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Anti-Hypercholesterolemic and Hepatoprotective effect of Aqueous Leaf Extract of *Moringa oleifera* in Rats fed with Thermoxidized Palm Oil Diet

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Abstract: *In the impact of Moringa oleifera leaf extract on the lipid profile and key liver function enzymes of female rats fed with palm oil diets, twenty-five (25) female rats were divided into five groups (A-E). The rats in group A served as the control, fed with rat chow and water ad libitum, group B (FPO) and C (TPO) were fed with Fresh palm oil and thermoxidized palm oil diet respectively, while group D (FPO + MO) and E (TPO+MO) were fed with fresh palm oil diet and thermoxidized oil with 600mg/kgbody weight of Moringa leaf extract added in each respectively. Each of the palm oil diet contained 15 %(w/w) thermoxidized or fresh palm oil. At the end of 28 days administration, blood samples were collected for the analysis of lipid profile and key liver function enzymes. The result showed that HDL was significantly ($P<0.05$) increased in all the test samples except TPO, while LDL followed a reverse trend. The Serum alanine amino tranferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) were significantly ($P<0.05$) reduced in all the test samples except TPO which was significantly ($P<0.05$) increased. Serum gamma glutamyl transpeptidase (GGT) showed decrease in all the test samples except TPO which was significantly ($P<0.05$) increased. The results indicate that Moringa oleifera has anti-hypercholesterolemic effect. The serum enzyme levels also show that the liver tissue integrity was stabilized by the addition of Moringa oleifera leaf extract to thermoxidized palm oil diet hence its hepatoprotective activity.*

Keywords*: anti-hypercholesterolemic, antioxidative, hepatoprotective, Moringa oleifera, thermoxidized palm oil,*

I. Introduction

Medicinal plants have been used in Africa for many centuries and today almost every part of the world use herbal plants for the treatment of different diseases [1]. In fact, natural medicines from plant sources of wide diversity have been used effectively in the treatment of higher lipid level [2-5].*Moringa oleifera* is one of the most widely cultivated species of the genus *moringa* and family *moringaceae*, which is native to sub-Himalayan tribe of India, Pakistan, Bangladesh and Afghanistan. This rapid-growing *moringa* tree was utilized by the ancient Romans, Greeks and Egyptians. It is now widely cultivated and has become naturalized in many locations in the tropics [6]. *Moringa oleifera* tree is commonly known as drumstick tree in English language,'okwe oyibo' in Igbo,'gawara or Habiwai' in Hausa, 'Adagbamaloye or ewe igbele' in Yoruba [7]. It is called Ofiarifiada in the Northern part of Cross River, precisely Okuku. A number of medicinal properties have been ascribed to various parts of this tree mostly the root, leaves, roots, bark, stem, gum, fruits, flowers, seed and seed oil. These parts have been used in folk medicine in Africa and south Asia [6] and have been advocated for traditional, medicinal and industrial uses [8]. *Moringa oleifera* leaf also contains a profile of important phytochemicals [9]. Crude leaf extract was reported to reduce cholesterol in the liver and kidney [10]. The leaf exhibits free radicals scavenging property while the ethanolic fraction showed considerable metal chelation properties with potential to protect against DNA nicking [11-13]. Leaf juice extracts has a stabilizing effect on blood pressure [14]. On the other hand, palm oil, derived from the fruit of *Elaesis guineensis* is the most widely used as cooking oil in Africa. It is consumed fresh and thermally oxidized. It is fresh when obtained from the pulp of the palm fruits by squeezing and boiling at low temperature to remove debris. Traditionally palm oil is used as cooking oil [15]. Carotenoids in palm oil acts as biological antioxidants, protecting cells and tissues from the damaging effect of free radicals and the build-up of free radicals in the body is associated with degenerative diseases such as heart disease, arthritis and Alzheimer's disease [16]. Palm oil may lower blood pressure [17]. A derivative of palm oil, palmolein, has been reported to increase platelet aggregation [18]. Palm oil is used in cardiac pills, the manufacturing of skin care medicinal products for external use and also used in the manufacturing of paints and polish [19]. Palm oil increases the level of good cholesterol and reduces the

level of bad cholesterol in the blood [20], thus reducing the risk of arterial thrombosis, atherosclerosis, inhibition of cholesterol biosynthesis and platelet aggregation [21].

