Haya: The Saudi Journal of Life Sciences **ISSN 2415-623X (Print)**
Scholars Middle East Publishers **ISSN 2415-6221 (Online)**
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

Antioxidant Properties Associated with the Biochemical Changes in the Development of African Walnut (*Tetracarpidium conophorum*) Fruit

Esosa Samuel Uhunmwangho[1], Ehimwenma Sheena Omoregie[2]
[1]Department of Biochemistry, Faculty of Basic Medical Science, Ondo City, Nigeria
[2]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

***Corresponding author**
Esosa Samuel Uhunmwangho

Article History
Received: 15.09.2017
Accepted: 23.09.2017
Published: 30.09.2017

DOI:
10.21276/haya.2017.2.6.2

Abstract: The antioxidant properties of oil and fruit extracts of *Tetracarpidium conophorum* (African walnut) from four weeks after anthesis(WAA) to fruit maturation were assessed in this study. The oils from the fruits were extracted with chloroform-methanol 1:2 (v/v), and the methanol extracts of the fruit seeds were also prepared using standard procedures. The *in vitro* antioxidant capacity of the fruits and oil extracts was determined by spectrophotometric methods using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, H_2O_2 and malondialdehyde (MDA) assays. Results from the study indicated that DPPH and H_2O_2 scavenging capacity as well as percentage inhibition of MDA were significantly ($P<0.05$) higher inoil 20 WAA (48.34±0.12%,46.9±1.03%,53.5±0.46%)and whole fruit extracts at 20 WAA (31.84±0.33%, 19.0±1.12%, 51.7±0.26%) respectively,but with significant ($P<0.05$) lesser percentage of inhibition at 4-12WAA in both the oil and the whole extracts. The hepatoprotective effects of the extracts were examined *in vivo* in male wistar rats challenged with sodium arsenate. Results showed that the rats fed with the oil and whole fruit extracts had significant reduction ($p<0.05$) in lipid peroxidation, increase in superoxide dismutase (SOD) and catalase (CAT) activities especially at 16-20 WAA relative to control values. African walnutextracts treated rats also showed similar decrease in serum AST (122.7±2.58U/L), ALT (59.1±2.49U/L), ALP (20.4±0.15U/L) and GGT (229.0±0.36U/L) levels at 20 WAA when compared to 4 WAA extracts treated rats, AST (129.3±0.64U/L), ALT (72.3±2.59U/L), ALP (34.4±0.08U/L) and GGT (299.7±1.27U/L). The results suggest that the various fruit extracts possess varied degrees of potent antioxidant activity both *in vitro* and*in vivo* and may serve as important sources of antioxidants in food, cosmetics and pharmaceutical industries.

Keywords: *Dacryodes edulis*, *Tetracarpidium conophorum*, antioxidants, sodium arsenate, hepato-protective

INTRODUCTION

For decades, the screening of medicinal plant materials for their therapeutic values has continued to represent potential sources of new effective medicine. Besides, evidence from epidemiological studies have suggested that high consumption of fruits and vegetables may be linked to reduced risk of developing most oxidative stress induced diseases such as cancer, diabetics mellitus, protein energy malnutrition (PEM), cataract, infections and other degenerative diseases of aging [1, 2]. Naturally, there is a dynamic balance between the amounts of free radicals produced in the body and antioxidants to scavenge or quench them to protect the body against deleterious effects. The amount of antioxidant principles present under normal physiological conditions and may be insufficient to neutralise free radical generated under pathological conditions. Therefore, it is important to enrich our diets with antioxidants to protect against harmful with diseases. Hence, there has been an increased interest in the food industries and preventive medicine in the development of ‘’natural antioxidants’’ from plant materials [2].

African walnut is an edible seed of any tree of the genus *Euphorbiaceae*, especially the *Tetracarpidium conophorum*, found in Nigeria and Cameroon. Itis a climbing shrub of 10-20ft long, cultivated principally for the nuts which are cooked and consumed as snacks or dessert [3]. It is known in the South-East Nigeria as *ukpa* (Igbo), Western Nigeria as *awusa* or *asala* (Yoruba), South-South Nigeria as*Okhue* (Edo) [4, 5].

Previous study on *Tetracarpidium conophorum* has shown that consumption of its seeds increases protection against proliferous diseases, oxidative stress and endothelial dysfunction [6]. *Tetracarpidium conophorum* leaves and roots extracts are applied in certain skin conditions such as eczema, psoriasis, warts, and parasitic skin conditions [7].

It has been reported that African walnut contain juglone (5-hydroxy-1,4-naphthoquinone, alpha(α) hydroxyjuglone and its glycoside, beta(β) hydrojuglone, caffeic acid, plumbagum, hyperin, kaempferol and tannin. Ellagic acid is also present [7]. The oil extract from *Tetracarpidium conophorum* seed showed the fatty acids and triacyl content with linoleic acid [7]. Walnuts have sufficiently higher amounts of omega-3-fatty acids as compared to other nuts [8]. Raw walnuts contain glyceryltriacylates of the n-3 fatty acid, alpha linolenic acid (ALA) [8]. This present study investigated the proximate, antinutritive and mineral composition of *Tetracarpidium conophorum* seed nuts at different stages of fruit development.

MATERIALS AND METHODS

Plant materials

Maturedfruits of *Tetracarpidium conophorum* were collected from private farm land in Okada Town of Ovia North-East LGA of Edo State, Nigeria. These fruits were authenticated by the Department of Botany, University of Medical Sciences, Ondo City. A voucher specimen of each plant was there after deposited in the herbarium of the same Department.

Preparatory of plant extracts

Methanol: chloroform extracts of each plant fruits was prepared according to previous method of Bligh and Dyer, [9] as reported by Bafor and Osagie, [10]. The sterilised mesocarp was macerated three times in 500ml of $CHCl_3$/MeOH (1:2 v/v) using MSE homogeniser. The homogenate was filtered each time and the filtrate residue re-blended with the$CHCl_3$/MeOH solution. Appropriate volume of chloroform and 0.72% NaCl solution were added to the combine filtrate to form a biphasic system chloroform/methanol/water in volume 1:1:09. The chloroform layer was withdrawn, and concentrated to dryness on a rotary evaporator at 30^0C under reduced pressure and then stored at 40^0C for subsequent analysis.

Animals and treatment

30 male rats of Wister strainweighing between (100-120) g were obtained from the animal house unit of the Department of Biochemistry, University of Medical Sciences, Ondo City, Nigeria.

The animals were divided into three sub-groups having five animals each. Animals in sub-group 1 were fed with 50mg/kg of the extract for seven days, while sub-group 2 and 3 was given normal saline. At the 8^{th} day, sub-group 1 and 2 rat were injected intraperitoneally with 1mg/kg of Sodium Arsenate, while sub–group 3 was given distilled water. Twenty-four hours later, the animals were sacrificed by cervical dislocation. The livers were removed at once, blotted dry, weighed and stored at 4^0C.

