International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 05 Issue 06 June 2025

Page No : 371-380

DOI: https://doi.org/10.47191/ijpbms/v5-i6-02, Impact Factor: 8.163

The Impact of Aqueous Leaf Extract of Justicia Carnea on Liver Enzymes and Histological Features of the Liver in Phenylhydrazine-Induced Anemic Rats

Suzan N. Ejikeme[1], F.C. Akpuaka[2], Uzozie C. Ofoego[3], Onoriode A. Eyeghre[4], Lotanna S. Akudu[5]

[1]Department of Anatomy, College of Nursing Sciences, Adazi Nnukwu, Anambra State

[2]Department of Anatomy, Abia State University, Uturu, Abia State

[3]Department of Anatomy, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra state.

[4]Department of Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra state.

[5]Department of Anatomy, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra state.

ABSTRACT

Anemia a global health issue affects both developed and developing nations, which is characterized by low levels of haemoglobin in the blood and is linked with hepatic damages. Medicinal plants are rich sources of phytonutrients, which play a dominant role in alleviating toxicity linked to vascular toxicity and hepatic and renal impairment. The study aimed to ascertain the impact of crude aqueous extract of Justicia carnea leave (ALJC) extract on liver enzymes and histoarchitectural changes of the liver in phenylhydrazine-induced anemic female wistar rats. Thirty-(30) female Wistar rats weighing 130-150g were used in the study and were divided into five groups of six animals each. Group A received feed and water ad libitum, Group B received 0.1ml of Phenyl-hydrazine (PHZ) only, and group C received 500mg/kg of ALJC for 8-weeks. Group D, E, and F received 1ml of PHZ for two weeks and treated with 250 mg/kg and 500 mg/kg of ALJC and 20mg /kg of Vitamin B12 respectively for 6-weeks through oral gavage. Data obtained for liver functions and bilirubin levels were analysed using SPSS version 25 using ANOVA followed with post Hoc LSD. Body weight was analysed using T-test and values were considered significant at $P \leq 0.05$. The result shows ALT, AST, and ALP levels showed a significant increase following PHZ only, while administration of ALJC revealed significant decline in treated groups compared to group A. Total protein had a significant increase and bilirubin indicated a significant decline following PHZ only intake, while treatments with the ALJC and Vitamin B12 showed an improved levels of total protein and bilirubin levels. The PHZ only had a deleterious impact on liver architecture revealing infiltration of hepatocytes, treatments with ALJC and vitamin B12 indicate improved levels of hepatic tissue. The study concludes that ALJC and Vitamin B12 improved hepatic functions and architectural changes, while PHZ only caused an impaired hepatic function.

KEYWORDS: phenylhydrazine, Anemia, Justicia carnea, liver enzymes, Vitamin B12

ARTICLE DETAILS

Published On:
05 June 2025

Available on:
https://ijpbms.com/

1.0 INTRODUCTION

Anaemia is a public health issue that causes decreased erythrocyte mass and haemoglobin concentration, affecting oxygen carrying capacity. Treatment involves diet changes and iron supplementation (1). Anaemia is clinical condition characterised by decrease in RBC, PCV, hemoglobin, and other RBC components; it is a significant public issues of concern affecting children under the age of five (2). Anaemia is a condition with dysfunctioning of red blood cells within the body, which ends up in lowered transfer of oxygen to the body organs (3). However, their etiologies are of different sources and have several mechanisms to elicit its destruction of RBC components in both experimental model and human model (4). Phenyl hydrazine (PHZ) is a potent chemical and

antipyretic agent known for toxicity in causing hemolytic anemia. It is a remarkable potent drug to meet blood disorders (5).

Chronic liver disease is accompanied by multiple haematological abnormalities. Iron deficiency anaemia is a frequent complication of advanced liver disease, and its aetiology is multifactorial, mostly due to chronic haemorrhage into the gastrointestinal tract (6). However, the liver functions in a sporadic way by protecting the body harmful substances assimilated from the gastrointestinal tracts through metabolism within the lobules (7). It is known for its xenobiotic activities through the cytochrome P-450 enzyme system and acts through phase-I reaction; with Phase II being conjugation of the substrates such as glucuronide, glutathione, sulfate, etc. (8,9). The enzymes of the hepatocytes occurring in cluster are the alanine aminotransferase (ALT) and aspartate aminotransferase (AST); however, hepatic compromise leading to liver dysfunction result in the burst of these enzymes into circulation causing deleterious effects in the liver architecture as well as its physiochemical function (10,11).