Palm oil when used in oxidized state possesses potential dangers to the physiological and biochemical functions of the body. Oxidized palm oil induces adverse effect on plasma lipid profile, free fatty acids, phospholipids and cerebrosides [21]. Following ingestion of thermally oxidized oil, there is a concomitant evolution of very cytotoxic and destructive by-products which are injurious to cells, tissues and organs [22]. Long term consumption of oxidized oils and fats has been reported to cause growth retardation, thrombosis, fatty livers, essential fatty acid deficiency, nucleic acid deficiency and micronutrients malnutrition leading to deactivation of key metabolic enzymes [23]. People use repeated, heated palm oil for the preparation of various meals like Beans cake 'akara', fried meat, fried yam, and fried plantain among others in Nigeria. The plant Moringa oleifera has been associated with several attributes from rich phytochemicals constituent to acting as an antioxidant as well as other health enhancing properties. Therefore, paucity demands the investigation into whether or not the leaf extract of *Moringa oleifera* will ameliorate the injurious effect of thermoxidized palm oil, possibly from the production of free radicals generated by thermoxidized palm oil in a rat model.

II. Materials and Methods

2.1 Preparation of Aqueous Extract *Moringa oleifera* Leaf

The fresh leaves of *Moringa oleifera* were collected from a home garden at Okuku in Yala Local Government, Cross River State, Nigeria in February, 2013. The leaves were washed under running tap water and air dried for 8 days. With the aid of a grinder, the dries leaves were pulverised to fine powder and stored in air tight glass. The powdered leaf (200g) was soaked in 500ml deionized water for 24hours to aid the extraction. Thereafter it was filtered through Whatman filter paper No.1. The filtrate was concentrated using rotary evaporator and stored at 10^{o} c for further use.

2.2 Experimental Animals and Design

Twenty-five (25) female albino Wistar rats weighing between 150-200g were obtained from the animal house of the Department of Human Physiology, Cross River University of Technology, Nigeria. The animals were acclimatized for one week (7days) and their weight noted before the commencement of experimental treatment. The rats were randomly assigned into five (5) groups of five rats each. Group A animals served as the control group and the animals were given normal feed and water. Group B animals were fed with fresh palm oil diet (diet I). Group C animals were fed with thermoxized palm oil (diet II).Group D animals were fed with fresh palm oil diet and 600mg/kg body weight aqueous leaf extract *Moringa oleifera*.Group E animals were fed with thermoxidized diet and aqueous leaf extract of *Moringa oleifera*. All administration was done orally for 28days using orogastric tubes. The animals were housed in stainless steel cages at atmospheric temperature (28 ± 2^{0}C) and had 12hrs light-dark cycle. They were fed and allowed access to water *ad libitum*. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily. The study was approved by the Animal Welfare and Ethics Committee of CRUTECH, Cross River State, Nigeria. All conditions of animal use were also as approved by United States National Institute of Health (NIH) guide for Care and Use of Laboratory Animals and in accordance with the recommendation of IASP [24]

2.3 Sources and Preparation of Thermoxidized Palm Oil

Twenty (20) litres of fresh palm oil was purchased from Okuku market, in Yala Local Government of Cross River State, Nigeria. The oil was divided into two equal portions. One portion was thermally oxidized and the other was used in its fresh form since these are the two forms of palm oil used for cooking. Fresh palm oil was heated at 150^{0}C in a stainless steel pot intermittently for five times with each lasting twenty minutes. At the end of each heating session, the oil was allowed to cool for five hours.