Preparation of homogenates

1g of the liver tissue was homogenised in 10ml of ice-cold physiological saline to obtain 10% (w/v) homogenates. The resulting homogenates were centrifuged at 5000g for ten minutes and the supernatants obtained were used for determination of superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation.

Chemicals

DPPH (2, 2-diphenyl-2-picrylhydrazyl), Gallic acid, folin-ciocalteau's reagent were obtained from Sigma Chemical company, USA. All other reagents and chemicals were of analytical grade and obtained locally from BDH and Aldrich in Nigeria.

Determination of *in vitro* antioxidant activities

TBARS

TBARS (Thiobarbituric acid reactive substances) modified assay was used to measure the lipid peroxide formed, using egg yolk homogenates as lipid rich media. Malondialdehyde (MDA), a secondary end product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA) yielding a pinkish red chromogen with an absorbance maximum at 532nm [11].

Inhibition of lipid peroxidation (%) by concentrates was calculated with formula:

(C-E) / Cx100 where C is the absorbance value of the fully oxidized control and E is ($Abs_{532\ TBA} - Abs_{532\text{-}TBA}$)

DPPH Radical Scavenging Activity

The radical scavenging activity of the fruit extracts against 2, 2-diphenyl-2-picryl-hydrazyl radical was determined by measuring UV absorbance at 532nm. Radical scavenging activity was measured according to the method of Blois [12]. The following 0.02, 0.04, 0.06, 0.08 and 0.1mg/ml concentration of samples were placed into test tubes and 0.5ml of 1mM DPPH solution in methanol was added after. The experiment was carried out in triplicate. The test was incubated for 15 minutes at room temperature and the absorbance was read at 517nm. A blank solution was prepared and measured containing the same amount of methanol and DPPH. Lower absorbance of reaction of the reaction mixture indicates higher radical scavenging activity. The radical scavenging activity was calculated using the following formula:

$$\text{DPPH scavenging activity \%} = \frac{[AB - AA]}{AB} \times 100$$

Where AB = Absorption of blank sample and AA is the absorption of tested extract solution.

Hydrogen peroxide (H_20_2) Determination

The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch *et al*., [13] as described by Rajesh *et al*., [14]. Crude extract at 20µ/ml concentration in 3.4ml phosphate buffer (0.1M, pH 7.4) was added to 0.6ml of H_2O_2solution (43Mm). The absorbance was taken at 230nm. Blank solution contain phosphor buffer without H_2O_2. Theconcentration of hydrogen peroxide (mM) in the assay medium was determined using a standard curve.

Calculations:

% scavenging of hydrogen peroxide = $\frac{(A_0 - A_1) \times 100}{A_0}$

A_0 - Absorbance of control
A_1 - Absorbance in the presence of plant extract

Determination of in vivo antioxidant activity

Superoxide dismutase (SOD) assay

SOD was assayed in the liver according to the method of Misra and Fridovich [15], based on the rapid auto-oxidation of adrenalin due to the presence of superoxide anions. This is measured spectrophometrically at 420nm and SOD concentration is expressed as units/g tissue.

Catalase activity

Catalase activity in the liver was determined as residual H_2O_2 after incubation with the enzyme according to the method described by Cohen *et al*., [16].

Estimation of Lipid Peroxidation

Lipid peroxidation in the tissue involves the determination of thiobarbituric acid reactive species (TBARS) which is the indicator of membrane lipid peroxidation. Values for TBARS were reported as Malondialdehyde (MDA) and quantified using a Molar extinction coefficient of 1.5 X 10^5 Mcm^3 and expressed as mmole $MDAg^{-1}$ of tissue [17].

STATISTICAL ANALYSIS

Data were expressed as mean ± standard error of mean (SEM). One way analysis of variance (ANOVA) was performed to test for differences between the groups mean. Significance differences between the mean were determined by Duncan's multiple range test and P-value <0.05 were regarded as significance [18].

RESULTS

The antioxidant activity of the fruits were evaluated by determining the percentage inhibition of thiobarbituric acid reactive species (TBARS), 1,1-diphenil-2-picrylhydrazyl (DPPH) and hydrogen peroxide (H_2O_2) scavenging activity by the plants whole fruit sample and the extracted oil from the plants. The oil extract were found to possess the highest inhibitive activity of TBARS, DPPH and H_2O_2at the matured stage (20 WAA) of the fruit (Fig. 1-6).

The effects of methanolic extracts of *Tetracarpidium conophorum* fruits on liver function of Wister rats were assessed by evaluating the serum alanin aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutaryl aminotransferase (γ-GT) and alkaline phosphatase (ALP) are showed in (figure 7 – 10).

The effects of the methanolic extract of *Tetracarpidium conophorum* fruits on oxidative damage were assessed by estimating the liver superoxide dismutase (SOD), catalase (CAT) and malondiadehyde (MDA) levels.The liver catalase levels in wister rats treated with the methanolic extracts of *Tetracarpidium conophorum* fruits are presented in (figure 11-13).

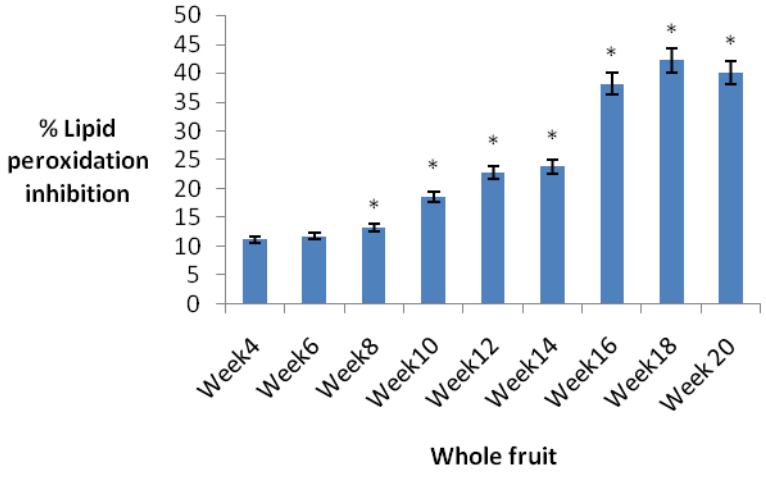

Fig-1: Inhibition of lipidperoxidation by *Tetracarpidium conophorum* at 4-20 WAA of fruit development. Values are mean ± SEM (* = P<0.05)