The use of medicinal plants in the management of anemic conditions is attributed to the phytochemicals present in them that enables several pharmacological or therapeutic actions they elicit. Justicia carnea is a flowering plant belonging to the Acanthaceae family and is widely distributed in the tropics and subtropics. It is used in traditional medicine for the treatment of anaemia, inflammation, fever, diarrhoea, liver diseases, arthritis, respiratory disorders, and gastrointestinal disorders (12,13). It has been reported that the species possess cardioprotective properties, are antioxidants, and are rich in vitamins and minerals (14). Its anti-anemic activity has been documented on hematological indices (13,15). but lacks pieces of literature on the hepatic function and histological activities.

PHZ has shown to cause an increased levels of ALT, AST, and ALP levels in anaemic rats (16); and a significantly higher levels of AST, ALT, and ALP were documented following PHZ induced anaemia (17). Kolawole et al. (18) reported that PHZ reduces all erythrocytes parameters in anemic rats. Despite reports on the PHZ on liver enzymes, there are limited literatures on the bilirubin; however, following treatments with aqueous leaf extract of J. carnea on PHZ-induced anaemic rats, the study tends to investigate this, with dearth to literatures on the hepatic function following administration of J. carnea and vitamin B12.

2.0 MATERIALS and METHODS

Ethical Approval and Study location: Ethical approval was obtained from the Animal Ethics Committee, Faculty of Basic Medical Sciences, College of Medicine and Health Sciences, Abia State, Uturu. Rats handling and treatments conform to the National Institute of Health guidelines for laboratory animal care and use. The study was carried out in the Animal House of the Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medicine and Health Sciences, Abia State, Uturu.

Materials and Reagents Used: Phenyl-Hydrazine (Sigma Aldrich, USA), Justicia carnea leaves, 30-Female wistar rats, S. Pyrex Beakers (Techmel, USA), Measuring cylinder (MINGHE), 2ml hypodermic syringe, electronic weighing balance (M-Metlar M311L; China), Oral cannula, Slide, Microscope (Olympus XSZ-107BN), Plain Container and EDTA Bottle (Fantastik, China), Heparinized Capillary Tube and Spectrophotometer (Model 721). Filter paper (Whatman Qualitative Filter Paper No. 1, Sigma Aldrich WHA1001042), Distilled water, Standard Plastic Cages and water can, Cotton wool (KENS LINT, Benin City, Nigeria), Latex Medical Hand gloves (Supermax Gloves, Selangor, Malaysia), Chloroform (Guondghuo, China), Vital feed grower (JOS, Nigeria), and Dissecting kits. Randox reagent Kits (England), Automatic Water distiller (SZ-1 Search Tech Instrument), Nexus Refrigerator, Rotary evaporator (Digital) TT-52 (Techmel and Techmel, USA), and Thermostat Oven (DHG-9023A, PEC MEDICAL USA).

Plant procurement, identification, and Extraction: Samples of Justicia carnea leaves was purchased from Nwko market in Nnewi, Anambra state. The botanical identification and authentication were confirmed by the herbarium of Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State.

The Justicia carnea leaves was washed in running tap water in a basin to remove dirt and air dried under ambient temperature. The dried leaves were milled into coarse powder using Local grinder. 250g of the dried leaves was macerated in 1500mls of Luke-warm water for 48hours. It was then filtered using a clean handkerchief and further filtration using Whatman No 1 filter paper. The filtrate was concentrated using a rotatory evaporator and was further dried using a laboratory oven at 50ºC into a gel-like form into air-tight container. The extract was preserved in a refrigerator at 4ºC for further usage. The extraction method was done with modifications as described by Quek et al. (19).