2.3.1 Formulation of Diet

Since the level of palm oil in most West African dishes is about 15% [25], fifteen gram of the cooled thermoxidized palm oil was mixed with eighty-five grams of rats feed and was designated test diet II while Fifteen grams of fresh palm oil was mixed with eighty-five grams rat feed and was designated as test diet I. The diets were stored in black containers at 4^{0}C to prevent further oxidation of the oil component.

2.4 Assay kits

Randox assay kits were used for the assay of alkaline phosphatase (ALP), Aspartate Amino Transaminase (AST), Alanine Amino Transferase (ALT) and gamma Amino Transferase (GGT) in the serum. Lipid profile; cholesterol, HDL-cholesterol and Triglyceride was assayed by a colorimetric method–enzymatic and point method (CHOD – PAP) and precipitation colorimetric method, while LDL was assayed using direct immune – Inhibition method. Automated analyzer (902) machine was used.

2.5 Collection of Blood and Serum Samples

After twenty-eight (28) days of feeding, the rats were subjected to an overnight fast, and were anaesthetized with chloroform/ether mixture in ratio 1:1. Their thoracic cavities were opened and blood withdrawn by cardiac puncture using a 5ml syringe into properly labelled non-heparinized tubes. The sample bottles were allowed to stand for 1hr after which they were spun at a speed of 3000 revolutions per minutes (rpm) for five minutes using MSE centrifuge (England). At the end of the spinning process, the sample bottles were removed from the centrifuge and the uppermost straw-coloured serum was drawn out using syringes. The serum samples were emptied into a different sample bottles properly labelled for the determination of serum lipid profile and liver function enzymes.

2.6 Statistical Analysis

Data are expressed as mean ± SEM (standard error of mean). Data were analysed using one way ANOVA (analysis of variance), followed with a post hoc (LSD) test for significant values. P-values of less than 0.05 were considered statistically significant

III. Results

The TC increased significantly ($P<0.05$) in all the test groups with the exception of the TPO which showed a significant ($P<0.05$) decrease compared with the control, Fig 1

The TG concentration showed a significant increase ($P<0.05$) in all the test groups when compared with the control, Fig 2. The HDL-C reveals a significant ($P<0.05$) elevation in all the experimental groups with the exception of TPO which showed a significant ($P<0.05$) decrease when compared with the control, Fig 3. The results of low density lipoprotein-cholesterol (LDL-C) showed a significant ($P<0.05$) reduction in LDL-C in all the test groups except TPO which showed a significant ($P<0.05$) increase when compared with the control, Fig 4 The results of very low density lipoprotein-cholesterol (VLDL) showed a significant ($P<0.05$) increase in all the test groups when compared with the control, Fig 5

The serum ALT, AST and ALP followed a similar pattern as there was a significant ($P<0.05$) decrease in all the test groups except TPO which was significantly ($P<0.05$) increased when compared with control, TABLE 1. The results of serum GGT showed a decrease in the test groups which were not significant ($P<0.05$) however TPO showed a significant ($P<0.05$) increase when compared with the control, TABLE 1

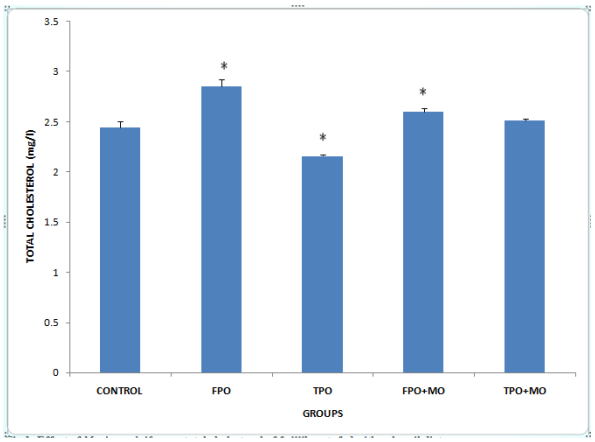

Fig 1: Effect of *Moringa oleifera* on total cholesterol of female rats fed with palm oil diets