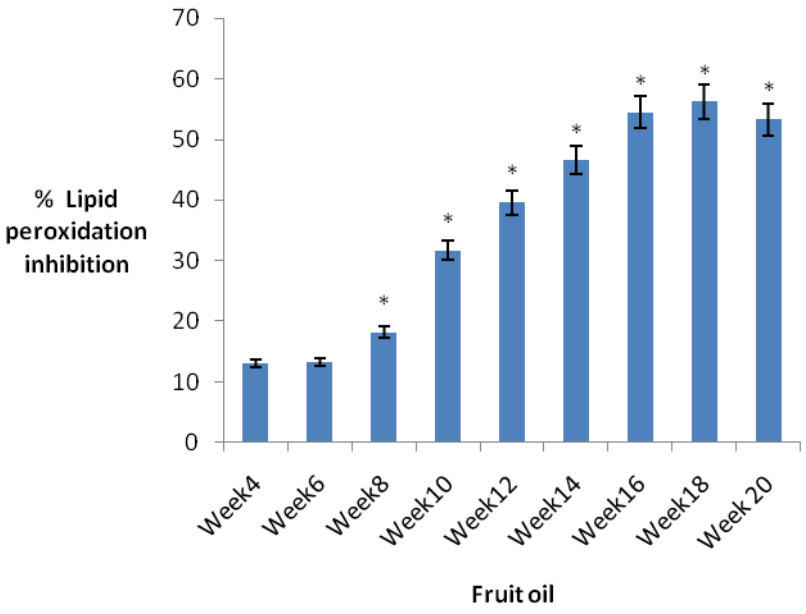

Fig-2: inhibition of lipid peroxidation by *Tetracarpidium conophorum* fruit oil at 4-20 WAA of fruit development. Values are mean ± SEM (* = P<0.05)

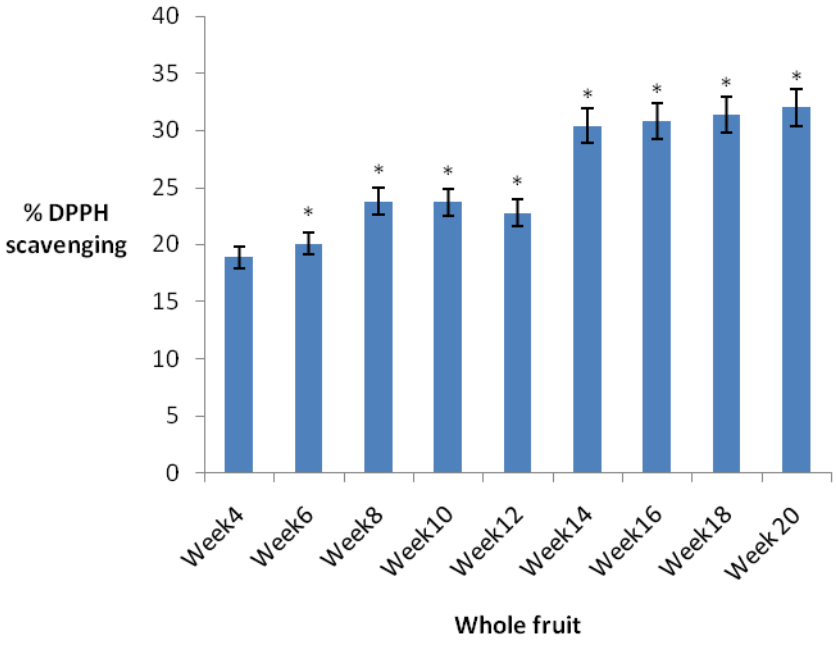

Fig-3: % DPPH scavenging capacity of *Tetracarpidium conophorumwhole* fruit at 4-20 WAA of fruits development. Values are mean ± SEM (* = P<0.05)

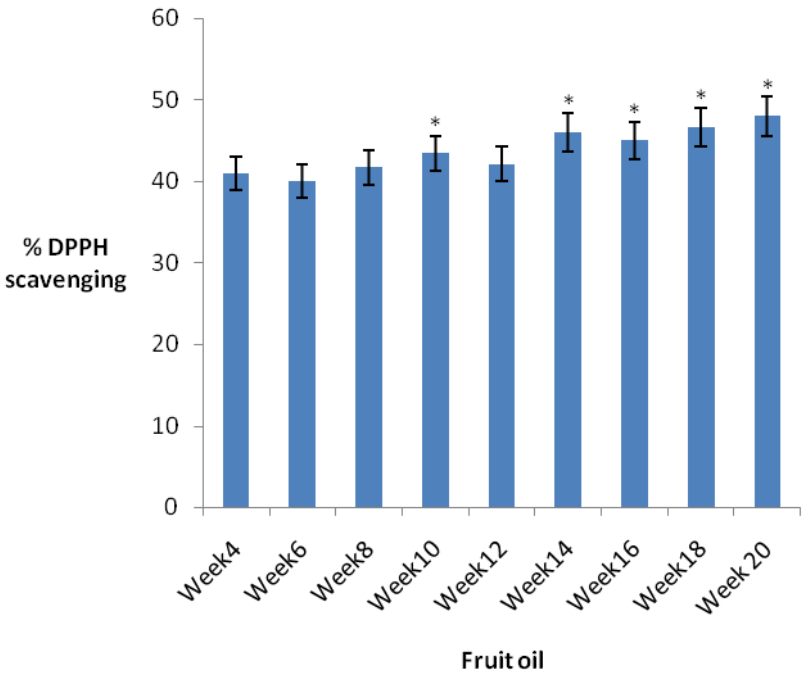

Fig-4: % DPPH scavenging capacity by oils of *Tetracarpidium conophorum* at 4-20 WAA of fruit development. Values are mean ± SEM (* = P<0.05)

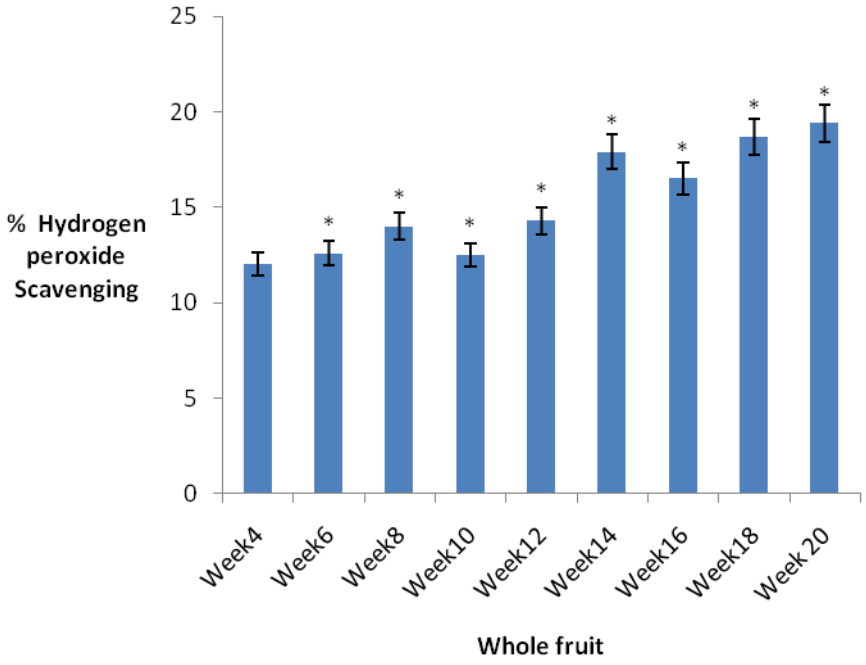

Fig-5: % Hydrogen peroxide scavenging capacity of *Tetracarpidium conophorum* 4-20 WAA of fruit development. Values are mean ± SEM (* = P<0.05)

