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Comparative effects of palm and moringa seed oils on some biochemical and haematological parameters in rats

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ABSTRACT

This study carried out a comparative evaluation of the effects of palm and Moringa oils as fat component in rat feed on weight, heamatology, platelets aggregation and lipids parameters. Adult male albino Wistar rats (18) were grouped into three groups of six rats each; one group served as baseline. The other two groups were fed formulated diet with 10% of either palm oil (PO) or Moringa oil (MO) as the fat component of the diet for 28days and weight was measured weekly. The haematological parameters, platelets aggregation, cholesterol and triacyglycerols levels were evaluated. There was an increase ($p < 0.05$) in the weight of the PO group. Haemoglobin (HGB), haematocrit (HCT), red blood cell count (RBC) and white blood cell count (WBC) was increased ($p < 0.05$) in both diets. However, the mean cell haemoglobin count (MCHC) and platelet count (PLT) were unchanged ($p > 0.05$). The platelet aggregation study showed no platelet aggregation in the three groups. There was an increase ($p > 0.05$) in the serum cholesterol and triacylglycerol in MO group, while in for the PO group triacylglycerols was elevated ($p < 0.05$) and serum cholesterol decreased ($p >0.05$). This study indicates that although palm oil and Moringa seed oil had comparable haematological effect, palm oil had higher values for erythropoetic parameters than moringa seed oil. The high value of WBC recorded for MO could be helpful in immuno-compromised conditions with prospects for cancer management.

Key words: Palm oil, *Moringa* oil, haematology, platelets aggregation, lipids.

INTRODUCTION

Dietary fat plays an important role in influencing blood lipid profile, thrombotic tendency and susceptibility to cardiovascular diseases [1]. Thus, estimation of total cholesterol and triglyceride have been used in accessing lipid disorder. High cholesterol levels are strongly associated with cardiovascular disease because they promote atheroma development in arteries (atherosclerosis). This disease process leads to myocardial infarction, stroke and peripheral vascular diseases [2]. For this reason, it is important that fat be evaluated for their effects on various biochemical parameters in *vivo* that may predispose towards these diseases.

In the mediterranean regions of the world, olive oil has long been the sole source of dietary-fat intake. Olive oil is reported to have high content of the monounsaturated fatty acid - oleic acid [3]. Studies support the lower incidence of heart related diseases among medditerreans to the high content of oleic acid in olive oil [4].

In India and much of Asia, *Moringa oleifera* Lam. (Moringaceae) seed oil is one of the major dietary fats. Oleic acid is also the main monounsaturated fatty acid (67.9-70%) in *Moringa* seed oil [5].

There are several reports on the beneficial effects of the consumption of high oleic acid fat, which include hypocholesterolemic and hypotriacylgycerolemic effects [3]. Furthermore, oils with high concentration of oleic acid are desirable in terms of nutrition and stability during cooking and frying [5].

However, in West Africa and much of the tropical forest of Africa, red palm oil derived from *Elaeis guineensis* Jacq (Arecaceae) is the most important dietary fat. It is the second largest vegetable oil produced in the world [6]. Palm oil has high content of saturated fatty acid – palmitic acid, making up about 50% of its fatty acid composition [6].

The detrimental effects of the intake of high palmitic acid diets especially in the development of CHD and atherosclerosis have been reported [7]. Nevertheless, several studies have also shown the beneficial effects of palm oil on serum total cholesterol despite its high SFA content [8-10]. In this regard, it is noteworthy that palm oil has a high content of oleic acid also. Studies have shown that oleic acid content of 50.40% have been obtained in countries like Sierra Leone, Liberia and Ivory Coast, while 42.50% has been obtained in the far East, the Congo, Nigeria and the Cameroons [11].

Red palm oil is richly fortified in antioxidant vitamins, especially the carotenoids, tocopherols and tocotrienols [12]. These antioxidants play scavenging roles in protecting oxidative damage to tissues [10]. Oxidative damage to tissues may cause cancer, atherosclerosis, inflammatory disease and ageing [13]. This unique antioxidant potential of red palm oil notwithstanding its controversial lipidemic effects makes it a good candidate for vitamin supplementation in the developing world.

