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Dietary Olive Oil Effect on the Histopathological Alterations Caused by Mixture of Saturated Fats in Both Aorta and Liver of Rat

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ABSTRACT

This study aimed to study the antioxidant effect of olive oil against the histopathological alterations induced by cholesterol 4%+cholic acid 1%+thipresentacil 05% on the aorta and Liver of rat. Olive oil and/or a mixture of cholesterol 4%+cholic acid 1%+thipresentacil 05% were given to male rats for 16 week, 12 h after the last diet animals were killed and the selected tissues obtained and prepared for histological study. The results demonstrated that treating rat with cholesterol 4%+cholic acid 1%+thipresentacil 05% induced sever histopathological changes in the liver, these changes included distribution of liver architecture as it lost the normal radiating pattern, cellular infiltration and cells turned into large foam cells contained numerous internal cytoplasmic vesicles. Also, aorta with straight tunica intimae has large sub endothelial lipid deposits; disorganized myofibrils with dense irregular nuclei and tunica media height increased till 58.70 μm. Co-administration of olive oil+mixture of cholesterol 4%+cholic acid 1%+thipresentacil 05% lessened most histopathological changes in aorta and liver as compared to animals treated only with mixture of cholesterol 4%+cholic acid 1%+thipresentacil 05%. This indicated that olive oil showed antioxidant effects and improvement in the structure of the aorta and liver of rat.

Key words: Light microscope, internal organs, unsaturated fat, antioxidant

INTRODUCTION

Most artery flow disrupting events occur at locations with less than 50% lumen narrowing (Glagov et al., 1987) and this can be occurred when Cholesterol is delivered into the vessel wall by cholesterol-containing low-density Lipoprotein (LDL) particles. The process is worsened if there is insufficient High-Density Lipoprotein (HDL) particle, that removes cholesterol from tissues and carries it back to the liver to be metabolized and excreted or reused (Wald and Law, 2003).

Olive oil is the main source of fat in the Mediterranean diet which has been shown to be effective against oxidative stress associated diseases. It has been reported also that olive oil is able to reduce the risk of Coronary Heart Disease (CHD) by decreasing levels of artery-clogging lipids in the blood (Kok and Kromhout, 2004). 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 (Huang and Sumpio, 2008). In fact, the beneficial effects of olive oil on CHD risk have been attributed to its high Monounsaturated Fatty Acid (MUFA) content, mostly in the form of oleic acid (18: 1n-9) which ranges from 70 to 80% of total fatty acids (Lopez-Mirandaa et al., 2010). Nevertheless, evidences have accumulated on the

beneficial properties of minor though highly bioactive components of olive (Granados-Principal *et al.*, 2010). MUFA-enriched diets have shown no long-term ill effects and are associated with reduced rates of CHD. Healthy effects of dietary MUFA were also attributed to decreased endothelial activation (Massaro *et al.*, 2002; Massaro and Caterina, 2002) and tendency of LDL to oxidation (Bonanome *et al.*, 1992). Also, Extra Virgin Olive Oil (EVOO), Lipophilic Fraction (OOLF) and Hydrophilic Fraction (OOHF) had protective effect on oxidative stress and fatty acid profile of erythrocytes in 2,4-D treated rats (Nakbi *et al.*, 2010). So it is become clear, that the diet and lifestyle of the Mediterranean populations have led to decreased rates of cancer, diabetes and heart disease (Menotti *et al.*, 1997) as the protective properties of the Mediterranean diet are in part due to the consumption of antioxidant-rich olive oil (Bogani *et al.*, 2007).

The objective of this study was to evaluate the antioxidant effect of dietary supplementation with olive oil against the histopathological alterations in the aorta and Liver in an experimental rat model fed an atherogenic diet cholesterol 4%+cholic acid 1%+thipresentacil 05%.

MATERIALS AND METHODS

Animals: Animals were maintained under the experimental protocols complied with the Guide for care and use of Laboratory Animals (NRC, 1985).