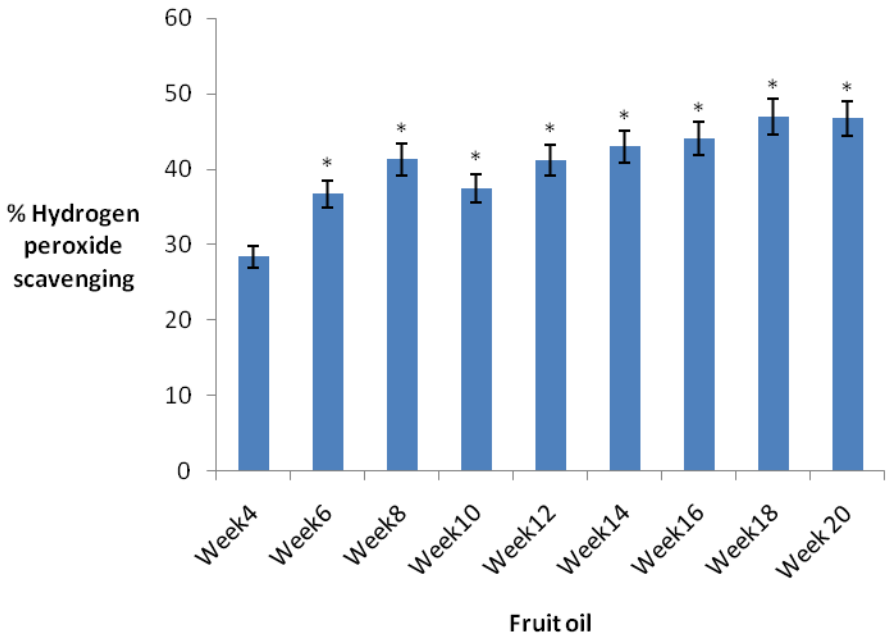

Fig-6: % Hydrogen peroxide scavenging capacity of *Tetracarpidium conophorum* 4-20 WAA of fruit development. Values are mean ± SEM (* = P<0.05)

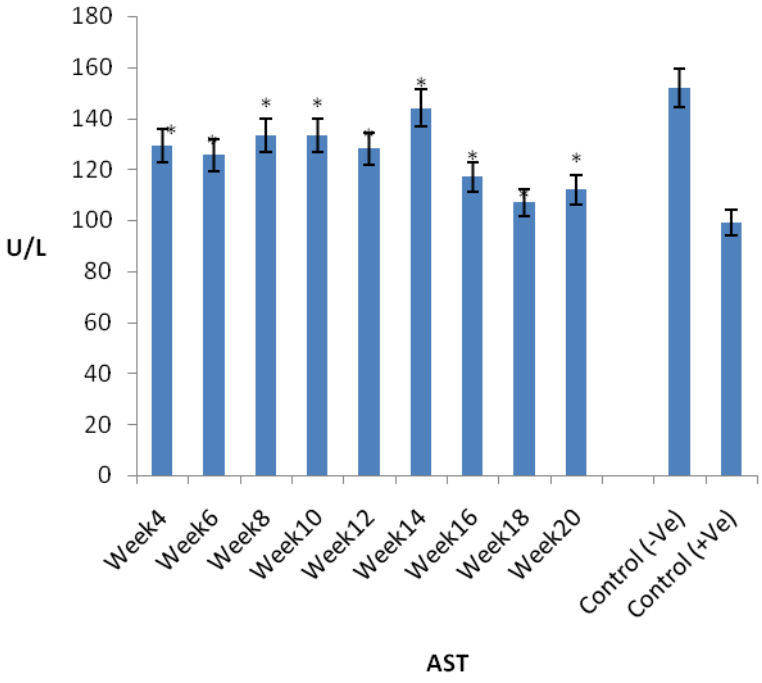

Fig-7: Serum Aspartate Aminotranferase (AST) levels in Wister rats administered extracts of *Tetracarpidium conophorum* fruits4-20 WAA of fruit development. Values are mean ± SEM (* = P< 0.05)

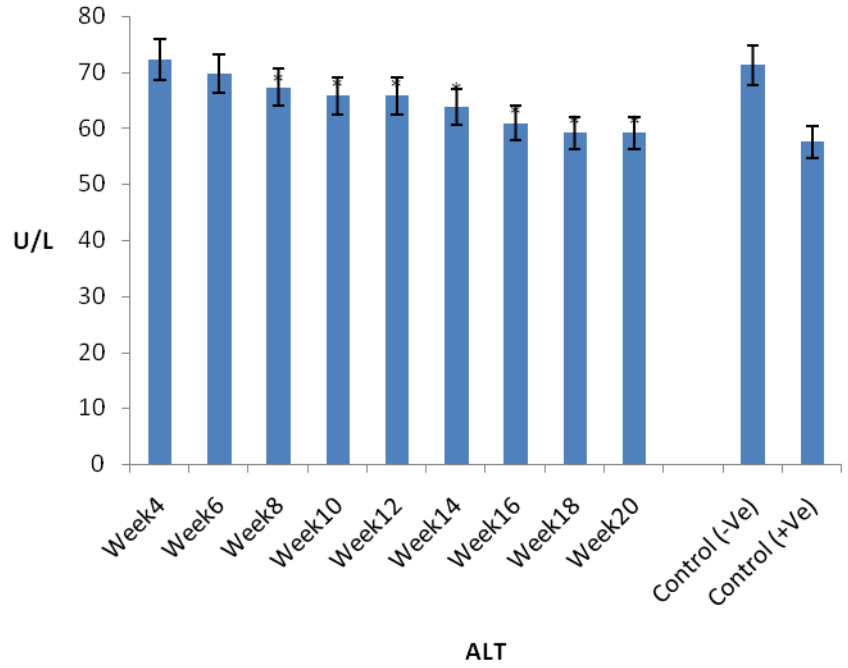

Fig-8: Serum Alanine Aminotransferase (ALT) levels in Wister rats administered extracts of *Tetracarpidium conophorum* fruits 4-20 WAA of fruit development. Values are mean ± SEM (* = P< 0.05)