These reports therefore, gave the impetus for this study, which is set to carry out a comparative study of the effect of Palm and *Moringa* seed oils on key markers of the status of the cardiovascular system and haematological functions. The objectives of the work is to evaluate the effect of Palm and *Moringa* seed oils formulated feeds on haematological parameters, platelets aggregation, cholesterol and triacylglycerides levels in rats.

MATERIALS AND METHODS

Sample collection:
The seeds of *M. oleifera* were purchased from the Department of Agricultural Engineering, Nnamdi Azikiwe University, Awka, Anambra State,Nigeria.

Palm oil derived from the flesh of the fruit was purchased from the local market Eke-Awka in Awka, Anambra State, Nigeria.

Oil extraction:
M. oleifera (418.48g) whole seeds were dehusked and ground using a grinding machine (Corona, Landers YCIA, Medellin-Colombia). Oil was extracted from the ground seeds with n-hexane (2.5L) for 16hrs using soxhlet extraction method. The solvent (n-hexane) was evaporated over a water bath for 12hrs or until completely evaporated.
Feed formulation: Mixtures of corn starch (1180g) (Orient empire, China), casilan (520g) (casilan 90, H.J. Heinz, England), vitamin (20g) and minerals (80g) (Premix, Animal care, Nigeria) were the component of the prepared feed. For the Palm oil diet, 10% (200g) of the total (2.0Kg) feed formulated was palm oil. Moringa seed oil constituted 10% of the total feed in the moringa diet.

Table 1: COMPOSITION OF EXPERIMENTAL DIET (g/100g DIET)

Group	Corn starch	Casilan	Vitamins	Minerals	Palm oil	Moringa seed oil
Control	59	26	1	4	10	-
Moringa	59	26	1	4	-	10

Experimental animals:
18 adult male Wistar rats were bought from Veterinary Medicine Department, University of Nigeria, Nsukka. After 7days acclimatization on commercial feed and water ad libitum, the rats were divided into three groups Control

(PO), Moringa (MO) and the Baseline of 6rats each. The baseline group were sacrificed (baseline values), while the control and moringa groups' rats were fed with the formulated experimental diets for 28 days.

Sacrificing and Blood Collection: The animals were weighed, fasted for 12hrs and anaesthetized. Blood sample (0.5 ml) was collected by cardiac puncture into EDTA bottles for heamatological parameters. Samples (1 ml each) were collected into plain dry centrifuge tubes for triacylglycerols and cholesterol estimation. Another set of samples (1 ml each) was collected into trisodium citrate centrifuge tubes for platelets aggregation studies.

For cholesterol and triacylglycerols estimation, the blood samples were centrifuged at 3500 rpm for 10 min while for platelets aggregation studies the samples were centrifuged at 500 rpm for 15 min to obtain platelet-rich plasma.

Estimation of Full Blood Count (FBC): The full blood using a BC-2800 fully automated Haematology Analyzer. The parameters analyzed include; White blood count (WBC), Red blood count (RBC), Haemoglobin (HGB), Haematocrit (HCT), Mean cell haemoglobin concentration (MCHC) and Platelet count (PLT).

Platelet Aggregation Studies:
The method of Born and Cross as modified by Nwodo, (1981) was employed. Blood samples were drawn by heart puncture. Platelet-rich plama (PRP) was obtained by centrifuging 1 ml of blood and 0.1ml of 3.8 % trisodium citrate at 500rpm for 15 min. The platelet aggregation evaluation was carried thus: To 0.5 ml PRP, 2.0 ml of normal saline and 0.1 ml of 4 mM $CaCl_2$ the mixture was incubated for I min. The absorbance was read at 600nm against the reagent blank (no PRP) at 1 min interval for 5 min.

Estimation of Serum Cholesterol and Triacylglycerols: The serum cholesterol and triacylglycerols were estimated using RANDOX kits (RANDOX laboratories, UK). Absorbance of the test sample and standard were measured against the reagent blank using Ultrospec 3100 pro UV/ Visible spectrophotometer at 546 nm.

