ORIGINAL ARTICLE

Effect of Palm Oil and Nigella Sativa in Aorta and Coronary Arteries of Albino Rats

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ABSTRACT

Aim: To study the effect of palm oil and Nigella sativa on the aorta and coronary arteries of albino rats.
Methods: Sixty albino rats including equal number of males and females were obtained from PCSIR Laboratories, Lahore. After overnight fasting of 12-14 hour all the animals were weighed and zero and 24 weeks. The rats were divided into five groups of twelve animals each. Each group had six males and six females kept in separate cages. At this stage the twelve animals of group C_0 were sacrificed as the control group for all the other groups to compare age changes. Then the experimental diet was started for the remaining four groups labeled as C_1, E_1, C_2 and E_2 based on the diets fed: C_1 (control group fed on 3% palm kernel oil), E_1 (experimental group fed on 3% palm kernel oil with Nigella sativa), C_2 (control group fed on 20% palm kernel oil with cholic acid and thiouracil acid) and E_2 (experimental group fed on 20% palm kernel oil with cholic acid, thiouracil acid and Nigella sativa). First sample was collected as baseline before starting the experimental and control diets (0 week) and the second blood sample was collected at the end of the experiment i.e. after 24 weeks. Two cc blood was drawn from each rat. At 24 weeks, after collecting blood samples, the rats were placed in an ether jar till death. The abdominal and thoracic cavities were opened through a median incision. The hearts and aortae were cleared. Biopsy specimens were kept in labeled jars for fixation containing 10% neutral buffered formalin. Then the specimens were subjected to gross and microscopic examination.
Results: Weight gain was statistically significant when experimental group E1 was compared with control group C1 and highly significant when experimental group E2 was compared with control group C2. When the aortae were opened with there were neither fatty streaks nor atheromatous plaques in the aortae of all experimental and group animals. Narrowing of coronary arteries was not observed in any of the groups. Palm oil increased the total cholesterol and low density lipoprotein cholesterol, decreased the high density lipoprotein cholesterol levels in all groups at 24 weeks as compared to 0 week samples. Nigella sativa decreased serum total cholesterol and low-density lipoprotein cholesterol and increased high density lipoprotein cholesterol levels which were highly significant.
Conclusion: Although there was significant weight gain and elevation of total cholesterol and lipids, these failed to produce atherosclerotic lesions in the experimental rats on Palm kernel oil in 24 weeks,
Keywords: Aorta, coronary arteries, albino rats, palm kernel oil, nigella sativa

INTRODUCTION

The impact of an excess of fatty acids in the diet on cardiovascular diseases has been studied and discussed both in human and animal studies. Generally, excessive saturated fats increase the risk, while unsaturated fats are considered less harmful.[1] It is well established that plant phenolics elicit various biological activities, with positive effects on health. Palm oil production results in large volumes of aqueous by-products containing phenolics[2]. Water-soluble phenolics from the oil palm possess significant biological properties[3]. Palm oil is enriched in vitamin E in the form of alpha-, gamma-, and delta-tocotrienols. Dietary tocotrienol supplements have been shown to prevent atherosclerosis development in patients and preclinical animal models[4]. The link between dietary fats and cardiovascular disease has created a growing interest in dietary red palm oil research. Also, the link between nutrition and health, oxidative stress and the severity or progression of disease has stimulated further interest in the potential role of red palm oil (a natural antioxidant product) to improve oxidative status by reducing oxidative stress in patients with cardiovascular disease, cancer and other chronic diseases[5]. Oxidized unsaturated fatty acids may contribute to the pathogenesis of atherosclerosis[6]. The palm fruit (Elaies guineensis) yields palm oil, a palmitic-oleic rich semi solid fat and the fat-soluble minor components, vitamin E (tocopherols, tocotrienols), carotenoids and phytosterols.[7] Studies have found that Nigella sativa essential oil is more effective in reducing the extent of potassium bromate

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induced multiple organ toxicity (cardiac and liver enzymes imbalance) that will ultimately helpful in reducing the extent of myocardial and liver necrosis.[8] Therefore, NS is a potential protective natural agent against atherosclerosis and cardiovascular complications in these patients.[9,10]

