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African Walnut (*Tetracarpidium conophorum*) Seed Oil Ameliorates CCl_4 Induced Liver Injury in Wistar Rats

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Abstract

The increase in the incidence of hepatotoxicity resulting from exposure of individuals to toxic substances from the environment especially industrial hazards as well as toxicity from drug intake calls for search of more sources of hepatoprotective substances. African walnuts (*Tetracarpidium conophorum)* have been widely used not only as food but for various acclaimed medicinal purposes. The protective effects of *Tetracarpidium conophorum* seed oil against carbon tetrachloride (CCl_4) - induced liver injury in wistar rats was evaluated. The oil was extracted with n-hexane from the walnut seed using Soxhlet apparatus. Forty eight male Wistar rats (100-120g) were used. These were divided into four groups of 12 rats each. Groups A and D were fed with normal rats' feed and water while Groups B and C were fed with diet containing 10% extracted *Tetracarpidium conophorum* seed oil throughout the experiment. Group C and D received 200mg/kg CCl_4 intraperitoneally once after 30 days of feeding. The administration of the African walnut oil attenuated the levels of serum aspartate aminotransferase, alanine aminotransferase, and liver lipid peroxidation in CCl_4 – treated rats. Histopathological studies of the rats' liver revealed that pretreatment of the animals with African walnut oil reduced the incidence of liver lesions induced by CCl_4. African walnut oil also increased the antioxidant capacity of the rats by increasing reduced glutathione (GSH) content and decreased malondialdehyde (MDA) that was formed due to CCl_4 administration in CCl_4-treated rats. The results show that *Tetracarpidium conophorum* seed oil has hepatoprotective effect against CCl_4 - induced liver toxicity.

Keywords: Carbon Tetrachloride (CCl_4), *Tetracarpidium Conophorum,* Hepatotoxicity, Hepatoprotective, Reduced Glutathione (GSH), Malondialdehyde (MDA).

1.0 INTRODUCTION

One of the most serious diseases is hepatotoxicity which is liver damage or injury caused by exposure to drugs (Andrade *et al*., 2007). Liver disease is a main cause of death in many developing countries and very deadly at that, not only because liver plays a significant role in metabolism and detoxification of exogenous toxins and therapeutic agents but also in the bio-regulation of fats, carbohydrates, amino acids, proteins, blood coagulation and immunomodulation (Juza and Pauli, 2014). Many of the drugs that should be employed in the treatment are most often than not, also toxic and sometimes more toxic to the liver, hence, there is need to delve into the natural environment for plants that can be effective in the treatment of liver injury.

Medicinal plants have always been rich sources of biologically active compounds vital to human health (Wolf *et al*., 2008). One of such plants is Walnut.

Walnuts are edible seeds that are widely cultivated for their delicacy. Prominent species include *Juglans regia* (L.), known as the English walnut and belonging to the family Juglandaceae (Burkill, 1985). The tropical African walnut, known as *Tetracarpidium conophorum* (Oyekale *et al*. 2015), belongs to the family Euphorbiaceae (Edem *et al*., 2009). The plant is popularly known as African walnut, black walnut and Nigerian walnut (Ekwe and Ihemeje 2013; Nwaichi *et al*., 2017). In Nigeria, among the Yoruba tribe, the walnut is known as *awusa* or *asala*, *ukpa*, or *oke okpokirinya* in Igbo and *gawudi bairi* in Hausa; and it is known as *okhue* or *okwe* among the Bini tribe of Edo State (Chijoke *et al*., 2015; Kanu *et al*. 2015). Walnuts are edible seeds that are widely cultivated for their delicacy. Walnut is highly rich in mono unsaturated fatty acids and polyunsaturated fatty acid. Walnut is a perfect source of Omega 3 (which is essential fatty acid) and a source of arachidonic acid. Phytochemical screening of

Tetracarpidium conophorum nuts, leaves and roots shows that it contains bioactive compounds such as oxalates, phytates, tannins, saponins, alkaloids, flavonoids and terpenoids (Ojobor *et al*., 2015). The presence of these phytochemicals partly shows the reason why the use of *Tetracarpidium conophorum* in herbal medicine is high.

The nutritional analysis of *Tetracarpidium conophorum* reveal it has a fair source of carbohydrate and fibre with appreciable protein content but significantly rich in edible and industrially useful oil as well as dependable quantity of essential dietary minerals for both children and adults. It also has amazing medicinal benefits. Considering the available reports on the health benefits of *Tetracarpidium conophorum* seed oil such as anti-cancer activity, anti-ulcer activity and anti-microbial activity from several published studies (Tchiegang *et al*., 2007; Ezealisiji *et al*., 2014; Ajaiyeoba and Fadare, 2006), there is scanty information on the hepatoprotective effect of the seed oil. This study therefore sought to give scientific basis for n-hexane extract of *Tetracarpidium conophorum* seed oil to be used as a hepatoprotective agent in carbon tetrachloride induced liver injury.

2.0 MATERIALS AND METHODS

2.1 Chemical Reagents

N-haxane and carbon tetrachloride and were gotten from Sigma Aldrich. All other chemicals were obtained from standard chemical suppliers and were of analytical grade.

2.2 Animals

Male adult Wistar rats (100 - 120g) were used. The animals were used according to the standard guidelines of the Committee on Care and Use of Experimental Animal Resources.

