

Research Article

Effect of aqueous extract of *Spondias mombin* leaves on Nitric Oxide level and some liver biomarkers in dietary palm oil supplement-fed Wistar rats.

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ABSTRACT

Background: Consumption of thermally oxidized palm oil over a period of time may lead to altered biochemical indices. Exposure to extract of *Spondias mombin* leaves has been reported to improve biochemical indices in rats. This study was designed to examine the effects of aqueous extract of *Spondias mombin* (SPM) leaves on nitric oxide level and some liver biomarkers in dietary oil supplemented Wistar rats. **Methods:** Thirty-six male Wistar rats weighing between 180-200 g, were used. The animals were randomly divided into six groups containing six rats each. Group 1 (control) received normal rat chow. Group 2 received normal rat chow+ SPM (300 mg/kg body weight orally), Group 3 was fed on feed mixed with fresh palm oil (FPO) (15% w/w), Group 4 was fed on feed mixed with FPO (15% w/w)+SPM (300mg/kg body weight orally), Group 5 was fed on feed mixed with thermoxidised palm oil (TPO) (15% w/w), Group 6 received feed mixed with TPO(15% w/w)+SPM (300 mg/kg body weight orally) for five weeks. **Results:** Result obtained shows a significant ($p<0.05$) increase in NO level in *Spondias mombin* (SPM) group when compared with the control. Serum glucose was significantly ($p<0.05$) high in FPO and TPO. However, SPM, FPO+SPM and TPO+SPM serum glucose levels were decreased by 24.07%, 10.93% and 39.28% respectively when compared with their untreated groups. Total protein and globulin concentration were increased significantly ($p<0.05$) in SPM, FPO+SPM and TPO+SPM. Albumin level was raised in FPO and FPO+SPM compared to control. Total Cholesterol (TC), Triglyceride (TG), Low density lipoprotein (LDL-c) and Very low density lipoprotein (VLDL) were significantly ($p<0.05$) elevated in TPO but Significantly ($p<0.05$) decreased upon treatment with SPM. **Conclusion:** We conclude that aqueous *Spondias mombin* leave extract promote liver protein synthesis and increases nitric oxide level in normal condition but not in consumption of thermally oxidized oil.

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INTRODUCTION

Palm oil is one of the most widely consumed vegetable oil in the world. This is attributable not only to its use as food supplement, but its value in terms of nutritional content (Edem 2002). Palm oil has been reported to contain both saturated and unsaturated fats assumed to be in a ratio of 1:1 (Cottrel, 1991; Edem 2002). Despite the presence of saturated fatty acids up to 50%, it does not promote atherosclerotic plaque formation and thrombosis in the vessels. Some identified components

available in fresh palm oil include vitamin C and Vitamin E such as tocopherols, tocotrienols and β -carotenes. The major carotenoids in palm oil are α - and β -carotene (Edem, 2002, Adam *et al.*, 2008). These constituents act as antioxidants that have the potentials to protect against lipid peroxidation/oxidative stress, protein oxidation as well as blood pressure reduction. Subjecting palm oil to repeated heating leads to thermoxidation and production of free radicals that are cytotoxic and may lead to decreased membrane fluidity and loss of enzyme and receptor activity (Leong *et al.*, 2008). In quest for therapeutic approaches to contribute to handling of the developing cardiovascular and other prevalent diseases, the use of folk medicine has been sort. *Spondias mombin*, commonly known as hog plum

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in English, 'akika' ('Iyeye') in Yoruba, ijikara in Igbo, tsardarmaser in Hausa and nsukakara in Efik, is a fructiferous tree in the family *Anacardiaceous* (Gill, 1992). It is mostly found in rain forests and coastal areas, attaining a height of 15-22 meters (Ayoka et al., 2008). *Spondias mombin* leaves have been reported to contain some bioactive substance such as tannins, saponins, flavonoids, phenolics and anthraquinone glycosides antioxidant vitamins; alpha-tocopherol and ascorbic acid (Abo et al., 1999; Maduka et al., 2014). These medicinal potential attributes of the plant extracts may makes it efficacious and therapeutic in protecting against hepatic toxicity and altered plasma protein following consumption of thermally oxidized palm oil.

