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Research Article

Comparative effects of fresh and multi-boiled peanut and olive oils on cholesterol fed Wistar rats

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Abstract

Oils are usually used for cooking purposes and claimed to protect against many disease like coronary heart disease, hypercholesterolemia, obesity, cancers and hypertension. This study was carried out to evaluate the effect of fresh and repeatedly- boiled peanut and olive oils (boiled five times) on blood lipid profile (High density lipoprotein HDL, low density lipoprotein LDL, Triglyceride TG and cholesterol) and their effect on weight and hematological parameters. Thirty Wistar rats were divided to five groups, control, fresh olive oil, boiled olive oil, fresh peanut oil, boiled peanut oil; in doses of 1 mg/kg/day plus cholesterol in 2 mg/kg/day being dissolved in bile and diluted 50% in distilled water, given orally for three weeks except control group which did not received neither cholesterol nor oil. All groups of rats showed decreased body weight when compared to the controls. Significant changes on WBCs, lymphocytes and neutrophils were also observed. On the other hand, lipid profile analysis showed significant increase ($p<0.05$) in serum cholesterol of fresh oils fed groups of rats, but significantly decreased in repeatedly- boiled oils. Significant increase ($p<0.05$) of triglycerides was observed in fresh oils fed groups where as no change was seen in stressed oil fed groups. Higher values of HDL were shown in fresh oils fed groups but no change was seen in repeatedly-boiled groups. Significant decrease of LDL was observed in all test groups when compared to the controls.

Introduction

Human health considered to be the point around which science are circling, one of the major aspect is to avoid disorders before they occur as the old say (prevention is better than cure). Scientist early attempts to cure disorders were established by using food, so healthy nutrition is the base for a healthy life.

Notably Mediterranean population suffers less from diseases associated with lipids. They use nutritional style rich in healthy oils, among which is the olive oil, which may explain the low prevalence of disease associated with high cholesterol and other lipid profile parameters (Aguila *et al.,* 2005).

Peanuts are grown in the warm climates of Asia, Africa, Australia, and North and South America. Sudan is among the major peanut growing countries. Peanuts are rich in energy and contain health benefiting nutrients, minerals, antioxidants and vitamins that are essential for optimum health. Peanut oil is the most common used in cooking in the Sudanese society. It is extracted from peanuts, it is also known as ground nut or arachis oil. The oil is available in refined, unrefined, cold pressed and roasted forms. Peanut oil has a high smoke point relative to many other cooking oils, so is commonly used for frying foods. Gas-liquid chromatographic analysis of the oil fatty acid methyl esters revealed the occurrence of palmitic (12.22 to 13.30%), stearic (3.17 to 3.67%), oleic (37.94 to 41.90%) linoleic (34.59 to 37.51%), arachidic (1.63 to 1.85%)

eicosaenoic (0.99 to 1.22%), behenic (3.24 to 4.36%), and lignoceric (1.08to 1.44%) as the major fatty acids (Berry, 1982).

Peanut oil is used to lower cholesterol and prevent heart disease; it is also used to decrease appetite as an aid to weight loss. Some people use it to help prevent cancer, sometimes applied directly to the skin for arthritis and joint pain, dry skin, eczema, scalp and for treating constipation (Eigenmann *et al.*, 1996 ; Menendez *et al.*, 2008).

Antioxidants such as Vitamin E are sometimes added, to improve the shelf life of the oil. Besides, peanut oil can also be used in manufacturing of skin care products and baby care products, pharmaceutical companies produce peanut oil for internal and external use as well as pea nut oil is considered as a domestic product. Refined peanut oils remove the peanut allergens and have been shown to be safe for the vast majority of peanut allergic individual (Eigenmann *et al*, 1996).

Olive oil is obtained from olive fruits (*Olea Europea*). It is produced by grinding whole olive fruits, extracting the oil by mechanical or chemical means. It far less used in the Sudanese home society for cooking. It is commonly used cosmetics, pharmaceuticals, and soaps and as a fuel for traditional oil lamps, there are a number of health benefits . Olive oil is also known to be gentle on the digestive system, and even may help prevent gallstones and soothe ulcers. Good quality olive oil contains a high concentration of monounsaturated fatty acids, valuable vitamins and nutrients, and it is loaded with antioxidants, which many believe help protect the body from cancer.