2.3 Plant Materials

Matured fruits of *Tetracarpidium conophorum* were collected from private farm land in Ondo Town, Ondo State, Nigeria. The fruits were authenticated by the Department of Plant Biotechnology, University of Medical Sciences, Ondo.

2.3.1 Preparation of Sample

The collected fruits were cleaned with a moist soft cotton wool and then the seeds were carefully separated from the fruits. Part of the separated nuts were immediately used for oil extraction, while the remaining part was dried at 65^0C for 4 hours in an oven, crushed with a laboratory mortar and pestle and kept in a well labelled air tight polythene bags.

2.3.2 Extraction of Oil from *Tetracarpidium Conophorum* Seeds

The seeds of *Tetracarpidium conophorum* were removed from their shells, cut into small pieces, and air dried at room temperature for 2 weeks. The method of extraction used in this study was mirrored after the soxhlet extraction method reported by Sankeshwari *et al*., (2018). Using this method, the dried seeds of *Tetracarpidium conophorum* were grounded into powdered form using a blender and further air dried. The ground sample (50 g) was weighed and placed in the thimble. The thimble was placed in the extraction chamber of the soxhlet extractor. The solvent (n-hexane) was measured to 500ml and poured into separate round bottom flasks. The apparatus was then fitted with the help of clamps and stand to support the soxhlet extractor, round bottom flask, and condenser, all of which was placed on a heating mantle. The solvent was heated and extraction under reflux was carried out. At the end of extraction, the extract (oil) was collected in the round bottom flask and was placed in a rotary evaporator to evaporate the solvent leaving a concentrated form of the oil. The extraction was carried out in the laboratory of the Department of Biochemistry, University of Medical sciences, Ondo, Ondo state.

2.4 Experimental Design

In this study, the rats procured were divided into 4 groups, which are;

Group A: Rats were fed with rat pellet and water only (Control)

Group B: Rats were administered n-hexane extract of *Tetracarpidium conophorum* seed oil only

Group C: Rats were pre-treated with n-hexane extract of *Tetracarpidium conophorum* seed oil and administered carbon tetrachloride (200 mg/kg intraperitoneally).

Group D: Rats were administered toxicant only (Carbon tetrachloride) (200 mg/kg).

The *Tetracarpidium conophorum* seed oil was administered by mixing it with the rat pellets and the toxicant was administered intraperitoneally.

2.5 Preparation of Homogenates

2 g of the liver tissue stored in normal saline was homogenized in 10 ml of Tris buffer. The homogenized samples were then centrifuged at 10000g for about 10 minutes after which the supernatant was collected and used for subsequent analysis (Mohamed *et al*., 2014).

2.6 Biochemical Enzyme Analysis

Blood samples were collected and spinned at 1000 g for 10 min at 25^0C to obtain serum. The clear supernatants collected were stored at -20^0C for further estimation of biochemical parameters.

2.6.1 Evaluation of Alanine Aminotransferase (ALT) Activity

The Alanine aminotransferase (ALT) activity was determined using commercial kits and carried out as described in the kit instruction leaflet (Product code: BXC0212).

Procedure

0.5 ml of R1 buffer containing phosphate buffer (100 mmol/L, pH7.4), L-alanine (200 mmol/L) and alpha-oxoglutarate (2.0 mmol/ L) was added to 0.1 ml of sample. The mixture was incubated for 30 minutes at 37^0C. 0.5 ml of R2 Dye Reagent containing 2,4-Dinitrophenyl Hydrazine (2.0 mmol/L) was added to the reaction mixture and allowed to stand for 20 minutes at 20-25^0C. 5 ml of 4.0 mol/L sodium hydroxide was then added and the absorbance was read against the sample blank after 5 minutes at 546nm.

2.6.2 Evaluation of Aspartate Aminotransferase (AST) Activity

The Aspartate aminotransferase activity was carried out using commercial kit as described in the kit instruction leaflet (Product code: BXC0202).

Procedure

0.5 ml of R1 AST buffer containing phosphate buffer (100 mmol/L, pH7.4), L-aspartate (200 mmol/L) and alpha-oxoglutarate (2.0 mmol/L) was added to 0.1 ml of sample. The mixture was incubated for 30 minutes at 37^0C. 0.5 ml of R2 Dye Reagent containing 2,4-dinitrophenyhydrazine (2.0 mmol/L) was added to the reaction mixture and allowed to stand for 20 minutes at 20-25^0C. 5 ml of sodium hydroxide (4.0 mol/L) was then added and the absorbance was read against the reagent blank after 5 minutes at 546nm.

2.6.3 Estimation of Reduced Glutathione (GSH) Concentration

The level of reduced glutathione (GSH) was estimated by using the method of Beutler *et al*., (1963). In this method, thiol residues of GSH reduced Ellman's reagent to 2-nitro-5-benzoic acid was read at 412 nm.

Procedure

The tissue homogenate (0.2 mL) was added to 1.8 mL of distilled water and 3 mL of the precipitating solution (trichloroacetic acid TCA) was gently introduced into the mixture. The mixture was then allowed to stand for 5 minutes and then filtered. Thereafter, 1 mL of filtrate was added to 4 mL of 0.1 M pH 7.4 phosphate buffer. Finally, 4.5 ml of the Ellman reagent was added. A blank was prepared with 4 ml of 0.1 M phosphate buffer, 1mL of diluted precipitating solution (3 parts to 2 parts of distilled water) and 4.5 mL of the Ellman's reagent. The optical density was measured at 412 nm. GSH concentration was proportional to the absorbance at that wavelength and the estimate was obtained from the GSH standard curve.