MATERIALS AND METHODS

Sixty albino rats including equal number of males and females were obtained from PCSIR Laboratories, Lahore. At the start of the experiment, they varied from 12-14 weeks of age and of 150-250 grams weight. They were kept in animal's house of Postgraduate Medical Institute, Lahore. The atmospheric temperature was kept at 24±2°C on an average with food and water available all times. The animals were weighed at zero and twenty four weeks. purified maintenance diet. After overnight fasting of 12-14 hour all the animals were weighed and zero. The rats were divided into five groups of twelve animals each. Each group had six males and six females kept in separate cages. For the identification of the individual, the ear hole method of marking was adopted. A rat ear punch was used for this purpose. Before the start of experimental diets, a four weeks acclimatization period was given during which they fed and blood samples were taken. At this stage the twelve animals of group C_0 were sacrificed as the control group for all the other groups to compare age changes. Then the experimental diet was started for the remaining four groups labeled as C_1, E_1, C_2 and E_2 based on the diets fed: C_1 (control group fed on 3% palm kernel oil), E_1 (experimental group fed on 3% palm kernel oil with Nigella sativa), C_2 (control group fed on 20% palm kernel oil with cholic acid and thiouracil acid) and E_2 (experimental group fed on 20% palm kernel oil with cholic acid, thiouracil acid and Nigella sativa). First sample was collected as baseline before starting the experimental and control diets (0 week) and the second blood sample was collected at the end of the experiment i.e. after 24 weeks. Two cc blood was drawn from each rat. Clean and dry test tubes were labeled for each sample and standard 0.05 ml (50 µl) of each sample and standard were taken and put into tubes labeled "test" and "standard". 2.0 ml (2000 µl) of sulphuric acid was added to the tubes. The tubes were mixed well, plugged with cotton wool and allowed to stand in boiling water for 10 minutes then cooled in acid water for 5 minutes 0.1 (100 µl) of solution was taken from "test" and "standard" labeled tubes. 0.1 ml (100 µl) of sulphuric acid was taken into a tube labeled as blank. 0.2 ml (200 µl) of color reagent was added into all tubes. All tubes were allowed to stand for 30 minutes at 37°C and tests were measured against reagent blank at 500 nm using Lab system Chemistry Analyzer FP-901. Mean values of lipid profiles in a group at 0 and 24 weeks and between different groups at 24 weeks were compared with the help of student's 't' test. At 24 weeks, after collecting blood samples, the rats were placed in an ether jar till death. The abdominal and thoracic cavities were opened through a median incision. The hearts and aortae were cleared. Biopsy specimens were kept in labeled jars for fixation containing 10% neutral buffered formalin. Then the specimens were subjected to gross and microscopic examination. Each aorta was rolled on itself with the intimal surface our, starting with the thoracic end while the hearts were opened transversely, grossly examined and 2 mm thick slices were cut for processing. The processing of tissues was done in an automatic processor. The paraffin blocks were made in L-shaped moulds. Sections of 3-4µm thickness were cut by rotary microtome. The sections were taken on albumenized slides. The sections were stained with hematoxylin and eosin, van Gieson, Verhoeff's elastic tissue, oil red o among other stains.

RESULTS

Difference of weight gain at 24 weeks was compared with control and relevant experimental groups. This was statistically significant when experimental group E1 was compared with control group C1 and highly significant when experimental group E 2 was compared with control group C2 (table 1). When the aortae were opened with there were neither fatty streaks nor atheromatous plaques in the aortae of all experimental and group animals. Narrowing of coronary arteries was not observed in any of the groups. Microscopic examination of sections from aorta and coronary arteries of different groups at 24 weeks was done and these revealed normal histology both in the control group and experimental group. Specifically in all groups no Stary's lesion was seen, van Gieson stain revealed normal pattern of collagen. Verhoeff's stain revealed normal pattern and distribution of elastic fibers. None of the aortae and coronary arteries in any group showed intracellular or extracellular subinitmal lipid on oil red O staining. The mean serum total cholesterol, serum triglycerides, total serum lipids among the control and experimental groups are shown in table 2.

Table 1: Comparison of mean±SD body weight (grams) in different groups at 0 and 24 weeks

Group	Male Rats (0 week)	Male Rats (26 weeks)	Female Rats (0 weeks)	Female Rats (24 weeks)	Total (0 weeks)	Total (24 weeks)
C1	200.8±18.8	381.6±11.3	174.2±12.4	288.0±16.3	187.5±20.6	335.0±50.5
E1	202.5±15.4	323.3±24.6	165.0±7.1	276.6±6.1	183.8±22.6	300.0±29.7
C2	205.8±17.4	305.8±8.6	160.0±10.5	273.3±8.3	182.9±27.6	289.5±18.8
E2	206.6±18.6	295.0±12.2	168.3±10.3	260.0±7.4	187.5±24.6	277±20.3

