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Effects of Ethanol Leaf Extract of Moringa Oleifera on Anacin–Induced Hepatotoxicity in Adult Male Wistar Rats

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ABSTRACT

Introduction: *The liver is an important organ which is essential for bodily functional processes in all vertebrates. Hepatotoxicity is a known characteristic of over 1000 medications. Moringa oleifera contains very high concentrations of antioxidants, hence this study aims to explore the antioxidant property of this plant.*

Materials and Methods: *Thirty adult Wistar rats were randomly assigned into six groups (1-6) of five rats each, they were acclimatized for one week. These groups received different treatments; Group 1 (normal control) rats were given 1 ml/kg N/Saline and fed on standard laboratory rat chow throughout the experimental period. Groups 2, 3, 4 and 5 were treated with 75 mg/kg Anacin dissolved in N/Saline administered Orally for four weeks after which ELEMO dissolved in N/Saline was administered at 200, 400 and 800 mg/kg to groups 3, 4, and 5 respectively for three weeks, while group 6 was treated with high dose ELEMO (800 mg/kg) for three weeks, followed by freshly prepared Anacin administration solution for 4 weeks under fasting condition. Animals in group 2 were sacrificed via cervical dislocation immediately after the hepatotoxicity induction, at the end of the experimental periods, animals in groups 1, 3, 4, 5, and 6 were also sacrificed. A midline incision was made along the anterior abdominal wall, blood was taken by cardiac puncture, the liver specimens were perfused with isotonic saline, excised, blotted dry and weighed. Liver tissues were fixed in 10% formol saline and processed for routine paraffin embedding method. Sectioned tissues were stained with Haematoxylin and Eosin for demonstration of general liver histoarchitecture, Masson's trichrome stain for demonstration of collagen fibers.*

Results: *The results of this study showed markedly elevated levels of liver enzymes (ALT, AST, ALP) and reduced levels of serum proteins (TP, Albumin, and Globulin), which are all indicators of liver dysfunctions. The histological findings revealed hepatic swelling and vacuolization with centrilobular hepatic necrosis in group 2 and partly in groups 3 and 4, whereas groups 5 and 6 showed significant improvement after intervention with high doses of ELEMO after and before hepatotoxicity respectively. There were normal collagen fibres distribution observed around the portal vein in group 1, 5 and 6, meanwhile, the collagen distribution in group 2 was denser when compared with the normal control around the portal vein, similar observation was seen in groups 3 and 4.*

Conclusion: *This study concluded that, Anacin induced hepatotoxicity in the liver of adult Wistar rats, was able to be reversed by the antioxidant and anti-inflammatory properties of ELEMO which may be a reliable therapeutic option for treating liver toxicity in human, in the nearest future.*

Keywords

Moringa oleifera, Hepatotoxicity, Anacin-Induced Liver Injury, Antioxidant Activity, Hepatoprotection.

The liver is a crucial organ responsible for numerous physiological functions in vertebrates. It plays a pivotal role in metabolism, including digestion, deamination, and detoxification. It manages macromolecules such as proteins, carbohydrates, and lipids. Additionally, the liver facilitates the excretion of both endogenous and exogenous substances, bile production, urea synthesis, and detoxification of xenobiotics. As a central metabolic organ, it is frequently targeted by toxins [1].

Nearly all drugs or toxins introduced into the human body undergo hepatic metabolism. Due to their lipophilic nature, most drugs are readily absorbed across cellular membranes. Within hepatocytes, these substances are converted into hydrophilic forms through biochemical processes, aiding in their inactivation and elimination. Drug metabolism typically occurs in two phases: Phase I involves oxidation or hydroxylation to increase polarity, while Phase II may follow directly in some cases, bypassing Phase I [2].

Over 1,000 medications and herbal agents are known to cause hepatotoxicity [1,2], with pharmaceuticals accounting for 20–40% of acute liver failure cases. Approximately 75% of idiosyncratic drug reactions culminate in liver transplantation or mortality [3]. Clinical presentations of drug-induced liver injury (DILI) vary widely, from asymptomatic elevations in hepatic enzymes to fulminant hepatic failure (National Institute of Diabetes and Digestive and Kidney Diseases).