2.6.4 Assessment of Lipid Peroxidation

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) present in the test sample according to the method of Varshney and Kale (1990).

3.0 RESULTS

3.1 Alanine Aminotransferase (ALT) Activity

There was a significant increase ($p<0.05$) in the serum ALT activity in untreated group (B) compared to control group (A). A significant decrease was also observed in the serum ALT activity in the pre-treated group (D) relative to the untreated group as shown in Figure 1.

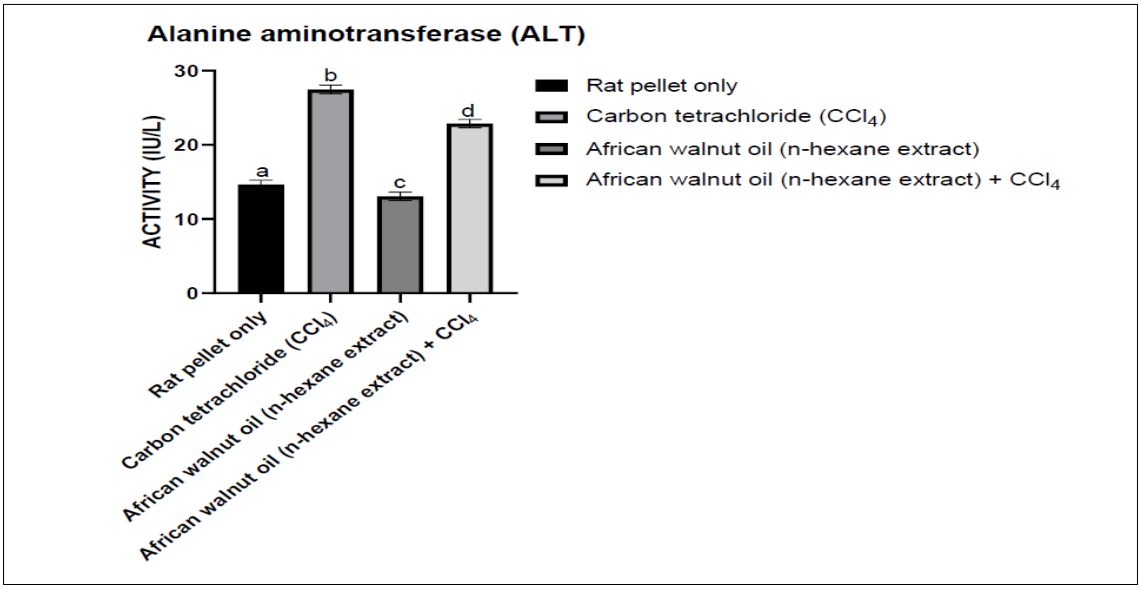

Figure 1: Effect of feeding *Tetracarpidium conophorum* seed oil on serum Alanine aminotransferase (ALT) activity

3.2 Aspartate Aminotransferase (AST) Activity

There was a significant increase ($p<0.05$) in the serum AST activity in untreated group (b) compared to control group (a). There was a significant decrease in the serum AST activity in the pre-treated group (d) relative to the untreated group as shown in Figure 2.

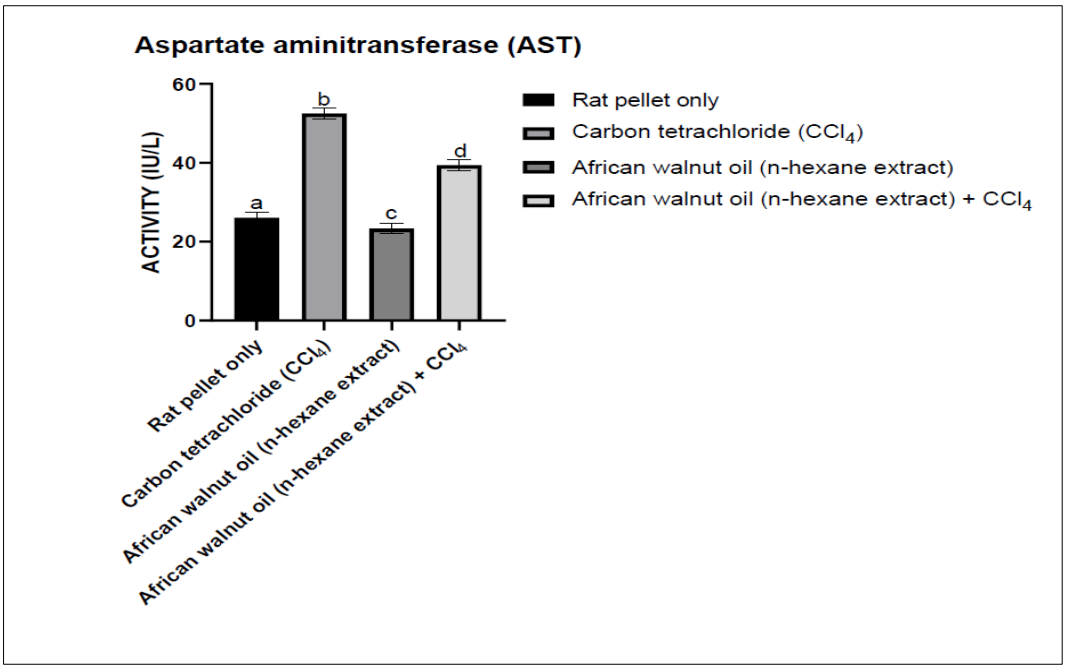

Figure 2: Effect of feeding *Tetracarpidium conophorum* seed oil on serum Aspartate aminotransferase (AST) activity