Experimental Animals and Design: Thirty-(30) female Wistar rats weighing 130-150g was obtained from the Animal House, Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine and Health Sciences, Abia State, Uturu. Animals were kept in standard cages at a room temperature of 27±2°C. The animals were maintained with normal laboratory chow (Grower feed) and water ad libitium. The animals were acclimatized for two weeks and before administering the Phenyl-hydrazine (PHZ), Vitamin B12, and aqueous leaf extract of Justicia carnea (ALJC). The animals were kept on 12hours light and dark cycles. A Quasi-experimental animal design was employed in the study. The animals were divided into 5-groups of six animals each as follows; Group A received feed and distilled water only,

Group B received 0.1ml of Phenyl-hydrazine only, Group C received 500mg/kg of ALJC for 8-weeks, Group D received 0.1ml of PHZ for two-weeks and treated with 250mg/kg of ALJC for 6-weeks, Group E received 0.1ml of PHZ for two-weeks and treated with 500mg/kg of ALJC for 6-weeks , and Group F received 0.1ml of PHZ two-weeks and treated with Standard drugs for 6-weeks. All experimental protocols were observed under strict supervision, the experiment last for 8-weeks, and administration was done through oral gavage.

Sample Collection: At the end of the experiment, animals in the different groups were anesthetized using chloroform in an enclosed container after 24-hours of the last administered dose of the PHZ, vitamin B12, and ALJC. Blood was collected from the animals using a heparinized capillary tube through ocular puncture as described by Parasuraman, Raveendran, and Kesavan (20). Blood was obtained by puncturing the retro-orbital sinus of the rats using heparinized capillary tube and was put in a plain bottle and allow to clot for the biochemical assays of liver enzymes and centrifuged for 10-minutes at 3000rpm. The serum was retrieved using a micropipette from the plain bottle. The serum retrieved from the plain container was used to assay for serum liver enzymes (AST, ALT, and ALP) and total protein and bilirubin. The liver was harvested through intra-abdominal incision and the weighed and washed using normal saline. The liver was fixed in 10% formo-saline as a primary fixative for histopathological studies.

Estimation of Liver enzymes: Alkaline Phosphatase (ALP) Estimation: This test was carried out according to the method described by (21). **Aspartate Amino Transferase (AST) Estimation and Alanine Amino Transferase (ALT) Estimation:** This test was carried out according to the method described by (22).

Total serum protein: The most widely used method for measuring serum protein is the biuret reaction (George, 2009). This reaction's principle is that serum proteins react with copper sulphate in sodium hydroxide to form a violet-biuret complex. The violet colour intensity was measured using a UV-VIS 752N Spectrophotometer and is proportional to the concentration of protein (Bjorsten et al., 2007).

Estimation of Bilirubin level: The quantitative determination of bilirubin are based on the reaction between bilirubin and diazotised sulfanilic acid. In aqueous solution only the direct bilirubin (conjugated) bilirubin will react in this manner. On the other hand, in order to estimate total bilirubin the unconjugated bilirubin must be freed from attachment to albumin and rendered water soluble. In Malloy-Evelyn method methanol is used while caffeine/sodium benzoate is used in the Jendrassik-Grof method (Kosaka et al., 1987). The series of reactions involved in the assay system is as follows: Conjugated (direct) and unconjugated (indirect) bilirubin in the sample react with diazotized sulfanilic acid to form the red colored azobilirubin in presence of caffeine. The same reaction, but by using normal saline in the absence of caffeine, is used to measure direct bilirubin. After adding tartarate for total bilirubin measurement the red colored azobilirubin converted to yellow to green color.

Sulfanilic acid + $NaNO_2$ $\xrightarrow{HCl}$ diazotized sulfanilic acid

Bilirubin + diazotized Sulfanilic acid $\rightarrow$ azobilirubin

The intensity of the color produced is directly proportional to bilirubin concentration. It is determined by measuring the increase in absorbance at 578 nm. For direct bilirubin, it is determined by measuring the increase in absorbance at 546 nm.

Histological Procedure of the Liver: The liver was fixed in 10% formosaline and were dehydrated in four (4) concentrations of Isopropyl alcohol, i.e., 70%, 80%, 90%, 100% for 1hr each and then cleared in xylene before embedded in molten paraffin wax to remove the isopropyl alcohol. Micro sections of 5micrometer using Leica RM 212 Rt. Rotary Microtome, tissues was stained using Haematoxylin and Eosin (H&E) to demonstrate general tissue structure. Tissues sectioned was examined and interpreted using Leica DM 750 binocular microscope with photomicrographic facilities and then photomicrographed by a histopathologist (23).

