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Full Length Research Paper

Atherogenicity of *Cucumeropsis mannii* and *Cucumis sativus* oils from Cameroon

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The atherogenicity of *Cucumeropsis mannii* and *Cucumis sativus* oils was compared to that of corn oil and palm oil. Female wistar albino rats of 6 weeks old weighing 58 – 65 g were randomly assigned to one of four diet groups: *C. mannii* and *C. sativus* oils (test diets) and corn and palm oil (control diets). There was weight gain in all the groups amounting to 128.65% (palm oil), 132.75% (*C. mannii*), 140.8% (*C. sativus*) and 153.45 (corn oil) with no significant difference. The weights of the livers ranged from 4.22 (palm oil) to 5.17 g (corn oil), ratio of weights of liver to that of rat, from 0.029 - 0.034 and percentage weight gain from 128.65 (palm oil) to 153.45% (corn oil). There was no significant difference in the values. For the atherogenic parameters measured, the triglyceride level ranged from 73 (palm oil) to 79.4 (*C. sativus* oil) with no significant difference. Total cholesterol levels ranged from 49.6 (corn oil) to 64.2 mg/dl (*C. sativus* oil) with significantly lower values in the corn oil group but similar values in the rest of the groups. HDL ranged from 18.94 (*C. mannii* oil) to 32.8 (palm oil) which was significantly high, LDL from 6.2 (palm oil) to 25.06 mg/dl (*C. mannii* oil) and atherogenic ratio (AR) from 0.2 (palm oil) to 1.61 (*C. mannii*) which was significantly high. The levels of these atherogenic parameters are far below the borderline level for oils to cause atherosclerosis, indicating that they could be potential good edible oils for reducing cardiovascular illnesses.

Keywords: Atherogenicity, oils, *Cucumeropsis mannii*, *Cucumis sativus*.

INTRODUCTION

In Africa, obesity has become a major problem in line with HIV. Though obesity is traditionally seen as a sign of wealth, it is becoming a very serious health issue due to its complications (BBC News, 2004). In Cameroon, according to information from an Obesity Clinic in the Yaounde Central Hospital, 35% of the adult population is either overweight or obese and this often leads to diabetes and cardiovascular diseases. In addition to exercises and drugs (which usually have undesirable side effects), one of the ways of overcoming this health problem can be through the use of diets and foodstuffs, especially those that are locally available. Cucurbitaceae (egusi) seeds are one of such foodstuffs. These seeds have been shown to be rich in proteins and oils and the oils contain mostly linoleic (an essential fatty acid which can only be got from the diet) followed by oleic acid (Silou et al., 1999; Murkovic et al., 1996; Younis et al., 2000; Achu et al., 2005; Achu, 2006). Essential fatty acids are important for normal foetal and infant growth and development, brain development and visual acuity (FAO, 1994). The other essential fatty acids; linolenic acid, can be got from linoleic acid, and arachidonic acid from linolenic acid. Arachidonic acid is a component of the liver, plasma phospholipids and cholesterol esters. It is an important precursor of prostaglandins, thrombosanes and prostacyclins. Prostaglandins stimulate contraction of smooth muscles. Prostacyclins have an antagonistic effect to that of thrombosanes by inhibiting platelet aggregation, relaxing coronary arteries and lowering blood pressure (Ottaway and Apps, 1984). Linoleic acid (polyunsaturated) moderately reduces serum cholesterol and LDL levels. Oleic acid (monounsaturated) appears to be

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neutral in regard to LDL but modestly raises HDL (FAO, 1994). There is little data on the atherogenicity of oils from Cucurbit seeds cultivated in Cameroon. The main objective of the study was to investigate the atherogenic property of oils extracted from *Cucumeropsis mannii* and *Cucumis sativus* seeds from Cameroon which can be exploited at the alimentary levels. This was in order to determine the levels of serum triglycerides, total cholesterol (TC), high density (HDL) and low density (LDL) lipoprotein cholesterol, atherogenic ratio (AR) and proteins of rats fed with oils extracted from the seeds of *C. mannii* (egusi melon) and *C. sativus* ("Ibo" egusi) seeds compared to rats fed with palm and corn oils diets.