Hepatotoxicity results from exposure to medicinal, chemical, dietary, or herbal toxins [4]. Globally, over fifty million individuals are affected by hepatotoxicity [5].

In the United States, around 2,000 acute liver failure cases are reported annually, with medications responsible for over half—39% due to acetaminophen toxicity and 13% from idiosyncratic drug reactions. Drug-induced liver injury (DILI) contributes to 2–5% of hospitalizations for jaundice and approximately 10% of acute hepatitis cases [6].

Research indicates that within six months of developing idiosyncratic DILI, 10% of patients may experience severe hepatic outcomes, including acute liver failure, liver transplantation, or death [7]. The estimated annual incidence of idiosyncratic DILI in the U.S. is 14–19 per 100,000 individuals, equating to roughly 60,000 cases. However, global data on the prevalence of such adverse hepatic reactions remain limited [7].

Traditional medicine encompasses a wide array of culturally rooted health practices passed down through generations. The World Health Organization defines traditional medicine as a collection of diverse health practices, knowledge systems, and beliefs incorporating plant, animal, and mineral-based remedies, spiritual therapies, manual techniques, and exercises used individually or in combination to maintain health and treat or prevent illness [8].

Anacin is an analgesic, a combination of a salicylate and a stimulant. It works by reducing substances in the body that cause pain, fever and inflammation. Caffeine is used in this product to increase the pain relieving effects of aspirin. It is used to treat headaches, muscle pain, arthritis or body pain and fever caused by common cold. Anacin is used to prevent heart attack, strokes and Angina [1,9]. Anacin is known to cause acute hepatotoxicity or frank Reyes syndrome, bleeding stomach ulcer or general gastrointestinal bleeding. Other side effects include; severe allergic reaction, hives, wheezing, swelling of the face, lips, tongue or throat.

Moringa oleifera L. (also known as Moringa pterygosperma G.), commonly referred to as the "drumstick" or "horseradish" tree, is indigenous to Northwest India and is cultivated in regions such as South and Northeast Africa, Madagascar, Tropical and Southwest Asia, Latin America, and West Africa, including Nigeria [10]. This small tree, belonging to the Moringaceae family, can grow up to 8 meters tall. It is known locally as okweoyibo (Igbo), Zogale gandi (Hausa), and Eweigbale (Yoruba). The leaves, flowers, and pods of Moringa oleifera are edible and widely utilized across various cultures [11]. The plant is rich in phytochemicals such as vanillin, omega fatty acids, carotenoids, ascorbates, and flavonoids, along with essential minerals like magnesium, iron, selenium, and zinc (Verma et al.). These constituents contribute to its antioxidant, anticancer, immunomodulatory, antidiabetic, antiatherogenic, and hepatoprotective properties [12].

When compared to other plants, 100 grams of dried Moringa oleifera leaves yield seven times more vitamin C than oranges, ten times more vitamin A than carrots, seventeen times more calcium than milk, nine times more protein than yogurt, fifteen times more potassium than bananas, and twenty-five times more iron than spinach [13].

Materials and Methods

Equipments

Hot plate (A39-CVL004, Japan), Floating Bath (Water Bath: MH-8504), Microtome (Laboid: LBM- RM2), Centrifuge (Denly, Model BS 400), Top Loader Balance (Metlet Telodo, Mg 126). Rotary evaporator (Buchi), dissecting kit, Soxhlet Extractor, oral cannula, Petri dish, Motic Scanner, 2 and 5 ml sterile disposable syringes and needles, cotton wool, 5 ml plain bottles.

