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Full Length Research paper

Palm and Egusi melon oils lower serum and liver lipid profile and improve antioxidant activity in rats fed a high fat diet

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Many studies have suggested that oils high in saturated fatty acids are responsible for the cholesterol-raising effect of saturated fat. The aim of this study was to compare the effect of palm oil (rich in saturated fatty acid) and egusi melon oil (rich in unsaturated fatty acids) on serum and liver lipids and their antioxidant status. Lipid profiles, malondialdehyde (MDA) levels and activities of glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-Rx) were determined in serum and liver of rats fed a high cholesterol diet for six weeks. Palm oil- and egusi melon-fed rats exhibited improved serum and liver lipid profiles and had a more significant reduction in serum and liver MDA levels as compared to control rats. Similarly, GSH-Px and GSSG-Rx activities were significantly higher in rats fed palm and egusi melon oils compared to the control. It could thus be concluded based on these findings, that palm oil and egusi melon improve serum and liver lipid profile in rats fed a high fat diet and their consumption could thus offer protection against lipid-related disorders.

Key words: Palm oil, egusi melon oil, saturated fatty acids, antioxidant.

INTRODUCTION

It is clearly established that long-term consumption of a high fat diet accelerates the development of Coronary Heart Disease (CHD). Therapeutic agents which control the levels of serum cholesterol have proven to be effective in the treatment of CHD (Bays and Stein, 2003; Linsel-Nitschke and Tall, 2005). While agents exist that can modulate circulating levels of cholesterol-carrying lipoproteins by inhibiting cholesterol synthesis, these agents have little or no effect on the intestinal absorption of cholesterol.

Dietary cholesterol can increase the level of serum cholesterol to levels which can place an individual at increased risk for the development or exacerbation of atherosclerosis (Onyeneke et al., 2007; Oluba et al., 2008a). CHD increases dramatically as the plasma concentration of LDL cholesterol increases (Berg et al., 1986). Consequently, the development of methods for lowering LDL cholesterol levels has become a major focus of medical research. The approach of reducing dietary cholesterol suffers from two limitations. The first is that cholesterol is present in all animal fats and many people are unwilling to scarify their preferred diet. The second is that the liver and other tissues synthesize cholesterol *de novo* if the dietary supply is inadequate.

Palm oil (PO), obtained from a tropical plant *Elaeis guineensis*, represents the second largest volume of vegetable oil produced in the world. It is rich in saturated fatty acids, antioxidant and vitamins and is widely used as an oil in diet. An earlier study by Oluba et al. (2008b) showed that PO is more hypocholesterolemic than soybean oil when both are fed as supplements (5% v/w) to a high fat diet in rats. Moreover, crude palm oil is considered to be the richest source of carotenoids and tocotrienols. The human body uses carotenoids as vitamin A. Carotenoids are also believed to enhance immune function by a variety of mechanisms, and can improve cardiovascular health (Ebong et al., 1999). Carotenoids and tocotrienols also play significant roles by acting as biological antioxidants, protecting cells and

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tissues from the damaging effect of free radicals. On the other hand, egusi melon oil (EMO), obtained from the seeds of *Citrulus lanatus* and used as a common component of daily meals in West Africa, is predominantly composed of unsaturated fatty acids (percentage composition by weight of oil: lauric, 0.21%; myristic, 0.78%; palmitic, 13.45%; stearic 13.71%; oleic, 14.50%; linoleic, 56.94% and linolenic, 0.46%) (Oluba et al., 2008c). Based on these findings, we sought to investigate whether PO (a saturated fatty acid source) and EMO (an unsaturated fatty acid rich source) have the same, similar or different effects on serum and tissue lipid profiles and their antioxidant activity in rats fed a high fat diet.