METHODS

Experimental design

Thirty-six male Wistar rats weighing between 150-200 g were used in this study and they were randomly divided into six groups containing six rats each. The animals were housed in plastic cages and kept under standard laboratory condition. Group 1 (Control) received normal rat chow. Group 2 received normal rat chow+ SPM (300 mg/kg body weight orally), Group 3 was fed on feed mixed with fresh palm oil (FPO) (15% w/w), Group 4 was fed on feed mixed with FPO (15% w/w)+SPM (300 mg/kg body weight orally), Group 5 was fed on feed mixed with thermooxidised palm oil (TPO) (15% w/w), Group 6 received feed mixed with TPO (15% w/w)+SPM (300 mg/kg body weight orally) for five weeks. All groups had free access to water and feed *ad libitum*. Ethical approval for the study was obtained from the Faculty animal research ethical committee of the Cross River University of Technology, Calabar (CRT/ARC/16/021),

Preparation of leaf extract of Spondias mombin.

The modified method of extraction according to Eno and Itam (1998) was used. Fresh leaves of *Spondias mombin* were collected and washed free of dirt. The leaves were dried on laboratory table for 5 days, after which they were grounded to powder. A quantity of the ground sample (20g) was weighed and Soxhlet extraction was done with 300 ml distilled water at 100 °C for 12 hours. The liquid extract obtained was slowly evaporated to dryness in vacuo. The total yield was 8.0 g. The extract was stored till further use.

Preparation of Oil diets

Palm oil used for this study was purchased from Okuku market, in Yala Local Government, Cross River State, Nigeria and used fresh or heated five times, according to the modified method of Owu, et al. (1998). To obtain the

five times heated palm oil, 1 kg of sliced yams with 2.5 L of palm oil in a stainless was heated. Oil was allowed to cool for five hours and then the heating process was repeated with a fresh batch of yams. This heating process was repeated four times to obtain the five times heated oil. At the course of heating, no oil was topped to make up for losses. The diet was formulated by mixing 15% (w/w) of fresh or heated oils respectively with the feed.

Measurement of biochemical parameters

Blood samples were collected orbital sinus into EDTA sample bottle, centrifuge at 4000 rpm for 10 minutes to prepare the plasma used for biochemical assays and Fasting Blood Glucose (FBS) was determined via tail puncture after overnight fast by using Accu-Check ActiveGlucometer (Roche Diagnostics Germany). Total cholesterol level in blood was determined as described by Siedel *et al.*, (1983). Triglyceride was determined by the method of Sullelian *et al.*, (1985). The equation of Friedwald *et al.*, 1972 ($LDL = TC - HDL + TG/2.23$) was used to estimate LDL-c level Serum total protein and albumin levels were estimated using the method of Lowry, (1951 and Doumas *et al.*, (1971) respectively

Determination of NO

Plasma nitric oxide levels were determined by the presence of nitrite metabolites according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA). Samples of 50 µL each were put into a microtiter plate and then 50 µL of modified Griess reagent added (Sigma-Aldrich, St. Louis, MO, USA). The nitrite concentration was then measured after 15 minutes of incubation at 540 nm using an Emax ELISA microplate reader. The procedures for NO determination were carried out in a dark environment. The nitrite concentrations were quantified with standard curve generated using known concentrations of sodium nitrite (Sigma-Aldrich, St. Louis, MO, USA).

Statistical Analysis

All results were presented as mean ± SEM. One-way analysis of variance (ANOVA) was done using Graph pad prism version 5 statistical software. Bonferroni's multiple comparison test was used for pair wise comparison and differences were considered significant at $P < 0.05$.

RESULTS

Nitric Oxide Level, Serum Blood Glucose Level and Lipid Profile in Rats Fed on Dietary Oils

The results obtained showed that Nitric oxide level was significantly ($p < 0.05$) reduced in the TPO group. Co-

TABLE 1: Effects of aqueous extract of *Spondias mombin* leaves on blood lipid profile

Variables (mmol/L)	Control Mean ± SEM	SPM Mean ± SEM	FPO Mean ± SEM	FPO+SPM Mean ± SEM	TPO Mean ± SEM	TPO+SPM Mean ± SEM
Total Cholesterol	0.75±0.009	0.71±0.01	0.70±0.02*	0.63±0.009*#	0.85±0.007*#	0.70±0.006* ^a
Triglyceride	0.43±0.024	0.42±0.008	0.41±0.009	0.40±0.019	0.58±0.016*#	0.44±0.009 ^a
HDL-Cholesterol	0.22±0.003	0.18±0.003*	0.20±0.004*#	0.19±0.003*	0.16±0.003*#	0.21±0.002 ^{#a}
VLDL-cholesterol	0.22±0.009	0.18±0.009*	0.21±0.004 [#]	0.22±0.007 [#]	0.28±0.007*	0.20±0.006 ^a