3.3 Estimation of Reduced Glutathione (GSH) Concentration

There was significant decrease ($p<0.05$) in the GSH level in the untreated group (B) compared to other groups. In contrast, the level of reduced glutathione across *Tetracarpidium conophorum* oil pretreated group (D) showed a significant increase relative to the untreated group (B) as shown in Figure 3.

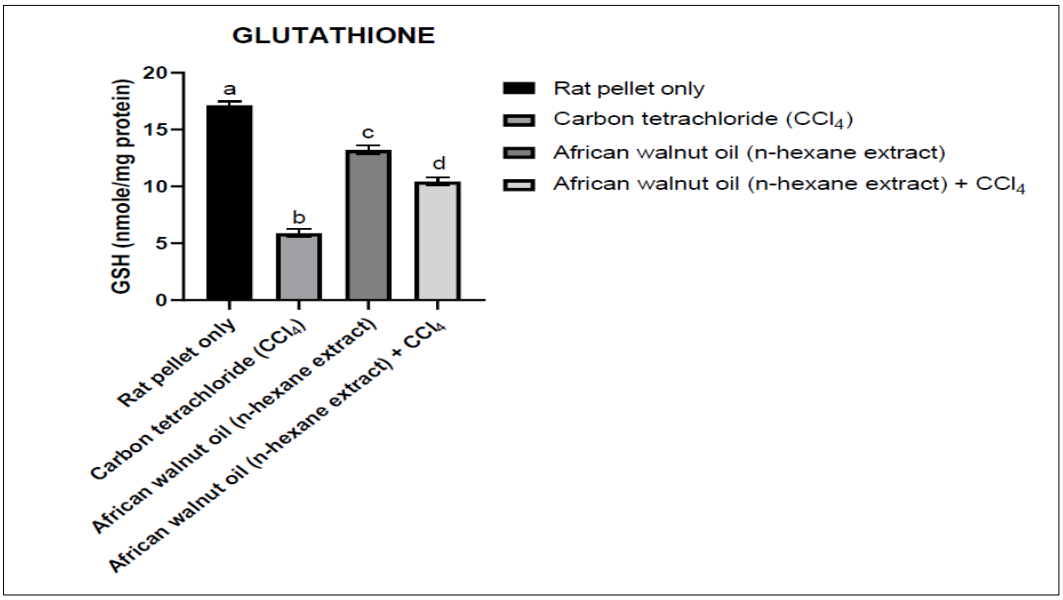

Figure 3: Effect of feeding *Tetracarpidium conophorum* seed oil on reduced glutathione (GSH) content

3.4 Assessment of Lipid Peroxidation

Figure 4 shows that *Tetracarpidium conophorum* seed oil inhibit the lipid peroxidation in hepatocyte as there was significant increase ($p<0.05$) in MDA level in the untreated group (B) compared to control (A) but a sharp decrease in the MDA formed in the pretreated group with *Tetracarpidium conophorum* seed oil.

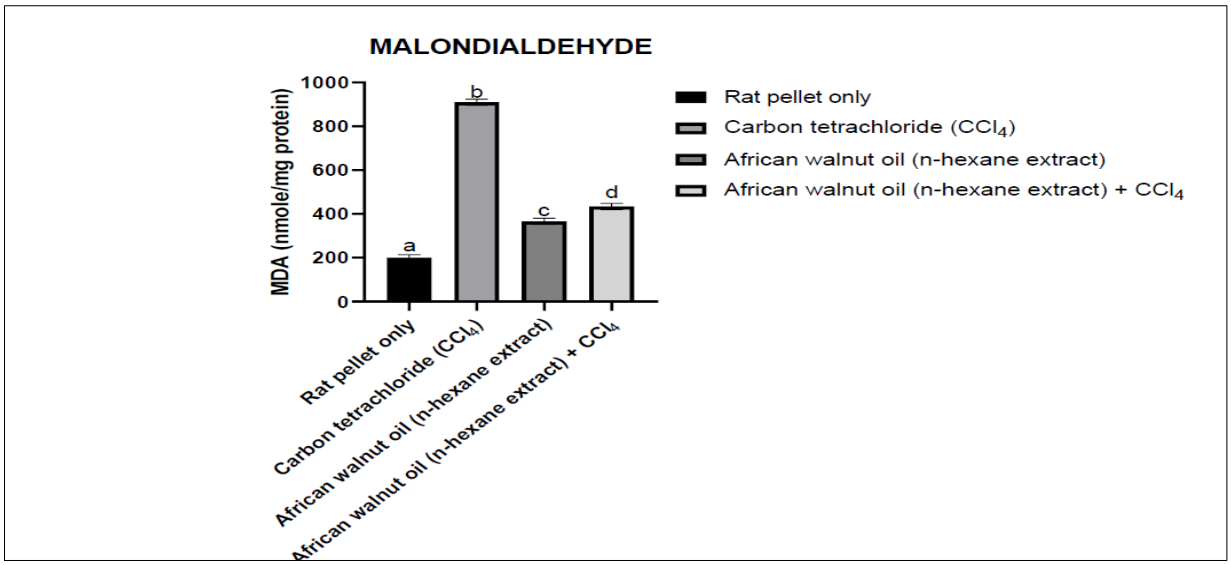

Figure 4: Effect of feeding *Tetracarpidium conophorum* seed oil on malondialdehyde level (MDA)