MATERIALS AND METHODS

Collection and preparation of egusi melon seeds sample

The egusi melon seeds used for this study were obtained from a local market in Iwaro-Oka Akoko, Ondo State, Nigeria and were identified as *C. lanatus* (egusi melon) by a taxonomist in the Department of Crop Science, Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria. The seeds were screened to remove bad ones, shelled manually and further screened. The seeds were then dried to constant weight in an oven at 70°C, ground using mechanical grinder, put in air-tight containers and stored in desiccators for further analyses while some of the seeds were subsequently deposited at the herbarium of the Faculty.

Oil extraction

Oil from the seeds of egusi melon was extracted by continuous extraction in a Soxhlet apparatus (Cehmglass) for 8 h using petroleum ether (60 to 80°C boiling range) as solvent according to the method of AOAC (1980). At the end of the extraction, the extraction solvent was evaporated in a rotary evaporator (Cehmglass). The extracted oil was used for feed formulation and the remaining stored in a light proof, airtight and moisture proof container at -4°C.

The palm oil sample used for this study was obtained from Okitipupa Oil Palm Mill, Okitipupa, Ondo State (Nigeria). The oil was stored in a light-proof, airtight and moisture-proof container at -4°C.

Experimental animals

Six-week old Male Wistar rats having a mean weight of 72.4 ± 4.8 g were obtained from the Nigerian Institute of Medical Research, Lagos and housed individually in stainless steel cages with raised wire flour in an environment of 24 to 25°C, 50 to 60% relative humidity and a 12 h light-dark cycle. Rats were acclimated to individual cages for one week before the diet treatment. During the acclimatization period, the rats were fed with a commercial rat chow (Guinea Feed Ltd, Nigeria) and water *ad libitum*. All experimental protocols complied with the NIH guidelines (NRC, 1985).

Diets

At the end of the first week, animals were assigned by weight into three groups of ten each (n = 10/group) and placed on the experimental diet for six weeks. The diet groups included control (CO), palm oil (PO), and egusi melon oil (EMO). The experimental diets were prepared according to the recommendations of the America Institute of Nutrition (AIN) and contained (% weight) casein 20%, fat 10%, vitamin mixture (AIN-76) 3.5%, choline bitartrate (BDH, England) 0.2%, DL-methionine (Sigma, England) 0.3%, corn starch 15% and sucrose 100%. In addition, fresh palm oil (5%) and egusi melon oil (5%) were added and mixed with PO and EMO diets, respectively.

Experimental design

Before the commencement of the experiment, animals were deprived food overnight but allowed access to water *ad libitum*. Five rats from each group were then sacrificed and blood and liver samples taken to establish the baseline levels of the studied parameters. At the end of the diet treatment, the remaining animals in each group were fasted overnight and sacrificed by decapitation. Animals were allowed free access to food and water for the duration of the study. Diet consumption and body weight were measured weekly, correcting for food spillage.

Serum preparation

At pre- and post- diet treatment periods, about 2 ml blood samples were collected from each animal by cardiac puncture into plain tubes. The blood was allowed to clot at room temperature for 1 h, and then centrifuged at 3000 g for 10 min with the serum separated into plain tubes and frozen at -20°C until analysis.

Liver lipid

Total lipids were extracted from the liver according to Folch et al. (1957).

Cytosolic and microsomal fractions

Liver cytosolic and microsomal fractions were prepared by the method of Speir and Wattenberg (1975). Briefly, rat livers were immediately removed, washed in ice cold 1.15% KCl solution, blotted, weighed, cut into small pieces in 1.15% KCl at a volume of 3 ml of KCl per gram liver and homogenized in 4 volumes of the homogenizing buffer (pH 7.4) using a Potterelvegin homogenizer. The homogenate was centrifuged at 900 g at 4°C for 20 min in a Sorvall RC-5B superspeed centrifuge. The supernatant was pipetted into a clean centrifuge tube and centrifuged further at 105,000 g at 4°C in a Beckman L5- 50B ultracentrifuge for 45 min. The cytosol was used for GSH-Px and GSSG-Rx activities.

