

## Effect of Saturated Fat Diet and Olive Oil in Thoracic Aorta of Albino Rats

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### ABSTRACT

**Aim:** To see the effect of use of oils and fats in the control and experimental groups regarding morphological changes in the thoracic aorta of experimental animals.

**Materials and methods:** 60 albino rats of 32 week old were included in this study, with equal ratio of males and females at optimum atmospheric and hygienic conditions with sufficient quantity of food and water available twenty four hours. A ventral midline abdominothoracic incision was made after replacing albino rats in the ether till death. Aorta was then dissected out and biopsy specimens were kept in labeled jar for fixation, containing formalin and formal calcium.

**Results:** A total 60, 32 weeks albino rats were studied for morphological changes in thoracic aorta. The changes of control and experimental groups were examined.

**Conclusion:** we concluded that the experimental animals which were fed olive oil had less atheromatous lesions, and the group fed with saturated fat diet especially butter showed atheroma formation. Thus olive oil at least can be recommended for the high risk patients of IHD.

**Key words:** Olive oil, thoracic aorta, albino rats

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### INTRODUCTION

Atherosclerosis is a special type of thickening and hardening of the medium sized and large arteries that account for a large proportion of heart disease<sup>1</sup>. While Chazove et al<sup>2</sup> stated that the earliest and most prominent sign of atherosclerosis at the cellular level is an accumulation of lipids in the cytoplasm of arterial sub-endothelial cells. Castelli<sup>3</sup> reported that three fourth of the people on earth do not get atherosclerosis and therefore do not get CHD, like people living in the rural areas of Asia, Africa and South America, due to their low fat diet, but soon as they move to Singapore or Cape Town, the incidence of CHD begins to rise due to their high fat diet.

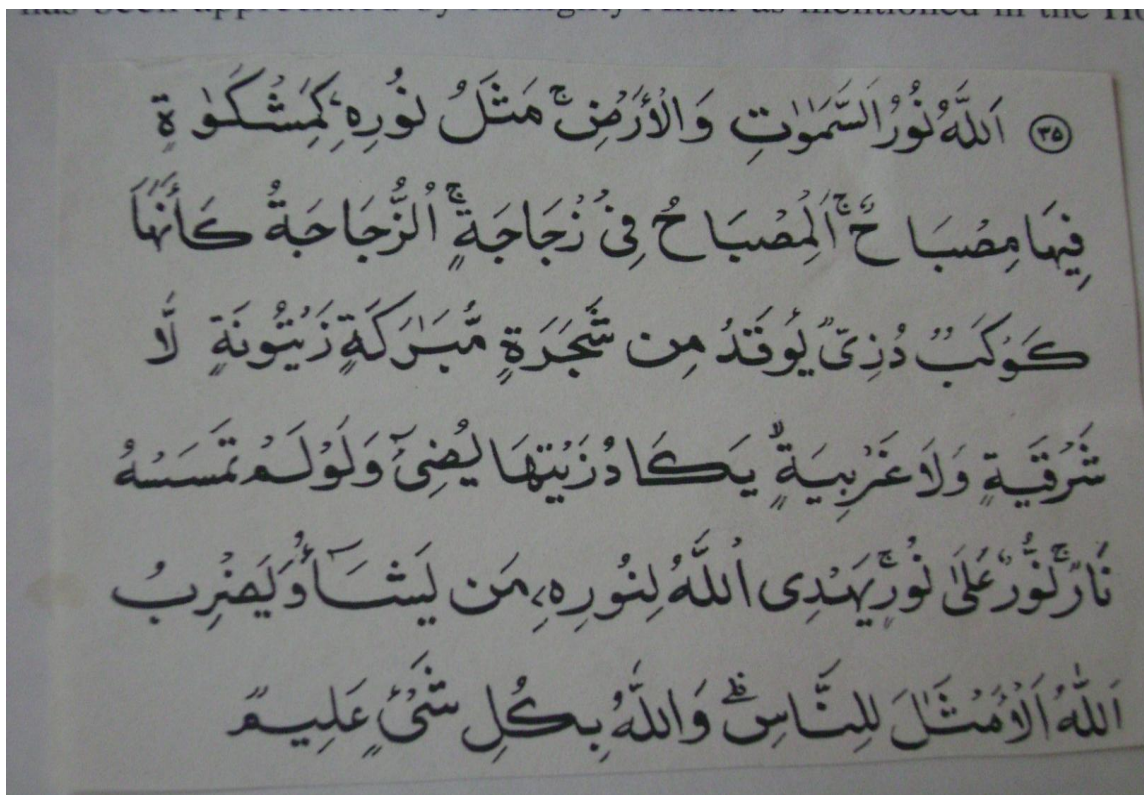
The first morphological change in the initiation of atherosclerosis is the migration of monocytes through an intact endothelial surface into the intima, these monocytes subsequently take up low density lipoprotein and become lipid filled macrophages called foam cells<sup>6</sup>. Whereas Camejo et al<sup>7</sup> refers the progressing atherosclerotic lesion as to a continuous extracellular and intracellular accumulation of lipoproteins and lipids. The atherosclerotic plaque then grows by the production of collagen fibers and smooth muscle cells<sup>8</sup>. Stary<sup>9</sup> clarified the segmental ultrastructural changes that occur in spontaneous