Statistical analysis: Data obtained from this study was analyzed using Statistical Package for Social Sciences (SPSS) version 25 (IBM, USA, 2018). Data obtained for liver enzymes (AST, ALT, and ALP), Total protein, Bilirubin level, and kidney enzymes (serum creatinine and urea levels) was analysed using One-Way ANOVA followed by post Hoc LSD multiple comparison, and body weight was analysed using T-test, and data was considered significant at $p \leq 0.05$.

3.0 RESULTS

Table 1: effect of aqueous extract of Justicia carnea on Liver enzymes (AST, ALT, and ALP) and relative liver weight following phenyl hydrazine toxicity

	Aspartate Transaminase (IU/L) MEAN±SEM	Alanine Transaminase (IU/L) MEAN±SEM	Alkaline Phosphatase (IU/L) MEAN±SEM
Group A (Control)	36.00±1.52	19.67±2.02	272.42±30.09

Group B (PHZ only)	56.33±0.88*	48.67±5.23*	515.39±13.09 *
Group C (500 mg/kg of ALJC)	36.33±1.45*	25.67±1.86*	360.39±5.72*
Group D (PHZ + 250 mg/kg of ALJC)	39.00±0.57 *	40.00±7.51ª	423.56±5.91ª
Group E (PHZ + 500 mg/kg of ALJC)	43.00±0.58*	39.33±5.54ª	402.35±65.18*
Group F (PHZ + 50 mg/kg of Vitamin B12)	27.67±1.76*	12.00±1.52*	217.90±12.36*
F-value	61.04	9.45	12.48

SEM: Standard error of mean. ALJC: aqueous leaf extract of Justicia carnea, PHZ: Phenyl hydrazine, significant (*) and not significant (ª)

Table 1 result revealed a significant increase in the AST level in group B when compared to A (p=0.002), while groups C, D, E, and F (p=0.010, p=0.001, p=0.000, p=0.021) had a significant decrease in the AST level when compared to group B. The ALT result demonstrated a significant increase in group B compared to A (p=0.001), groups C and F (p=0.004, p=0.001) had a significant decrease and groups D and E (p=0.203, p=0.173) had a non-significant decrease compared to group B. The ALP result showed a significant increase in group B compared to A (p=0.001), while groups C, E, and F (p=0.004, p=0.022, p=0.000) had a significant decrease and group D had a non-significant decrease compared to group B.

Table 2 effect of aqueous extract of Justicia carnea on total protein and bilirubin level following phenyl hydrazine toxicity

	Total protein (g/dl)	**Conjugated bilirubin (mg/dl)**	**Unconjugated bilirubin (mg/dl)**
	MEAN±SEM	**MEAN±SEM**	**MEAN±SEM**
Group A (Control)	8.33±0.44	0.82±0.29	0.32±0.06
Group B (PHZ only)	5.60±0.49*	3.93±0.52*	0.86±0.04*
Group C (500 mg/kg of ALJC)	7.36±0.37*	1.87±0.26*	0.45±0.11*
Group D (PHZ + 250 mg/kg of ALJC)	9.60±0.66 *	1.39±0.30*	0.56±0.03*
Group E (PHZ + 500 mg/kg of ALJC)	10.50±0.51*	1.62±0.18*	0.39±0.03*
Group F (PHZ + 50 mg/kg of Vitamin B12)	8.76±0.78*	1.39±0.30*	0.44±0.11*
F-value	9.41	10.86	6.67

Data was analyzed using ANOVA followed by post Hoc LSD multiple comparison, and values considered significant at p<0.05. SEM: Standard error of mean. ALJC: aqueous leaf extract of Justicia carnea, PHZ: Phenyl hydrazine, significant (*) and not significant (ª)

Table 2 result revealed a significant decrease in the total protein level in group B compared to A (p=0.005), while groups C, D, E, and F (p=0.046, p=0.001, p=0.000, p=0.002) had a significant increase compared to group B. The conjugated bilirubin level showed a significant increase in group B compared to A (p=0.001), while groups C, D, E, and F (p=0.001, p=0.002, p=0.011, p=0.000) had a significant decrease compared to group B. The unconjugated bilirubin level indicated a significant increase in group B compared to A (p=0.000), while groups C, D, E, and F (p=0.002, p=0.015, p=0.001, p=0.002) had a significant decrease compared to group B.