Lipid assays

Total cholesterol (TC), HDL-cholesterol and triacylglycerol (TAG) were quantified using enzymatic kit (Randox Laboratories, San Francisco, USA) methods according to the manufacturers instructions. Non HDL-cholesterol was calculated by the Friedwald formula.

Estimation of lipid peroxidation products: Lipid peroxidation was estimated in the liver by assessing malondialdehyde (MDA) levels using the Thiobarbituric Acid Reactive Substances (TBARS) method of Varshney and Kale (1990).

Enzyme assay: Glutathione peroxidase (GSH-Px) activity was

Table 1. Weekly body weight gain (g).

Time (Week)	CO	PO	EMO
0	72.0 ± 8.4	72.4 ± 5.5	72.2 ± 9.0
1	79.5 ± 10.3	79.2 ± 4.8	79.0 ± 12.5
2	86.4 ± 6.2	85.7 ± 9.0	85.2 ± 8.2
3	97.7 ± 17.5	94.5 ± 5.2	95.5 ± 10.6
4	125.1 ± 20.4	109.2 ± 17.0*	114.2 ± 20.2*
5	148.5 ± 11.9	129.5 ± 10.7*	131.8 ± 15.0*
6	164.3 ± 32.5	141.3 ± 8.9*	154.2 ± 22.2*

Values are mean ± SEM of five determinations. CO, control group; PO, palm oil group; EMO, egusi melon group. * Significantly different ($p < 0.05$) from control (CO).

measured according to the method described by Rotruck *et al*. (1973). Glutathione reductase (GSSG-Rx) activity was measure by the method of Racker (1955) with slight modifications. The assay mixture consisted of 0.1 M phosphate buffer (pH 7.6), 0.1 mM NADPH, 0.5 mM EDTA, 1 mM GSSG and cytosol in a final volume of 1.0 ml. Enzyme activity was quantitated at 29°C by measuring the disappearance of NADPH at 340 nm. Specific activity for GSSG-Rx was defined as the oxidation of 1 µmol of NADPH per min per mg protein. Protein estimation was conducted by Lowry et al.'s (1951) method.

Statistical analyses

Data are mean ± SEM of five determinations. Statistical analysis was done by Analysis of Variance (ANOVA) and Duncan's Multiple Range Tests (DMRT) using SPSS 10.0. p values of < 0.05 control was considered significant.

RESULTS

No significant differences in food intake (23.1 ± 3.2 g/day) among the three diet groups during the study. At the end of the experiment, rats fed PO and EMO diets gained less weight than rats fed control diet. However, rats fed PO diet gained less weight than those fed EMO diet ($p < 0.05$) (Table 1). The difference in weight gain could be attributed to the low total body fat content in the group fed PO in comparison with the group fed EMO (Table 1).

Serum lipids

Serum TC concentrations was about 30 and 20% significantly lower ($p < 0.001$) in the groups fed PO and EMO respectively than in the CO group. Although rats fed PO had higher reduction than rats fed EMO diet in serum TC, the difference was not significant ($p > 0.1$) (Table 2). Serum TG concentrations were about 30% significantly lower in both PO and EMO fed rats compared to CO group. No significant difference ($p > 0.1$) was observed in serum TG levels between PO and EMO groups (Table 2). No significant differences ($p > 0.1$) in serum HDL-C were detected among the three groups at the end of the study. However, serum nHDL-C was significantly lower ($p < 0.001$) in both PO and EMO than in the CO group. A non-significant ($p > 0.1$) lower concentration in serum nHDL-C was also observed in PO compared to EMO group (Table 2).

