

Morphological Changes in Aorta and Coronary Arteries of Albino Rats Fed on Palm Kernel Oil and Nigella Sativa

ZAFAR IQBAL¹, ABDUL SATTAR², SAJJAD HAIDER³, ⁴MUHAMMAD TAYYAB, ⁵NASEER AHMED CHAUDRHY

ABSTRACT

Objective: To study the morphological changes in aorta and coronary arteries of albino rats fed on palm kernel oil and Nigella sativa

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. The rats were divided into four groups labeled as C₁, E₁, C₂ and E₂. Each group had 15 rats. Two cc blood was drawn from each rat. A rat ear punch was used for this purpose. All tubes were allowed to stand for 30 minutes at 37°C and tests were measured against reagent blank at 500 nm using Labsystem Chemistry Analyser FP-901.

Results: The mean serum total lipid levels±SD showed increase in serum total lipid levels at 0 week and 24 weeks. The difference was statistically very highly significant.

Conclusion: Palm kernel oil increased the serum 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.

Keywords: Aorta, coronary arteries, albino rats, palm kernel oil, nigella sativa

INTRODUCTION

Atherosclerosis accounts for more than half of all deaths in the western world. No disease in the United States or other developed countries is responsible for more deaths. Although any artery may be affected, the major targets are the aorta, the coronary and cerebral arteries. Coronary atherosclerosis induces ischaemic heart disease (IHD) and when these lesions are complicated by thrombosis, myocardial infarction occurs, which alone is responsible for 20-25% of all deaths in United States¹. When atherosclerosis is compounded by obesity, hypertension, smoking and diabetes, the death toll rises abruptly by arterial wall^{2,3}. Out of these risk factors hyperlipidaemia is of prime significance because atherosclerosis starts from childhood^{4,5}. The hyperlipidaemias may be primary or secondary and the prevalence of primary hyperlipidaemias as compared to secondary is meager one, so secondary type gets the upper hand in causing atherosclerosis⁶.

Intake of palm kernel oil increases secretion of very low density lipoprotein (VLDL) particles⁷. Animals fed on palm kernel oil showed higher levels of plasma total cholesterol (TC) than animals fed on

corn oil⁸. Human beings feel very satisfied and interiorly confident when being treated with some traditional medicine. Out of the traditional remedies, the prescriptions of Nigella sativa for the treatment of various diseases are well known since prehistoric era⁹. Amongst these various pharmacological effects of Nigella sativa, its anti-hyperlipidaemic effect was studied in the present experimental design. Palm kernel oil which contains mainly saturated fatty acids was included in the diet of experimental animals. The antiatherogenic effect of Nigella sativa associated with hyperlipidaemic state was thus studied.

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 rats were divided into four groups labeled as C₁ (control group fed on 3% palm kernel oil), E₁ (experimental group fed on 3% palm kernel oil with Nigella sativa), C₂ (control group fed on 20% palm kernel oil with cholic acid and thiouracil acid) and E₂

^{1,2}Department of Pathology, Khawaja Muhammad Safdar Medical College Sialkot, ³Department of Pathology, Allama Iqbal Medical College Lahore, ^{4,5}Department of Pathology, Postgraduate Medical Institute Lahore
Correspondence to Dr. Zafar Iqbal,
e-mail: ziqbalguman@gmail.com

(experimental group fed on 20% palm kernel oil with cholic acid, thiouracil acid and *Nigella sativa*). Each group had 15 albino rats. 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. 2cc blood was drawn from each rat. For the identification of the individual animals, the ear hole method of marking was adopted (Arrington 1978). A rat ear punch was used for this purpose. Clean and dry test tubes were labeled for each sample and standard 0.05ml (50 μ l) of each sample and standard were taken and put into tubes labeled "test" and "standard". 2ml (2000 μ l) 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 μ l) of solution was taken from "test" and "standard" labeled tubes. 0.1ml (100 μ l) of

sulphuric acid was taken into a tube labeled as blank. 0.2ml (200 μ l) of colour 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 Labsystem Chemistry Analyser 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.

RESULTS

The mean \pm SD values (mg/dl) of serum lipid profile in different groups, at 0 and 24 weeks are given Table 1. The mean serum total lipid levels \pm SD showed increase in serum total lipid levels at 0 week and 24 weeks. The difference was statistically very highly significant (Table 2).