SPM- *Spondias mombin* group, FPO (Fresh Palm Oil Group) TPO Thermoxidized Palm Oil Group) *,#, a P<0.05-significantly different from the control, SPM, and TPO respectively

treatment with SPM does not significantly improve the level of Nitric oxide. *Spondias mombin* only group had a significant (p<0.05) increase in nitric oxide level when compared with the control, TPO and TPO+SPM groups. Nitric oxide level was not significantly different in FPO and FPO+SPM (FPOT) when compared with the control (Figure 2).

compared to all other groups. Treatment with the extract significantly (p<0.05) reduced the lipid profile level in the TPO.

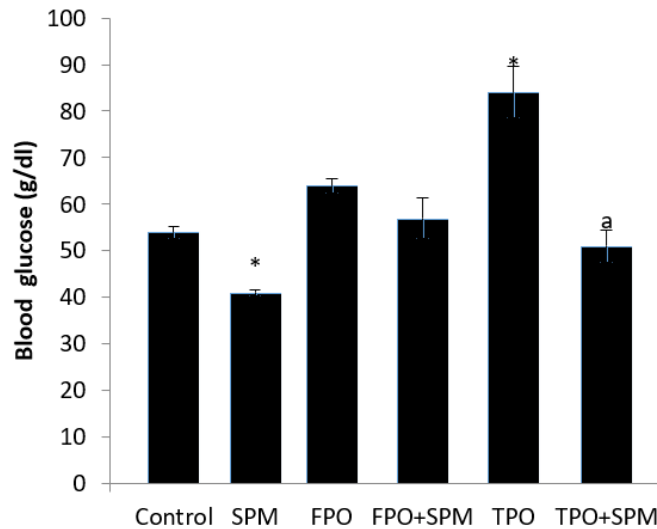


Fig.1: Effects of aqueous extract of *Spondias mombin* leaves on serum blood glucose. SPM- *Spondias mombin* group, FPO (Fresh Palm Oil Group) TPO Thermoxidised Palm Oil Group) *, #, a P<0.05-significantly different from the control, SPM, and TPO respectively

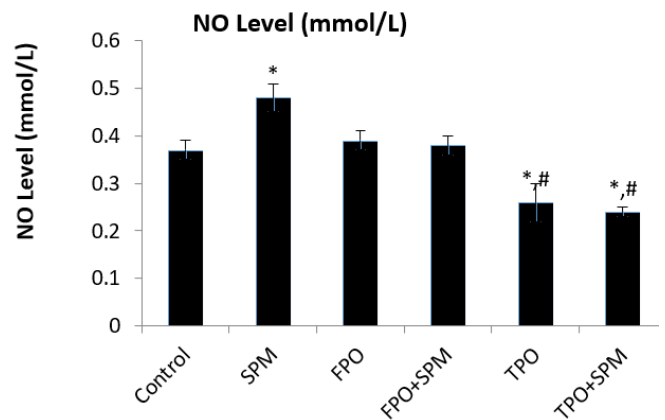


Fig. 2: Effects of aqueous extract of *Spondias mombin* leaves on NO. SPM- *Spondias mombin* group, FPO- (Fresh Palm Oil Group) TPO- (Thermoxidised Palm Oil Group) *, #, a P<0.05-significantly different from the control, SPM, and TPO respectively

Blood glucose level was significantly (p<0.05) raised in TPO group. Treatment with SPM extract significantly (p<0.05) reduce the blood glucose level in both SPM and TPO+SPM groups (Figure 1). The results of lipid profile are also presented in table 1. Serum TC, TG, LDL-c and VLDL were significantly (p<0.05) elevated in TPO

Total Protein, Albumin and Globulin in Rats Fed on Dietary Oils

In Figure 3, total protein level in all the treated groups were significantly (p<0.05) different from the Control. Co-treatment of TPO with extract significantly (p<0.05) increased the total protein level compared to TPO only group. Globulin concentration in SPM group and TPO +SPM groups were significantly (p<0.05) raised compared to all other groups. Albumin concentration in the TPO+SPM was increased by 8.1% compared to TPO only group. There was no significant difference between FPO and FPO+SPM compared to control.