atherosclerosis, particularly in the early lesions. In an autopsy study of the coronary arteries and aorta in young people, The presence of foam cells in the intima in type I; addition of smooth muscle cells containing lipid droplets and by scattered extracellular lipid in type II; The appearance of multiple extracellular lipids in type III and the appearance of atheromata in type IV starry lesions. Fatty streak is the earliest lesion of atherosclerosis, commonly found in children and is grossly flat, lipid-rich lesion consisting of both macrophages and some smooth muscle fibres<sup>10</sup>. While Bondjers et al<sup>11</sup> stated that the atheromatous lesion which develops in the arterial intima, reveals lipid deposition, connective tissue formation, smooth muscle cell proliferation and infiltration of inflammatory cells. Apgar et al<sup>12</sup> observed saturated fats and cholesterol in the American diet to be the cause of CHD. About Olive oil almighty Allah has mentioned in the holy Quran<sup>13</sup> containing God is the light of the heavens and the earth, the parable of his light. Is as if there were a niche And within it a lamp: The lamp enclosed in glass: The glass as it were A brilliant star: Lit from a blessed tree, An olive, neither of the east Nor of the west, Whose oil is well-nigh Luminous, Though fire scarce touched it Light upon light ! God doth guide, whom he will, to his light God doth set forth parables, for men and God, Doth know all things, Many experimental studies were carried out on albino rats at post graduate medical institute Lahore, and were encouraging, which encourages us to see the effect of olive oil and saturated fat diet in Aorta of albino rats.

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## MATERIALS AND METHODS

Sixty albino rats of 32 weeks age having similar ratio of males and females were kept into five groups of 12 rats each, and were maintained under optimum atmospheric and hygienic conditions, with food and water available twenty four hours. The initial experimental diet (table 1) was given for 12 weeks and then atherogenic supplement, namely cholesterol, propylthio-uracil and bile salt was added to the experimental diet for 12 weeks as shown in (table 2). At the end of the experiment i- e after 32 weeks aorta was then dissected out, biopsy

specimen were kept out in labeled jar for fixation formal saline and formal calcium.

Table 1: Percentage composition of synthetic Diet for Rats.(n=100) (Malathi et al 1963).

Ingredients	%age
Casein	20.0
Maize Starch	60.0
Cane Sugar	10.0
Corn Oil	5.0
Choline & Methionine	0.5
Mineral Mixture	3.5
Vitamin Mixture	1.0

Table 2: Percentage composition of the experimental Diets (g).

Ingredients	I (Control)	II Control	III (Control)	IV (Control)	V (Control)
Wheat starch	60.0	58.6	43.6	43.6	43.6
Casien	20.0	20.0	20.0	20.0	20.0
Cane sugar	0.0	10.0	10.0	10.0	10.0
Olive oil	1.0	1.0	20.0	-	-
Ghee	1.0	1.0	-	20.0	-
Butter	1.0	1.0	-	-	20.0
Choline & methionine	0.5	0.5	0.5	0.5	0.5
Mineral mixture	3.5	3.5	3.5	3.5	3.5
Vitamin mixture	1.0	1.0	1.0	1.0	1.0
Cholesterol	-	1.0	1.0	1.0	1.0
Propylthiouracil	-	0.1	0.1	0.1	0.1
Bile Salt	-	0.3	0.3	0.3	0.3

RESULTS

Gross and microscopic changes were observed in the thoracic aorta of the animal after 32 weeks of experimental diet (table 3, 4). In control group I atherogenic diet, on gross examination, no fatty streak nor atheromatous plaque was seen on microscopic examination no atherosclerotic lesion was seen. Control group II with atherogenic diet revealed on gross examination, fatty streaks (photograph No: 1). Seen in eight out of twelve animals including five. (41.66%) male and three (25%) females (table 5).