The concentration of cholesterol (or triacylglycerols) (mmol/L) in samples was calculated using the formula:

$$= \frac{\Delta_A \text{ sample}}{\Delta_A \text{ standard}} \times \frac{\text{Conc. of standard}}{1}$$

RESULTS AND DISCUSSION

The present study focused on the effects on weight, lipid profile, platelet aggregation and haematological functions in rats fed with Moringa oil in comparison to palm oil, in relation to the indices implicated in pathogenesis of cardiovascular diseases and degenerative diseases.

The decrease in weight of the MO-fed rats in contrast to the increase in the weight of the PO-fed rats is quite significant and could be attributed to the differing effects of the lipid component (oils) of the feed on serum total triacylglycerol. Similar report was made by Rebole *et al.* [15] that substitution of saturated fat with monounsaturated fatty acids (MUFA) resulted in a decrease in abdominal fat. This could also be related to an increase in Lipoprotein Lipase (LPL) activity and consequently, with a reduction in plasma triacylglycerol concentrations. Ayeleso *et al.* [10] further supports this finding that palm oil fed rats had an increase in weight.

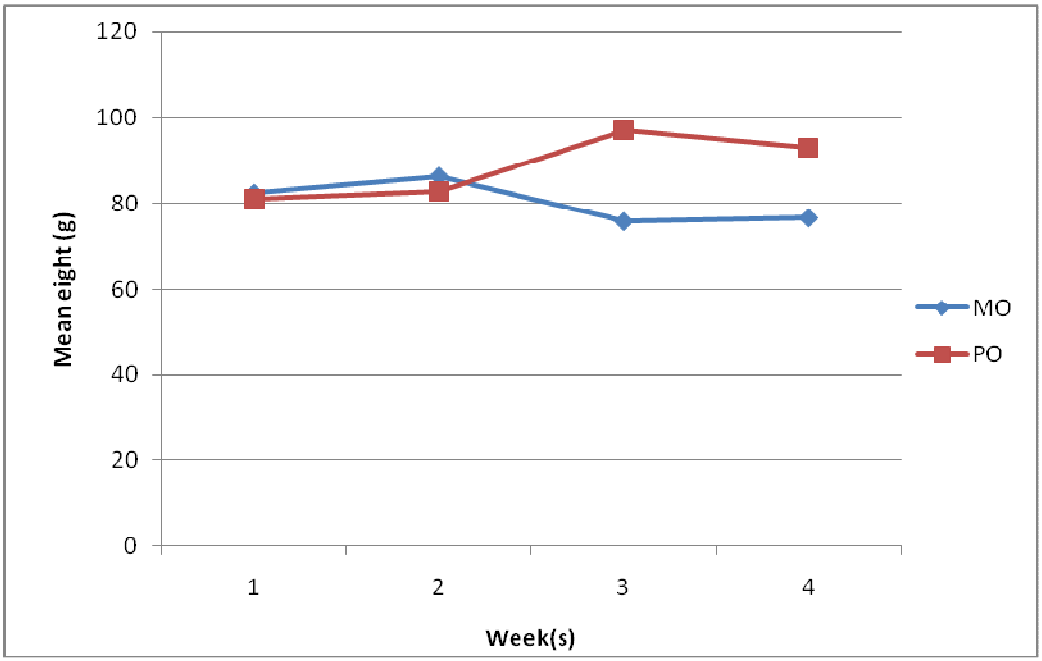

Fig. 1: Mean weight of MO and Control (PO)