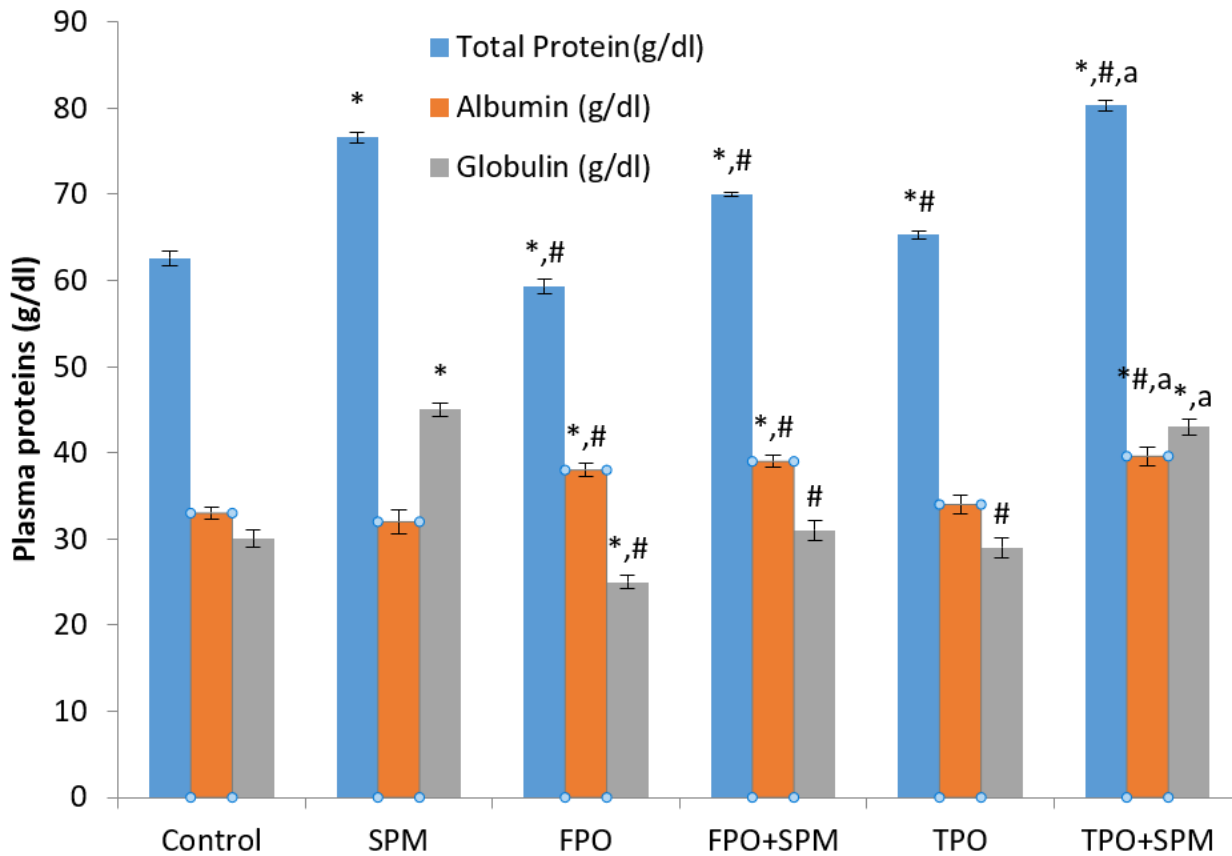


Fig. 3: Effect of aqueous extract of *Spondias mombin* leaves on total protein and liver biomarkers level in Wister rats fed on dietary oils. SPM- *Spondias mombin* group, FPO- (Fresh Palm Oil Group) TPO- (Thermoxidized Palm Oil Group) *,#,a P<0.05-significantly different from the control, SPM , and TPO respectively.