Table 3: Morphological changes in thoracic aorta of 60 cases in relation to different groups and sex (Gross Findings)

Groups	Fatty streak		Atheromatous plaque	
	M	F	N	F
I	-	-	-	-
II	5	3	-	-
III	-	-	-	-
IV	6	6	-	-
V	-	-	6	6

Key:

Group I= Control without atherogenic diet  
 Group II= Control with atherogenic diet  
 Group III= Olive oil diet  
 Group IV= Ghee  
 Group V= Butter

Table 4: Morphological changes in thoracic aorta of 60 Cases in Relation to different Groups and sex

Group	Sex	H&E Stain				Oil Red O Stain		Van Gieson Stain			Verhoeff's Elastic Stain					
		Number of cases with stray lesion.				Fat		Proliferation of Collagen Fibers			Thickness of intima			Total wall thickness		
		I	II	III	IV	Number Of Cases Present	Number Of Cases Absent	Mild	Moderate	Severe	Mild	Moderate	severe	Mild	Moderate	Severe
I	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II	M	1	5	-	-	5	1	6	-	-	6	-	-	6	-	-
	F	3	3	-	-	3	3	6	-	-	6	-	-	6	-	-
III	M	6	-	-	-	-	6	6	-	-	6	-	-	6	-	-
	F	6	-	-	-	-	6	6	-	-	6	-	-	6	-	-
IV	M	6	-	-	-	-	-	-	6	-	-	6	-	-	6	-
	F	6	-	-	-	-	-	-	6	-	-	6	-	-	6	-
V	M	-	-	-	6	6	-	-	-	6	-	-	6	-	-	6
	F	-	-	-	6	6	-	-	-	6	-	-	6	-	-	6



Table 5: Number and Percentage Distribution in Morphological Changes of Aorta in Relation to Group II with Atherogenic Diet (Gross findings) figures in parenthesis indicate Percentage.

Blood vessels	Sex	Fatty Streak	Atheromatous plaque
Thoracic	M	5(41.66%)	-
Aorta	F	3(25%)	-

## ORIGINAL ARTICLE

Table 6: Number & percentage Distribution of Morphological changes in aorta in Relation to control group- II with Atherogenic Diet (Microscopic findings) figures in Parenthesis indicate percentage.

	Sex	H & E Stain				Oil red stain		Van Gieson stain			Verhoeff's Elastic Stain					
		No. of cases with stray lesion				Fat		Proliferation of Collagen fibres			Thickness of intima			Total wall Thickness		
		I	II	III	IV	Present	Absent	Mild	Mod	Sev	Mild	Mod	Sev	Mild	Mod	Sev
Thoracic	M	1 (8.33%)	5 (41.66%)	-	-	(41.66%)	(8.33%)	6 (50%)	-	-	6 (50%)	-	-	6 (50%)	-	-
Aorta	F	3(25%)	3(25%)	-	-	3(25%)	3(25%)	6 (50%)	-	-	6 (50%)	-	-	6 (50%)	-	-

On microscopic examination H&E stain revealed four cases 1 (8.33%) male and 3 (25%) female, of stary I lesion and 5 (41.66%) male and 3(25%) female of staryII lesion, van-Gieson stain showed mild internal wall thickness in all the twelve cases. Oil red O stain showed presence of fat in eight out of twelve cases (table 6). Photograph (2+3). The results were statistically very highly significant as compared to control group I.