For medicinal uses, olive oil is used to prevent cardiovascular disease, breast cancer, rheumatoid arthritis, and migraine headache, also used to treat constipation, high cholesterol, high blood pressure, blood vessel problems associated with diabetes, and pain associated ear infections, and gallbladder disease. Olive oil is also used to treat jaundice, intestinal gases (Tess, 2007, and Servili *et al.*, 2009). This oil is an imported product. When vegetable oils are used in frying purpose, several chemical reactions occur affecting the quality and validity of oil for human consumption. The reactions are of oxidative stress which comprises the oil free radicals with subsequent formation of polymers (one of carcinogens) (Grootveld *et al*, 2001)

The use of unrefined peanut and olive oils may cause a deposit of cholesterol (steroid of high molecular weight) in the blood circulation; which may eventually adversely affect the heart and its blood system. Repeated use of boiled oil in frying has urged us to investigate the consequences of misuse of the boiling oil on human health.

The present study was conducted to study the effect of fresh and thermally oxidized olive and peanut oils on lipids profiles (High density lipoprotein HDL, Low density lipoprotein LDL, Triglyceride TG and cholesterol) in cholesterol fed Wistar rats, and to study the effect of these oil on weight and haematological parameters

Materials and Methods

Materials

Peanut oil and olive oil were obtained from local market in Khartoum, Sudan. Cholesterol powder was obtained from Biochemistry lab. Bile was obtained freshly from the slaughter house and stored in the fridge at 4 °C. 2g of cholesterol was dissolved in 4ml of bile salts and stored as stock solution.

Experimental animals

Thirty male and females Wistar rats with average body weight ranged from 120 to 128g were used in this study. The rats were apparently clinically healthy and housed within the premises of Faculty of Science and Technology, El-Neelain University Animal House under standard husbandry conditions (30°C ± 2°, 60–70% relative humidity and 12h: 12h day-night cycle) and fed on the rat diet (flour 55.3%, meat 35%, edible oil 7.5%, sodium chloride 1.5% and vitamins and minerals 0.7) and water provided *ad. Libitum.* Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee.

Experimental Design

The rats were allotted randomly to five groups, each of 3 rats. Group 1 received 4ml/kg of bile only and served as control. Group 2 was given cholesterol (2g/kg/day) via oral route, in addition to fresh olive oil, group 3 was given cholesterol (2g/kg/day) orally in addition to boiled olive oil, Group 4 was given cholesterol (2g/kg/day) via oral route, in addition to fresh peanut oil and Group 5 was given cholesterol (2g/kg/day) via oral route, in addition to boiled peanut oil. All rats were dosed their designated experimental oral doses for 3 weeks.Body weight and weight gain for each group were recorded weekly. Blood samples of all rats were collected at slaughter.

Haematological parameters

Blood samples were collected in an EDTA container (Ethylene diamine tetra acetic acid) for determination of hemoglobin concentration (Hb), packed cell volume (PCV), red blood cells (RBCs) counts, white blood cells (WBCs), and differential WBC counts, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

Serobiochemical parameters

Blood samples were collected at slaughter in plain containers, and serum was separated and stored at -20 °C until analyzed

for the levels of cholesterol, triglycerides, cholesterol HDL (high density lipoprotein) and LDL (low density lipoprotein).

Methods

Preparation of boiled oils

500 ml of olive and peanut oil were heated separately until boiling (232°C) for peanut oil and 191°C for olive oil then cooled down to room temperature and heated again, this operation was repeated five times without the addition of fresh oil.

Haematological methods

These techniques were performed according to an Automated Haematology Analyzer (Sysmex KX-21. Japan, 1999). The parameters measured were Hemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cells (RBCs), platelets count, White Blood Cells (WBCs), differential WBCs counts and erythrocytes indices; Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

Serobiochemical methods

Blood samples were allowed to clot and serum were separated by centrifugation at 3500 rpm for 5 min and stored at -20°C until analyzed. The following methods for lipid profile level of control and tested rats were performed according to the instructions in automatic analyser (ACCENT 200, Polnda, 2010).

Statistical analysis

The values were analyzed by one way analysis of variance (ANOVA) followed by Independent sample T-test. The significance of differences between means (mean ± Standard error (M± S.E)) was compared at each for all groups, $p<0.05$ was considered statistically significant (Snedecor and Cochran, 1989). Analysis was performed by Statistical Package for Social Science (SPSS) software, version 16.

Results

Growth changes

The effect on body weight and body weight gain of rats given daily oral doses of fresh and multi-boiled oils (peanut and olive) + cholesterol for three weeks are represent in Table 1. No significant difference ($p<0.05$) was observed in body weight gain in group 2 given fresh olive oil. After the end of week one and two and at the end of the experimental period, there was a significant decrease ($p<0.05$) in body weight gain of rats of groups 3, 4 and 5 when compared to the control group (group 1).