Liver lipids

Analysis of liver lipid composition showed that animals in PO and EMO groups had significantly lower ($p < 0.001$) levels of TC compared to those in the CO group at the end of the study. Though TC concentration was lower in the PO group than in the EMO group, the decrease is not significant ($p > 0.1$) (Table 3). Hepatic TAG concentrations were significantly ($p < 0.001$) reduced in both PO and EMO rats compared to rats in the CO group. The decreased level observed in liver TAG in PO rats compared to EMO rats was not significant ($p > 0.1$) (Table 3). Lipoprotein fraction analysis of the liver revealed a significant increase in HDL-C in PO group compared to CO group and a non-significant increase ($p > 0.1$) in the EMO group compared to the CO group (Table 3). The nHDL-C levels were significantly reduced in both PO and EMO groups compared to CO group. The nHDL-C was also significantly reduced ($p < 0.001$) in the PO group compared to the EMO group (Table 3).

Antioxidant status

Serum concentration of MDA was significantly reduced in both PO and EMO groups compared to the CO group. A significantly lower value was also observed when MDA level in the PO group was compared to the EMO group (Table 4). However, serum activities of GSH-Px and GSSG-Rx were significantly elevated in both PO and EMO groups compared to the CO group. A significantly higher difference was also observed when activities of both enzymes in PO rats were compared to those of EMO animals (Table 4). Liver concentrations of MDA were significantly lower in both PO and EMO groups when compared to the CO group. However, liver activities of GSH-Px and GSSG-Rx were significantly elevated in both PO and EMO groups compared to the CO group. A non-significantly ($p < 0.1$) higher difference was also observed when activities of both enzymes in PO rats were compared to those of EMO animals (Table 4).

DISCUSSION

Body weight gain differed despite the similar cumulative energy intake throughout the study in the three groups. The difference in weight gain could be attributed to the low total body fat content in the group fed PO in comparison with the group fed EMO (Tables 2 and 3).

Table 2. Serum lipid composition and serum cholesterol lipoprotein fractions (mg/dl).

Time (Week)	TC (mg/dl)			TAG (mg/dl)		
	CO	PO	EMO	CO	PO	EMO
0	52.3 ± 2.2	54.0 ± 3.0	50.8 ± 5.1	21.0 ± 1.3	21.7 ± 1.2	2.5 ± 1.0
6	104.6 ± 12.2	70.4 ± 8.2*	81.5 ± 5.5*	42.5 ± 2.3	30.3 ± 1.2*	31.1 ± 2.2*
	HDL-C			nHDL-C		
	CO	PO	EMO	CO	PO	EMO
0	6.3 ± 1.2	7.0 ± 1.2	6.1 ± 1.5	46.0 ± 5.3	47.0 ± 3.6	44.7 ± 2.2
6	11.6 ± 2.0	10.6 ± 1.5	9.8 ± 1.1	93.0 ± 10.5	59.8 ± 7.2*	71.7 ± 3.8*

CO, control group; PO, palm oil group; EMO, egusi melon group; TC, total cholesterol; TAG, triacyl glycerol; HDL-C, high-density lipoprotein cholesterol; nHDL-C, non high-density lipoprotein cholesterol.*Significantly different ($p < 0.05$) from control (CO).

Table 3. Liver lipid composition (mg/g wet tissue) and lipid cholesterol lipoprotein fractions (mg/dl).

Time (Week)	TC			TG		
	CO	PO	EMO	CO	PO	EMO
0	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
6	15.5 ± 3.6	8.4 ± 0.5*	10.8 ± 2.6*	8.8 ± 2.2	5.4 ± 1.5*	6.2 ± 1.3*
	HDL-C			nHDL-C		
	CO	PO	CO	PO	CO	PO
0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.2
6	1.7 ± 0.1	2.4 ± 0.2*	2.0 ± 0.2	13.8 ± 1.2	6.0 ± 0.5*	8.8 ± 1.2*

CO, control group; PO, palm oil group; EMO, egusi melon group; TC, total cholesterol; TG, triacyl glycerol; high-density lipoprotein cholesterol; nHDL-C, non high-density lipoprotein cholesterol. *Significantly different ($p < 0.05$) from control (CO).