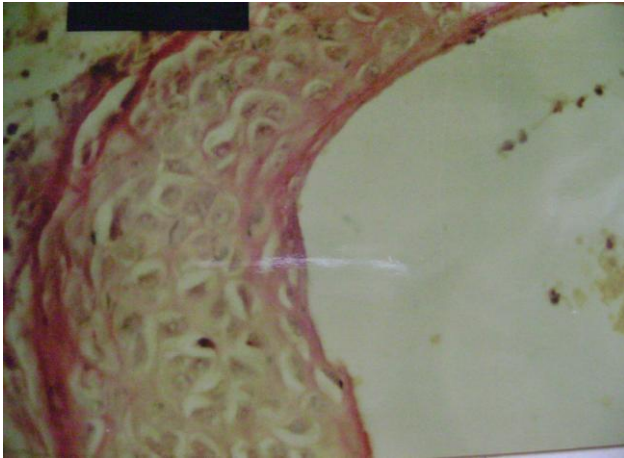
Olive oil group on gross examination showed no fatty streak nor atheromatous plaque in any of the twelve cases, on microscopic examination H+E stain revealed stray I lesion in all the twelve cases. Van-Gieson stain revealed mild proliferation of collagen fibres, verhoeffs elastic stain, showed mild intimal wall thickness and total wall thickness in all the twelve cases. Oil red O stain showed absence of fat in all the twelve cases (table 7) and photograph 4 and 5.

Among saturated fatty diet hydrogenated vegetable oil i-e Ghee on gross examination showed fatty streaks in all the twelve cases. On microscopic examination H+E stain revealed stray II lesion in all the twelve cases, van Gieson stain revealed moderate proliferation of collagen fibres, verhoeffs elastic stain showed moderate intimal as well as total wall thickness in all the cases. Oil red O stain showed presence of fat in all the twelve cases (table 8).

Butter group on gross examination of thoracic aorta showed atheromatou plaque in all the twelve cases, on microscopic examination of thoracic aorta on H+E stain revealed stary IV lesion in all the twelve cases, van-Gieson stain revealed severe proliferation of collagen fibres, verhoeffs elastic stain severe intimal wall and total wall thickness in all the twelve cases. Oil red O stain showed presence of fat in all the twelve cases. (table 9)and (photograph- 6,7,8,) The results were statistically very highly significant as compared to control group II.

Table 7: Number and percentage Distribution of Morphological changes in Aorta in relation to Group- III I-E. Olive oil diet. (Microscopic Finding) Figures in Parenthesis indicate percentage.

	Sex	H & E Stain				Oil red stain		Van Gieson stain			Verhoeff's Elastic Stain					
		No. of cases with stray lesion				Fat		Proliferation of Collagen fibres			Thickness of intima			Total wall Thickness		
		I	II	III	IV	Present	Absent	Mild	Mod	Sev	Mild	Mod	Sev	Mild	Mod	Sev
Thoracic	M	6(50%)	-	-	-	-	6(50%)	6(50%)	-	-	6(50%)	-	-	6(50%)	-	-
Aorta	F	6(50%)	-	-	-	-	6(50%)	6(50%)	-	-	6(50%)	-	-	6(50%)	-	-



Photograph 2: Van Gieson Stain of cross section of aorta. Fibroblast and collagen fibers proliferation) x 40. Control group II.



Photograph 3: Oil Red O Stain of Aorta (lipids droplets) x 40. Control group II.

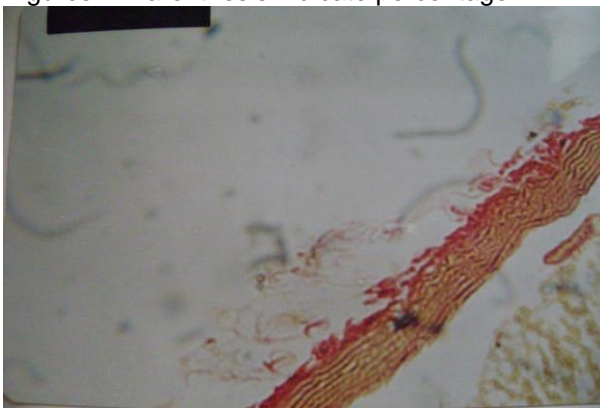
Table 8: Number and percentage Distribution of Morphological changes in Aorta in relation to Group- IV I-E. Ghee diet. (Microscopic Finding) Figures in Parenthesis indicate percentage.