Haematological changes

The hematological changes of rats given daily oral doses of fresh and multi-boiled oils (olive and peanut) + cholesterol for three weeks are represented in Table 2. No significant changes were observed in the haematological parameters of the treatment groups, except the values of neutrophils which significantly decreased ($p<0.05$) in groups 2, 3 and 4 when compared to the control group. On the other hand significant increase in the values of WBCs and lymphocytes was recorded in the same groups when compared to rats of group 1, only group 5 showed a significant decrease ($p<0.05$) decrease in the value of WBCs when compared to the controls and other test groups.

Changes in lipid profile

The effects on lipid profiles of rats given daily oral doses of fresh and multi-boiled oils (olive and peanut) + cholesterol for three weeks are represent in Table 3. Cholesterol and HDL levels in group 2 and 4 showed significant increases ($p<0.05$); while it was significantly decreased ($p<0.05$) in the experimental groups 3 and 5 when compared to the control rats. Also, significantly ($p<0.05$) lower values of LDL, in all test groups, and triglycerides of groups 2, 4 and 5 were recorded when compared to the controls.

Table.1. Body weight and Body weight gain in rats orally given olive and peanut oils for three weeks

Treatment groups	Body weight (g) 0 week	Body weight gain(g) one week	Body weight gain (g) Two weeks	body weight gain (g) Three weeks
1. Control (normal diet)	124 ± 3.9	133 ± 4.3	142 ± 4.3	146 ± 4.2
2. Fresh olive oil	122 ± 2.8	124 ±0.24 ^{NS}	140 ± 2.4 ^{NS}	138 ± 0.8^{NS}
3. Boiled olive oil	123 ± 3.8	124 ±3.9*	119 ± 4.5*	115 ± 5.5*
4. Fresh peanut oil	122 ± 3.4	108 ± 1.1*	110 ±0.9*	123 ± 1.0*
5. Boiled peanut oil	124 ±0.2	126 ± 1.2*	107 ± 1.6*	114 ±2.0*

Values are expressed as means ± SE; NS= not significant; *Significant = ($P<0.05$)

Table.2. Hematological analysis of rats given olive and peanut oils and cholesterol orally for three weeks

Groups / Parameters	1.Control	2.Fresh olive oil +cholesterol	3.Boiled olive oil +cholesterol	4.Fresh peanut oil +cholesterol	5.Boiled peanut oil +cholesterol
Hb (g/dl)	12.4±1.0	13.3±0.8 ^{NS}	12.1±0.6 ^{NS}	13.6±0.3 ^{NS}	12.7 ±1.0 ^{NS}
RBC ($X10^6$ mm^3)	7.3±0.5	7.9±0.4 ^{NS}	7.7±0.1 ^{NS}	7.8±0.1 ^{NS}	7.7 ±0.8 ^{NS}
PCV (%)	39.4±3.1	42.7±3.0 ^{NS}	41.7±0.7 ^{NS}	43.1±0.9 ^{NS}	41.0 ±3.89 ^{NS}
MCV (cm^3)	53.8±0.3	53.9±1.1^{NS}	53.9±0.1 ^{NS}	55.0±0.2 ^{NS}	53.6 ±0.7 ^{NS}
MCH (pg)	11.9±5.1	16.7±0.1 ^{NS}	16.9±0.1 ^{NS}	17.4±0.1 ^{NS}	16.7 ±0.5 ^{NS}
MCHC (%)	31.6±0.2	31.1±0.4 ^{NS}	31.4±0.8 ^{NS}	31.6±0.1 ^{NS}	31.1±0.5 ^{NS}
WBC ($X10^3$ mm^3)	10.4±3.0	16.1±1.8 *	17.7±0.5*	15.8±1.3*	7.9 ±2.3 *
Lymphocytes (%)	47.3±3.0	58.4±3.3 *	61.9±1.3 *	55.8±3.33 *	48.0 ±3.4^{NS}
Neutrophils (%)	52.7±2.9	41.6±3.3 *	36.0±0.6 *	44.2±3.3 *	51.0 ±3.5 ^{NS}

Values are expressed as means ± SE; NS= not significant; *Significant = (P<0.05)

Table.3. Lipid profile of rats given oral olive and peanut oils with cholesterol for three weeks

