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EFFECT OF COMBINED THERMO-TREATED PALM OIL AND GARLIC ON THE LIPID PROFILE OF WISTAR ALBINO RAT

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ABSTRACT
Some dietary fats pose negative effect on the human cardiovascular system. These effects over time have been reported to be preventable using plant extracts. *Allium sativum* have been found to posses many therapeutic effects. The aim of this study is to investigate garlic extraction for their hypolipidemic effect on hyperlipidemia induced by thermally treated palm oil which is a cholesterol rich diet. Twenty adult male wistar albino rats were used in these experiments. The animals were divided into five groups (4 each). Group I, the Control group (CO) where the animals were fed with basal diet. Group II, the Fresh Palm Oil group (FPO), the animals were fed with basal diet containing fresh pure palm oil (10ml/300g diet). Group III, the thermally treated palm oil group (TTPO) where the animals were fed with the basal diet containing thermally treated palm oil at a dose of (10ml/300g of diet). The group (Group IV) were fed with 300g of basal feed and they received 1m each of the garlic extract orally simultaneously. The last group (Group V) received the basal feed and a combination of thermally treated palm oil (10ml/300g diet) and 1ml of Garlic extract. Blood samples were collected via cardiac puncture, sera was separated from clotted samples for biochemical analysis. The data were analysed using analysis of variance (ANOVA) where p 0.05 is considered statically significant. The findings showed that the effects of Basal Feed + Thermally treated palm oil were significant in CHO and LDL at p value of 0.002 and 0.0364 levels respectively. Also, the main effects of Basal Feed + Garlic were respectively significant in on CHO and HDL at .p<0.05 level of significance. The results of Thermally Treated Palm Oil + Garlic were significant in TG and LDL at p<0.05 level of significance. Thus, evidence obtained from this study indicates that garlic extraction has potential effects in the prevention and control of hyperlipidemia complications and is beneficial when taken as a dietary supplement.

KEYWORDS: *Allium sativum*, hypolipidemic, hyperlipidemia.

INTRODUCTION
Palm oil, obtained from the mesocarp of the fruit of African oil palm (*Elaeis guineensis)*, is naturally reddish in color because of high beta-carotene content. It is different from palm kernel oil derived from the kernel of the same fruit, (Poku, 2002) or coconut oil derived from the kernel of the coconut palm (*Cocos nucifera*). The differences occur in their color (raw palm kernel oil lacks carotenoids and is not red) and in saturated fat content. Palm oil contains about 41% saturated fat, while palm kernel oil and coconut oil contain about 81% and 86% saturated fat respectively (Harold, 2004). Palm oil is a common cooking ingredient in the tropical belt of Africa, Southeast Asia and parts of Brazil. Its use in the commercial food industry in other parts of the world is widespread because of its lower cost and the high oxidative stability (saturation) of the refined product when used for frying (Matthäus, 2007).

Compositely, palm oil, like all fats, is composed of fatty acids, mainly esterified with glycerol. Palm oil has an especially high concentration of saturated fat, specifically, of the 16-carbon saturated fatty acid palmitic acid, to which it gives its name. Monounsaturated oleic acid is also a major constituent of palm oil. Unrefined palm oil is a significant source of tocotrienol, part of the vitamin E family (Ahsan *et al.*, 2015).

Palm oil is thermally treated when the fresh form is subjected to several rounds of heating at high temperatures. High temperature is known to cause decomposition of fatty acids, peroxides and carotenoids. Thermal treatment of palm oil results in its oxidative deterioration. When oxygen from air and water from the food being fried, are mixed and heated, the rate of oxidation increases. The cooked food absorbs this oxidized oil so it becomes part of our diet (Ammu *et al.*, 2000). The consistent heating of oil minimizes its antioxidant properties due to oxidation progression as antioxidants (e.g. vitamin E) are highly sensitive and destroyed by heat (Adam *et al*., 2007). Destruction of antioxidants may lead to increased formation of free radicals in the oil following recurrent thermal exposure.

Thermal oxidative breakdown of oils results in formation of lipid peroxidation compounds, volatile and nonvolatile decomposition compounds, enzyme inhibitors, antinutritional factors, mutagens and carcinogens. The changes that occur depend on the degree of unsaturation, existence of unsaponifiables compounds and antioxidants. During the frying or heating process, polyunsaturated fatty acids (PUFA) of oil become oxidized, hydrolyzed and polymerized. The nonvolatile compounds formed in the oil are polymers and polar substances. When frying oils are heated at 70°C, polar compounds such as hydroperoxides and aldehydes are formed but heating at 150 °C produce aldehydes, giving rise to rancidity. Thus long term consumption of oxidized oils and fats has been reported to cause growth retardation, thrombosis, essential fatty acid deficiency and artheroslerosis leading to deactivation of key metabolic enzymes (Leong *et al*., 2008). It also causes significant rise in blood pressure and hyperlipidemia.