This study started at 2008 where forty male Sprague-Daweiey rats (150-200 g) (supplied from King Fahd Medical Research Center) maintained under a 12 h photoperiod (08.00-20.00) at an ambient temperature of 22C, fed the appropriate rat chow diet (commercial rat cubes consisting of 23% crude protein, 4.3% crude oil, 3.1% crude fiber, 7.1% ash, 1.22% sand silica). Water was available ad lib.

Experimental design: Four groups of 10 animals each, were studied Group 1(control): Supplemented with normolipemic diet, Group 2: Supplemented with normolipemic diet+10% olive oil, Group 3: Supplemented with atherogenic diet or saturated fatty acid-enriched diet cholesterol 4%+cholic acid 1%+thipresentacil 05% and Group 4: Supplemented with atherogenic diet+10% olive oil. The animals were fed the experimental diets for 16 weeks. All animals were sacrificed by cervical decapitation, thoracic aorta and liver sections were subjected to histological examination by H and E. Microscopic images of the liver and the vascular tissue were studied.

About a total of 10 longitudinal sections of arterial wall randomly selected from each mouse for measuring the height of tunica media using a calibrated eye piece (x 400) ocular micrometer in order to draw their mean value for individual mice. The results were analyzed by using the program SPSS version 15 where One way ANOVA (Mould, 1989) was used to assess the significance of changes between control and treated mice. The results are Mean±Standerd Error. Significantly different from control group at $p < 0.0001$.

RESULTS

Control group: The light micrographs of both control liver (Fig. 1) and control aorta (Fig. 5) showed the normal pattern of rats liver and aorta (Junqueira *et al.*, 1998).

Treated groups
The liver: Concerning histological examination of the liver sections of (normolipemic+10% olive oil) group, it consists of normal radiating cells with few small vacuoles and dark stained nuclei around a central vein. The liver strand are altering with narrow sinusoids with stasis of few blood cells (Fig. 2).
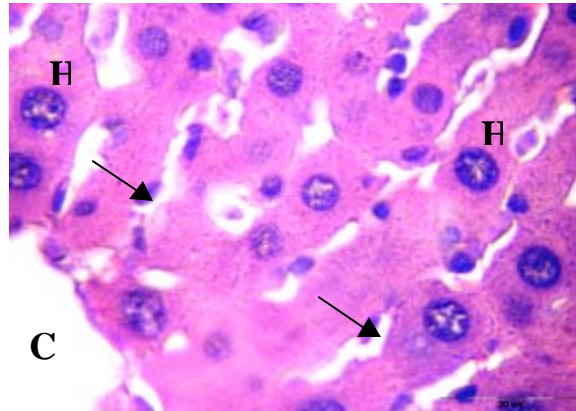
Fig. 1: Photomicrograph of control liver, illustrating Central vein (C), Hepatocytes (H) and sinusoidal spaces with Kupffer cells (arrow) (H and E) (scale bar: 20 µm)
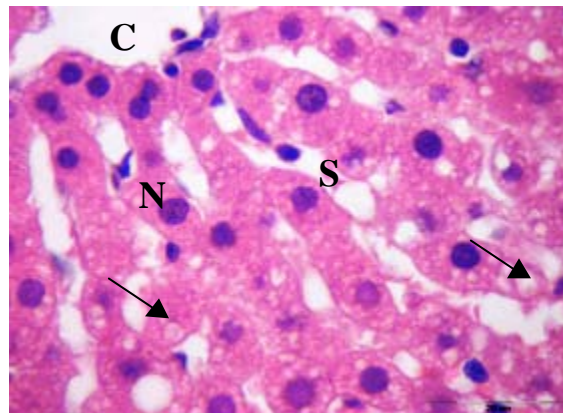

Fig. 2: Photomicrograph of the liver of (normolipemic+10% olive oil) rat ,illustrating few small vacuoles (arrows), dark stained Nuclei (N) and wide Sinusoids (S). Central vein (C). (H and E) (scale bar: 20µm)

Under the microscope, liver sections of atherogenic group revealing loss of the normal radiating pattern with cells slowly turning into large foam cells so-described because of their changed appearance resulting from the numerous internal cytoplasmic vesicles. The cells contained high lipid content and pyknotic nuclei with lost of their polyhedral shape. Foam cells eventually die and further propagate the inflammatory process (Fig. 3).