Table 3 effect of aqueous leaf extract of Justicia carnea on body weight following phenyl hydrazine toxicity

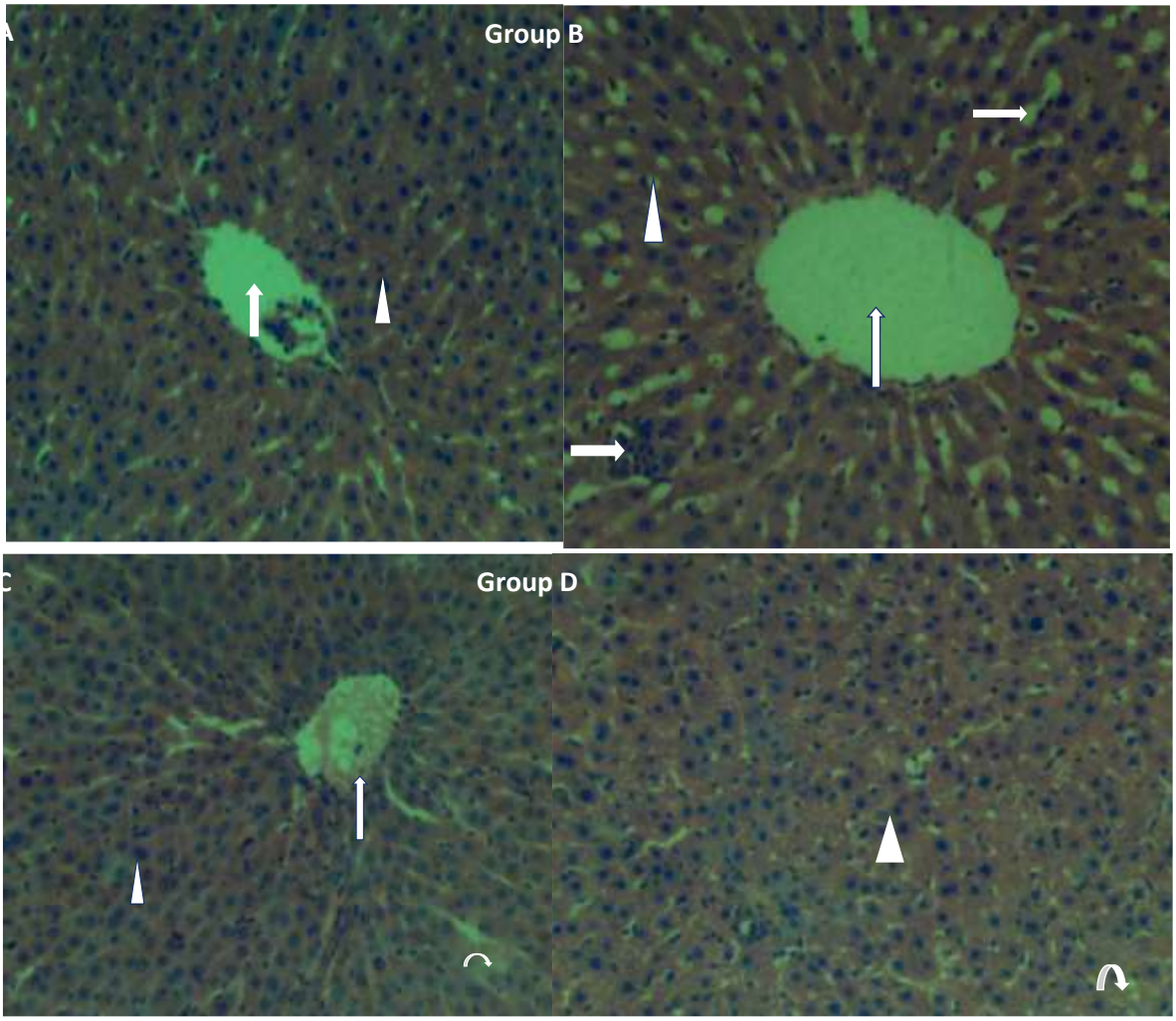
	Initial weight (g)	**Final weight (g)**	**P-value**	**T-value**
	MEAN±SEM	**MEAN±SEM**		
Group A (Control)	168.50±21.50	218.50±33.50	0.000*	-4.167