The rats that consumed moringa seed oil had a slight increase in the cholesterol level over the baseline. However, the PO-fed group had a decrease in their cholesterol level in comparison to the baseline and a lower cholesterol concentration than the MO-fed group. These changes however, are not significant. Studies have shown that groups having diets with high levels of monounsaturates had lower cholesterol and decreased incidence of coronary heart diseases, CHD [16]. Evaluation of the effect of dietary fatty acids on oxidative modification of LDL in healthy normolipidemic patients was made. In vitro analyses of the isolated LDL shows, that oleic acid protect the lipoprotein from oxidative modification. LDL from the oleate group after incubation with the oxidizing system had less uptake of macrophages possibly indicating that dietary monounsaturates may protect the progression of CHD by providing a diet, which shall decrease the susceptibility of LDL to oxidative changes [16]. The PO-fed and MO-fed groups obviously share common benefits of oleic acid content. However, the PO-fed group may have the added advantage of high tocotrienols content, which is anti-thrombotic [17]. The tocotrienols content of palm oil is 525mg/kg, which is the highest for any other oil in commerce and is more than twofold over than the next commercial tocotrienol rich oil, corn oil with a value of 225mg/kg [18]. Tocotrienols function as antioxidants and may have reduced the oxidized LDL-cholesterol concentration with the concomitant reduction of foam cells and fatty streak in the PO-fed group.

Table 2: The Concentration (mMol/L) of Cholesterol and Triacylglycerols in the Serum of Rats

Groups	Cholesterol	Triacylglycerides
MO	1.361±0.235	1.163±0.453
PO	1.038±0.425	1.813±0.539*
Baseline	1.256±0.208	0.479±0.129

**Significant at p<0.05*

Serum triacylglycerol levels are an independent risk factor for coronary heart disease and are strongly determined by very low-density lipoprotein (VLDL) composition, which can be specifically modified by dietary lipid source [19]. The non-significant increase in triacylglycerol concentration in MO-fed animals, is corroborated by Viveros *et al*. [20] that MUFA-enriched feed had lower triglyceride and VLDL concentrations than did feeds rich in saturated fatty acids in broilers. In addition, Mcnamara [21] reported that the intake of PUFA and MUFA may reduce plasma triacylglycerol-rich lipoproteins, changing the composition and the catabolism of VLDL. The Control feed (PO) elevated ($p<0.05$) total serum triacylglycerols finds support in reports that palm oil is high in saturated fatty acid – palmitic [6]. Palmitic acid has been implicated in the pathogenesis of cardiovascular diseases such as atherosclerosis

[7]. However, interactions with other variables such as the tocotrienols and carotenoids will ultimately determine if progression could be made to atheromatous state.

Table 3: Full blood count (FBC) of Palm Oil and *Moringa* Seed Oil formulated Feed-fed Rats

Groups	HGB (g/dL)	RBC (10^{12}/L)	HCT (%)	MCHC (g/dL)	PLT (10^{9}/L)	WBC (10^{9}/dL)
MO	12.017±0.644*	7.5±0.434*	40.8±1.906*	29.35±0.257	543±63.284	13.633±2,732*
PO	12.2±0.585*	7.808±0.318*	42.8±1.764*	28.425±0.229	419±33.011	13.175±0.440*
Baseline	6.317±2.287	3.162±1.362	16.8±7.7732	28.525±3.065	333±101.185	4.733±2.252

**Significant at p<0.05*

The increase (p>0.05) in the red blood cell indices is suggestive of a positive erythropoetic effect and thus, enhancing the normal physiological function of the red cells [22]. Furthermore, the increased WBC count indicates that the oils to an extent had an effect on the immune system of the rats [23]. However, Moringa seed oil increased the WBC count more than palm oil indicating that Moringa seed oil could be helpful in pathological conditions with compromised immune system. In malignancies, the immune system is compromised not just by therapies to treat the cancer, but also by the direct effects of the cancer itself. In addition, the presence of mucous membrane abnormalities, malnutrition, prolonged exposure to antibiotics and frequent hospitalization all add to the risk for infection in people with malignancies.

However, the main abnormality of the immune system associated with infection in people with cancer is neutropenia [24]. Haematopoietic growth factors currently in clinical use are erythropoietin, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage-colony stimulating factor (GM-CSF) and interleukin II. G-CSF and GM-CSF act on various myeloid progenitor cells. G-CSF has been shown to dramatically accelerate (within 12days) the rate of neutrophil recovery after dose-intensive myelo-suppressive chemotherapy [25].These haematopoietic growth factors are not only expensive but also relatively scarce. MO raised the level of all the blood cells especially the white blood cells and the red blood cells in the rats and could therefore play important roles in the correction of neutropenia in cancer patients. Consequently, the differential count should be assayed to elucidate the properties in which different white blood cells are elevated by *Moringa oleifera*.