Some medicinal plants have been proven to pose a reduction or preventive effects on atherosclerosis and hyperlipidemia. A good example is Garlic (*Allium sativum*). According to Sanjay and Subir (2002), garlic and its preparations have been widely recognized to prevent and treat cardiovascular and other metabolic diseases, atherosclerosis, hyperlipidemia, thrombosis, hypertension and diabetes. The potency of garlic has been acknowledged for more than 500 years. Garlic contains or has at least 33 sulfur compounds, several enzymes and the minerals germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc as well as vitamins A, B1 and C, fiber and water. It also contains 17 different amino acids which include; lysine, histidine, arginine, aspartic acid, threonine, swine, glutamine, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tryptophan and phenylalanine (Josling, 2005). Garlic products or preparations are used as sources of medicine in many ways in human beings in their day-to-day life (Gebreselema and Mebrahtu, 2013).

Many researchers are interested in the medicinal values of garlic and its broad-spectrum therapeutic effect with minimal toxicity. Garlic extract has antimicrobial activity against many genera of bacteria, fungi and viruses. Garlic contains a higher concentration of sulfur compounds which are responsible for its medicinal effects. The potency of garlic has been acknowledged for more than 500 years. In the ancient times, garlic was used as a remedy for intestinal disorders, flatulence, worms, respiratory infections, skin diseases, wounds, symptoms of aging, and many other ailments. Through the middle ages into World War II, the use of garlic to treat wounds surfaced repeatedly. It was ground up or sliced and was applied directly to wounds to inhibit the spread of infections (Pennington Nutrition Series, 2005).

The effect of garlic on lipid profile has been investigated in numerous trials and summarized in some studies, with variable results. It has been demonstrated that garlic has a reducing effect on blood lipids. Ali *et al*., (2000) showed that, in consideration of effects of thermally treated palm oil and garlic, garlic is beneficial in reducing blood cholesterol, triglyceride levels and systolic blood pressure in hypercholesterolemia animals. More so, garlic may beneficially affect two risk factors of atherosclerosis, that is, hyperlipidemia and hypertension. S-allyl cysteine sulfoxide, isolated from garlic, is more or less as active as gugulipid in controlling hypercholestermia, obesity and derangement of enzyme activities in cholesterol diet fed rats.

The beneficial effects of the drugs are partly due to their inhibitory effects on transaminases, alkaline phosphatase, lipogenic enzymes and HMG CoA reductase and partly due to their stimulatory effects on plasma lecithin-cholesterol acyl transferase lipolytic enzymes and fecal excretion of sterols and bile acids. Furthermore, oxygen free radicals are involved in the genesis and maintenance of hypercholesterolemic atherosclerosis and that use of garlic can be useful in preventing the development of hypercholesterolemic atherosclerosis. However, the commercial garlic oil preparation investigated had no influence on serum lipoproteins, cholesterol absorption, or cholesterol synthesis. It is proposed that, the mechanism of the hypolipidemic effect of the oil involves the active principle, diallyl disulphide, inactivating enzymes and substrates containing thiol groups in an exchange reaction; increased hydrolysis of triacylglycerols as increased lipase activity is induced by the oil; and the reduction in the biosynthesis of triacylglycerols as NADPH is made unavailable for the process by the metabolism of the oil.

Dietary factors have continued to play a key role in the development of various human diseases, including cardiovascular disease and alteration in serum lipids. Increased blood lipids are closely linked with intake of fatty substances. Worthy of mention among the fatty foods is thermo-oxidized or thermally treated palm oil. Consumption of thermally treated palm oil products and fats over a period of time has been reported to cause growth retardation, thrombosis, fatty livers, essential fatty acid deficiency, nucleic acid deficiency, hyperlipidaemia and micronutrient malnutrition leading to deactivation of key metabolic enzymes. Epidemiological studies have also shown that diets rich in fruits, herbs and spices are associated with a low risk of cardiovascular disease. Garlic acquired a reputation in the folklore of many cultures over centuries as a formidable prophylactic and therapeutic medicinal agent. Garlic has attracted particular attention of modern medicine because of its widespread health use around the world, and the cherished belief that it helps in maintaining good health, warding off illnesses and providing more vigor. To date, many favorable experimental and clinical effects of garlic preparations, including garlic extract, have been reported. However, no concrete report on combined effect of thermally treated palm oil and garlic has been documented in our immediate environment. It is on this background that this work was carried out to determine the combined effects of thermally treated palm oil and garlic on lipid profile of wistar albino rats.