MATERIALS AND METHODS

Sample collection and treatment

C. mannii seeds were collected from Ebolowa (in the South Province) and *C. sativus* seeds from Bafia (in the Centre Province), which are amongst the regions of great cultivation of these seeds in Cameroon. The seeds were bought already sun-dried by the farmers, transported in polyethylene bags to the laboratory, wiped with filter paper and dried in an Oven at 70°C to constant weight. They were ground in an electric grinder, put in airtight bottles and stored in the desiccator for analyses.

Extraction of the oils

Oils were extracted from the ground seeds by continuous extraction in a Soxhlet apparatus for 8 h using hexane as solvent (AOAC, 1980). The hexane was evaporated on a rotary evaporator and the oil obtained was dried in an oven at 60°C for 24 h to remove all traces of solvent.

The Experimental design

The experimental design was 4 types of oils tested x 4 groups of rats x 5 rats per group. That is: 4 oils x 4 groups = 4 x 4 groups.

Formulation of the diets given to rats

The diet was formulated by weighing the various components according to the American Institute of Nutrition-76 (AIN-76) composition, modified for casein level as follows: casein 10%, starch 36%, α-cellulose 5%, vitamin mixture 1%, choline chloride 0.1%, DL-methionine 0,3%, sucrose 27.6%, salt mixture 5%, oil 5% (Oil = corn oil, palm oil, oil extracted from *C. mannii* and *C. sativus* seeds). This diet was mixed with 10% of water to moisten the food for easy consumption by the rats. The food was given to the rats in the form of a paste of 20 g of food per rat.

Treatment of the rats

20 wistar albino rats of 6 weeks old weighing 58 – 65 g were used. One rat was put in a metabolic cage for an adaptation period of 5 days. During this time, they received the same food (that is, the food that was consumed by rats of the corn oil group which is the corn oil diet) and water *ad libitum*. They were then weighed and randomly distributed into 4 groups of 5 rats each, receiving 4 types of food differing only by the type of oil (corn oil and palm oil for the control groups and *C. mannii* and *C. sativus* oils for the experimental or test groups). The feeding took 3 months. This was to allow enough time to follow up the effect of the oils tested on the metabolism of the rats. The rats were weighed every 3 days. 12 h before sacrifice, these rats were left to fast. They only took water. On the morning of the sacrifice, they were weighed and sacrificed by decapitation. The blood of each rat was collected into dry 10 ml test tubes and centrifuged at 3000 rpm/min for 5 min. The separated serum was collected into 2 ml ependoff tubes. The serum was divided into 2 parts. The first part was preserved in aliquots at 4°C for HDL cholesterol analysis and the second part at -28°C for the rest of the analysis. The liver of each rat was equally collected and weighed in order to see the effect of the oils tested on the size of the liver.

Analysis of serum samples

Triglycerides, total and HDL cholesterol were assayed enzymatically according to the method on the Randox kits used, while LDL levels were calculated using the formula of Friedewald et al. (1972). The AR ratio was also calculated and the serum protein levels were assayed according to the method of Gornall et al. (1949).

Statistical analysis

The Kruskal-Wallis test (for the distribution of the sample as shown by the Kolmogorov test was non normal) was used to find differences between the parameters measured in the rats that received diets prepared with the different oils. The Student-Newman-Keuls (S-N-K) test was used to locate these differences. The tests were done at 5% level of significance, using SPSS 10.1.

RESULTS

Table 1 shows the amounts of food ingested, the evolution of the body weights of the rats and the ratio, weight of liver/weight of rat. The amounts of food ingested per rat per day ranges from 9.68 (corn oil) to 11.06g (*C. mannii* oil). At the start of the experiment, the weights of the rats ranged from 58.28 (*C. sativus* oil) to 64.91 g (*C. mannii*). At the end of the experiment, they ranged from 139 (*C. sativus*) to 151.07g (*C. mannii*). There is an increase in weight in all the groups with a gain of 80 (palm oil) to 90.52 g (corn oil) amounting to 128.65% (palm oil), 132.75% (*C. mannii*), 140.8% (*C. sativus*) and 153.45% (corn oil), showing no significant difference. The weights of the livers range from 4.22 (palm oil) to 5.17g (corn oil), ratio of weights of liver to that of rat, from 0.029 (palm and *C. mannii* oils) to 0.034 (corn oil). There is no significant difference between the food ingested and the weights of these rats that received diets prepared with the different types of oils.