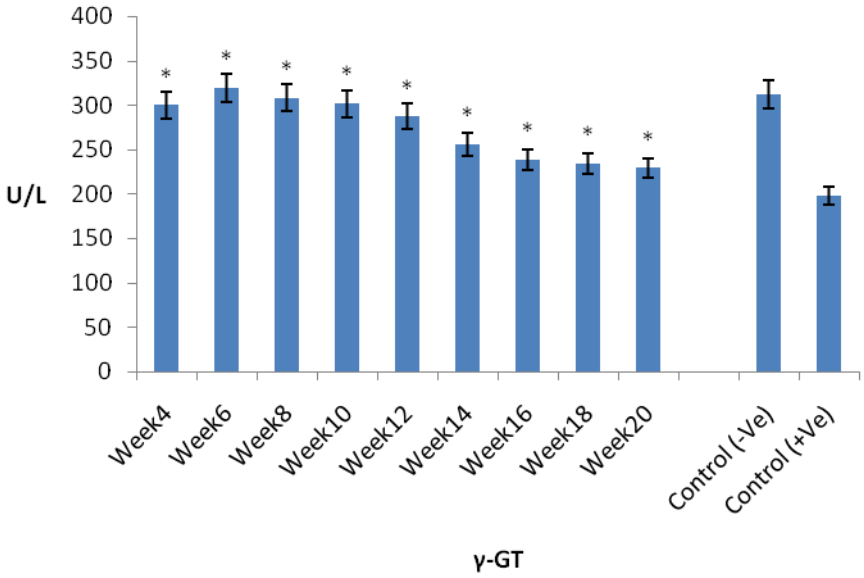

Fig-9: Serum L-$^{\gamma}$-Glutamyltransferase (GGT) Level in Wister rats administered extracts of *Tetracarpidium conophorum* fruits 4-20 WAA of fruit development. Values are mean ± SEM (* = P< 0.05)

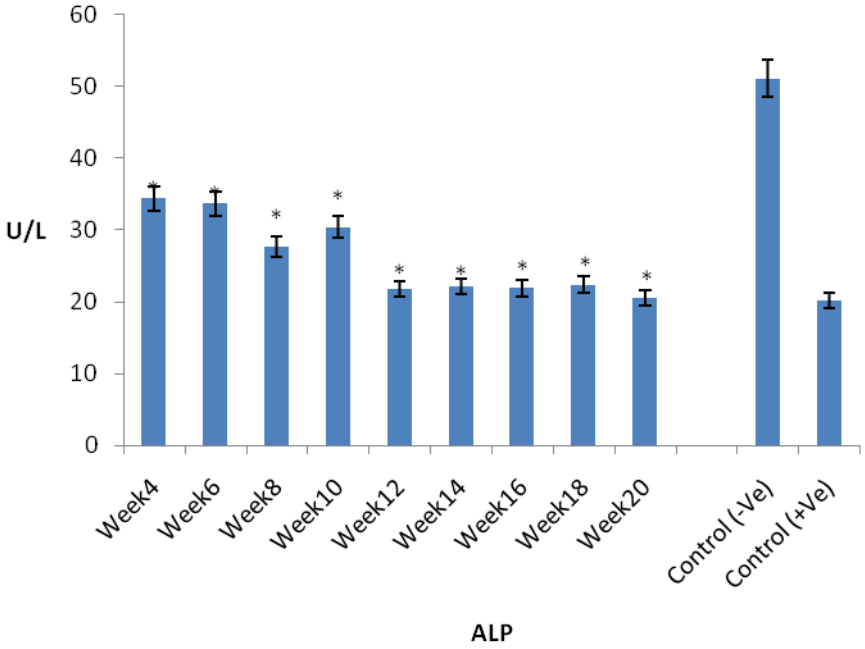

Fig-10: Serum Alkaline Phosphate (ALP) Level Level in Wister rats administered extracts of *Tetracarpidium conophorum* fruits 4-20 WAA of fruit development. Values are mean ± SEM (* = P< 0.05)

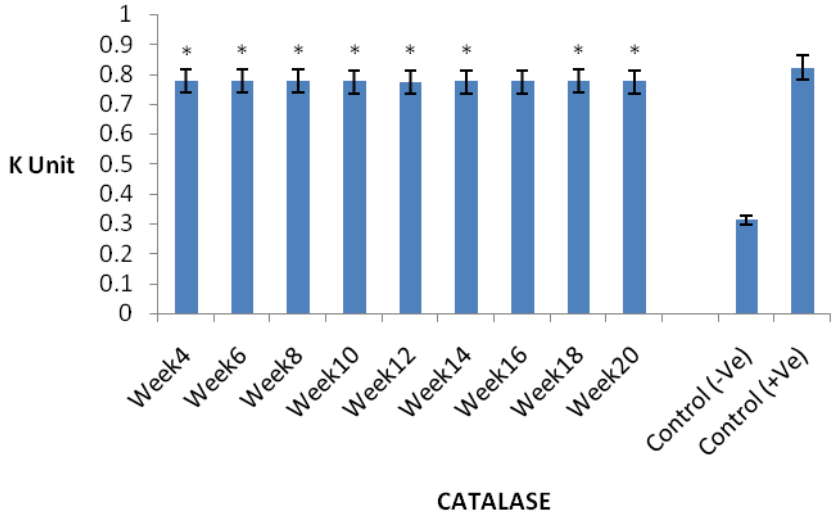

Fig-11: Liver Catalase Level Level in Wister rats administered extracts of *Tetracarpidium conophorum* fruits 4-20 WAA of fruit development. Values are mean ± SEM (* = P< 0.05)

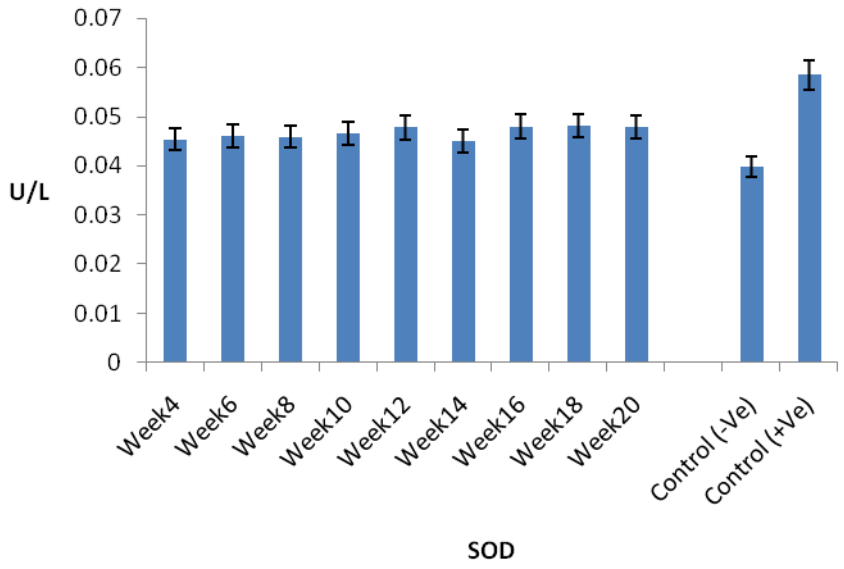

Fig-12: Superoxide Dismutase (SOD) level in Wister rats administered extracts of *Tetracarpidium conophorum* fruits at 4-20 WAA of fruit development. Values are mean ± SEM (* = P< 0.05)