3.5 Histopathological Analyses

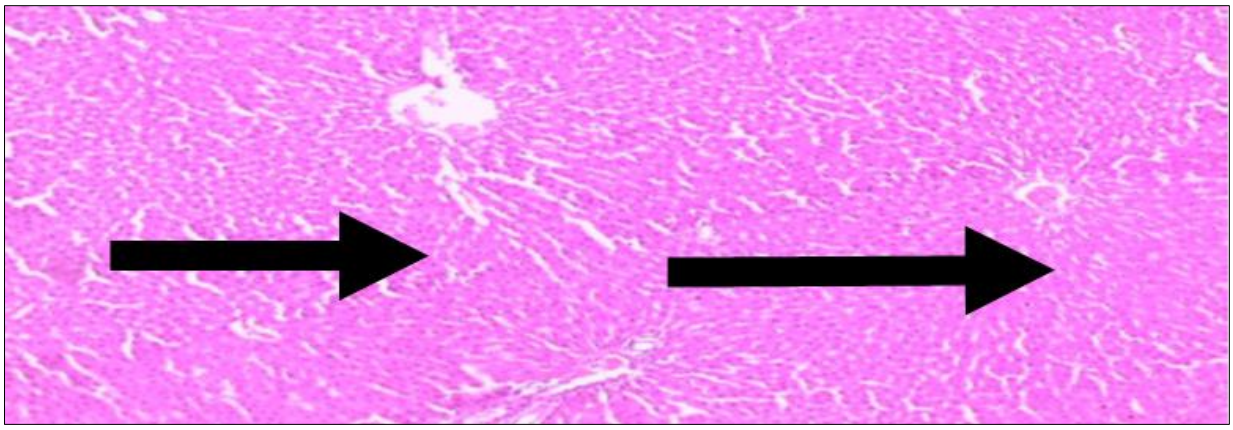

Plate 3.1: Photomicrograph of a liver section of control group (fed water and rat pellet only) stained by Haematoxylin and Eosin. The photomicrograph shows a normal central vein and plates of hepatocytes (black arrow)

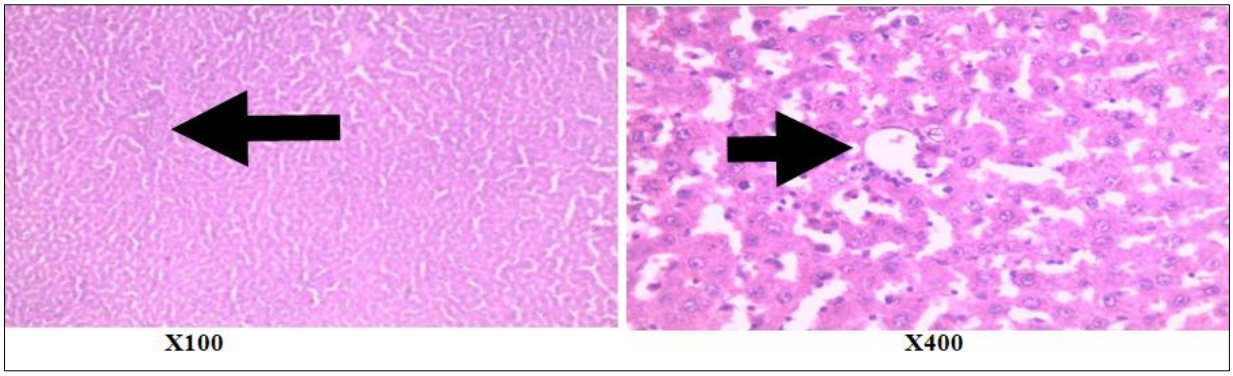

Plate 3.2: The photomicrograph of a liver section of group fed with n-hexane extract of *Tetracarpidium conophorum* seed oil stained by Hematoxylin and Eosin. The photomicrograph shows normal central venules (white) and mild fatty change involving the hepatocytes