DISCUSSION

Palm oil is commonly used for cooking either in its fresh state or fried. Depending on the frequency of frying, it loses its unsaturation status. The altered nature of these oils affects the body's physiologic function (Osim *et al.*, 1996; Owu *et al.*, 1998). The aim of this study therefore was to investigate the effect of aqueous extract of *Spondias mombin* leaves NO level and some liver biomarkers in oil diet supplemented Wistar rats. In our study, thermally oxidized oil remarkably reduced nitric oxide level. This result is in accordance with earlier reported works by Jaarin *et al.* (2011). Generally, repeated heating of oils at high temperatures predisposes to fatty acid oxidation and release of free radicals including peroxides, and superoxide anions. These products of oxidation down regulates the synthesis of nitric oxide (Carr & Frei 2000; Gao & Lee, 2001; Hayashi *et al.*, 2004; Jaarin *et al.*, 2011) thereby limiting its physiologic function. That nitric oxide level depreciated in this study is therefore not surprising. Even though nitric oxide concentration was significantly

increased in the rats that received *Spondias mombin* extracts only, it was observed that treatment of the thermally oxidized group (TPO) with the same extract had no significant effect. The probable and possible reason for this observed inactivity is that the presence of the free radicals may be counteracting the effect of the extract on the site of synthesis of the cell signaling molecule or acting to inhibit the nitric oxide producing cells. According to Adams *et al.* (2007), heated palm oil reduced the vitamin E content of palm oil. Vitamin E is an important dietary free radical scavenger.

Data presented in this study also showed that prolonged consumption of thermally oxidized palm oil significantly raised serum blood glucose concentration. Similarly, the lipid profile status in TPO was challenged. The total cholesterol (TC), triglyceride (TG), Low density lipoprotein (LDL-c) and VLDL were significantly increased while the HDL-c often referred to 'good' cholesterol was reduced. This result is in line with earlier observations reported by Osim *et al.*, (1996)

and Owu *et al.*, (1998). This may suggest the presence of lipid metabolism disorder that could result in systemic diseases such as high blood pressure.

Interestingly, treatment with *Spondias mombin* leave extract reasonably decreased serum glucose concentration in FPO+SPM and TPO+SPM as well as the lipid profile in TPO +SPM. This hypoglycemic and hypolipidemic effect of the leave extract may be linked to its medicinal attributes occasioned by the rich phytochemical constituents and the reported free radical scavenging property (Maduka *et al.*, 2014). In the liver, overproduction of reactive oxygen species may result in oxidative stress. This affects hepatic functions. Our study has shown that total protein, albumin, globulin, concentrations were significantly decreased in TPO. The recorded significant decrease in total protein and albumin of the groups fed on thermally oxidized palm oil diets shows that this form of oil may alter protein synthesis in the liver. Similar result had earlier been reported by Ayodeji *et al.*, (2015). Treatment with extract recorded a tremendous increase in these parameters, suggesting ameliorating and protective property. Research has shown that bilirubin and albumin are pivotal in detoxification and antioxidant protection by scavenging superoxide, peroxides and hypochlorous acids (Frei *et al.*, 1988; Wu *et al.*, 1994). Hypoalbuminemia is associated with liver disorder (Ayodeji *et al.*, (2015). With the increase in these liver biomarkers recorded in this study following treatment with *Spondias mombin* leave extract, there is a strong indication that the available phytochemical constituents may be promoting liver hepatocyte function.

In conclusion, aqueous extracts of *Spondias mombin* leaves promote liver protein synthesis, hypoglycemic, and hypolipidemic activity but does not influence NO level in thermally oxidized oil consumption.

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REFERENCES

Abo, K.A., Ogunleye, J.O. and Asindi, J.S. (1999). Antimicrobial potential of *Spondias mombin* *Croton zambesicus* and *Zygotritoniacrocea*. *Phytother Res.* 13: 494-497. 2.
 Adam, S.K., Sulaiman, I.N., Umar, N.A., Mokhtar, N., Mohammed, N., Jaarin, K.(2008).Effect of repeatedly heated palm oil on serum lipid profile, lipid

peroxidation and homocysteine levels in postmenopausal rat model. *McGill J Med* 11(2): 145-151.