Chemicals and Reagents

ANACIN (WHITEHALL LABORATORIES (NEW YORK), PTE LTD) was purchased at SKG Pharmaceutical Limited, 7/9 Sapara Street, Off Oba Akran Avenue, Ikeja, Lagos. Varying doses of Ethanol, Normal saline and Distilled water. The markers of liver enzyme Test Kits were purchased from Nano Technologies, Austin Texas, USA. All other chemicals were of Analytical grade and were procured from Sigma Aldrich (St. Louis. MO USA)

Plant Material

Fresh leaves of *Moringa oleifera* were obtained from GIWA'S GARDEN, OAU Road 7 Ile-Ife, and authenticated by a taxonomist in the Department of Botany, Obafemi Awolowo University, Ile Ife; a Voucher Specimen Number (IFE 18343) was assigned and plant deposited in the Department herbarium for reference purpose.

Plant Extraction & Preparation

The fresh leaves of *Moringa oleifera* were removed from the stem, washed in distilled water, air dried at room temperature, weighed at intervals to obtain a constant weight to ensure appropriate dryness, this was then blended to powdery form with the use of an electric blender. The extraction of the plant constituents was done using Soxhlet Extraction method with ethanol as solvent. The extract

was filtered using Whatman No. 1 filter paper (0.2 mm) to remove residues. The filtrate was concentrated with a rotary evaporator and lyophilized. The dried extract was stored in a desiccator for use.

Animal Care and Management

Thirty adult Wistar rats with an average weight of (180 – 200) g were used for this study. The rats were procured from the animal holding of College of Health Sciences, OAU, Ile-Ife. The rats were kept in plastic cages, under standard laboratory condition of temperature, humidity and light in the Animal House at the Department of Anatomy and Cell Biology of OAU, Ile-Ife; fed on standard laboratory rat chow procured from Sunshine Mill, Oshogbo, Osun State, Nigeria and given free access to clean water.

Experimental Design

Thirty adult Wistar rats were randomly assigned into six groups (1-6) of five rats each, they were acclimatized for one week. These groups received different treatments; Group 1 (normal control) rats were given 1 ml/kg N/Saline and fed on standard laboratory rat chow throughout the experimental period. Groups 2, 3, 4 and 5 were treated with 75 mg/kg Anacin dissolved in N/Saline administered Orally for four weeks after which ELEMO dissolved in N/Saline was administered at 200, 400 and 800 mg/kg to groups 3, 4, and 5 respectively for three weeks, while group 6 was treated with high dose ELEMO (800 mg/kg) for three weeks, followed by freshly prepared Anacin administration solution for 4 weeks under fasting condition.

Animals in group 2 were sacrificed via cervical dislocation immediately after the hepatotoxicity induction, at the end of the experimental periods, animals in groups 1, 3, 4, 5, and 6 were also sacrificed. A midline incision was made along the anterior abdominal wall, blood was taken by cardiac puncture, the liver specimens were perfused with isotonic saline, excised, blotted dry and weighed. Liver tissues were fixed in 10% formol saline and processed for routine paraffin embedding a semi quantitative method described by Pilette et al., [14].

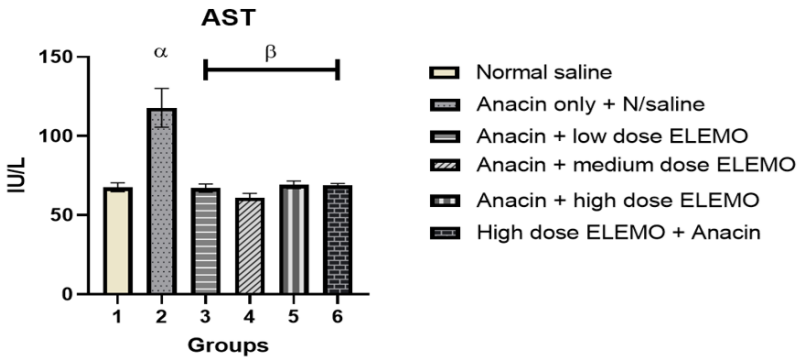
Histological Staining

Sectioned tissues were stained with Haematoxylin and Eosin for demonstration of general liver histoarchitecture, Masson's trichrome stain for demonstration of collagen fibers.