MATERIALS AND METHODS

Unpackaged palm oil and Garlic were acquired from local market in Port Harcourt City Local Government. Palm oil was used because it is to some extent cheap and available to consumers, particularly in rural areas. Chemical kits for measuring total lipids, triglycerides, total cholesterol, phospholipids and HDL-C were obtained from medical diagnostic stores in Port Harcourt.

Experimental Animals

Twenty adult wistar Albino rats (weighing approximately 130 to 140 g) were used for this experiment. The rats were obtained from the animal house of the Department of Zoology, Faculty of Science, University of Port Harcourt, and acclimatized for two weeks before starting the experiment. During this period, they were housed in metal cages at a laboratory temperature of 23±3°C, maintained under a 12 hours light/dark cycle and fed on a standard diet and water. All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

Preparation of Thermally Treated Palm Oil

For 2 liters of the palm oil, pan frying was done in an uncovered stainless steel pan fryer for 12.3 minutes. The frying process was repeated 5 times at 175 ± 5°C twice daily for 4 successive days. No renewal of oil was done. At the end of the experiment, the heat treated was transferred into a bottle and stored in the refrigerator at 4°C.

Preparation of Garlic Supplementation

Fresh garlic bulbs were obtained from the local market and cut into small pieces. 50g of garlic was homogenized in 100 ml of cold distilled water and crushed in a mixing machine. The resultant slurry was squeezed and filtered through a fine cloth and the filtrate was quickly frozen until used (Emmanuel and James 2011).

Experimental Design

The animals were divided at random into five groups with 4 animals in each group. In Group I, the Control group (CO), the animals were fed with basal diet. In Group II, the Fresh Palm Oil group (FPO), the animals were fed with basal diet containing fresh pure palm oil (10ml/300g of diet). In Group III, the Thermally oxidized Palm Oil group (TPO), the animals were fed with the basal diet containing thermally treated palm oil at a dose of (10ml/300g of diet).In Group IV the animals were fed with basal feed and they received 1m each of the garlic extract orally simultaneously. In Group V the animals received the basal feed combined with thermally treated palm oil (10ml/300g of diet) and 1ml of Garlic extract. The experiment lasted for 14 days.

Sample Collection

At the end of the experiment, all animals were not given water for 12 hours and thereafter blood samples were collected via cardiac puncture. Blood samples were left to clot. Sera were separated from the clotted blood samples by centrifugation at 3000 rpm and stored at -20°C until used for biochemical analysis of total cholesterol, high density lipids, triglycerides and low density lipids.

RESULTS

Comparative Analysis of Lipid Parameters of Control Rats (NC: BF) and Rats Treated with Basal Feed + Fresh Palm Oil (BF+FPO).

Parameters	CHO (mg/dl)	TG	HDL-C	LDL-C
NC:BF(n=4)	52.08±2.57	38.75±6.20	29.80±3.65	14.53±2.281
BF+FPO(n=4)	65.63±4.60	49.40±5.53	35.33±2.26	20.42±2.09
p value	0.0021	0.0427	0.0422	0.0089
t value	5.144	2.563	2.573	3.811
Remark	S	S	S	S

S= significant, NS= Not Significant. NC:BF :Negative Control: Basal Feed Only; BF+FPO: Basal Feed + Fresh Palm Oil. N= no of rats.

Table 4.2: Comparative Analysis of Lipid Parameters of Control Rats (NC: BF) and Rats Treated with Basal Feed + Thermally treated oil (BF+TTO).

Parameters	CHO	TG	HDL-C	LDL-C
NC:BF(n=4)	52.08±2.57	38.75±6.20	29.80±3.65	14.53±2.281
BF+TTO(n=4)	64.40±3.86	47.58±5.55	33.63±2.61	21.25±4.47
P VALUE	0.002	0.0782	0.1394	0.0364
T VALUE	0.0018	2.121	1.703	2.682
REMARK	S	NS	NS	S

S= significant, NS= Not Significant. NC:BF: Negative Control: Basal Feed Only; BF+TTO: Basal Feed + Thermally Treated Oil.

Table 4.3: Comparative Analysis of Lipid Parameters of Control Rats (NC: BF) and Rats Treated with Basal Feed + Garlic (BF+G).