Groups / Parameters	1. Control (normal diet)	2. Fresh olive oil + cholesterol	3. Boiled olive oil +cholesterol	4. Fresh Peanut oil + cholesterol	5. Boiled Peanut oil + cholesterol
Cholesterol mg/dl	127 ±2.0	136 ± 6.1 *	117 ± 0.4 *	148 ± 0.6 *	104 ± 0.2 *
T.G mg/dl	84.7 ± 0.1	50 ± 2.6 *	78 ± 0.5 ^{NS}	63.3 ± 0.7 *	60.0 ± 0.5 *
HDL mg/dl	38.3 ± 0.8	64 ± 3.2 *	40 ± 0.3 ^{NS}	73.0 ±1.1 *	40.0 ± 0.2 ^{NS}
LDL mg/dl	73.0 ± 1.3	61 ± 3.2 *	56.0 ± 0.4 *	69.0 ± 0.4 ^{NS}	56.0 ± 0.1 *

Values are expressed as means ± SE; NS= not significant; *Significant = (P<0.05)

Discussion

The use of repeatedly heated oil is a common practice that can pose a human health risk through the generation of free radicals (Adam *et al.,* 2008). Several studies demonstrated the adverse effects of dietary uses of oxidized oils in human and experimental animals. It can cause Increase in blood glucose as well as decrease in vitamin E and vitamin A in the liver of rats (Benedetti *et al*., 1987), acceleration of fatty streak formation in rabbits (Staprans *et al.,* 1996) and liver dysfunction (Izaki *et al*., 1984; Owu *et al.,* 1998)

Using olive oil in the diet instead of saturated fat can significantly reduced total cholesterol levels. However, some research suggests other dietary oils such as sunflower and rapeseed (canola) might reduce high and low-density lipoprotein (LDL) cholesterol and another type of cholesterol called apolipoprotein B better than olive oil (Mensink and Katan, 1989).

In the present study, the level of cholesterol in experimental rats that received short term (three weeks) repeated - boiled olive and peanut oils in addition to an oral dose of cholesterol were reported to be relatively lower than the values of the control group of rats which was limited to the normal diet only. Narasimhamurthy and Raina (1999) studied the effects of heated and fried oils (peanut oil, sesame oil and coconut oil) on growth, plasma and tissue

lipids in rats and their results showed no deleterious effect on growth but higher plasma cholesterol levels were observed in heated oil fed group of rats compared to corresponding fried oil groups. Low levels of HDL-c and increased LDL-c were noted in heated/fried oil groups with significantly low levels ($p<0.001$) of triglyceride in heated/fried sesame oil group of rats. No significant change in phospholipid was observed in any of the groups.

Battino *et al.*, 2002 have stated that the virgin olive oil possesses specific features for modulating the damage occurred by indigenous and exogenous oxidative stress being particularly rich in antioxidant molecules. Dietary intake of the fried oil, did not affect neither the body weight not the weight of the liver and normal values of cholesterol and triglycerides were obtained. Chacko and Rajamohan 2011 reported that in heated oil (Coconut, mustard and sunflower) fed groups, plasma lipids levels were significantly elevated. HDL cholesterol levels showed a reduction in all heated oil fed groups relative to the fresh oil fed groups. Only heated coconut oil fed group showed lower tendency towards hyperlipidemia.

On the other hand, five- times heated palm oil was found to cause a significant increase in serum Thiobarbituric Acid Reactive Substances (TBARS) and total cholesterol when compared to the controls (Adam *et al.*, 2008).

In this study performance changes expressed as body weight gain were decreased significantly with time compared with the control in groups of rats fed on repeatedly- boiled cooking oils. Alexander *et al.*, 1983 reported that animals derived less metabolizable energy from diets containing heated or hydrogenated fats with subsequent decrease in body weight

Also, Ruiz-Gutierrez *et al*, 1992 stated that dietary heated oils accelerate the conversion of palmitic to palmitoleic acid by activating the Δ^9 desaturase activity and slow down the synthesis of arachidonic from linoleic acid by inhibiting the Δ^6 and Δ^5 desaturase activities in the rats liver microsomes.

It is worth mentioning that the results of serum Cholesterol and HDL obtained from rats treated with fresh oils and cholesterol has shown significant increase as compared to those treated with boiled oil. Alderson *et al*. (1986) reported that the group of monkeys fed on 2.0% cholesterol and 20% Peanut oil was associated with significant increase in the HDL cholesterol concentration; but the total plasma cholesterol decreases. This discrepancy between these results could be ascribed to differences in production and storage conditions which may influence the characteristics (validity and shelf life) of the produced peanut oil.

The haematological picture recorded in the present study showed rather similar picture in all experimental and control groups of this study.

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