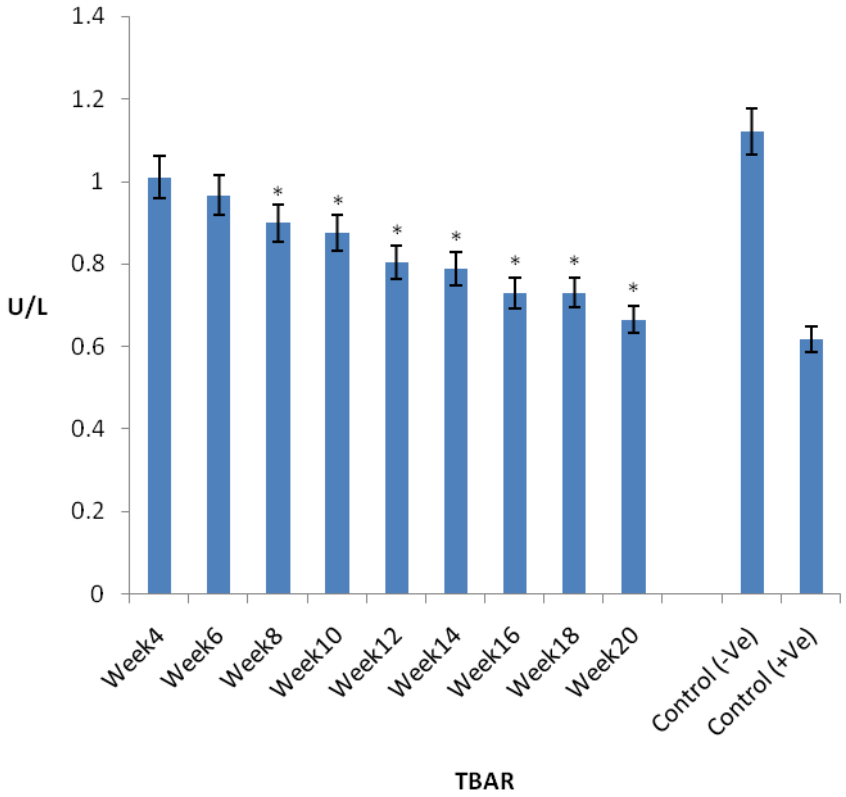

Fig-13: Malondiadehyde (MDA) Level in Wister rats administered extracts of *Tetracarpidium conophorum* fruits at 4-20 WAA of fruit development. Values are mean ± SEM (* = P< 0.05)

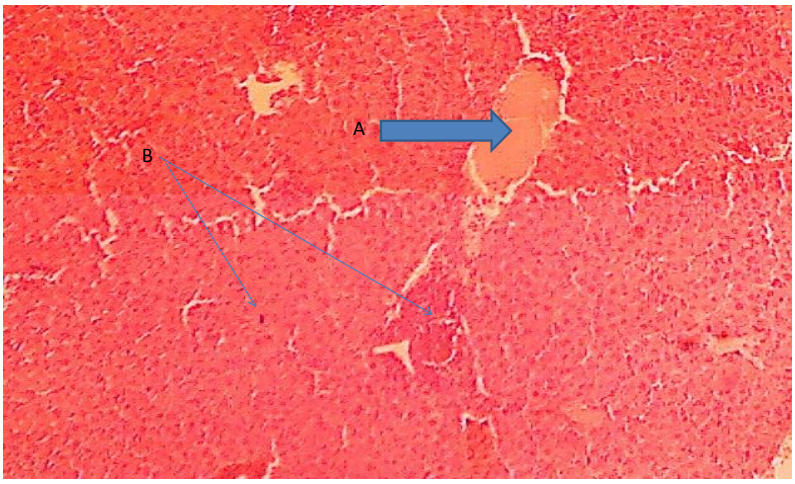

Plate 3.1 (week6): Microscopic representation of liver (x100) section of Wister rats treated with extract of *Tetracarpidum Conophorum*. The histoarchitecture of the liver tissue shows **(A)** prominent central vein and **(B)** hepatocytes prominently visible and no degeneration observed.

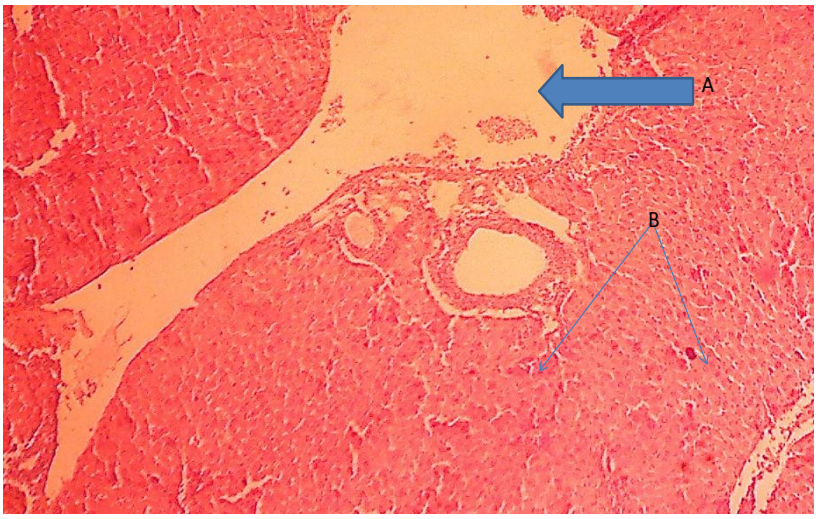

Plate3.2 (week12). Microscopic representation of Liver (X40) section of Wister rats treated with oils extracted from *Tetracarpidium conophorum* fruits of the premature stage of development.
The histoarchitecture of the liver is well organized, **(A)** prominent central vein, (**B**) hepatocytes compactly prominent and no degeneration noticed.