Liver sections of (atherogenic+10% olive oil) group compared with atherogenic group showed less severe pathological lesions of the liver tissue, as it exhibited nearly the normal radiating pattern of regenerated arenchyma with markable reduction of fatty droplets and reappearance of sinusoids. No inflammation was detected (Fig. 4).
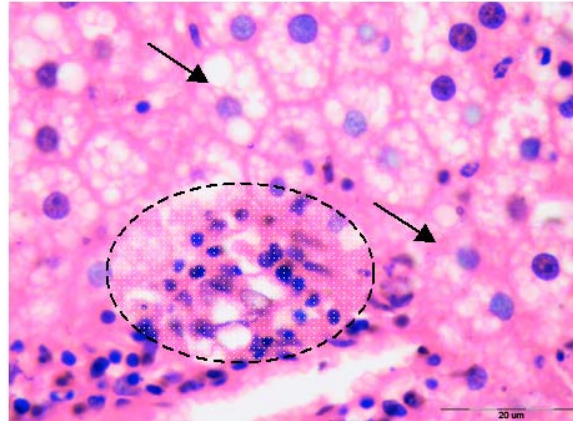
Fig. 3: Photomicrograph of the liver of atherogenic rat, revealing loss of the normal liver radiating pattern with pyknotic nuclei, foamy cells (arrows) and cellular infiltration (detached-line) (H and E) (Scale bar: 20μm)
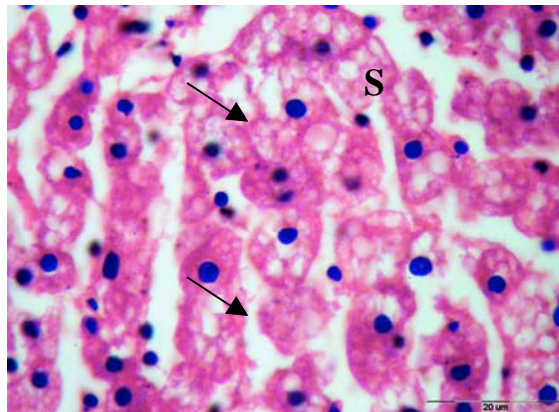

Fig. 4: Photomicrograph of the liver of (atherogenic+10% olive oil) rat, revealing liver with radiating pattern in between wide Sinusoid (S) and variable sized-microvacuolation (arrows) (H and E) (scale bar: 20 μm)

The aorta: Sections of (normolipemic+10% olive oil) group, illustrating nearly normal irregular tunica intimae with significantly decrease tunica media (35 μm) in compare with the height of control tunica media (45.57 μm) (Table 1). Also, the tunica media contained smooth muscle fibers with normal appearance and elastic fibers were markedly thick, continuous and wavy. Note loose tunica adventitia was recorded (Fig. 6).

