.68, when compared to the untreated anaemic group 66.69 ± 1.68, *(Ref range 32-84 µ/L)*
ALT (µ/L) 43.46 ± 1.34 ,39.62 ± 1.34 and 34.84 ± 1.34 when compared to the untreated anaemic group 49.14 ± 1.34, *(Ref range 30-58 µ/L)*
ALP (µ/L) 112.24 ± 3.1, 101.98 ± 3.1, 90.00 ± 3.1 and 77.35 ± 3.1, when compared to the normal control 99.04 ± 1.98 *(Ref range 0-500 µ/L)*
No statistically significant difference at $p<0.05$ and all values fall within the normal reference range.
Haemolytic anaemia produced by PHZ (33)was used as an experimental model for the study of haematinic effects of *J. carnae* in line with the work of (14) (12).

The result in Fig 1 showed that plant extract significantly ($p<0.05$) restored to their normal ranges, of the (Hb), PCV, and RBC which experimentally were depleted by Phenylhydrazine when compared with the anaemic untreated group. This haematinic effect of this plant extract occurred in a dose-dependent manner, which implies that increasing the dose of the extract from 100 to 400, significantly, and increased the haematinic effect of the extract. These values remained significantly low in the untreated rats. However, the induction with phenyl-hydrazine (PHZ) did not significantly alter the MCV but caused increased values of MCH, MCHC, and TWBC as observed in Fig 1. Erythrocytes that have a normal size or volume (normal MCV) are called normocytic, whereas high and low mean values indicate macrocytic and microcytic respectively. Erythrocytes with normal of haemoglobin concentration (MCHC) are normochromic**,** whereas, abnormally high and low mean values indicate hyperchromic and hypochromic conditions respectively, though there is no hyperchromic condition. So the MCV, MCH and MCHC values in this work were normal suggesting normocytic normochromic anaemic condition

The result presented in Fig 3 showed that *J. canae* extract-treated groups progressively returned the TP value to mean values compared with the normal value in a dose-dependent manner. The liver and kidney biomarkers which were significantly elevated by the PHZ agent as shown in the untreated rats Fig 4 and 5, were gradually brought back to normal reference range comparable with the normal control rats

following treatment with the plant extract.

Studies have shown that intravascular hemolysis in any condition may damage the liver and other vascular organs(34) (35) , apart from haemolysis induced liver injury.

The result in Fig 3 showed that liver enzymes were restored to the normal reference range. The mechanism of action of *J. carnae* may solely depend on the restoration of these hematological parameters thereby preventing damage to the liver and/or restoration of injured hepatocytes indicating that *J. carnae* extract could be hepatoprotective against hemolytic anemia and/or phenylhydrazine induced hepatotoxicity in rats.

Our research agrees with (36) that *J. carnae* can revive anaemic condition. In this work also, the extract of *J. carnae* is safe to liver and kidney cells at dose <400 mg/kg body weight.

Rats showed significant recovery at a higher dose of *J. carnae.* The significant recovery as shown in Fig 1-3 could be due to the administration of *J. carnae* extract, and not by the natural physiological compensation of the bone marrow.

Conclusion

Therefore, it can be concluded that *J. carnae* extract increased the PCV, Hb and RBC levels and caused a reduction in AST, ALT, and ALT with the restoration of hepatocytes after phenylhydrazine induced haemolytic anaemia. This suggests that the extract may be beneficial in the treatment of haemolytic anaemia induced by phenylhydrazine or haemolysis as a result of infectious agent.

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