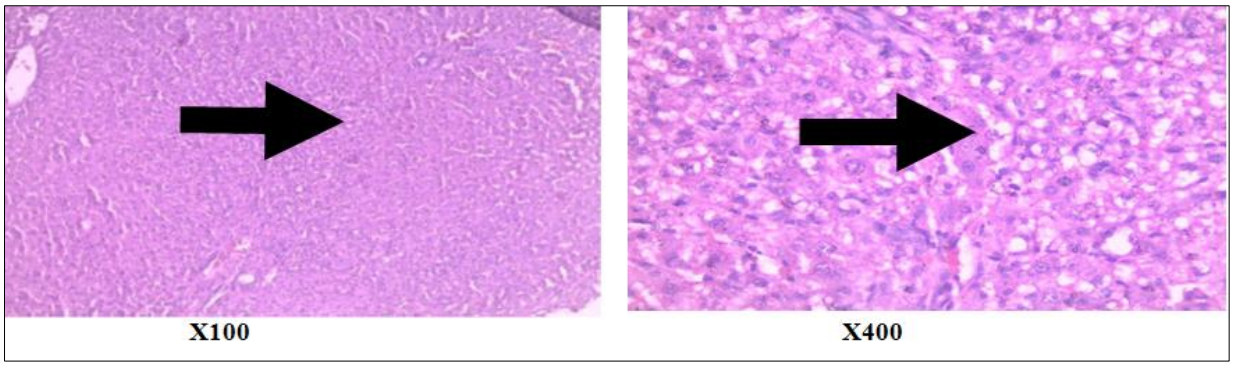

Plate 3.3: The photomicrograph of a liver section of group pre-treated with n-hexane extract of *Tetracarpidium conophorum* seed oil and administered carbon tetrachloride to induce liver injury stained by Hematoxylin and Eosin. The photomicrograph shows mild fatty change (black arrow) involving the hepatocytes with no inflammation

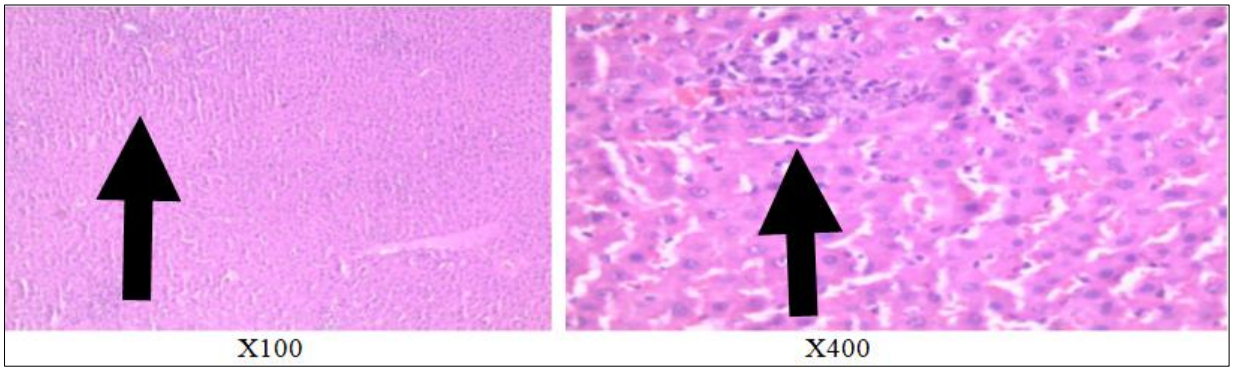

Plate 3.4: The photomicrograph of a liver section of group administered carbon tetrachloride only (negative control) to induce hepatic damage stained by Haematoxylin and Eosin. The photomicrograph shows plates of hepatocytes that are separated by sinusoids. There is mild presence of chronic inflammatory cells (black arrow) extending from the portal tract into the sinusoids. The morphology of the hepatocytes shows necrosis

4.0 DISCUSSION

African walnut seed oil (*Tetracarpidium conophorum*) has been reported to possess bioactive components including monounsaturated fatty acids (MUFA); stearic and palmitic acid and polyunsaturated fatty acids (PUFA); Omega-3 (Alpha-linolenic acid) and Omega-6 fatty acids (Linoleic acid) (Uhunmwangho and Omoregie, 2017). The oil extract was observed to boost antioxidant system, hence, increasing its potential to handle threats posed by exposure to the toxicant. These results are consistent with previous research on the antioxidant effects of monounsaturated fatty acids (MUFAs) demonstrated that oleic acid-rich diets were less susceptible to oxidative damage (Solà *et al.,* 1997). Besides, the MUFAs significantly reduced almost all of the cellular insults that are caused by saturated fatty acids, including oxidative stress, mitochondrial dysfunction, apoptosis, and inflammation in both human and rat hepatocytes (Chen *et al.,* 2018). PUFAs have numerous benefits, particularly the omega-3 group. This includes eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that lower oxidative stress and blood pressure, while also improving immune response and insulin resistance. They are also effective in the prevention of heart disease and other chronic diseases (Das, 2004). Another category of beneficial fatty acids is omega-6, which includes linoleic acid (LA), gammalinolenic acid (GLA), arachidonic acid (AA) and dihomo-gamma-linolenic acid (DGLA). The MUFAs and PUFAs which are detected in *Tetracarpidium conophorum* seed oil could be partly responsible for the observed ameliorative effects of the extracts on the damage caused by CCl_4.

Liver enzymes (AST and ALT) are predominantly present in the liver. Upon pathological damage of the liver, the enzymes leaks out into the blood stream, raising serum concentrations of these enzymes (Dolai *et al*., 2012). Thus elevated serum ALT, AST and ALP levels in CCl_4 administered rats is suggestive of cellular leakage and loss of functional integrity of cell membrane (Eidi *et al*., 2014). In this study, serum activity of Alanine aminotransferase (ALT) was significantly elevated ($p<0.05$) in the rats administered with CCl_4 only which was suggestive of hepatic injury. Rats pre-treated with feed containing n-hexane extract of *Tetracarpidium* seed oil however, showed significant decrease in the serum ALT activity (Fig 1). Aspartate aminotransferase (AST) is present in all tissues except bone, with highest levels in liver and skeletal muscle (Kalas *et al.,* 2012). Serum activity of Aspartate aminotransferase (AST) was significantly elevated ($p< 0.05$) in the rats administered with CCl_4 only which was suggestive of liver injury. Rats pre-treated with feed

containing n-hexane extract of *Tetracarpidium conophorum* seed oil however, showed significant decrease in the serum AST activity (Fig 2). This suggests the hepatoprotective effect of African walnut seed oil. These findings are similar to those of Oriakhi and Uadia (2020) which shows that various fractions of crude methanolic extract of *Tetracarpidium conophorum* extract diminishes the increased enzyme activities in the plasma caused by CCl_4. This research provides added information that hexane extracts of seed of *Tetracarpidium conophorum* also possesses hepatoprotective effect.