P<0.001(Highly significant)

Table 2: Lipid profile in different groups at 24 weeks in mg/dl (Mean±SD)

Groups	Total Cholesterol	HDL	LDL	Total glycerides	Total lipids
C1	94.9±4.3	20.8±6.5	50.3±6.6	118.7±6.3	447.2±8.7
E1	95.6±3.5	26.4±4.3	45.9±4.1	121.6±4.1	447.1±8.9
C2	198.1±7.6	27.4±1.9	140.5±8.3	150.7±4.3	545.7±5.9
E2	195.3±6.3	38.7±4.0	126.0±4.0	148.3±4.7	546.7±7.6

DISCUSSION

Atherosclerotic disease (AD) is the leading cause of death worldwide.[11] South-Asians (SAs) show an increased risk of atherosclerosis and have the highest mortality rates from CAD than any other ethnic group[12]. The leading independent predictors of coronary events and death [systolic blood pressure, total/HDL-cholesterol ratio, followed by diabetes and (central) obesity] are related to the metabolic syndrome, estimated to prevail in 3-4% of adults aged 30 or over, and to underlie one-eighth of cases of coronary disease.[13] The increase in weight that occurred in rats fed high fat diet has been found in other studies as well[14,15]. No aortic plaques were found in the studied rats, this was true across all the groups- controlled or uncontrolled. This finding has also been substantiated by other studies, which did not find the occurrence of these plaques after 6-12 weeks of palm oil.[16-18] The beneficial effect of palm oil on coronary arteries is well known[19,20].

It is true that higher levels of TC and LDL-c were obtained which play a positive role in atherogenesis but at the same time, HDL-c level was also raised which has a protective role in the genesis of atherosclerosis. This has been found in the work done by other colleagues[21-23]. Other workers have shown that different dietary components can beneficially modulate free radical mediated oxidative stress induced by lipid peroxidation.[24] They also demonstrated that palm oil consumption reduces blood cholesterol in comparison with the traditional sources of saturated fats such as coconut oil, dairy and animal fats. In addition, palm oil consumption may raise HDL levels and reduce platelet aggregability[25]. Ramachandran and other workers found that serum lipid analysis showed significant increase in cholesterol, LDL-c+ VLDL-c and decrease in HDL-c levels in all the treated group of animals[24].

The allegation that palm oil consumption leads to raised blood cholesterol levels and is therefore atherogenic is without scientific foundation. Examination of the chemical and fatty acid composition of palm oil or its liquid fraction should convince most nutritionists that the oil has little cholesterol-raising potential.[25] Elson and other workers have found that a group of antioxidants, the tocotrienols, are present in both palm olein and red palm oil. These vitamin E-active constituents are potent suppressors of cholesterol biosynthesis; emerging data point to their anticarcinogenic and antithrombotic activities.[26] It has been shown that palm oil, obtained from a tropical plant, Elaeis guineensis contains 50% saturated fatty acids, yet it does not promote atherosclerosis and arterial thrombosis. The saturated fatty acid to unsaturated fatty acid ratio of palm oil is close to unity and it contains a high amount of the antioxidants, beta-carotene, and vitamin E[27].

REFERENCES

1. Stanescu R, Stanescu MR, Bold A, Mateescu GO. Cerebral microvascular changes induced by rich cholesterol and saturated fatty acid diet in Wistar rats. Romanian journal of morphology and embryology. Revue roumaine de morphologie et embryologie. 2013;54(1):151-6.
2. Sambanthamurthi R, Tan Y, Sundram K, Hayes KC, Abeywardena M, Leow SS, et al. Positive outcomes of oil palm phenolics on degenerative diseases in animal models. Br J Nutr 2011;106(11):1664-75.
3. Leow SS, Sekaran SD, Sundram K, Tan Y, Sambanthamurthi R. Oil palm phenolics attenuate changes caused by an atherogenic diet in mice. Eur J Nutr 2013;52(2):443-56.
4. Li F, Tan W, Kang Z, Wong CW. Tocotrienol enriched palm oil prevents atherosclerosis through modulating the activities of peroxisome proliferators-activated receptors. Atherosclerosis 2010;211(1):278-82.
5. Oguntibeju OO, Esterhuyse AJ, Truter EJ. Red palm oil: nutritional, physiological and therapeutic roles in improving human wellbeing and quality of life. Br J Biomed Sci 2009;66(4):216-22.