Cholesterol feeding has often been used to elevate serum cholesterol concentration in studying the etiology of hypercholesterolemia related metabolic disturbances such as atherosclerosis. In this study, the elevated serum and liver TC and TG concentrations following the consumption of a high cholesterolemic control diet were similar to the responses reported in rats fed diets with similar amounts of cholesterol (Onyeneke et al., 2007; Oluba et al., 2008a). It is observed from this study that palm oil, a saturated fatty acid - rich oil, better improves serum lipid profile than egusi melon oil. According to a report by Oluba et al. (2008c), egusi melon oil like soybean oil, is predominantly composed of unsaturated fatty acids especially linoleic acid. The results of this study invalidate the general misconception that oils rich in saturated fatty acid increase serum cholesterol compared to those rich in unsaturated fatty acids. Data generated in this study are in accordance with the findings of Oluba et al. (2008b) which show that palm oil lowers serum TC and TG compared to soybean oil when both are fed as supplements to a high cholesterol diet in rats. Similarly, Scholtz et al. (2004) in a separate study reported that palm oil lowers serum TC and nHDL-C concentrations than coconut oil.

Another interesting finding from this study is the effect of palm oil and egusi melon oil on both serum and liver antioxidant status. Cholesterol inclusion in the diet has been reported to decrease the circulating levels of insulin and serum glutathione (Scholtz et al., 2004), thus predisposition to tissue lipid peroxidation. The elevated concentrations of tissue TC, TG and nHDL-C following a high cholesterol diet presumably could increase the susceptibility of tissues to lipid peroxidation unless adequate amounts of antioxidants are present. Thus, the observed decreases in serum and liver MDA concentrations and increases in GSH-Px and GSSG-Rx activity (which are important lipid peroxide decomposing enzymes in rats) could be attributed to the hypocholesterolemic effect of these oils in the serum and liver.

Several mechanisms have been advanced to explain the unexpected hypocholesterolemic action of palm oil. Of importance is its rich content of the antioxidant, tocotrienols. Studies have confirmed that tocotrienols inhibit cholesterol synthesis *in vivo* (Karaji-Bani et al., 2006), thus enhancing the receptor-mediated transport of serum cholesterol to the liver. Tocotrienols could also participate in the clearance of lipid peroxides which may

Table 4. Serum and liver antioxidant status indicators.

Time (Week)	Serum								
	MDA			GSH-Px			GSSG-Rx		
	CO	PO	EMO	CO	PO	EMO	CO	PO	EMO
0	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
6	5.6 ± 0.4	2.7 ± 0.5*	3.2 ± 1.1*	3.5 ± 1.0	0.8 ± 0.2*	1.5 ± 0.4*	1.2± 0.2	0.6 ± 0.2*	1.0 ± 0.2*
	Liver								
	MDA (mM/cm^3)			GSH-Px (mol NADPH/min/mgprotein)			GSSG-RX (µmol NADPH/min/mg protein)		
	CO	PO	EMO	CO	PO	EMO	CO	PO	EMO
0	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
6	10.2 ± 3.7	5.7 ± 1.2*	6.5 ± 2.0*	2.8 ± 1.0	4.2 ± 1.2*	3.9 ± 1.2*	2.2 ± 0.2	3.9 ± 0.5*	2.7 ± 0.2*

CO, control group; PO, palm oil group; EMO, egusi melon group; MDA, malondildehyde; GSH-Px, glutathione peroxidase; GSSG-Rx, glutathione reductase. *Statistically different ($p < 0.05$) from control (CO).

be generated as a result of cholesterol feeding. Human studies have also confirmed that palm oil tocotrienols have the ability to reverse the blockage of carotid artery and platelet aggregation (the clumping together of cells) thereby reducing the risk of stroke, arteriosclerosis, and ischemic heart disease (Chow, 1992). On the other hand, the effect of egusi melon (though has not received much attention in terms of its nutritional potential) on serum cholesterol may be attributed to its rich content of essential fatty acids. Since the results obtained form this study correlate with those obtained using human studies, it therefore infers that these results (from this study) can be explored in humans.