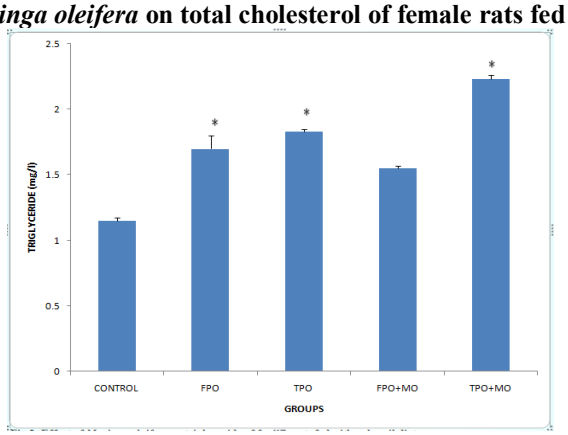

Fig 2: Effect of *Moringa oleifera* on triglyceride of female rats fed with palm oil diets

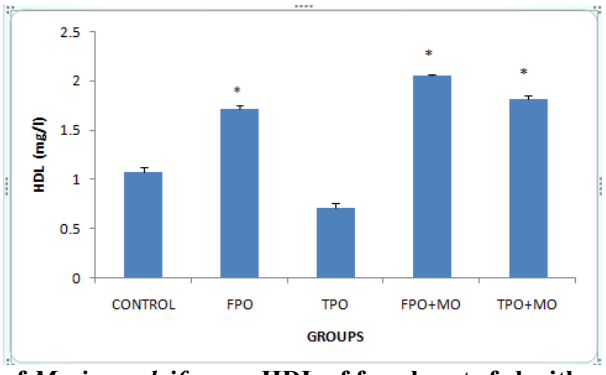

Fig 3: Effect of *Moringa oleifera* on HDL of female rats fed with palm oil diets.

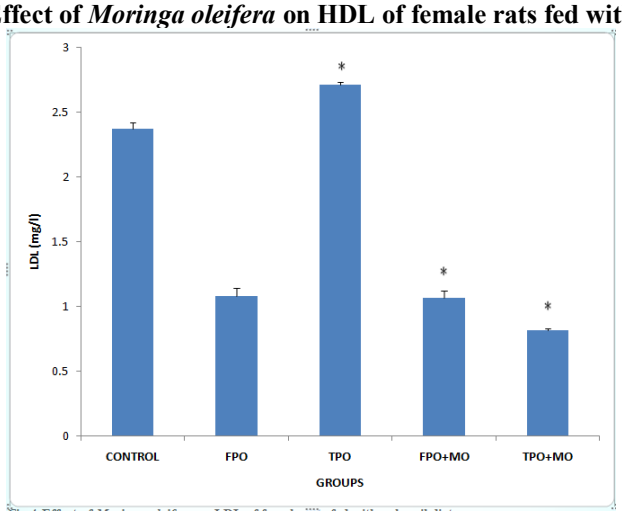

Fig 4:Effect of *Moringa oleifera* on LDL of female rats fed with palm oil diets.

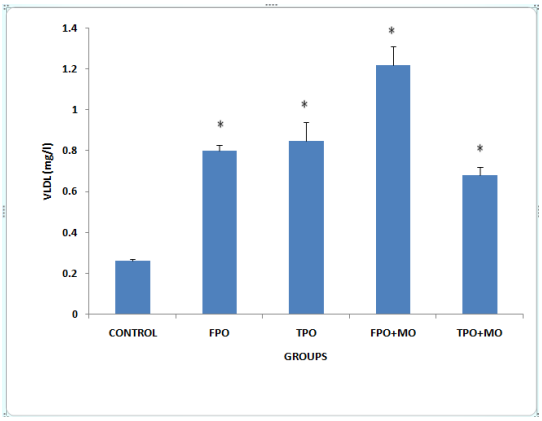

Fig 5: Effect of Moringa oleifera on VLDL of female rats fed with palm oil diets.