Parameters	CHO	TG	HDL-C	LDL-C
NC:BF(n=4)	52.08±2.57	38.75±6.20	29.80±3.65	14.53±2.281
BF+G(n=4)	45.15±3.57	46.58±7.86	23.38±2.43	12.46±0.70
p value	0.0199	0.1692	0.0263	0.1342
t value	3.148	1.562	2.930	1.731
Remark	S	NS	S	NS

S= significant, NS= Not Significant. NC:BF: Negative Control: Basal Feed Only; BF+G: Basal Feed + Garlic.

Table 4.4: Comparative Analysis of Lipid Parameters of Control Rats (NC: BF) and Rats Treated with Thermally Treated Palm Oil + Garlic (TTO+G:).

Parameters	CHO	TG	HDL-C	LDL-C
NC:BF(n=4)	52.08±2.57	38.75±6.20	29.80±3.65	14.53±2.281
TTO+G(n=4)	45.75±5.05	52.48±3.82	26.40±4.18	8.84±1.14
p value	0.0670	0.0093	0.2667	0.0043
t value	2.233	3.767	1.224	4.462
Remark	S	S	NS	S

S= significant, NS= Not Significant. NC: BF: Negative Control: Basal Feed Only; TTO+G: Thermally Treated Palm Oil + Garlic.

Table 4.5: Analysis of Variance (ANOVA) of Lipid Parameters of Rats Treated with Varying brand of palm oil and additives.

Parameters	CHO	TG	HDL-C	LDL-C
NC:BF (n=4)	52.08±2.67[a]	38.75±6.20[a]	29.80±3.65[a]	14.53±2.28[a]
BF+FPO (n=4)	65.63±4.60[bc]	49.40±5.53[ac]	35.33±2.26[ab]	20.42±2.09[bc]
BF+TTO (n=4)	64.40±3.86[bce]	47.58±5.55[acd]	33.63±2.61[abd]	21.25±4.47[bce]
BF+G (n=4)	45.15±3.57[adfg]	46.58±7.87[acde]	23.38±2.43[acef]	12.46±0.70[adfg]
TTO+G (n=4)	45.75±5.05[bdfg]	52.48±3.82[bcde]	26.40±4.18[acef]	8.835±1.14[bdfg]
p-value	<0.0001	0.0550	0.0004	<0.0001
F value	24.18	2.957	10.06	17.87
Remark	S	NS	S	S

DISCUSSION

Fatty diets have a major role in the prevention and treatment of cardiovascular disorders. Both polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) could affect the lipoprotein metabolism with hypocholesterolemic effect in the body. According to Ros (2000), triglycerides and cholesterol are important biological lipid parameters, and taking them excessively in the diet is relevant to the development of two common cardiovascular risk factors, obesity and hypercholesterolemia. This study was carried out on wistar albino rat to ascertain the effects of thermally treated palm oil on factors related to cardiovascular disease, particularly hyperlipidemia. These parameters have been attributed to atherosclerosis. Palm oil was chosen in this study because it is widely used as cooking oil. The oil is often used repeatedly for deep frying in many food outlets in order to minimize costs.

Results from this study showed that oily foods has the tendency of inducing hyperlipidaemia, while garlic extract supplementation have potential effects in preventing hyperlipidemia. With respect to cholesterol level, there was an increase in serum total cholesterol and low density lipids in all fresh palm oil-fed groups and thermally treated palm oil groups. The percentage increase in these cholesterol components of the lipid profile determined was significant compared to control that was fed with the basal diet. The increase in serum cholesterols in the study groups is probably due to the high cholesterol level in the diet. This finding is in agreement as feeding rabbits with cholesterol- enriched diet caused a significant increase in total cholesterol and LDL-cholesterol levelsin blood. Also, Adams *et al.*, (2008) reported that cholesterol-rich food like fresh palm oil and thermally treated palm oil diets increased plasma total cholesterol (T-CHOL) and LDL levels in rats fed with fat-rich food. Similarly, Ali *et al.*, (2000) and Bennani *et al.*, (2000) reported that, rats fed with cholesterol- enriched diet showed severe hypercholesterolemia, elevated plasma LDL and VLDL-cholesterol compared to the control group of rats fed on a normal diet.

Elevation of total cholesterol may also be attributed to the palm oil which is rich in palmitic acid. Farag *et al*, (2010) reported that a palmitic acid-rich diet led to higher total plasma cholesterol concentration than an oleic acid-rich diet.

Results in this study shows that feeding rats with fresh palm oil had little or no effect on the plasma triglycerides and HDL levels. This finding is in agreement with that of Staprans *et al*., (1996), where it was reported that there was no difference in serum TG concentrations between control and oxidized-diet group but poses little or insignificant effect on HDL. The reason for this could not be ascertained. Kamsiah *et al*., (2004) posited that intake of oily food do not trigger a significant increase in HDL-C. However, this finding contrasts the findings of Rueda-Clausen *et al*. (2007), in which it was reported that consumption of fresh palm oil and deep-fried palm oil increased serum TG and HDL level in humans. Also, Adam *et al*. (2008) demonstrated that there was a decreasing trend in serum HDL-cholesterol in all heated oil-fed groups.