In the current study, sections of atherogenic group (Fig. 7) showed abnormal straight tunica media with small subendothelial lipid deposits entered the artery wall. Also, some atrophied muscular fibers and dense irregular nuclei were detected. The elastic fibers were thin, straight and interrupted. There is also smooth muscle proliferation and migration from tunica media to tunica intimae. The proliferation of cells within the wall of the artery resulting in thickening and expansion of the wall with significant increase (58.70 μm) (Table 1).
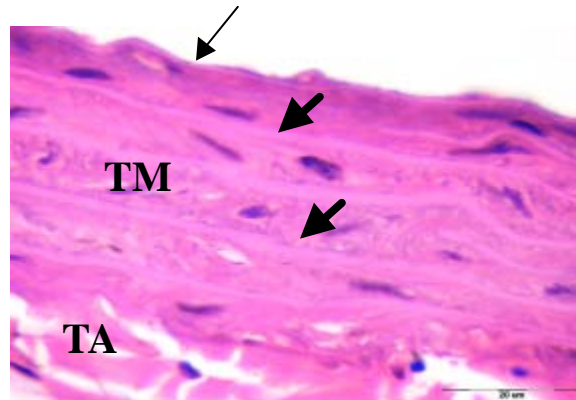
Fig. 5: Photomicrograph of control aorta of rat, illustrating irregular tunica intimae (thin-arrow), tunica media (TM) with elastic fibers (thick-arrows) and tunica adventitia (TA) (H and E) (scale bar: 20 μm)
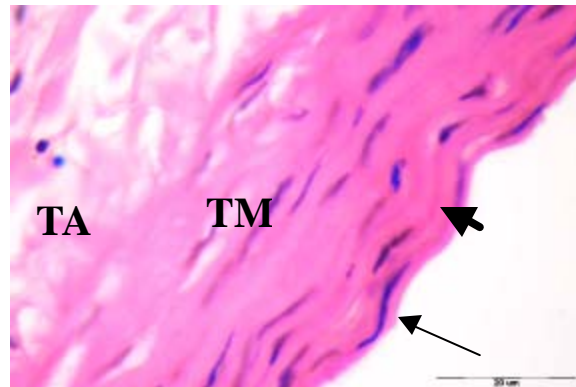

Fig. 6: Photomicrograph of the aorta of (normolipemic +10% olive oil) rat ,illustrating irregular tunica intimae (thin-arrow), less height tunica media (TM) with normal smooth muscle fibers and thick elastic fibers (thick-arrows). Note loose tunica adventitia (TA) (H and E) (scale bar: 20 μm)

Table 1: The height of tunica media of control and experimental groups

Variable	Experiment groups			
	Control	Olive oil	Atheroscol	Olive+Atheroscol
Tunica Albugina length μm^{-1}	45.57±0.18*	35.00±0.11*	58.70±0.15*	41.63±0.18*

Each value is given as the mean with standard deviations. *Significantly different from control group at p<0.0001. All parameters corresponding to a mean value of 10 animals

Histological examination of (atherogenic+10% olive oil) group (Fig. 8) showed less amount of lipid vesicles and less severe histological lesions on the endothelium and vascular wall. The tunica intimae still suffered and looked straight but tunica media with elongated nuclei reversed towards control structure with height (41.63 μm) (Table 1).
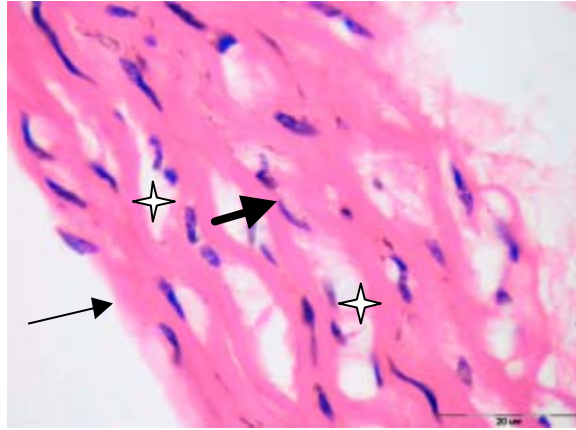
Fig. 7: Photomicrograph of the aorta of atherogenic rat, revealing straight tunica intimae (thin-arrow), disorganized tunica media with large lipid deposits (stars) and dense irregular nuclei (thick-arrows) (H and E) (scale bar: 20 µm)
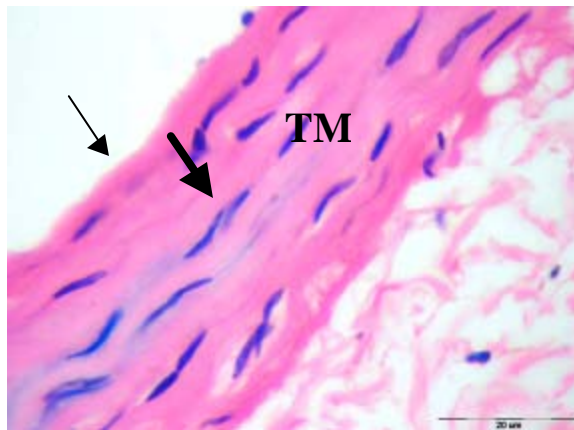