Table 2 shows the serum levels of triglycerides (TG), total cholesterol (TC), HDL and LDL cholesterol, atherogenic ratio (AR) and serum proteins. No significant differences were observed in the total TG levels among the four groups. However, total serum cholesterol was significantly higher in the rats on *C. sativus* compared to the rats on corn oil diet. There was no significant difference in serum HDL cholesterol among palm oil and *C.*

Table 1. Evolution of body weights of rats and the ratio, weight of liver/weight of rat.

Groups	Food ingested (g/rat/day)	Initial weight of rats (g)	Final weight of rats (g)	weight gain (g)	% weight gain	Weight of liver (g)	Liver/rat weight
Corn oil	9.68 ± 2.58	58.89± 3.82	149.40± 19.03	90.52± 16.67	153.45± 24.68	5.17± 1.26	0.034± 0.005
Palm oil	9.77 ± 1.93	63.06 ± 3.36	143.73 ± 6.11	80.66 ± 8.36	128.65 ± 19.09	4.22 ± 0.37	0.029± 0.002
***C. sativus* oil**	10± 1.67	58.28 ± 5.23	139.85 ± 6.37	81.57± 2.83	140.80± 12.58	4.42 ± 0.26	0.032 ± 0.001
***C. mannii* oil**	11.06 ± 1.72	64.91 ± 3.17	151.07± 12.86	86.16 ± 11.49	132.75 ± 16.76	4.32 ± 0.28	0.029± 0.002

No significant differences were observed between groups in the amount of food ingested and in the total weight gain.

Table 2. Serum levels of triglycerides, total cholesterol, HDL and LDL cholesterol, atherogenic ratio and serum proteins.

Groups	^{0}Triglycerides (mg/dl)	Total Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	Atherogenic Ratio, (LDL/HDL)	^{0}Total Proteins (g/l)
Corn oil	76.2± 12.66	49.6± 4.93^{b}	25.92± 2.52^{ab}	8.44± 1.14^{b}	0.33± 0.05^{b}	79.04 ± 13.4
Palm oil	73 ± 10.2	53.6± 7.99^{ab}	32.8± 6.53^{a}	6.2 ± 4.57^{b}	0.20± 0.13^{b}	81.57± 8.01
C. sativus oil	79.4 ± 10.06	64.2± 8.64^{a}	27.72± 4.18^{ab}	20.6± 8.65^{a}	0.77± 0.38^{a}	78.17± 11.83
C. mannii oil	75 ± 6.96	59 ± 6.82^{ab}	18.94± 7.14^{b}	25.06± 7.25^{a}	1.61 ± 1.05^{a}	84.71±15.37

Kruskal-Wallis test: ^{0} = there is no significant difference ($p>0.05$) between values in the same column.
Student-Newman-Keuls test: Values in the same column with different letter superscripts are significantly different ($p<0.05$).

sativus oil. However, HDL cholesterol was significantly higher in the rats on palm oil compared to those on *C. mannii* oil ($p<0.05$). LDL cholesterol was significantly higher in the rats on *C. mannii* and *C. sativus* oils compared to the control diets, corn oil and palm oil. Similarly the atherogenic ratio (AR) was significantly higher in the rats on *C. mannii* and *C. sativus* oils compared to corn and palm oil diets. Total serum protein was not signify-cantly different among groups.