Photomicrography

The photomicrographs of the tissue slides were scanned using Motic Easy scan scope Pro N6 in Virtual Slides SVS format. Slide Loading took up to 6 slides; these were placed in the motorized tray. Then One-Click Scanning was done which Initiated automated scanning and image capture. The autofocus system was employed to adjust focal plane dynamically based on selected mode and Image Capture with High-resolution camera (up to 12MP) was used to capture detailed images. The File Output were Saved in formats like MDS, SVS, JPEG, DICOM, TIFF and data

GRPS	TREATMENTS (N = 5)	DOSAGE	ROUTE	PERIOD (weeks)
1	NORMAL SALINE	1 ml/kg	Oral	4 + 3
2	ANACIN ONLY + N/SALINE	75 mg/kg	Oral	4 + 3
3	ANACIN + LOW DOSE ELEMO	75 mg/kg + 200 mg/kg	Oral	4 + 3
4	ANACIN+MEDIUMDOSE ELEMO	75 mg/kg + 400 mg/kg	Oral	4 + 3
5	ANACIN + HIGH DOSE ELEMO	75 mg/kg + 800 mg/kg	Oral	4 + 3
6	HIGH DOSE ELEMO + ANACIN	800 mg/kg + 75 mg/kg	Oral	3 + 4



Data were expressed as mean ± SEM. 'α' is significantly different from group 1 and 'β' is significantly different from group 2, using one-way ANOVA. Alpha level was set at p<0.05

Figure 1

were automatically uploaded by sending scanned images to a slide management server or local storage and the scanned slides were viewed thereafter, the degree of hepatic necrosis and fibrosis were determined by a semi-quantitative method.

Statistical Analysis

Data were analyzed using GraphPad prism 8 (Version 8.03; Graph Pad Inc., San Diego, CA, USA) was the statistical package used for data analysis. One-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons as post hoc. A value of $p < 0.05$ was considered statistically significant. Results were expressed as Means ± S.E.M.

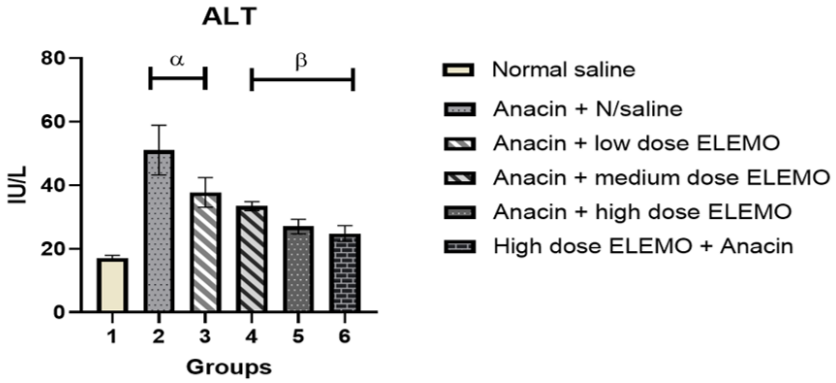
Results

Results of Biochemical Analysis

Discussion

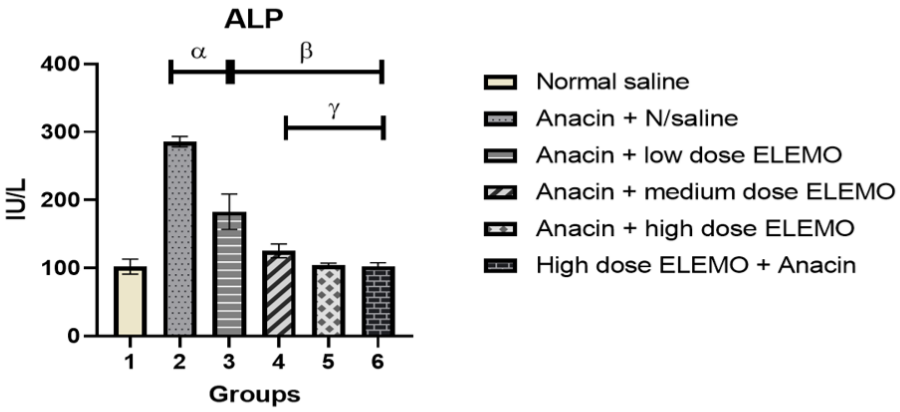
This study investigated the effects of ethanol leaf extract of *Moringa oleifera* on Anacin-Induced hepatotoxicity in adult male Wistar rats, with a view to provide histological evidence of the moringa oleifera which has been documented to possess hepatoprotective effect, which researches have provided sparse information about its biochemical and histomorphological effects.