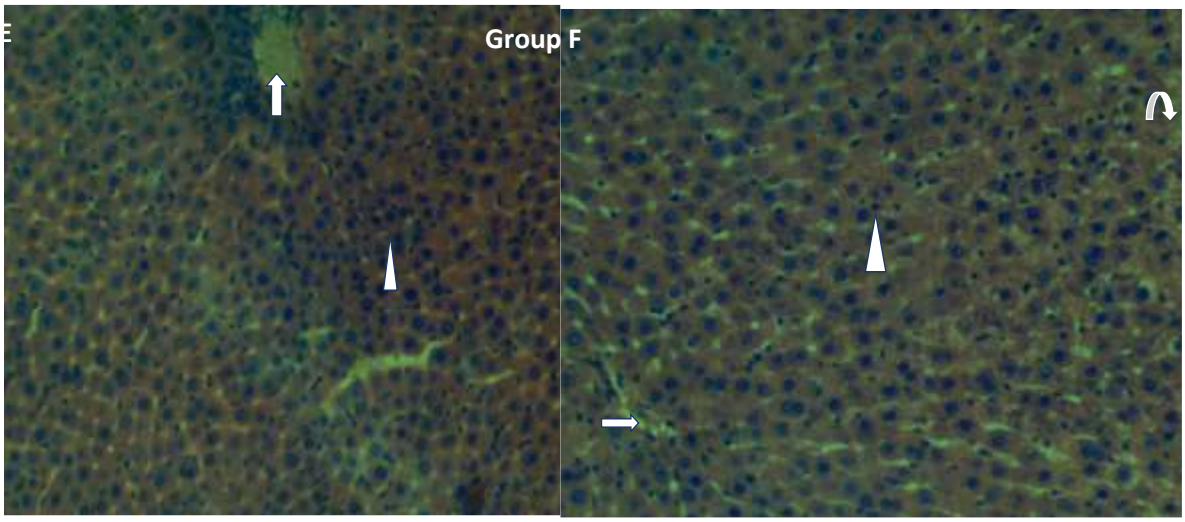
Group B (PHZ only)	169.00±13.61	189.67±10.33	0.071[a]	-1.694
Group C (500 mg/kg of ALJC)	169.00±13.07	215.00±16.64	0.009[*]	-1.765
Group D (PHZ + 250 mg/kg of ALJC)	169.00±4.10	221.25±15.10	0.005[*]	-2.769
Group E (PHZ + 500 mg/kg of ALJC)	169.67±7.26	200.00±4.51	0.007[*]	-2.613
Group F (PHZ + 50 mg/kg of Vitamin B12)	178.25±2.02	180.00±8.49	0.433[a]	-0.197

Data was analyzed using T-test, and values considered significant at p<0.05. SEM: Standard error of mean. ALJC: aqueous leaf extract of Justicia carnea, PHZ: Phenyl hydrazine, significant (*) and not significant (a)

Table 3 result revealed an increase in the body weight in groups B and F, while groups A, C, D and E had an increase in the body weight when the initial weight was compared to the final weight. However, the increase in the higher mean body weight in groups B and F had no significance, while groups A, C, and D had significance, which is an indicator that the ALJC could be used in the ameliorating anorexic effect.

Histological findings





Group A received feed and water only: Photomicrograph of liver tissue shows morphology consistent with normal liver histology. The hepatocytes (arrowhead) and central vein (arrow) appear normal (H&E X 400). **Group B received 1ml of Phenyl hydrazine only.** Photomicrograph of liver tissue shows morphology consistent with normal liver histology hepatocytes (arrowhead) and mildly hypertrophied central vein (arrow) are normal and moderate vacuolation of liver sinusoids (arrows) (H&E X 400). **Group C received 500 mg/kg of ALJC:** Photomicrograph of liver tissue shows morphology consistent with normal liver histology. The hepatocytes (arrowhead) appear normal, and central vein (arrow) shows mild lipid infiltration and mild healing in the liver (H&E X 400). **Group D (PHZ + 250 mg/kg of ALJC):** Photomicrograph of liver tissue shows morphology consistent with normal liver histology. The hepatocytes (arrowhead) appear normal and shows mild fatty changes in the background and moderate healing (H&E X 400). **Group E (PHZ + 500 mg/kg of ALJC)**: Photomicrograph of liver tissue shows morphology consistent with normal liver histology. The hepatocytes (arrowhead) appear normal and central vein (arrow) shows mild lipid infiltration and moderate healing (H&E X 400). **Group F (PHZ + 50 mg/kg of Vitamin B12)**: Photomicrograph of liver tissue shows morphology consistent with normal liver histology. The hepatocytes (arrowhead) appear normal and portal triad (arrow) shows mild shrinkage, mild fatty change (curved arrow) (H&E X 400).