Table 1: Lipid profile in different groups at 0 week and 24 weeks

Groups	0 week					24 weeks				
	TC	HDL-c	LDL-c	TG	T. lipids	TC	HDL-c	LDL-c	TG	T. lipids
C ₁	80.6 \pm 5.6	22.2 \pm 4.1	43.5 \pm 8.5	73.9 \pm 7.1	400.3 \pm 6.4	94.9 \pm 4.3	20.8 \pm 6.5	50.3 \pm 6.6	118.7 \pm 6.3	447.2 \pm 8.7
E ₁	80.8 \pm 4.5	20.8 \pm 3.3	44.3 \pm 4.9	75.8 \pm 6.6	395.2 \pm 6.9	95.6 \pm 3.5	26.4 \pm 4.8	45.9 \pm 4.1	121.6 \pm 4.1	447.1 \pm 8.9
C ₂	81.6 \pm 4.4	21.2 \pm 3.2	45.5 \pm 5.7	74.4 \pm 7.2	398.5 \pm 4.2	198.1 \pm 7.6	27.4 \pm 1.9	140.5 \pm 8.3	150.7 \pm 4.3	545.7 \pm 5.9
E ₂	80.4 \pm 5.1	22.1 \pm 3.8	42.3 \pm 6.5	74.1 \pm 6.8	398.3 \pm 5.9	195.3 \pm 6.8	38.7 \pm 4.0	126.9 \pm 4.0	148.3 \pm 4.7	546.7 \pm 7.6

Table 2: Comparison of mean serum total lipid levels (mg/dl) at 0 and 24 weeks in various groups

Groups	0 week	24 weeks
C ₁	400.3 \pm 6.4	447.2 \pm 8.7
E ₁	395.2 \pm 6.9	447.1 \pm 8.9
C ₂	398.5 \pm 4.2	545.7 \pm 5.9
E ₂	398.3 \pm 5.9	546.6 \pm 7.6

*p<0.001

DISCUSSION

In the second half of the twentieth century atherosclerotic coronary heart disease (CHD), has become an epidemic in most industrialized countries and threatens to overwhelm the developing countries as well. Atherosclerosis is the pathological process that underlies most cases of CHD, which produces extensive sickness, disability and mortality, much of it occurring in the prime of life, as well as in older persons¹⁰. It prevails from high to low class society and affects all ages especially the middle age group^{11,12}. Increased levels of serum low density lipoprotein cholesterol (LDL-c) with positive correlation and high density lipoprotein cholesterol (HDL-c) levels with negative correlation pertaining to atherosclerosis has been found by many workers^{13,14}.

The gross and microscopic examination of aortae and coronary arteries revealed no change in any of rats. These results reconcile with the reports of Weisgraber et al¹⁵ and Joris et al¹⁶ These workers

concluded that albino rat does not develop atherosclerosis in natural state and severe hypercholesterolaemia is required to induce atherosclerosis. 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. The results are in partial conformity with Newman et al¹⁷ and Solberg & Strong.¹⁸ There was no significant difference in mean serum levels of TC, TG and total lipids. Serum HDL-c level was increased in group E₂ as compared to C₂ which was very highly significant while serum LDL-c level was decreased which also was very highly significant. These results are in complete conformity with Dahri.¹⁹ On gross and microscopic examination of aortae and coronary arteries no lesion was seen. This may be due to decreased LDL-c and increased HDL-c levels as also reported by other workers.^{20,21} The atheroma formation was prevented by decreasing atherogenic LDL-c level and raising HDL-c level. The possible mechanism of LDL-c decrease may be the enhancement of LDL-receptors on hepatocytes. The LDL-c may have been taken up by the hepatocytes. As already reported by Ali and Blunden²², the hepatocytes might utilize cholesterol to form bile acids and then secreted into the bile. The possible mechanisms of HDL-c raising effect of *Nigella sativa* are not known.

CONCLUSION

Palm kernel oil (containing 75% saturated fatty acid) raises serum levels of total cholesterol and low density lipoprotein cholesterol. It also decreases the serum high density lipoprotein cholesterol level. So it has a strong atherogenic potential. *Nigella sativa* increases the serum levels of total cholesterol and low density lipoprotein cholesterol and it prevents the process of atherogenesis. Although modest hypercholesterolaemic state can be achieved with high fat (20% palm kernel oil) diet along with cholic acid and thiouracil but this is not of that intensity to produce the atherosclerotic lesions.