Glutathione (GSH) is a cellular antioxidant which plays a central role in maintaining the cells redox state (Ulusu and Tandoğan, 2007). From the result obtained in this study, there was significant reduction in the GSH levels (fig.3) in untreated group compared to the control group. It shows that the free radicals generated due to the CCl_4-induced liver injury were at higher concentrations compared to the enzyme antioxidant. The increased glutathione content in the pretreated group shows the antioxidant effect of this oil extract as it was capable of enhancing the activity of the enzyme in scavenging free radicals which cause hepatocellular damage. Lipid peroxidation is a chain phenomenon resulting in the formation of various active compounds that result in cellular damage (Valgimigli, 2023). Lipid peroxidation can be initiated by any chemical species that can extract a hydrogen atom from side chain of a polyunsaturated fatty acid (PUFA) which is generally present in the cell membranes (Yin *et al.,* 2011). Lipid peroxides, which are derived from polyunsaturated fatty acids, are unstable. They readily decompose to form a complex series of compounds, which include malondialdehyde (MDA) (Sahin *et al.,* 2024). MDA is the major metabolite of arachidonic acid and serves as a reliable biomarker for oxidative stress. According to this study, there was a significant increase ($p<0.05$) in the MDA level of rats treated with CCl_4 relative to the control group which was suggestive of increased lipid peroxidation causing cellular damage. However, rats pre-treated with n-hexane extract of *Tetracarpidium conophorum* seed oil showed a significant decrease in the MDA level suggesting the antioxidant potential of *Tetracarpidium conophorum* seed oil.

The result of histopathological studies of the liver samples show that the liver section of rats treated with CCl_4 (plate 4.4) developed chronic inflammatory cells (necrosis) as compared to the control group (plate 4.1). The photomicrograph of the liver section of group pre-treated with n-hexane extract of *Tetracarpidium conophorum* seed oil and administered carbon tetrachloride to induce liver injury shows mild fatty change (black arrow) involving the hepatocytes with no inflammation which shows that the seed oil has attenuated the hepatotoxic effect of CCl_4.

Taken together, the present study explains some of the possible mechanisms involved in the hepatoprotective action of *Tetracarpidium conophorum* seed oil that was earlier reported by Oriakhi and Uadia (2020). One of the possible mechanisms is its antioxidant ability as the study shows that the *Tetracarpidium conophorum* seed oils are able to boost glutathione oxidation reducing power.

Since hepatoxicity is closely linked to oxidative stress, we can conclude that the *Tetracarpidium conophorum* seed oil utilizes various antioxidative mechanisms which among others include inhibition of lipid peroxidation as evident in its ability to reduce the lipid peroxidation product, MDA.

REFERENCES

- Ajaiyeoba, E.O. and Fadare, D.A. (2006). Antimicrobial potential of extracts and fractions of the African walnut (*Tetracarpidium conophorum*). *African Journal of Biotechnology*, 5: 2322 – 2326.
- Andrade, R., Robles, M., Fernández-Castañer, A., López-Ortega, S., López-Vega, M.C. and Lucena, M.I. (2007). Assessment of drug-induced hepatotoxicity in clinical practice: A challenge for gastroenterologists. *World Journal of Gastroenterology*, 13: 329-340.
- Burkill, H.M., (1985). *A review of Dalziel's the useful plants of West Africa*, Royal Botanical Garden Kew, UK, p. 857.
- Chen, X., Li L., Liu X., Luo R., Liao G., Li L., Liu J., Cheng J., Lu Y. and Chen Y. (2018). Oleic acid protects saturated fatty acid mediated lipotoxicity in hepatocytes and rat of non-alcoholic steatohepatitis. *Life sciences*. 203: 291-304.
- Chijioke, O. C., Anosike Chioma, A., and Collins, A. C. (2015). Studies on the phytochemical and nutritional properties of *Tetracarpidium conophorum* (black walnut) seeds. *Journal of Global Bioscience*, 4(2): 1366-1372
- Das, U.N. (2004). Long-chain polyunsaturated fatty acids interact with nitric oxide, superoxide anion, and transforming growth factor to prevent human essential hypertension. *European Journal Clinical Nutrition.* 58: 195–203.
- Dolai, N., Karmakar, I., Kumar, R.B.S., Kar, B., Bala, A. and Haldar, P.K. (2012). Free radical scavenging activity of Castanopsis indica in mediating hepatoprotective activity of CCl4 intoxicated rats. *Asian Pacific Journal of Tropical Biomedicine,* 2: 243-251.
- Edem, C. A., Dosunmu, M. I., and Bassey, F. I. (2009). Determination of proximate composition, ascorbic acid and heavy metal content of African walnut (*Tetracarpidium conophorum*). *Pakistan Journal of Nutrition*, 8(3): 225-226.
- Eidi, A., Mortazavi, P., Moghadam, J.Z. and Mousar, P. (2014). Hepatoprotective effects of

Portulaca oleracea extract against CCl4-induced damage in rats. *Pharmaceutical Biology*, 1: 1-10.