6. Adam SK, Soelaiman IN, Umar NA, Mokhtar N, Mohamed N, Jaarin K. Effects of repeatedly heated palm oil on serum lipid profile, lipid peroxidation and homocysteine levels in a post-menopausal rat model. J Med 2008;11(2):145-51.
7. Sundram K, Sambanthamurthi R, Tan YA. Palm fruit chemistry and nutrition. Asia Pacific J Clin Nutr 2003;12(3):355-62.
8. Sultan MT, Butt MS, Ahmad RS, Pasha I, Ahmad AN, Qayyum MM. Supplementation of Nigella sativa fixed and essential oil mediates potassium bromate induced oxidative stress and multiple organ toxicity. Pak J Pharma Sci 2012;25:175-81.
9. Kaatabi H, Bamosa AO, Lebda FM, Al Elq AH, Al-Sultan AI. Favorable impact of Nigella sativa seeds on lipid profile in type 2 diabetic patients. J Fam Comm Med 2012;19(3):155-61.
10. Sabzghabaee AM, Dianatkhah M, Sarrafzadegan N, Asgary S, Ghannadi A. Clinical evaluation of Nigella sativa seeds for the treatment of hyperlipidemia: a randomized, placebo controlled clinical trial. Medicinski Arhiv 2012;66:198-200.
11. Al-Omran M. Atherosclerotic disease and risk factor modification in Saudi Arabia: a call to action. Vascular Health Risk Management 2012;8:349-55.
12. Bainey KR, Jugdutt BI. Increased burden of coronary artery disease in South-Asians living in North America. Need for an aggressive management algorithm. Atherosclerosis 2009;204(1):1-10.
13. Onat A. Risk factors and cardiovascular disease in Turkey. Atherosclerosis 2001;156(1):1-10.
14. Wong WY, Poudyal H, Ward LC, Brown L. Tocotrienols reverse cardiovascular, metabolic and liver changes in high carbohydrate, high fat diet-fed rats. Nutrients 2012;4(10):1527-41.
15. Earl LK, Baldrick P, Hepburn PA. A 13-week feeding study in the rat with shea oleine and hardened shea oleine. Inter J Toxicol 2002;21(1):13-22.
16. Matthan NR, Dillard A, Lecker JL, Ip B, Lichtenstein AH. Effects of dietary palmitoleic acid on plasma lipoprotein profile and aortic cholesterol accumulation are similar to those of other unsaturated fatty acids in the F1B golden Syrian hamster. J Nutr 2009;139(2):215-21.
17. Pereira TA, Sinniah R, Das NP. Effect of dietary palm oil on lipoprotein lipases: lipoprotein levels and tissue lipids in rat. Biochem Med Metabol Biol 1990;44(3):207-17.
18. Rand ML, Hennissen AA, Hornstra G. Effects of dietary palm oil on arterial thrombosis, platelet responses and platelet membrane fluidity in rats. Lipids 1988;23(11):1019-23.
19. Mozaffarian D, Clarke R. Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils. Eur J Clin Nutr 2009;63 Suppl 2:S22-33.
20. Fattore E, Fanelli R. Palm oil and palmitic acid: a review on cardiovascular effects and carcinogenicity. Inter J Food Sci Nutr 2013;64(5):648-59.
21. Lambert MS, Avella MA, Botham KM, Mayes PA. The type of dietary fat alters the hepatic uptake and biliary excretion of cholesterol from chylomicron remnants. Br J Nutr 2000;83:431-8.
22. Singer P, Hoffmann P, Beitz J, Forster W, Wirth M, Godicke W. Serum triglycerides and HDL cholesterol from SHR after evening primrose oil and other polyunsaturated fats. Prostaglandins Leukotrienes Med 1986;22(2):173-7.
23. Surra JC, Barranquero C, Torcal MP, Orman I, Segovia JC, Guillen N, et al. In comparison with palm oil, dietary nut supplementation delays the progression of atherosclerotic lesions in female apoE-deficient mice. Br J Nutr 2013;109:202-9.
24. Ramachandran HD, Kusum R, Raina PL. Modulatory effect of dietary factors on iron-induced in vivo lipid peroxidation in liver and serum of albino rats. Indian J Experi Biol 2006;44(1):32-8.
25. Chong YH, Ng TK. Effects of palm oil on cardiovascular risk. Med J Malaysia 1991;46(1):41-50.
26. Elson CE. Tropical oils: nutritional and scientific issues. Crit Rev Food Sci Nutr 1992;31:79-102.
27. Edem DO. Palm oil: biochemical, physiological, nutritional, hematological, and toxicological aspects: a review. Plant Foods Hum Nutr 2002;57(3-4):319-41.