Adam, S.K., Sulaiman, N.A., Mat Top, A.G., Joarin, K, (2007).Heating reduces vitamin E content in palm and soy oils. *Malaysia Journal of Biochemistry and Molecular Biology.* 15; 76-79.
 Ayodeji, O. F.,Ganiyu, O., Adedayo, O.A., Oluwatoyin, V. O.(2015). Consumption of thermally oxidized palm oil diets alters biochemical indices in rats. *Beni-suef University Journal of Basic and Applied Sciences.* 4 (2): 150-156
 Ayoka, A.O, Akomolafe, R.O, Akinsomisoye, O.S. and Ukponmwan, O.E. (2008). Medic inaland Economic Value of *Spondias mombin*. *Afri J Biomedic Res.* 11; 129 – 136. 5.
 Carr A, Frei B. The role of natural antioxidants in preserving the biological activity of endothelium-derived nitric oxide. *Free Radic Biol Med.* 2000;28: 1806–14. 10.1016/S0891-5849(00)00225-2
 Cottrell, R.C (1991). Introduction: nutritional aspects of palm oil. *Am J Clinical Nutri* 53: 989s-1009s
 Edem,D.O.(2002).Palmoil:biochemical physiological,nutritional haematological and toxicological aspects; a review. *Plant Food and Human Nutrition.*57 (3-4): 319-341.
 Eno, A.E. and Itam E.H. (1998): Stimulation of Cholinoreceptors in the rat uterus by a crude extract from *Elaeophorbia drupilera* leaves. *Pharmacol.* 36(2): 97-102
 Frei B., Stocker R., Ames B. N. (1988). Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc. Natl. Acad. Sci. U.S.A.* 85, 9748-9752. 10.1073/pnas.85.24.9748
 Friedwald, W.T., Levy, R.J., Friedricken, D.S.,(1972). Estimation of HDL-c in the plasma without the use of preparative ultracentrifuge. *Clinical Chemistry.*18: 449 -502
 Gao YJ, Lee RMKW. Hydrogen peroxide induces a greater contraction in mesenteric arteries of spontaneously hypertensive rats through thromboxane A₂ production. *Br J Pharmacol.* 2001;134:1639–46.
 Gill L.S. (1992). Ethnomedicinal uses of plants in Nigeria (UNIBEN press), Nigeria, pp. 222-223
 Hayashi Y, Sawa Y, Nishimura M, Fukuyama N, Ichikawa H, Ohtake S, et al. Peroxynitrite, a product between nitric oxide and superoxide anion, plays a cytotoxic role in the development of post-bypass systemic inflammatory response. *Eur J Cardiothorac Surg.* 2004;26:276–80
 Jaarin, Kamsiah,S-Nik,Aziz.,S-Tan.,Siew., I-syen., Zahir (2011). Changes in serum lipid profile and malondialdehyde following consumption of fresh

- or heated red oil. *Medical Journal of Islamic Academy of Science*.14 :(2), 79-86
- Leong, X.F., Aishah, A., Nor Aini, U., Das, S., Jaarin, K. (2008). Heated palm oil causes rise in blood pressure and cardiac changes in heart muscle in experimental rats. *Archives of Medical Research*. 39(6567-572
- Lowry, O.H., Rosebrough, N.J., Farr,A.L., Randall,R.J., (1951). Protein measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry* 193; 269-275.
- Maduka, H.C.C, Okpogba, A.N, Ugwu, C.E, Dike, C.C., Ogueche, P.N., Onwuzurike, D.T and Ibe, D.C (2014). Phytochemical, antioxidant and microbial inhibitory effects of *Spondias mombin* leaf and stem bark extracts. *J Pharm Biol Sci*. 9(2): 14-17. 27.
- Osim, E.E., Owu, D.U., Etta, K.M.(1996). Arterial pressure and lipid profile in rats following chronic ingestion of palm oil diets. *African Journal of Medicine and Medical Science*. 25:335-340.
- Owu, D.U., Osim, E.E., Ebong,P.E.(1998). Serum liver enzymes profile of Wistar rats following chronic consumption of fresh or oxidized palm oil diets. *Acta tropics* 69: 65-73.
- Siedel, J. Hagele, E.O. Ziegenhorn, J., Wahlefield, A.W.(1983). Reagent for the enzymatic determination of serum cholesterol with improved lipolytic efficiency. *Clinical Chemistry*.20:1075.
- Sullivan D.R. Kruijswijk Z, West, C.E, Kohlmeier M, Katan M.B. (1985): Determination of serum Triglycerides by an accurate enzymatic method not affected by free glycerol. *Clin. Chem*.31:1227-12
- Wu T. W., Fung K. P., Yang C. C. (1994). Unconjugated bilirubin inhibits the oxidation of human low density lipoprotein better than Trolox. *Life Sci*. 54, 477-48110.1016/0024- 3205(94)90140-146