4.0 DISCUSSION AND CONCLUSION

The study investigates the effect of crude aqueous extract of Justicia carnea leave extract and vitamin B12 on liver function and histological changes in phenyl-hydrazine anemic-induced female wistar rats. An elevation in the ALT activity, which is a critical hepatic marker, is a sign of liver toxicity; however, it is a sensitive indicator of hepatocellular indicator for the xenobiotic activities of the liver (24). The study findings showed that PHZ demonstrated a significant increase in ALT compared to control group A. However, the mechanism of action following the significant increase could be attributed to the presence of generation of reactive oxygen species production, which resulted to lipid peroxidation; thus, promotes haemolysis (increases ferrous, ferritin, and erythropoietin levels), liver enlargement, and chronic failure (through hypertrophy of liver cells) (25). The findings of Obayuwana et al. (25), Akara et al. (17), Okafor and Atsu, (16), Ezeigwe et al. (26) demonstrated a significant increase in ALT level following PHZ, which agrees with the study findings. Zangeneh et al. (27) has similarities to the study findings following PHZ induced hepatotoxicity, which revealed a significant increase in ALT levels. Further, treatments with Justicia carnea demonstrated a significant decline in ALT levels in the treated groups, which is attributed to the presence of flavonoids, alkaloids, saponins, and proteins. The report of Ani et al. (28) revealed a significant reduction in the ALT following administration of ethanolic leaf extract of J. carnea in diabetic rats, which agrees to the study outcomes. Also, Ukpabi-Ugo et al. (29) reported a significant decrease in ALT level following ingestion of methanol extract of J. carnea, which agrees to the study findings.

Aspartate transaminase is an enzyme of the liver cytosol and is present in a more significant amount both in the hepatocytes and mitochondria. Also, it is found in the heart, brain, and skeletal muscle and has specificity to liver dysfunction (30). The study findings demonstrated a significant increase in group B compared to A, groups C and F had a significant decrease and groups D and E had a non-significant decrease compared to group B. The physiology behind the significant increase in the AST level as shown by PHZ, is linked to lipid peroxidation and free radical generations. The study has accordance with the reports of Obayuwana et al. (25), Akara et al. (17), Okafor and Atsu, (16), Ezeigwe et al. (26), Zangeneh et al. (27), which indicated a significant increase in the AST level following PHZ hepatotoxicity. However, treatment with J. carnea demonstrated a significant decrease in group C, E, and F, which is linked to the flavonoids, alkaloids, saponins, and

proteins. The works of Ani et al. (28), reveals a significant reduction in the AST following administration of ethanolic leaf extract of J. carnea in diabetic rats, which agrees to the study outcomes. Ukpabi-Ugo et al. (29) report disagree with the study findings following a non-significant decrease in AST level following administration of methanol extract of J. carnea.

serum ALP activity may remain elevated even after biliary obstruction has been the source of elevated ALP in the liver. In addition to biliary obstruction and the liver, another possible source of elevated ALP is the increased osteoblastic activity in the placenta. This is particularly relevant in pregnant women, where the placenta is likely to be the main cause of elevated ALP levels, especially during the late third trimester (31,32). The study findings demonstrated a significant increase in the ALP levels in group B compared to A, while groups C, E, and F had a significant decrease and group D had a non-significant decrease compared to group B. The physiology behind the significant increase following PHZ is attributed to lipid peroxidation and ROS generation caused by PHZ. The study has accordance with the reports of these authors (16,17,25–27) indicating a significant rise in ALP levels following PHZ intoxication. The study reported a significant decrease in the ALP level, which is attributed to the presence of flavonoids. The study of Ani et al. (28), Falode et al. (2023), revealed a significant reduction in the AST following administration of ethanolic leaf extract of J. carnea in diabetic rats, which agrees to the study outcomes. However, Ukpabi-Ugo et al. (29) report a non-significant decrease in ALP level following J. carnea, which refutes the study findings. Onochie et al. (33) demonstrated a non-significant increase in ALP level following ethanolic leave extract of J. carnea, which disagree with the study findings.