REFERENCES

1. Kumar V, Cotran RS, Robins SL. Robins basic pathology. 7th ed. London: WB Saunders, 2005; 277-302.
2. Lewis B, Chait A, Oakley CMO, Wootton IDP, Krikler DM, Onitiri A, Sigurdsson G, February A. Serum lipoprotein abnormalities in patients with ischaemic heart disease: comparisons with a control population. *Br Med J* 1974; 3(5929): 489-93.
3. Ahmed I, Khan MN, Ahmed MS, Khurshid R, Naveed AK. Over all combined effect of various life styles on lipids and lipoproteins levels of CAD patients. *Pak J Pathol* 2001;12(2):27-30.
4. Laskarzewski P, Morrison JA, deGroot I, Kelly KA, Mellies MJ, Khoury P, Glueck CJ. Lipid and lipoprotein tracking in 108 children over a four-year period. *Pediatrics* 1979;64(5):584-91.
5. Hadfield SG, Horara S, Starr BJ, Yazdgerdi S, Marks D, Bhatnagar D, et al. Family tracing to identify patients with familial hypercholesterolaemia: the second audit of the Department of Health Familial Hypercholesterolaemia Cascade Testing Project. *Ann Clin Biochem* 2009;46(Pt 1):24-32.
6. Baruth M, Wilcox S, Egan BM, Dowda M, Laken M, Warren TY. Cardiovascular disease risk factor clustering among African American adults. *Ethn Dis* 2011;21(2):129-34.
7. Abdel-Fattah G, Fernandez ML, McNamara DJ. Regulation of guinea pig very low density lipoprotein secretion rates by dietary fat saturation. *J Lipid Res* 1995;36(6):1188-98.
8. Fernandez ML, Abdel-Fattah G, McNamara DJ. Dietary fat saturation modifies the metabolism of LDL subfractions in guinea pigs. *Arterioscler Thromb* 1993;13(10):1418-28.
9. Al-Tayyab AA. Treatment by *Nigella sativa*: experience and examples. *Annals* 1991;1:10-95.
10. WHO. Prevention in childhood and youth of adult cardiovascular diseases: time for action. Geneva: World Health organization, 1990.
11. Qureshi AA, Qureshi N, Wright JJ, Shen Z, Kramer G, Gapor A, Chong YH, DeWitt G, Ong A, Peterson DM, et al. Lowering of serum cholesterol in hypercholesterolemic humans by tocotrienols (palmvitee). *Am J Clin Nutr* 1991;53(4 Suppl):1021S-6.
12. Bhatti MS, Raja RJ, Shakoor M. Relation of cholesterol and triglycerides levels in smokers and obese individuals with ischaemic heart disease. *Pak J Health* 1993; 30: 3-6.
13. Grundy SM. Monounsaturated fatty acids, plasma cholesterol, and coronary heart disease. *Am J Clin Nutr* 1987;45(5 Suppl):1168-75.
14. Canoui-Poitrine F, Luc G, Bard JM, Ferrieres J, Yarnell J, Arveiler D, et al. Relative contribution of lipids and apolipoproteins to incident coronary heart disease and ischemic stroke: the PRIME Study. *Cerebrovasc Dis* 2010;30(3):252-9.
15. Weisgraber KH, Innerarity TL, Mahley RW. Role of lysine residues of plasma lipoproteins in high affinity binding to cell surface receptors on human fibroblasts. *J Biol Chem* 1978;253(24):9053-62.
16. Joris I, Zand T, Nunnari JJ, Krolikowski FJ, Majno G. Studies on the pathogenesis of atherosclerosis. I. Adhesion and emigration of mononuclear cells in the aorta of hypercholesterolemic rats. *Am J Pathol* 1983;113(3):341-58.
17. Newman WP 3rd, Freedman DS, Voors AW, Gard PD, Srinivasan SR, Cresanta JL, et al. Relation of serum lipoprotein levels and systolic blood pressure to early atherosclerosis. The Bogalusa Heart Study. *N Engl J Med* 1986;314(3):138-44.
18. Solberg LA, Strong JP. Risk factors and atherosclerotic lesions. A review of autopsy studies. *Arteriosclerosis* 1983;3(3):187-98.
19. Dahri AH. Study of morphological changes in aorta and coronary arteries of albino rats fed on atherogenic diet with *Nigella sativa* and palm oil. [Thesis] Lahore: University of the Punjab, 1996; 123-30.
20. Havel RJ, Rapaport E. Management of primary hyperlipidemia. *N Engl J Med* 1995;332(22):1491-8.
21. McCarthy M. Lipid-lowering therapy may reduce thrombosis risk. *Circulation* 1997;95: 825-30.
22. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. *Phytother Res* 2003;17(4):299-305.