In conclusion, the results of this study showed that palm oil and egusi melon oil improve serum and liver lipid profiles and offer better protection against resultant lipid peroxides from consumption of high fat diet.

REFERENCES

AOAC (1980). Official methods of analysis of the Association of Official Analytical Chemist. 13th edn, William Horwitz, Washington DC, USA, pp. 56-132.

Bays H, Stein EA (2003). Pharmacotherapy for atherogenic dyslipidaemia - Current therapies and future agents. Expert Opin. Pharmacother., 4: 1901-1938.

Berg KA, Borrensen H, Dawon G (1986). Serum high density lipoprotein and atherosclerotic heart disease. Lancet., 1: 499-501.

Chow CK (1992). Fatty acids in foods and their health implications. New York: Marcl Inc., pp. 237-262.

Ebong PE, Owu DU, Isong EU (1999). Influence of palm oil (*Elaesis guineensis*) on health. Plant Foods Hum. Nutr. (formerly Qualitas Plantarum), 53(3): 209-222.

Folch J, Lee M, Sloane-Stanley GH (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509.

Karaji-Bani M, Moritazeri F, Hashemi M (2006). Effect of palm oil on serum lipid profiles in rats. Pak. J. Nutr., 5(3): 234-236.

Linsel-Nitschke P, Tall AR (2005). HDL as a target in the treatment of atherosclerotic cardiovascular disease. Nat. Rev. Drug Discov., 4: 193-205.

Lowry OP, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with Folin phenol reagent. J. Biol. Chem., 193: 265-275.

National Research Council (1985). Guide for the care and use of laboratory animals. Publication no. 85-23 (Rev). National Institutes of Health, Bethesda, MD.

Oluba OM, Adeyemi O, Ojieh GC, Adebisi KE, Isiosio IO, Aboluwoye CO (2008a). Effect of dietary cholesterol on some serum enzymes. J. Med. Sci., 8(4): 390-394.

Oluba OM, Adeyemi O, Ojieh GC, Aboluwoye CO, Eidangbe GO (2008b). Comparative effect of soybean oil and palm oil on serum lipids and some serum enzymes in cholesterol-fed rats. Eur. J. Sci. Res., 23(4): 559-566.

Oluba OM, Adeyemi O, Ojieh GC, Isiosio IO (2008c). Fatty acid composition of *Citrullus lanatus* (egusi melon) and its effect on serum lipids and some serum enzymes. Internet J. Cardiovasc. Res., 5: 2.

Onyeneke EC, Adebisi KE, Eriyamremu GE, Ojeaburu SI, Asagba SO, Oluba OM (2007). Effect of lipid-based diet on some lipid-metabolizing enzymes. J. Med. Sci., 7(8): 1283-1289.

Racker E (1955). Glutathione reductase (liver and yeast). Methods Enzymol., 2: 722-725.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973). Selenium: Biochemical role as a component of glutathione peroxidase. Sci.. 179: 588-590.

Scholtz SC, Pieters M, Oosthuizen W, Jerling JC, Bosman MJ, Vorster HH (2004). The effect of red palm olein and refined palm olein on lipids and haemostatic factors in hyperfibrinogenaemic subjects. Throm. Res., 113: 13-25.

Speir CL, Wattenberg LW (1975). Alterations in microsomal metabolism of benzo(a) pyrene in mice fed butylated hydroxylanisole. J. Natl. Cancer Inst., 55: 469-472.

Varshney R, Kale RK (1990). Effects of Calmodulin antagonist on radiation induced lipid peroxidation in microsomes. Int. J. Rad. Biol., 58: 733-743.