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RESEARCH ARTICLE

HISTOLOGICAL CHANGES IN AORTIC WALL OF RABBITS FED ON DIETS RICH IN CHOLESTEROL AND OLIVE OIL

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ABSTRACT

Dietary fat is one of the most important environmental factors associated withthe incidence of cardiac diseases; diets high in cholesterol and saturated fat havebeen shown to promote atherosclerosis. Conversely, dietary polyunsaturated fats have been shown to reduce its development in several species. The role of various dietary lipids in the control of atherosclerosis is of considerable interest as dietary lipids play an important role in biochemical and physiological processes of vascular tissues and heart function. The present work aimed to study the protective effect of olive oil against the histopathological alterations induced by cholesterol in the aorta of rabbit. For the study 18 male Indian rabbits weighing 2-2.5 kg were randomly divided in 3 experimental groups; group A rabbits served as control, group B rabbits were fed on increased cholesterol diets and group C rabbits fed on olive oil. On histological examination after 60 days fibrofatty plaques were observed in aortic wall of only group B rabbits.

INTRODUCTION

Dietary fat is one of the most important environmental factors associated with the incidence of cardiac diseases; diets high in cholesterol and saturated fat have been shown to promote atherosclerosis (1). Conversely, dietary polyunsaturated fats have been shown to reduce its development in several species (2, 3). The dietary lipids play an important role in biochemical and physiological processes of vascular tissues and heart function.

This is surprising since traditional Mediterranean diets are followed in geographical areas that have a low incidence of coronary heart disease and they combine a relatively low cholesterol diet and the use of olive oil as the main source of fat (4). Support for this approach comes from several randomized controlled trials demonstrating that diet modification can reduce coronary events (5) and improve coronary anatomy relative to a Western diet (6).

Oleic acid is an isomer of elaidic acid and the dominant fatty acid in olive oil. TFA formation during the process of partial hydrogenation produces a considerable amount of elaidic acid (7, 8, 9). High consumption of olive oil may protect against myocardial infarction which is the hallmark of ischemic heart disease (10). More specifically, recent reviews have summarized the effects of olive oil on cardiovascular risk factors (11, 12).

Several components of olive oil have beneficial health effects on the atherosclerotic and thrombotic pathways, which include lipid oxidation, hemostasis, platelet aggregation, coagulation, and fibrinolysis (13). It was reported, that the diet and lifestyle of the Mediterranean populations have led to decreased rates of cancer, diabetes, and heart disease (14). As the protective properties of the Mediterranean diet are in part due to the consumption of antioxidant-rich olive oil (15, 16). Olive oil (which is the main source of unsaturated fatty acids in mediterranean region and also a nutritional regimen gaining ever increasing reknown for its beneficial effects on inflammation, cardiovascular diseases and cancer) is not an important part of tropical diet epecially in North Indian region, olive oil is very rarely used as a cooking medium.

We have used rabbits as study material as experiments on animals can be performed in relatively short time. The clinical results are supported by post mortem examination of the arterial system.

MATERIAL AND METHODS

A total of 18 male Indian rabbits weighing 2-2.5 kg were randomly divided in 3 experimental groups and kept one per cage, under a 12:12 hour light: dark cycle with free access to food (150 g/day) and water.

Group A (Control Rabbits)

This group served as control was fed for 60 days on a diet containing 97% *s*tandard chow and 3% lard.

Group B (Rabbits Fed on Increased Cholesterol Diets)

Rabbits of this group were fed for 60 days on 95.7% standard chow, 3% lard and 1.3% cholesterol.

Group C (Rabbits Fed on Olive Oil)

Rabbits were fed for 60 days on the diet of group A plus 1.75g /100 g diet /day. All diets were kept in darkness at 4 °C to avoid peroxidation until use. The animals will be weighed weekly.

Histological Analysis of Aortic Wall

Entire aortas were dissected out and 1 cm of each of the arch of aorta, thoracic and abdominal aorta was selected.
For histological examinations, buffered 4% formaldehyde-fixed, paraffin-embedded tissue sections were stained with hematoxylin and eosin (H&E). Samples were evaluated for the presence of the fatty streak, medial calcifications and development of fibrous plaque.