In Figures 1 and 2, the biochemical analysis of the study revealed a markedly elevated level of aspartate aminotransferase (AST)



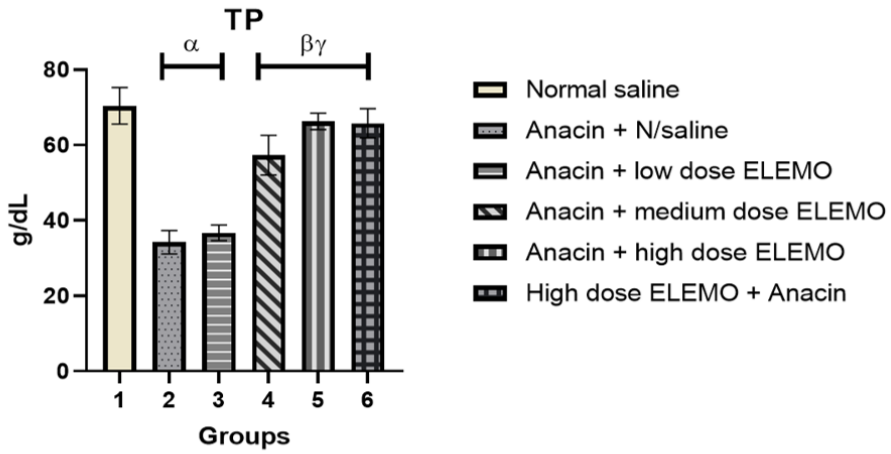
Data were expressed as mean ± SEM. 'α' is significantly different from group 1 and 'β' is significantly different from group 2, using one-way ANOVA. Alpha level was set at p<0.05

Figure 2



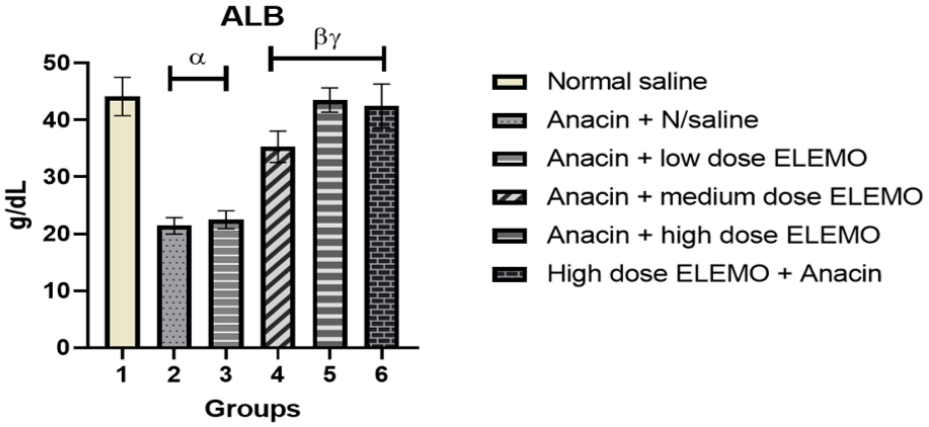
Data were expressed as mean ± SEM. 'α' is significantly different from group 1, 'β' is significantly different from group 2, and 'γ' is significantly different from group 3, using one-way ANOVA. Alpha level was set at p<0.05