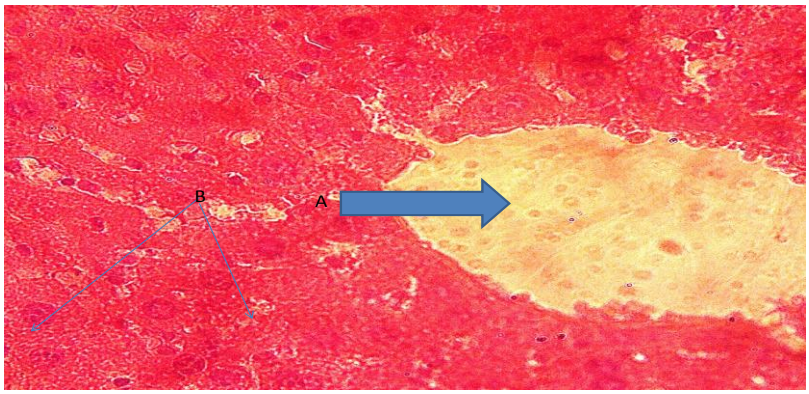

Plate 3.3 Microscopic representation of Liver (X40) section of Wister rats treated with oils extracted from *Tetracarpidium conophorum* fruits of the matured stage of development (week20).
The histoarchitecture of the liver tissue shows **(A)** prominent central vein and **(B)** prominently visible hepatocytes.

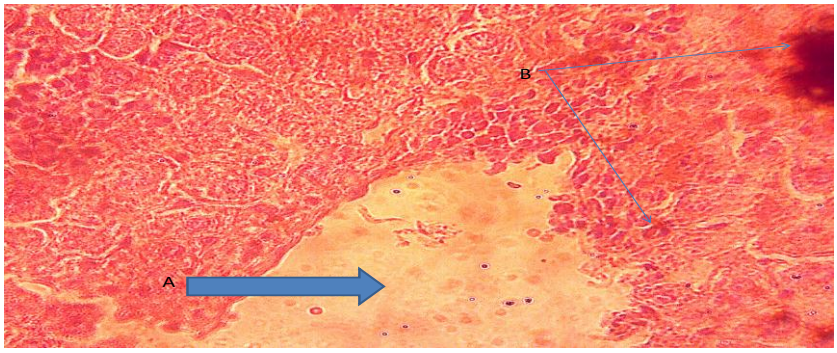

Plate 3.4 Negative control: Negative control: Microscopic representation of liver (X40) section of the Wister rats with Sodium Arsenate in distilled water at a dose of 1.0mg/kg. **(A)** The histoarchitecture of the liver is distorted and the hepatocytes visible and **(B)** degeneration noticed with patches of blood prominently visible in the central vein.

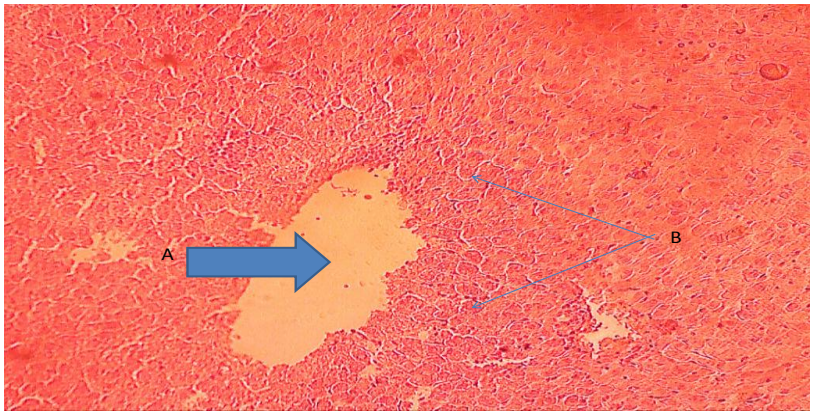

Plate 3.5 positive control. Microscopic representation of liver (x100) section of Wister rats treated with growers mash. The histoarchitecture of the liver is well organized, **(A)** prominent central vein, **(B)** hepatocytes visible and no degeneration noticed

DISCUSSION

In the immature stages of the fruits development of *Tetracarpidium conophorum*, there were characteristic depression of the antioxidant defend mechanism which attributed to the imbalance between the pro-oxidant load and the antioxidant defend system in the Wister rats. Similarly, the animals treated with the immature fruits extracts (4–10 WAA) showed appreciably reduced (inhibition) activities of the antioxidant enzymes- superoxide dismutase (SOD), Catalase (CAT) as well as high level of malondialdehyde (MDA)–an index of lipid peroxidation – in the liver. This might be possible because in the immature state, the concentration of antioxidant status

(phytochemicals and essential polyunsaturated fatty acids of the African walnut were low, exposing their antioxidant defence system of the rats to damages by the pro-oxidant (Sodium Arsenate). But the concentration of the antioxidant status of the both fruit plants increased there after till fully matured stages (14–20 WAA) and were able to inhibits or prevent oxidation in the animals. Hence, the SOD and CAT increased and the MDA level were drastically reduced in both fruit plants.

Malondialdehyde is the major oxidation product of peroxidised polyunsaturated fatty acids (PUFAs) and increase MDA levels an important indicator of lipid peroxidation. Catalase on the other hand, is an enzymatic antioxidant widely distributed in all animal tissues including the red blood cell and the liver. Catalase decomposes H_2O_2 and helps protect the tissues from highly reactive hydroxyl radicals. SOD, another antioxidant enzyme which removes superoxide radicals by converting it to H_2O_2 [1].

The enhanced oxidative stress in the animals treated with immature fruit extracts (4–10 WAA), were however significantly reduced ($P<0.05$) in the animals treated with matured fruits (16–20 WAA) when compared with the control. The overall antioxidative capacity of the oil suggest a positive correlation as well as synergistic effects with respect to flavonoids, anthocyanidins and essential polyunsaturated fatty acids components of the extracts.

The serum concentrations of aspartate aminotransferases (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and L-ɣ-glutaryl transferase (GGT) determines the functionality and cellular integrity of the liver [19]. The enzyme ALT is most prevalent in the liver relative to other tissues or organs, where as AST may be found in heart, skeletal muscle and liver to nearly the same extent [20, 21]. Significance increases in the transaminases are associated with liver diseases such as toxic hepatitis, acute liver necrosis or hepatic cirrhosis. High levels of AST are often seen in haemolytic anaemia, myocardial infarction and cholestatic diseases of the liver [20]. The fractional increase in serum AST and ALT or the ratio of AST: ALT may be a useful tool in assessing the extent of liver damage. The liver cells contain more AST than ALT and with the later confined largely to the cytoplasm in which its concentration is higher than that of the former. With inflammatory or ineffective conditions such as viral hepatitis, the cytoplasmic membrane sustains the greater damage and the relative increase in ALT is higher than that of the AST. The situation is reverse in infiltrative disorders in which both the cytoplasmic and mitochondrial membrane are affected, resulting in a proportionality greater increase in AST relative to ALT [20].