Microscopic Examination

Microscopic examination was done with light microscope under 10x and 40x power. After examination of different sections, findings related to different layers of aorta in different parts of sections were studied and compared.

Observations

Histological Findings

Figures 1A & B depict the haematoxylin and eosin stained section of aorta of Group A (control). Here normal histological findings of aorta can be appereciated. Tunica intima, media, and adventitia, all are well diferrentiated. No fibrofatty plaques are observed.

Figures 2 A represents haematoxylin and eosin stained section of Group B rabbits which were fed on high cholesterol diet. Here fibrofatty plaques are observed in tunca media. In figure 8 A, a fatty streak could also be seen in a small region in intima.

Figures 3A and B haematoxylin and eosin stained section of aorta of Group C rabbit (fed on olive oil) are observed. Normal histolgical finding of aorta can be observed in both these sections

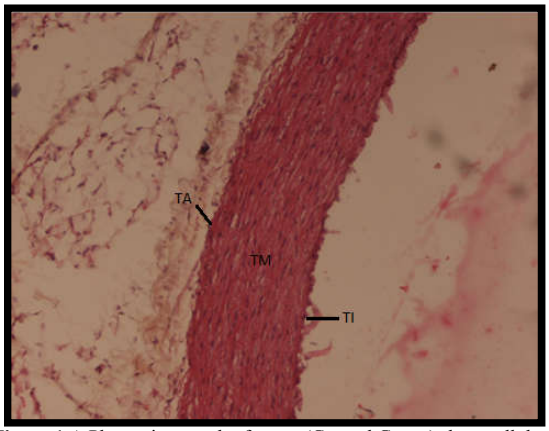

Figure 1 A Photomicrograph of aorta (Control Group) shows all the layers of aorta can be seen. Tunica intima, media &adventitia can be appreciated. TM (tunica media),TI (tunica intima), TA(tunica adventitia)Haematoxylin and eosin x 100X

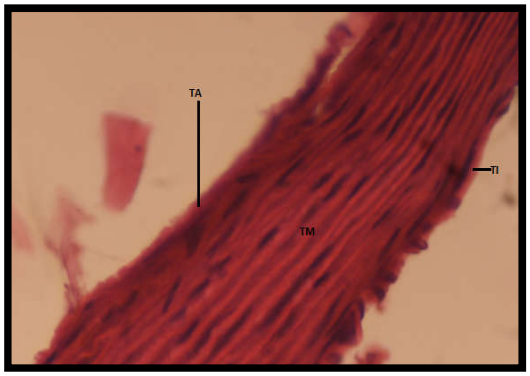

Figure 1 B Photomicrograph of aorta (control group) Same as above. No changes can be seen in any layer. Haematoxylin and eosin x 400X

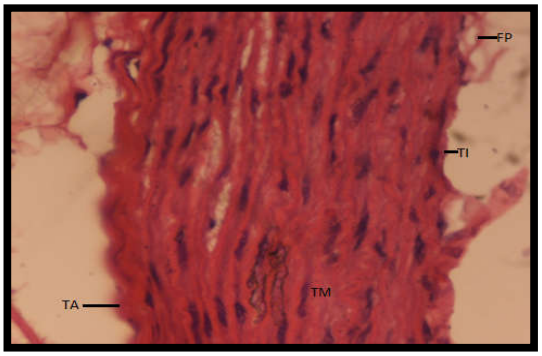

Figure 2 A Photomicrograph of aorta (group B) Fibrofatty plaque can be appreciated in tunica intima