Fig. 8: Photomicrograph of the aorta of (atherogenic+10% olive oil) rat showing, less severe morphological lesions of the vascular wall ,tunica intimae (thin-arrow), nearly normal tunica media (TM) with elongated nuclei (thick-arrows) (H and E) (scale bar: 20 µm)

DISCUSSION

The obtained results showed that, there was a relationship between average intake of dietary fat, its quality and the incidence of atherosclerosis with agree with Glagov *et al.* (1987) as we noticed in the current study that (Atherosclerotic) group showed markedly thick endothelium and vacuolation of the smooth muscle cells. Also we noticed smooth muscle proliferation and migration from tunica media to intimae with agree with Wald and Law (2003) who explained this as it is responding to cytokines secreted by damaged endothelial cells. In this study the co-administrated of olive oil with mixture of (cholesterol 4%+Cholic acid 1%+thipresentacil 05%) lessened most histopathological alterations in aorta with agree with Aguilera *et al.* (2002) who found that a

supplemental diet of extra virgin olive oil lowered atherosclerotic lesions in all aortic fragments isolated from the rabbits.

In atherosclerosis, oxidative stress generated free radicals which had harmful effects on every organ (Pandya *et al.*, 2006) and this might explain the present negative effects on liver of (Atherosclerotic) group which revealed drastic alteration in histo-architecture where the hepatocytes disrupted, vacuolated and lost their polyhedral shape as indicated before in liver treated with carbon tetrachloride (Omara *et al.*, 2006). The present study investigated the potential protective effect of olive oil supplementation to the liver of animals treated with olive oil+mixture of (cholesterol 4%+Cholic acid 1%+thipresentacil 05%) where good recovery as was evident from the well defined hepatic cords and polyhedral hepatocytes with round nuclei. Theses present results could be explained as coenzyme Q (Co Q) in food had a role in cellular bioenergetics and antioxidant-protection (Mataix *et al.*, 1997) and this was clear when virgin olive oil used in the dietary treatment of atherosclerosis, it appeared to be a valid alternative for maintaining adequate levels of CoQ_{10} and hydroperoxides in liver mitochondria (Ramirez-Tortosa *et al.*, 1997) which led to reduce plasma triacylglycerols and cholesterol which was desirable in many pathologic situations (Quiles *et al.*, 2003).

The previous studies showed that virgin olive oil in took during ethanol treatment in rats resulted in a higher antioxidant activity and inhibited toxicity to the liver, as monitored by the reduction of transaminases levels and hepatic lipid peroxidation (Kasdallah-Grissa *et al.*, 2008). Moreover, in Nonalcoholic fatty liver disease, exposure of murine or human hepatocytes to monounsaturated fatty acid resulted in lipid accumulation without changes in cell viability while cell incubation with saturated fatty acids significantly decreased cell viability and increased caspase activation and apoptosis (Li *et al.*, 2009). Nevertheless, evidences had accumulated on the beneficial properties of minor though highly bioactive components of olive oil (Granados-Principal *et al.*, 2010).

CONCLUSION AND RECOMMENDATION

Results from the present study supported the idea that, increased visceral fat was associated with further deposition of fat in the liver and muscle, the deposition of lipoproteins (plasma proteins that carry cholesterol and triglycerides) led to inflammatory response in the liver tissue.

Sections of liver and aorta of (olive oil+mixture of (cholesterol 4%+Cholic acid 1%+thipresentacil 05%) group showed less severe morphological lesions than (cholesterol 4%+Cholic acid 1%+thipresentacil 05%) group so, these results might be helpful in determining the effect of olive oil in the human thrombogenic system where the usage of olive oil was recommended for healthy life.

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