In this study, the AST, ALT, ALP and GGT levels were generally decreased in wistar rats administered with oil extract revealed reduction in serum AST, ALT, ALP and GGT levels that were significant ($P<0.05$) at the matured stages (14-20 WAA) of the *Tetracarpidium conophorum*fruits. The presence of the extracts and their metabolism in the experimental animals could cause changes of biochemical processes [23], thus decreasing the indicator of liver injuries [22]. The injuries which have resulted from the pro-oxidant (Sodium Arsenate) as revealed in the histopathological results are implicated in toxic liver injuries. ALP is located in the biliary duct of the liver and obstruction of the duct increases the levels of the enzymes in the serum [22]. The ALP result showed significant reduction of the enzymes in the animal serum with the administration of extracts from the fully matured fruits (18-20 WAA). The decrease in ALP levels suggest that the fruit oil of study significantly inhibit damage or obstruction of the duct, as reported by Nuhu and Aliyu, [24] that ALP increases is noticeable with most liver problems.

The results obtained from this study showed that African walnut is capable of protecting living cells from oxidative damage i*n vivo*. The increase in serum antioxidant status upon treatment of animals with *Tetracarpidium conophorum*, compared with control in a paired statistical analysis data, as seen in the decrease in lipid peroxidation in the sera which correlated with the protective capacity of the of the fruit extracts on the liver by reducing the ALT, AST ALP and GGT levels in the blood compared to the control, and increase in the antioxidant enzymes (SOD, catalase) may be link to the high content of phenolics, flavonoids, monounsaturated and polyunsaturated fatty acids present in these fruits. Phytochemical studies results showed that the plant is rich in alkaloids, flavonoids, tannins and saponins are also likely to possess antibacterial properties as well as physiological effects [25]. The histopathological pattern seen in the liver of Wister rats treated with extracts (oils) from *Tetracarpidium conophorum* fruit were characterised by well preserved hepatic parenchyma, central vein and hepatocytes.

These are suggestive to confer such potent antioxidant effect on their extracts and could be said to be responsible for its widely reported therapeutic relevance. Hence, African walnut remains a potential candidate with potent antioxidant properties that could help in the management of degenerative diseases in which oxidative stress have been implicated in their aetiology.

REFERENCES

1. Omoregie, E. S., & Osagie, A. U. (2007). Phytochemical screening and anti-anaemic effect of *Jatropha tanjorensis* leaf in protein malnourished rats. *Plant Arch*, *7*(2), 509 – 516.

2. Amaeze, A. O., Ayoola, G. A., Sofidiya, M. O., Adepoju-Bello, A. D., Adegoke, A. O., & Coker, H. A. B. (2011). Evaluation of antioxidant activity of *Tetracarpidiumconophorum*(*Mill-ArgHUIch&Dalziel*) leaves. *Oxidative medicine and Cellular Longevity*, *15*, 1155-1162.
3. Adebona, M. B., Ogunsuo, A. O., & Ologunde, M. O. (1998).Development of *conophor* nut-based cereal snack food I – biscuits.*J. Food and Africa*, *2*, 123-126.
4. Burkill, H. M. (1985). The useful plants of West Africa. *Royal Botanic Gardens Kew, 1*, 960.
5. Edem, C. A., Dosunmu, M. I., & Bassey, F. I. (2009).Determination of proximate composition, Ascorbic Acid and Heavy metal content of African walnut *Tetracarpidium conophorum*.*Pak. J. nutrit.*, *8*, 225-226.
6. Nwaoguikpe, R. N., Ujowundu, C. O., & Wesley, B. (2012).Phytochemical and biochemical compositions of African Walnut (*Tetracarpidium conophorum). Journal of pharmaceutical and biochemical Sc, 20* (9), 1-4.
7. Ogbe, A.O., & John, P. A. (2012). Proximate study, mineral and anti-nutrient composition of *moringaoleifera*leaves harvested from Lafia, Nigeria: Potential benefits in poultry nutrition and health. *Journal of Microbiology, Biotechnology and Food Sciences, 1* (3), 296-308.
8. Olusanya, J. O. (2008). Essentials of food and nutrition. 1st edition, Apex Books limited, Lagos, pp. 36-77.
9. Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification.Can. *J. Biochem. Physiol*., *37*, 911 – 917.
10. Bafor, M. E., & Osagie, A. U. (1988). Changes in some non-polar lipids composition of Deloping oil palm fruit (*Elaeis guineesis*) mesocarp, *Var. Duru. J. Sc. Food Agric*, *43*, 261-264.
11. Trease, G. E., & Evans W. C. (1989). *Pharmacognosy*, 13th. Braillière Tindall, London .290.
12. Blois, M. S. (1958). Antioxidant determinations by the use of stable radical. *Nature, 181*, 1199-1200.
13. Ruch, R. J., Cheng, S. J., & Claunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, *10*, 1003.
14. Rajesh, M., Screenivas, S. A., Sakarkar, D. M., & Avijit, C. (2011). Radical Scavenging and antioxidant activity of *Hibiscus rosasinensis* extract. *African Journal of Pharmaceutical and Pharmacology, 5*(17), 2027 – 2034.
15. Mishra, H., & Fridovich, I. (1989).The role of superoxide dismutase anion in the antioxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem*, *247*, 3170.
16. Cohen, J. M., Langer, L. C., Rosen, & Cameron, G. C. (1970). Neutron star models based on an improved equation of state.*Astrophys. Space Sci.*, *6*, 228-239.
17. Gutteridge, J. M. C., & Wilkins, S. (1982). Copper dependent hydroxyl radical damage to ascorbic acid. *FEBS Lett.*, *137*, 127-329.
18. Sokal, R. R., & Rohlf, F. J. (1995).Biometryl in the principle and practice of statistics in biological research.3rdEdn. Freeman W. II and co. new York. 887.
19. Shivaraj, G., Prakash, D., Vinayak, H., Avinash, M., Sonal, V., & Shruthi, K. (2009). A review on liver function test.*The Pan Afr. Med. J, 3*, 17-28.
20. Mayne, P. D. (1994). Clinical Chemistry in Diagnosis and treatment (6th Edition), London ELST with Arnold, 223-238.
21. Wallach, J. (1991). Interpretation of diagnostic tests.6th Edition little Brown and Co. New York. 33-87.
22. Edet, E. E., Atangwho, I. J., Akpanabiatu, M. I., Edet, T. E., Uboh, S., & David-Oku, E. (2011). Effects of *Gongronemalatifolium* leaf extract on some liver enzymes and protein levels in diabetic and non-diabetic rats. *J. Pharm. Biomed. Sci*, *1*, 104-107.
23. Udoh, F. E., Okon, I. E., & Ekong, M. B. (2010). Effect of aqueous extract of Psidiumguajava leaves on liver enzymes, histological integrity qandhemthological indices in rats. *Gastrolenterol. Res*, *3*, 32-38.
24. Nuhu, A. A., & Aliyu, R. (2008). Effects of *Cassia occidentalis* aqueous extract on biochemical markers of tissue damage in rats. *Trop. J. Pharm. Res*, *7*, 1137-1142.
25. Sofowora, A. (2008). Medicinal plants and traditional medicine in Africa. 2nd Edn., Spectrum books Ltd. Ibadan, Nigeria, 10-158.