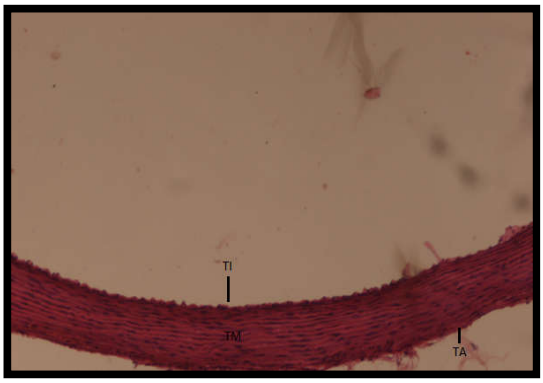

Figure 3A Photomicrograph of aorta (group C) No changes Observed in any layer Haematoxylin and eosin x 100X

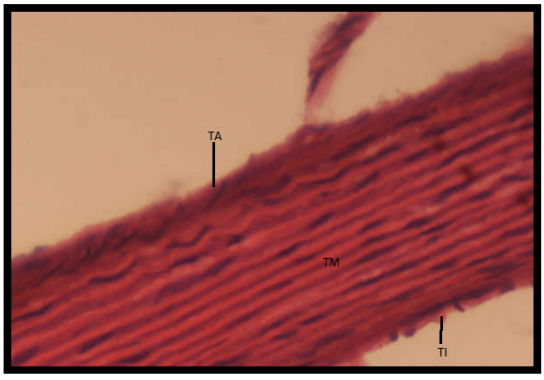

Figure 3 B Photomicrograph of aorta (group C) No changes observed in any layer Haematoxylin and eosin x 400X

Dissection of Rabbit Showing Aorta

DISCUSSION

Wojiciki J *et al* (17) found different degrees of vascular lesion development according to the section examined. Rudel *et al* (18) did not find a positive effect of the oleic acid enriched safflower oil on coronary artery atherosclerosis in African green monkey, here in present study when we compared the effect of olive oil rich diet (group C) and cholesterol rich diet (Group B) we found fibrofatty plaques and fatty sreaks only in the haematoxylin & eosin stained sections of aorta of rabbits fed on high cholesterol diet (fig 2 A) Normal histological findings of aorta were observed in contol and olive oil fed rabbits.

De la Cruz *et al* (19) have also shown that administration of virgin olive oil to atherosclerotic rabbits reduces vascular thrombogenicity, On the basis of present study it can be said that addition of olive oil (Group C) could prevent fatty streak formation (fig 3) . Others studies have reported regression of aortic lesions in olive oil fed animals from different models: hamsters done by Mangiapane *et al* (20), female ApoE-knockout mice done by Calleja L *et al* (21) and cholesterol-fed rabbits done by Nielson LB *et al* (22) and Mortensen A *et al* (23).

For prevention of atherosclerosis life style modification is very necessary step, which includes change in dietary fat intake. Our study included only histological findings to assess development of atherosclerosis. Other parameters can be included to completely confirm the protective effect of oilve oil as compared to other oils. Other clinical trials on human subjects will further help in the study.
One thing is certain from the present study that the olive oil is very beneficial in preventing atherosclerosis. Also its inclusion in North Indian diet can improve the quality of life in that region.

CONCLUSION

On histological examination of haematoxylin and eosin stained section of aorta we observed normal features of elastic artery in control rabbits. Similar findings were seen in olive oil fed rabbits. Rabbits which were given olive oil in combination with high cholesterol diet shown mild fatty deposition in media.Fibrofatty plaque were observed in tunica media of aorta of rabbits fed with increased cholesterol diet , alongwith fatty streak which was observed in tunica intima
From the above findings it can be concluded that replacement of increased cholesterol diet by olive oil can reduce the progression of atherosclerosis. This study also emphasises the fact that diet plays an important role in development of atherosclerosis and underlines the need of inclusion of olive oil in Indian diet.

Result of the present study further support experimental and epidemiological studies as well as randomised trials (6, 16, 17) and clearly demonstrate that use of olive oil reduces the rate of coronary artery disease. Its use can be recommended in tropical diet.

Moreover present study also paves the way for future studies regarding certain parameters associated with atherosclerosis and also studies on beneficial effects of olive oil.

This study finds its application in north Indian population where the use of olive oil as cooking medium can increase longevity.

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