DISCUSSIONS

The triglyceride (TG) levels were 75 in *C. mannii* and 79.4 in *C. sativus* oil rats compared to 73 in palm oil and 76.2mg/dl in corn oil rats, but with no significant differ-rence. However, from the results of analysis of the quality of *C. mannii* and *C. sativus* oils, *C. sativus* oil shows lower levels of free fatty acids (acid index: *C. sativus* oil = 1.76 and *C. mannii* oil = 4.56), higher degree of unsatu-ration (iodine index: *C. sativus* oil = 114.4 and *C. mannii* oil = 106.78; unsaturated fatty acids: *C. sativus* oil = 78.4% and *C. mannii* oil = 54.7%; saturated fatty acids or SFA: *C. sativus* oil = 21.7% and *C. mannii* oil = 42%), lower level of peroxides (peroxide index: *C. sativus* oil = 3.31 and *C. mannii* oil = 13.68), lower R_1 value (R_1 is the ratio of the sum of saturated to that of unsaturated fatty acids. The R_1 of *C. sativus* oil = 0.28 and *C. mannii* oil = 0.77) and lower linolenic acid level (linolenic acid: *C. sativus* oil = 0.1% and *C. mannii* oil = 0.3%) (Achu, 2006). These suggest that the quality of *C. sativus* oil is better than that of *C. mannii* oil and that *C. sativus* oil contains more unsaturated fatty acids than *C. mannii* oil. *C. sativus* and *C. mannii* oils have high levels of polyun-saturated fatty acids, PUFA (39.3% in *C. mannii* oil and 62.2% in *C. sativus* oil) as in corn oil (58.5%) while palm oil has a low level (8.5%) (Table 3). These results are similar to those of Noubissi in Djamen (1998), who after 2 months of experiment, found a non significant decrease in the TG level of rats fed with palm oil diet (rich in SFA) compared to those fed with cotton oil diet (poor in SFA).

However, these results show that these egusi oils can be good edible oils (do not cause hypertriglyceridaemia) because their TG levels which are 75 in *C. mannii* oil and 79.4 mg/dl in *C. sativus* oil rats, are far below 150 mg/dl, the borderline level of TG, above which the person has hypertriglyceridaemia (Randox Assayed Multisera, 2004b).

The total cholesterol (TC) level of *C. sativus* oil (64.2) is similar to those of *C. mannii* oil (59) and palm oil (53.6) but significantly higher than that of corn oil rats (49.6mg/dl). *C. sativus* and corn oils all have high levels of PUFA which are 62.2 and 58.5% respectively, due to their high linoleic acid levels. But *C. sativus* oil has higher levels of SFA (21.7%) than corn oil (16.5%) (Table 3). Hence, the high TC level in *C. sativus* oil compared to corn oil rats may be due to its higher SFA levels.

However, the TC levels of all these four groups of rats were low when compared to levels that cause hyperch-olesterolaemia. Secondly, other studies have shown that the hypercholesterolaemic effect of SFA is mostly due to C14:0 and C12:0 fatty acids rather than C16:0 (Ng, 1994). *C. sativus* and *C. mannii* oils contain little or no

Table 3. Fatty acid composition of oils used.

Fatty Acid	Symbol	Corn oil (FAO, 1981)	Palm oil (Ngogang et al., 1996)	*C. sativus* oil (Achu, 2006)	*C. mannii* oil (Achu, 2006)
Caprylic acid	C8:0	-	-	-	-
Capric acid	C10:0	-	-	-	-
Lauric acid	C12:0	-	-	-	1.5
Myristic acid	C14:0	1	3.5	-	4.7
Palmitic acid	C16:0	12	40.6	10.7	24.4
Palmitoleic acid	C16:1	0.5	-	-	0.8
Stearic acid	C18:0	2	4	10.6	11.2
Oleic acid	C18:1	24	43	16.2	14.6
Linoleic acid	C18:2	56.5	8.5	61.8	38.7
Linolenic acid	C18:3	2	-	0.1	0.3
Arachidonic acid	C20:0	1	-	0.4	0.2
Gadoleic acid	C20:1	0.5	-	-	-
Arachidonic acid	C20:2	-	-	0.3	0.3
Behenic acid	C22:0	-	-	-	-
Lignoceric acid	C24:0	0.5	-	-	-
Total Saturated fatty acids		16.5	48.1	21.7	42
Total MUFA		25	43.4	16.2	15.4
Total PUFA		58.5	8.5	62.2	39.3
Total unsaturated fatty acids		83.5	51.9	78.4	54.7
PUFA/SFA (R_2)		3.55	0.17	2.87	0.93

= Not found, MUFA = monounsaturated fatty acids, PUFA = poly unsaturated fatty acids.

C14:0 and C12:0 fatty acids. Their SFA are mostly C16:0 as in corn and palm oils (Table 3) which do not lead to much rise in cholesterol levels. Also, the non-hypercholesterolamic action of these oils is possibly due to their presence of tocotrienols, the unsaturated analogue of tocopherols. Tocotrienols inhibit cholesterol synthesis *in vivo*, exerting a hypocholesterolamic action in humans and animals. This has been shown in palm oil by Rukmini (1994). However, these results show that these oils are good edible oils (do not cause hypercholesterolaemia) because their TC levels (59 in *C. mannii* oil and 64.2 mg/dl in *C. sativus*) are far below 200 mg/dl, the desirable blood cholesterol level, above which the person has hypercholesterolaemia (Randox, 2004a).