	Sex	H & E Stain				Oil red stain		Van Gieson stain			Verhoeff's Elastic Stain					
		No. of cases with stray lesion				Fat		Proliferation of Collagen fibres			Thickness of intima			Total wall Thickness		
		I	II	III	IV	Present	Absent	Mild	Mod	Sev	Mild	Mod	Sev	Mild	Mod	Sev
Thoracic	M	-	6(50%)	-	-	6(50%)	-	-	6(50%)	-	-	6(50%)	-	-	6(50%)	-
Aorta	F	-	6(50%)	-	-	6(50%)	-	-	6(50%)	-	-	6(50%)	-	-	6(50%)	-

Table 9: Number and percentage Distribution of Morphological changes in Aorta in relation to Group- V I-E. Butter diet. (Microscopic Finding)

	Sex	H & E Stain				Oil red stain		Van Gieson stain			Verhoeff's Elastic Stain					
		No. of cases with stray lesion				Fat		Proliferation of Collagen fibres			Thickness of intima			Total wall Thickness		
		I	II	III	IV	Pre sent	Absent	Mild	Mod	Sev	Mild	Mod	Sev	Mild	Mod	Sev
Thoracic	M	-	-	-	6(50%)	6(50%)	-	-	-	6(50%)	-	-	6(50%)	-	-	6(50%)
Aorta	F	-	-	-	6(50%)	6(50%)	-	-	-	6(50%)	-	-	6(50%)	-	-	6(50%)

Figures in Parenthesis indicate percentage.



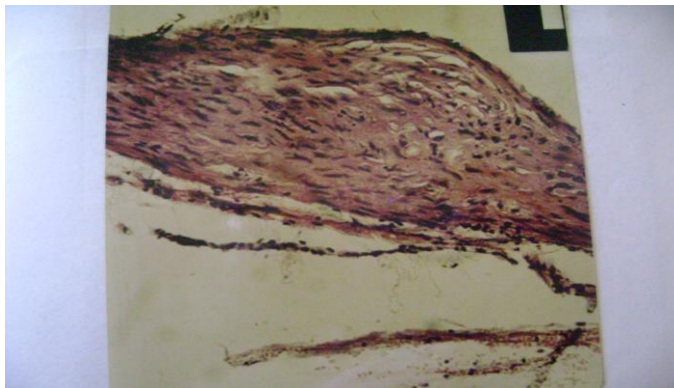
Photograph 4: Van Gieson Stain of Aorta x 20 olive oil group



Photograph 5: Verhoeff's elastic stain of aorta x 40. Olive oil group.



Photograph 6: Gross examination of Aorta with atheromatous plaque (arrowed) butter fat group.



Photograph 7: H+E Stain of Aorta (atheroma formation x 40 butter fat group).



Photograph 8: Verhoeff's elastic stain with van Gieson Counter stain x 40 butter fat group

## DISCUSSION

This study was conducted to examine the morphological changes of thoracic aorta in consequence to olive oil with saturated fat diet i.e hydrogenated vegetable oil (ghee) and butter fat. In this study on gross examination in thoracic aorta, the fatty streaks were present in (twenty) 20 out of sixty (60) animals and atheromatous plaques were seen in

twelve (12) out of sixty animals. Microscopic examination of the thoracic aorta showed stray I lesion 16 out of 60 animals, stray II lesions in 20 out of 60 animals, and stray IV lesion in 12 out of 60 animals, and severe intimal wall thickness in 12 out of 60 animals and presence of fat 24 out of 60 animals, and these results suggests that olive oil which show no presence of fat in the intimal wall of thoracic aorta has a protective role in atherosclerosis. Recently phillips and lough<sup>14</sup> in their experimental study in mice after 10-20 weeks on consuming a diet enriched with saturated fat and cholesterol, showed fatty streak like lesions in the ascending aorta, continuing the atherogenic diet for further 15 weeks led to the development of fibro fatty lesions which have many of the characteristics of human atheromatous plaque. Grundy<sup>15</sup> reported that populations with high rates of CHD show rich total fat, saturated fatty acids and cholesterol in their diet. The results of the present work are in agreement with the results of all the above reports.

The wide spread use of oils, and fats, and their relation to the higher incidence of coronary heart disease is of interest in the modern trend of life. The teachings of the creator Allah and his messenger Holy prophet Muhammad (peace be upon him) who took endless pains to guide humanity to the destination of ultimate success and who enabled us to recognize our creator, need not any scientific proof but their teachings provide an incentive to explore the un-explained and un-earth potentials. The scientific explanations would further strengthen the faith of the believers.

## CONCLUSION

The above facts and figures regarding olive oil in the diet has a favourable effect, so it can be recommended for the Ischemic heart disease individuals.

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