Figure 3



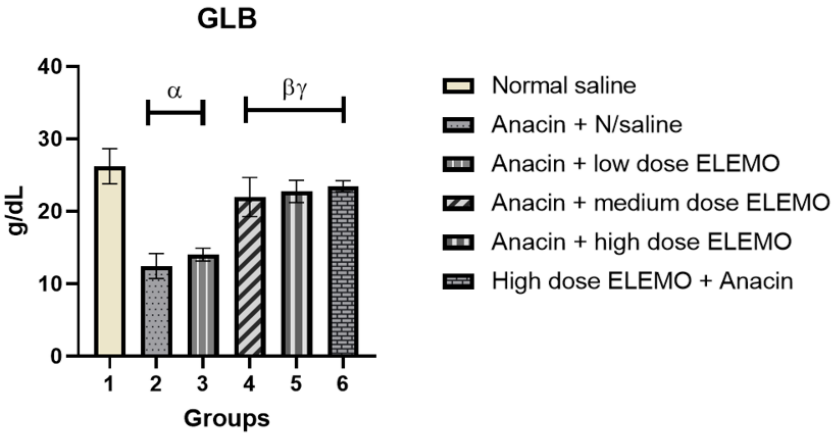
Data were expressed as mean ± SEM. 'α' is significantly different from group 1, 'β' is significantly different from group 2, and 'γ' is significantly different from group 3, using one-way ANOVA. Alpha level was set at p<0.05.

Figure 4



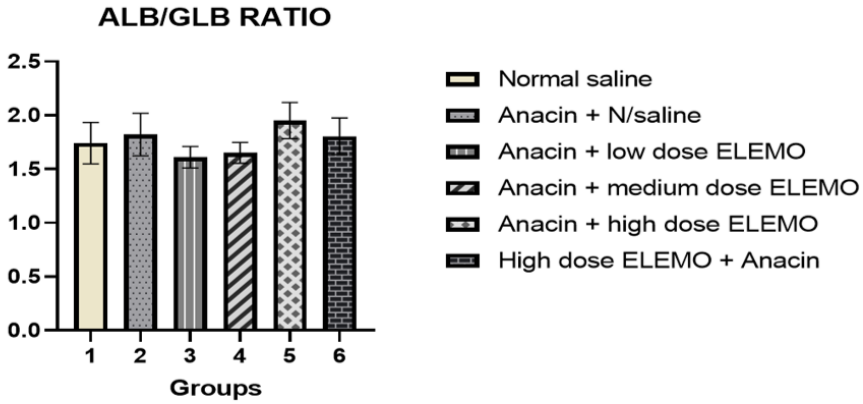
Data were expressed as mean ± SEM. 'α' is significantly different from group 1, 'β' is significantly different from group 2, and 'γ' is significantly different from group 3, using one-way ANOVA. Alpha level was set at p<0.05

Figure 5



Data were expressed as mean ± SEM. 'α' is significantly different from group 1, 'β' is significantly different from group 2, and 'γ' is significantly different from group 3, using one-way ANOVA. Alpha level was set at p<0.05

Figure 6



Data were expressed as mean ± SEM. No significant difference across all groups, using one-way ANOVA. Alpha level was set at p<0.05

Figure 7

Histology Reports

PLATE 1

Representative photomicrograph of the liver of anacin–induced hepatotoxicity in adult male wistar rats. H&e x 400.