The HDL level of *C. sativus* (27.72) is similar to those of *C. mannii* (18.94), corn oil (25.92) and palm oil (32.8 mg/dl) rats. The HDL level of *C. mannii* oil is similar to those of *C. sativus* and corn oil rats but significantly lower ($p<0.05$) than that of palm oil rats (Table 2). This lower HDL value in *C. mannii* compared to palm oil rats may be due to the lower level of MUFA in *C. mannii* (15.4%) than palm oil (43.4%) and higher PUFA in *C. mannii* (39.3%) than palm oil (8.5%) (Table 3). This is in line with the results of a study carried out by O'Callaghan et al. in During et al. (2000) who found that patients fed PUFA dairy products showed lower plasma HDL levels than patients fed MUFA dairy products. This might be the case with these oils where *C. mannii* had higher PUFA, lower MUFA and showed lower HDL levels than palm oil with lower PUFA, higher MUFA and high HDL levels. The LDL level of *C. mannii* oil rats (25.06) is similar to that of *C. sativus* (20.6) and these are significantly higher than those of corn (8.44) and palm (6.2) oil rats, with similar LDL levels. Apart from increasing HDL levels, high MUFA also reduce LDL levels. This high LDL level and the eventual higher atherogenic ratio in *C. mannii* and C. *sativus* oils may be due to their lower MUFA and higher PUFA levels as opposed to corn and palm oil rats. This is seen in the study carried out by During et al. (2000) where rats fed with experimental cheeses containing mostly MUFA resulted in a significant increase of HDL-cholesterol (11%) and a significant reduction of LDL-cholesterol (31%). Other findings have shown that, in addition to unsaturated TG, dietary phytosterols in vegetable oils can also reduce plasma LDL levels (Jones et al. in During et al., 2000). Hence, high HDL and low LDL levels are due to high MUFA (low PUFA) and dietary phytosterols (as in palm oil) and low HDL and high LDL levels are due to low MUFA, high PUFA and possibly low phytosterols (as in *C. mannii* and C. *sativus* oils).

However, the HDL levels of *C. mannii* (18.94) and *C. sativus* oil rats (27.72mg/dl) are below the desirable HDL level (>40 mg/dl) and their LDL levels 25.06 and 20.6 mg/dl respectively are far below the borderline (<130

mg/dl) (American Academy of Family Physicians, 2005). This means that these oils could be very good in reducing LDL and to a lesser extent, raise HDL levels. However, these oils need to be tested on humans in order to better confirm these findings that were carried out on *wistar albino* rats.

The atherogenic ratio, AR (LDL/HDL) in *C. mannii* oil rats (1.61) is similar to that of *C. sativus* (0.77) and these are significantly higher ($p<0.05$) than those of corn (0.33) and palm (0.2) oil rats with similar AR levels. These high AR levels in *C. mannii* and *C. sativus* oil rats are due to their high LDL levels, caused by their low MUFA levels. Although the atherogenic ratio is higher in the test groups (than the palm oil and corn oil groups), these ratios are good, for they are below 3.55 in men and 3.22 in women, the borderline levels for lipids to cause atherosclerosis. Above these values, the risk of appearance of cardiovascular illness is high (Laboratoires Fournier, 1981).

The serum protein levels are 84.71 g/l in *C. mannii* and 78.17 in *C. sativus* oil rats compared to 79.04 in corn and 81.57 g/l in palm oil rats but with no significant difference in all these values.

Conclusion

This study which was aimed at investigating the atherogenic property of oils extracted from *C. mannii* and *C. sativus* seeds from Cameroon showed that these oils are good in reducing LDL cholesterol levels thereby reducing the atherogenic ratio, for the AR levels were far below the threshold values expected to cause athero-sclerosis. *C. mannii* and *C. sativus* oils could be potential good edible oils for use in the reduction of cardiovascular illnesses. There is the need to carry out this study on humans to better confirm these findings that were carried out on *wistar albino* rats.

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