TABLE 1: Effect of FPO, TPO, FPO + MO and TPO +MO on serum enzymes of wistar albino rats

PARAMETERS	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)
CONTROL	31.8 ± 0.20	25.20 ± 0.20	94.4 ± 0.24	14.85 ± 0.08
FPO	22.8 ± 0.58*	23.5 ± 0.22*	45.6 ± 0.58*	12.43 ± 0.09
TPO	62.0 ± 0.22*	34.43 ± 0.53*	83.2 ± 0.74*	17.20 ± 0.18*
FPO+MO	25.1 ± 0.29	26.54 ± 0.26	30.02 ± 0.03*	12.45 ± 0.08
TPO+MO	24.68 ± 0.39*	25.14 ± 0.18	38.1 ± 0.19	13.02 ± 0.06

Results are expressed in mean ± SEM (n=5)*significant at P<0.05 compared with the control

IV. Discussion

This study was carried out to determine the anti-hypercholesterolemic and hepatoprotective effect of *Moringa oleifera* on rats fed with thermoxidized palm oil diet, hence the assay of lipid profile and key liver function enzymes. The assessment of the lipid profile showed significant increase in the TC in all the test groups except TPO. There was also a significant increase in TG of all the test groups including TPO. However, there was significant increase in the HDL-C of all the test groups but a reverse in the case of TPO and a significant decrease in LDL-C in all the test groups but a reverse effect by TPO. A rise in cholesterol and TG has been associated with CHD [26]. The increase in cholesterol in this case may have been as a result of formation of adrenocortical and sex hormones. Increased TG could cause the liver to form other types of lipids particularly the phospholipids [27]. However, the obvious significant decrease in the TC and HDL-C with a concomitant significant increase in the LDL-C level in TPO indicates a significant shift towards formation of bad cholesterol (LDL-C) but the addition of *Moringa oleifera* leaf extract was seen to reverse this shift in TPO + MO thereby stabilizing the production of good cholesterol (HDL-C). Clinically, increased HDL is beneficial to health since it reduces the risk of coronary heart disease (CHD) [28]. Thermoxidized oils contain substances such as core aldehydes and 9-oxononanoic acid which are injurious to the body by inducing lipid peroxidation and altering hepatic metaboilsm through production of free radicals causing increased level of LDL-C [29, 30]. LDL-C is known to be the primary marker for a number of degenerative diseases, particularly arteriosclerosis [16] The presence of phytochemicals in the *Moringa oleifera* such as glucosinolates, flavonoids and phenolic acids may have mopped up the free radicals produced by TPO [9, 31,32] restoring an improved HDL-C level as seen in TPO+MO. In addition, Ghasi *et al*. [10] has reported that *Moringa* contains beta-sitosterol which lowers blood cholesterol in rat. Further investigation on key liver function enzymes suggest that TPO has a deleterious effect on the liver compared to the FPO. It also suggest that this effect caused by TPO can be indeed as a result of free radicals produced as significantly increased serum levels of GGT was observed. However the addition of *Moringa oleifera* from results obtained was able to reverse this effect as seen in the decreased serum GGT levels of TPO+MO. Increase in key liver function enzymes vis a vis ALT, AST, ALP as well as GGT is suggestive of liver disease including hepatic necrosis, severe hepatocellular injury and cholestasis [33-36].

V. Conclusion

The result of the present study shows that Moringa oleifera leaf extract possesses anti-hypercholesterolemic effect. It also exercises hepatoprotection due to phytochemicals present in the leaf extract that were able to mop up free radicals produced from the thermoxidized palm oil. Therefore the extract can serve palliative measures in cases of possible toxicity from consumption of thermoxidized oil diets. Though the cardioprotective activity of this leaf extract was not the case of the present study, but indications of increased serum HDL-C and reduced AST are pointers that Moringa oleifera leaf extract might exhibit cardioprotective action as well.

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