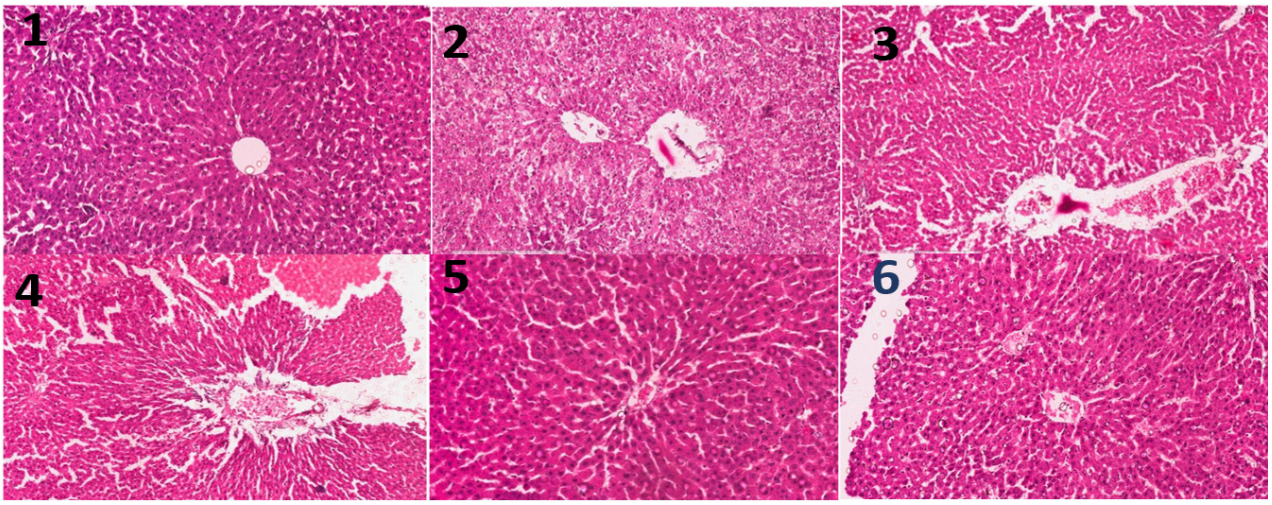
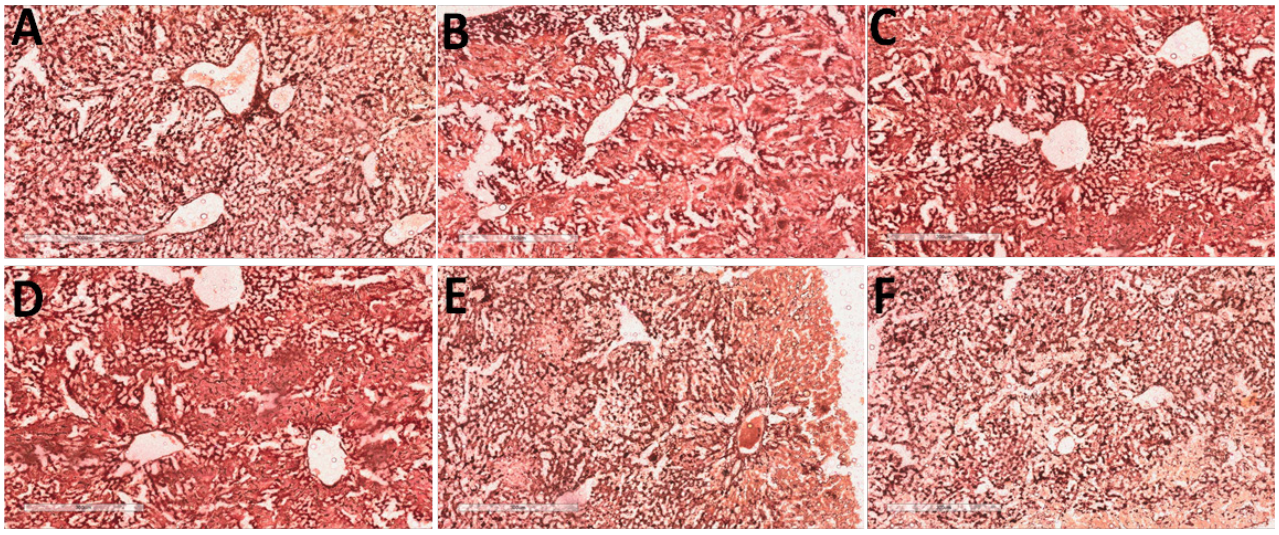