The study findings showed a significant decrease in the total protein level in group B compared to A, while groups C, D, E, and F had a significant increase compared to group B. The conjugated bilirubin level showed a significant increase in group B compared to A, while groups C, D, E, and F had a significant decrease compared to group B. The unconjugated bilirubin level indicated a significant increase in group B compared to A, while groups C, D, E, and F had a significant decrease compared to group B. However, the mechanism of action linked to PHZ induced hypoproteinemia and hyperbilirubinemia is associated to ROS formation. Baker et al. (34), Zangeneh et al. (27) and Ayoade et al. (35) reported significant decrease in total protein following PHZ toxicity, which aligns with the study findings. However, it showed similarities to the study report demonstrating a significant increase in conjugated and unconjugated bilirubin level. The study disagrees with the findings of Akintimehin et al. (13) demonstrating a significant decrease in total protein level following administration of J. carnea. Onochie et al. (33) reported a non-significant increase in total protein, bilirubin (conjugated and unconjugated) following ethanolic extract of J. carnea, which refutes the study findings.

The study findings showed a non-significant increase in the body weight in groups B and F, while groups A, C, D and E had a significant increase in the body weight when the initial weight was compared to the final weight. The mechanism of action following the non-significant increase in the PHZ group only is not fully elucidated. However, the findings of Onyeabo et al. (36) showed a significant increase in the body weight following phenyl hydrazine induced toxicity, which has disagreement to the study findings. Aloke et al. (37), Muhammadi et al. (38), de Souza et al. (39) reported a significant decline in the bodyweight following PHZ administration, which contradicts the study findings. Also, report of Ezeigwe et al. (26) reported a non-significant increase in the body weight following PHZ, which is in accordance with the study findings. Further, treatments with crude aqueous extract of Justicia carnea demonstrated a significant increase in the body weight, which is linked to high flavonoids content. The study agrees with the report of Onyeabo et al. (12) who reported a significant increase in the body weight following administration of J. carnea leaves extract following PHZ toxicity. The study demonstrated that PHZ showed mildly hypertrophied central vein are normal and moderate vacuolation of liver sinusoids, which is linked to lipid peroxidation and generation of free radicals. The study of Kale et al. (40) showed similarities to the study following PHZ toxicity. Okorie et al. (41) had similar findings to this study outcome revealing deleterious effect on the liver architecture following PHZ. However, treatments with J. carnea demonstrated a restorative effect on the hepatic cells and general architectural features of the liver, which is attributed to the presence of flavonoids. Akintimehin et al. (13) and Ukpabi-Ugo et al. (29) showed similar report to the study findings following ethanolic leaf extract of J. carnea on liver architecture. Further, the study findings showed that PHZ caused focal infiltration of the renal architecture, which is associated with increased lipid peroxidation and ROS formation through oxidative stress process (26,42). The report of Elaby and Ali, (43) showed similarity to the study findings following PHZ toxicity. Also, Ekweogu et al. (44), Akara et al. (2021), Umoren et al. (45) reported alterations in the renal architecture following PHZ toxicity, which is in accordance with the study report.

CONCLUSION

The study concludes that PHZ demonstrates a toxic effect on hepatic function resulting to a compromise in the liver enzymes and bilirubin level with no impact on body weight. Also, PHZ resulted in alterations in the histoarchitectural structure of the hepatocyte; thus, treatments with the ALJC and Vitamin B12 demonstrated improved function in hepatic function and histoarchitectural changes of the liver in female rats. However, it is therefore recommended that ALJC and

Vitamin B12 could improve the overall health outcomes for individuals with hepatic dysfunction in anaemic condition.

ACKNOWLEDGEMENTS

The authors showed their profound gratitude to the Head of laboratory unit, Department of Anatomy, College of Nursing Sciences, Adazi Nnukwu, Anambra State and their efforts towards the success of this study.

FUNDING

The authors funded this research within the time of investigation and there was no external grant available as at the time of the investigation.

CONTRIBUTIONS AMONG AUTHORS

Dr. Mrs. Ejikeme, S.N and Mr. Eyeghre, O.A drafted the manuscript, Prof. Akpuaka, F.C., and Dr. Ofoego, U. C. edited the manuscript and gave their immense contributions, the data analysis was done and edited by Mr. Eyeghre, O.A and Dr. Akudu, L.S.

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