- Ekwe, C. C., and Ihemeje, A. (2013). Evaluation of physiochemical properties and preservation of African walnut (*Tetracarpidium conophorum*). *Academic Research International*, 4(6): 501.
- Ezealisiji, K.M., Stanley, C.N. and Ekanem, E.S. (2016). Evaluation of anti-cholesterol activity of ethyl acetate and n- hexane extracts of *Tetracarpidium conophorum* (Mull. Arg.) Hutch and Dalziel (African walnut) towards hypercholesterolemic rats. *International Journal of Pharmacognosy and Phytochemical Research,* 8: 1372–1376.
- Juza, R.M. and Pauli, E.M. (2014). Clinical and surgical anatomy of the liver: a review for clinicians. *Clinical Anatomy*, 27: 764–769.
- Kalas M.A., Chavez L., Leon M., Taweesedt P.T. and Surani S. (2021). Abnormal liver enzymes: A review for clinicians. *World Journal Hepatology* 13(11): 1688-1698.
- Kanu, A.M., Kalu, J.E., Okorie, A.C., Olabinri, B.M., Eniyansoro, O.O., Okoronkwo, C.O. et al., (2015), 'Evaluation of chelating ability of aqueous extract of *Tetracarpidium conophorum* (African walnut) *in vitro*', *International Journal Applied Research in Natural Products* 3(3): 1–13.
- Mohamed, N. Z., Howaida, I. A., Hanan, F., Mantawy1, A. M., Nehal, and Hassan, S. A. (2014). CCl4-induced hepatonephrotoxicity: protective effect of nutraceuticals on inflammatory factors and antioxidative status in rat. *Journal of Applied Pharmaceutical Science*, 4: 87-100.
- Nwaichi, E.O., Osuoha, J.O. and Monanu, M.O. (2017). Nutraceutical potential of *Tetracarpidium conophorum* and *Buccholzia coriacea* in diet-induced hyperlipidemia", *Journal of Chemical Health Risks* 7(3): 157–170.
- Ojobor, C.C., Anosike, C.A. and Ani, C.C. (2015). Studies on the phytochemical and nutritiona properties of *Tetracarpidium conophorum* (Black walnut) seeds. *Journal of Global Bioscience*, 4: 1366 –1372.
- Oriakhi, K. and Uadia, P.O. (2020). Hepatoprotective effects of African walnut. *Archives of Basic and Applied Medicine*, 8: 45-48.
- Oyekale, K.O., Odutayo, O.I., Esan, E.B., Ogunwemimo, K.O., Denton, O.A. and Bolaji, D.T., (2015). Comparative studies on phytochemical and proximate composition of four morphological distinct segment of the conophor seedlings (*Tetracarpidum conopphorum* Hutch and Dalziel). *Brazilian Journal of Biological Sciences* 3: 91–100.
- Şahin, I.K., Sezer, C.V., and Ayhanci A. (2024). The effects of oxidative stress on cellular structures: lipid peroxidation. *Oxidative Stress and Antioxidant Defense Systems*, 15.
- Sankeshwari, R.M., Ankola, A.V., Bhat, K. and Hullatti, K. (2018). Soxhlet versus cold maceration: Which method gives better antimicrobial activity to licorice extract against Streptococcus mutans. *Journal of the Scientific Society*, 45: 67-71.
- Solà R., La Ville A.E., Richard J.L., Motta C., Bargalló M.T., Girona J., Masana L. and Jacotot B. (1997). Oleic acid rich diet protects against the oxidative modification of high density lipoprotein. *Free Radical Biology and Medicine.* 22 (6):1037-1045.
- Tchiegang, C., Kapseu C., Parmenter M. (2007). Chemical Composition of Oil from *Teracarpidium conophorum* (Mull.Arg, Hucth and Dalz) Nuts. *Journal of Food Lipids*. 8: 95-102.
- Uhunmwangho, E.S. and Omoregie, E.S. (2017). Evaluation of Nutritive, Antinutritive and Mineral Content of *Tetracarpidium conophorum* (African Walnut) Seed Oil at Different Stages of Fruit Maturation. *Haya: The Saudi Journal of Life Sciences,* 2: 210-216.
- Ulusu, N.N., and Tandoğan, B. (2007) Purification and kinetic properties of glutathione reductase from bovine liver. *Molecular and Cellular Biochemistry*, 303: 45–51.
- Valgimigli, L. (2023). Lipid Peroxidation and Antioxidant Protection. *Biomolecules* 13: 1291.
- Wolf, A.T., Maurer, R., Glickman, J. and Grace, N.D. (2008). Hepatic venous pressure gradient supplements liver biopsy in the diagnosis of cirrhosis. *Journal of Clinical Gastroenterology*, 42: 199–203.
- Yin, H., Xu, L. and Porter, N.A. (2011). Free radical lipid peroxidation: Mechanisms and analysis. *Chem. Rev.* 111: 5944–5972.