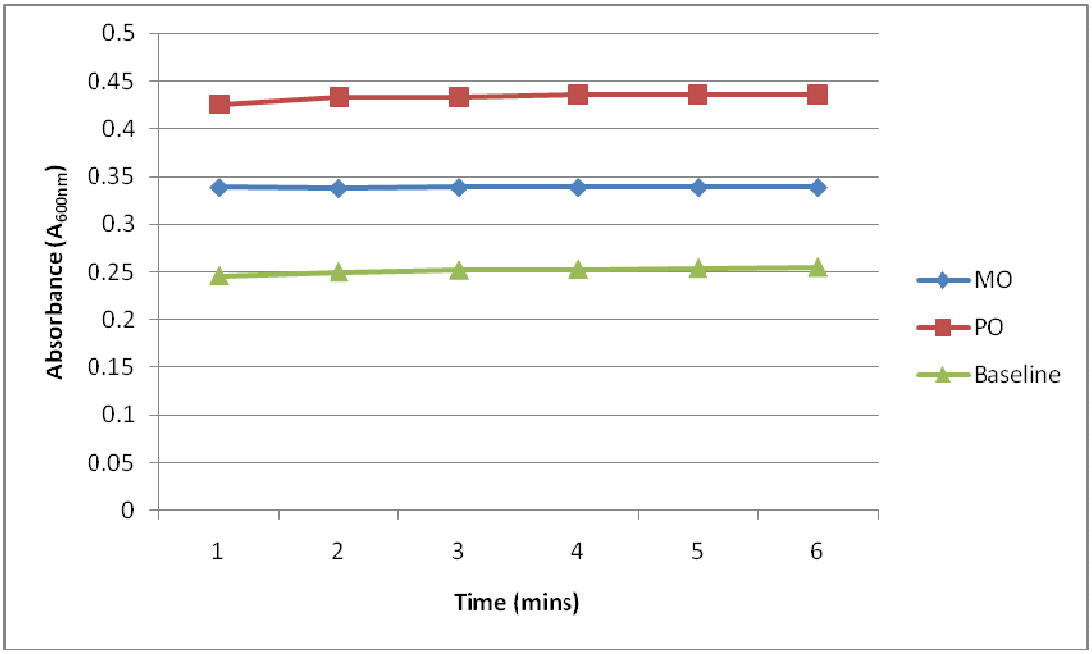

Fig 2: Platelet Aggregation of PRP of Palm and *Moringa* oils fed rats and Baseline

Platelet aggregation studies showed that there was no platelet aggregation in both diets. Previous studies by Nunez *et al*. [26] revealed that oleic acid is a potent inhibitor of platelet aggregating factor (PAF)-induced platelet aggregation. This is achieved by decreasing the level of phosphatidyl inositide (PIP) and PIP2, which are associated

with an inhibition of platelet aggregation induced by PAF. Furthermore, they opined that excess of oleic acid impairs the incorporation of arachidonic acid into platelet phospholipids. Arachidonic acid is an essential fatty acid (EFA) involved in the synthesis of prostaglandins and thromboxanes. Thus, impairment of its incorporation into platelet phospholipids poses a potent inhibition of platelet aggregation. Moreover, recent studies by Pantaleo *et al.* [27], have confirmed the anti-platelet aggregating effect of oleic acid against the significant platelet aggregating potentials of arachidonic acid administration in patients with cirrhosis. The higher platelet count in the MO group in comparison to the PO group suggests that moringa seed oil could cause more platelet aggregation than palm oil.

CONCLUSION

This study indicates that although palm oil and *Moringa* seed oil had comparable haematological effect, palm oil had higher values for erythropoetic parameters than moringa seed oil. The high value of WBC recorded for moringa seed oil fed rats could be helpful in immuno-compromised conditions with prospects for cancer management. While palm oil reduced serum cholesterol level, moringa seed oil reduced serum triacylglycerol. Hence, a blend of these two oils could have the prospects for the control of high serum lipids and cardiovascular diseases as their biochemical indices complement each other.

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