PLATE 2

Representative photomicrograph of the liver of anacin–induced hepatotoxicity in adult male wistar rats. Vvg x 400.



and Alanine aminotransferase (ALT) in group 2 animals, which is statistically significant when compared with groups 1, 3, 4, 5 and 6. However, there was no significant difference in the levels of AST within experimental groups (3,4,5 and 6) as shown above. Meanwhile there was a significant statistical difference between group 3 and the other test groups (4,5 and 6) in the level of ALT. This finding is in keeping with the work of Kumari et al., [15] who stated that AST increased significantly in Wistar rats treated with hepatotoxicants, likewise the level of ALT.

The biochemical analysis of the Alkaline phosphatase (ALP) in Figure 3, showed noticeably increased level of this enzyme in group 2 animals, when compared to the experimental groups (3,4,5 and 6), there was also a significant difference between group 3 and other test groups (4, 5 and 6). There was no significant difference between the normal control (group 1) and experimental groups 5 and 6. This corroborates the work of Khudhair et al., [16], which posited that liver enzymes including ALP showed significant elevations in the Wistar rats' serum in NSAID- Induced hepatotoxicity, which is consistent with impaired hepatic function and structural damage. This observed toxicity aligns with the proposed mechanisms of NAPQI- mediated oxidative stress, mitochondria dysfunction and eventual inflammatory cascades.

Total serum protein, albumin and globulin were represented in Figures 4, 5 and 6 respectively, which showed a statistically significant reduction in groups 2 and 3 when compared with groups 4, 5 and 6. However, the total protein level in group 4 was statistically reduced when compared to groups 5 and 6. This is in agreement with the work of Ekam et al., [17] who posited that hepatocellular damage in rats was established by significant decrease in serum total protein including albumin and globulin levels. No statistically significant difference was observed across all the groups (1, 2, 3, 4, 5, 6) in the albumin globulin ratio as

depicted in figure 7, which may likely be due to the fact that the Anacin-induced hepatotoxicity was in the acute stage, or the damage is mild and as such, it has not yet compromised the synthetic function of the liver.

The histological results as shown in plate 1 revealed the general layout of the histoarchitecture of the liver with H&E staining of Anacin-induced hepatotoxicity which is typically driven by oxidative stress, mitochondrial dysfunction, and inflammatory cytokine release. In this study the liver histology reflected hepatocyte swelling and hydropic degeneration of the hepatocytes in group 2, marked with cytoplasmic vacuolization with areas of hepatocyte necrosis, which was also noted in groups 3 and 4 to a lesser extent, whereas the normal control group showed typical liver histology with no swelling or vacuolization, groups 5 and 6 showed hepatic regeneration and other liver features very close to the normal control (group 1).

Markers of acute inflammation was noted by the presence of leukocytes' Infiltration which was noted in group 2, there were few neutrophilic infiltration in groups 3 and 4 as depicted above. There were no such features in the normal control (group 1) and groups 5 and 6 as represented in Plate 1 above. This is in keeping with the work of Taha et al., [18] who stated that, hepatocellular damage induced by NSAID was ameliorated based on the histological and biochemical results by the administration of Moringa oleifera leaves before the intake of the hepatotoxicant.

The histological results shown in plate 2 revealed the distribution of collagen fibers in the histoarchitecture of the liver with Verhoeff Van Giesson staining of Anacin-induced hepatotoxicity. The collagen fibres are seen surrounding the central vein in the normal control group while the density of the collagen fibres around the central vein areas in the experimental groups 2, 3 and 4 are denser when compared with groups 5 and 6, the collagen fibres distribution in groups 5 and 6 is almost similar when compared with group 1.

It can be concluded from this study that Anacin induced hepatotoxicity in the liver of adult Wistar rats, which ethanol leaf extract of Moringa oleifera was able to protect by reversing the injury inflicted via its antioxidant and anti-inflammatory properties. ELEMO may be a reliable therapeutic alternative, for treating liver toxicity in human, in the nearest future.

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