These summations suggest that thermally oxidized palm oil diets decreased plasma HDL-cholesterol which is the “good cholesterol”. HDL actually works to clear cholesterol from the blood (Tabas, 2002). Decreased HDL-cholesterol level has been associated with cardiovascular diseases.

Effect of fresh palm oil and thermally treated palm oil diets on the studied parameters showed increased total cholesterol and LDL cholesterol compared to controls (Table 4). This implies that thermally treated palm oil possesses the potential of inducing hyperlipidaemia to a greater extent when compared with fresh palm oil. Ingestion of thermally oxidized oil has been reported to cause concomitant production of cytotoxic and destructive by-products which may be injurious to cells, tissues and organs. Due to the fact that the main sites of triglyceride synthesis are the liver, adipose tissue and intestinal mucosa, damage to any of these could cause a decreased level of plasma triglyceride.

Considering the effect of garlic on the lipids profile of treated rats, results in this study showed that the combination fresh garlic and thermally treated palm oil significantly increased the serum HDL-C but significantly reduced the cholesterol level with mild lowering of LDL-C levels. Since the concentration of LDL-C was significantly changed compared to other groups, the cholesterol level as observed in the group was attributed to the elevation of HDL-C, making the most characteristic changes involved in cholesterol modulation by fresh garlic. It is widely believed that the antioxidant micronutrients obtained from fruits and vegetables afford significant protection against diseases. Many research studies have been published, which report beneficial properties of herbal mixtures. The results here demonstrated that the daily administration of garlic to rats fed with thermally treated oil had a beneficial effect on the lipid profile of the rats.

Several studies have suggested that garlic may have beneficial effects on plasma cholesterol levels, while other studies did not find the same. The composition and quantity of sulfur components of different protocol designs of garlic preparations and the different rat models and inappropriate methods of randomization, lack of dietary run in period, short duration, and inadequate statistical power could account in part for the inconsistent findings (Yeh and Liu, 2001). The mechanism by which garlic or garlic preparations reduce plasma lipids has not been fully investigated. The proposed mechanisms for lipid-lowering include inhibiting enzymes involved in cholesterol synthesis and deactivation of HMG-CoA reductase and also reducing the hepatic activities of lipogenic enzymes such as malic enzyme, fatty acid synthase and glucose-6 phosphate dehydrogenase. It is, therefore reasonable to infer that the hypolipidemic effect of garlic may stem in part from impaired cholesterol synthesis. Garlic can also contain high levels of tellurium and selenium compounds, which contribute to the overall block in cholesterol synthesis by inhibiting squalene monooxygenase. Squalene monooxygenase plays an important role in the overall regulation of cholesterol biosynthesis (Pereira *et al*., 2004).

Large population-based observational studies found that garlic fibers are associated with a reduced CVD risk. Soluble fibers in garlic are thought to bind bile acids during the intra luminal formation of micelles. This leads to increased bile acid synthesis, reduction in hepatic cholesterol content, up-regulation of LDL-C receptors, and increased LDL-C clearance (Pereira *et al*., 2004). Other potential mechanisms include increased intra luminal viscosity, decreased cholesterol absorption and entrapment of cholesterol in the small intestine. Most studies found that high fat diet results in increased body weight and food intake. Garlic could have interfered with satiety and influenced food choices, resulting in a reduction in dietary food intake and final body weight (Kannar *et al*., 2001).

The total lipids in each of the group varied significantly. A high total lipid was observed in the group fed with basal feed and fresh palm oil and thermally treated palm oil compared to the control group. Heat-treated palm oil did appear to be more hyperlipidemic than fresh palm oil so the increase in lipid profile parameters may be due to high dietary lipid content.

CONCLUSION

Intake of fatty or hyperlipidemic foods such as thermally treated palm oil diets poses deleterious effects on biochemical indices in rats, especially lipid profile. These effects were significant in rats fed with the thermally treated palm oil diets. Results of this study shows that consumption garlic extract had positive effects on lipid profile and hyperlipidaemia as co-administration of garlic on the palm oil fed rats resulted in significant decrease in the plasma lipids levels. Consumption of garlic had significant hypolipidaemic on the animals as garlic. Therefore, garlic supplementation in diet has potential hypolipidaemic effect on the hyperlipidaemia induced by the consumption of foods containing thermally treated palm oil.

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