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Efficacy of Olive Oil Diet on the Histopathological Changes induced in Hypercholesterolemic Rat.

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ABSTRACT

Olive oil is the main source of unsaturated fatty acids in the Mediterranean region**,** a nutritional regimen gaining ever-increasing renown for its beneficial effects on inflammation**,** cardiovascular disease, and cancer.

The present work aimed to study the protective effect of Olive Oil against the histopathological alterations induced by (cholesterol 4%+ Cholic acid 1% + thiouracil 05%) in the aorta and Liver of rat.

Olive oil and / or a mixture of (cholesterol 4%+ Cholic acid 1% + thiouracil 05%) were given to male rats for 16 wk, 12 h after the last diet animals were killed and the selected tissues obtained and prepared for histological study.

The obtained results demonstrated that treating rat with (cholesterol 4% + Cholic acid 1% + thiouracil 05%) induced sever histopathological changes in the liver ,these changes included disruption of liver architecture as it lost the normal radiating pattern, cellular infiltration and cells turned into large "foam cells" contained cytoplasmic vesicles. Also, aorta with straight tunica intimae has large subendothelial deposits of lipid; many myofibrils were disorganized and destroyed containing dense irregular nuclei, tunica media increased till 58.8µm.

Co-administration of olive oil+mixture of (cholesterol 4% + Cholic acid 1% + thiouracil 05%) lessened most histopathological changes in a aorta and liver as compared to animals treated with the mixture of (cholesterol 4% + Cholic acid 1% + thiouracil 05%) alone. This indicated that olive oil showed improvement in the structure of the aorta and liver of rat. So the usage of olive oil was recommended for healthy life.

Key words: Olive oil, saturated fat, histopathology, aorta, liver.

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). HDL particle are antiatherogenic. (Kratz *et al*., 2002)

Grundy (1989) reported that monounsaturated fatty acids, when substituted for saturated fatty acids in the diet, effectively reduce plasma LDL cholesterol concentrations and might be utilized in dietary modifications to lower plasma cholesterol concentration. The primary monounsaturated fatty acids (oleic acid) can be obtained from olive oil (Becker *et al*., 1983).

It was reported, 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, 2007). 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).

The objective of this study was to evaluate the effect of dietary supplementation with olive oil in aorta and liver of rats fed an atherogenic diet.

MATERIALS AND METHODS

Animals:

Animals were maintained and experimental protocols complied with the guide for care and use of Laboratory Animals (National Research Council, 1985.).

Forty male Sprague-Daweley rats (150-200g) (supplied from King Fahd Medical Research Center) maintained under a 12 h photoperiod (08.00-20.00) at an ambient temperature of 22ºC, were fed the appropriate rat chow diet (commercial rat pellets consisting of 23% crude protein, 4.3% crude oil, 3.1% crude fiber, 7.1% ash, 1.22% sand silica) until 12 h prior to the experiment, when food was withdrawn. Water was available *ad libitium.*

Experimental design:

Four different groups of 10 animals each, were studied: Group 1(G1) normolipemic diet (control).Group 2 (G2) atherogenic diet or saturated fatty acid-enriched diet (cholesterol 4% + Cholic acid 1% + thiouracil 05%). Group 3 (G3) normolipemic diet with 10% olive oil. Group 4 (G4) atherogenic diet with 10% olive oil.

The animals were fed the experimental diets for 16 weeks. All animals were sacrificed by cervical decapitation, arterial sections of thoracic aorta and liver were subjected to histological examination by heamatoxylin and eosin. Microscopic images of the liver and the vascular tissue were studied. Morphometric parameters in the arterial wall were done.

RESULTS

Control group (G1):

The light micrographs of both control liver (Fig.1) and control aorta (Fig.5), showed the normal pattern of rat's liver and aorta.

Treated group:

1-The liver:

Histological examination of the liver of (G2), revealed that 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). Under the microscope, liver of group (G3) 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) **.**Animals of (G4) showed, compared with (G3), less severe histological lesions of the liver tissue, as it exhibited the normal radiating

pattern of regenerated parenchyma with markable reduction in fatty droplets and reappearance of sinusoids. No inflammation was detected (Fig.4).

2-The aorta:

Animals of **(**G2**)**, illustrating nearly normal irregular tunica intimae with less height tunica media (35 µm) in compare with the height of tunica media of control group (45.7 µm). Also, the tunica media contained smooth muscle fibers with normal appearance and elastic fibers were markedly thick, continuous and wavy. Note loose tunica adventitia (Fig. 6).

Group (3) showed abnormal straight tunica media with small subendothelial deposits of lipid enter the artery wall. There is also smooth muscle proliferation and migration from tunica media to intimae .The proliferation of cells within the wall of the artery resulting in thickening and expansion of the wall till (58.8µm), sometimes, some atrophy of the muscular layer and appearance of dense irregular nuclei were detected. The elastic fibers were thin, straight and interrupted (Fig.7). Animals of (G4) showed, compared with (G3), an improved lipid and less severe histological lesions of the endothelium and vascular wall. The tunica intimae still suffer and look straight ,but tunica media with elongated nuclei reversed towards control structure with height (41.7 µm) (Fig.8). Data in figure (9) showed that the height of tunica media varied in the four experimental groups. The height decreased in G4 in comparison with G3.

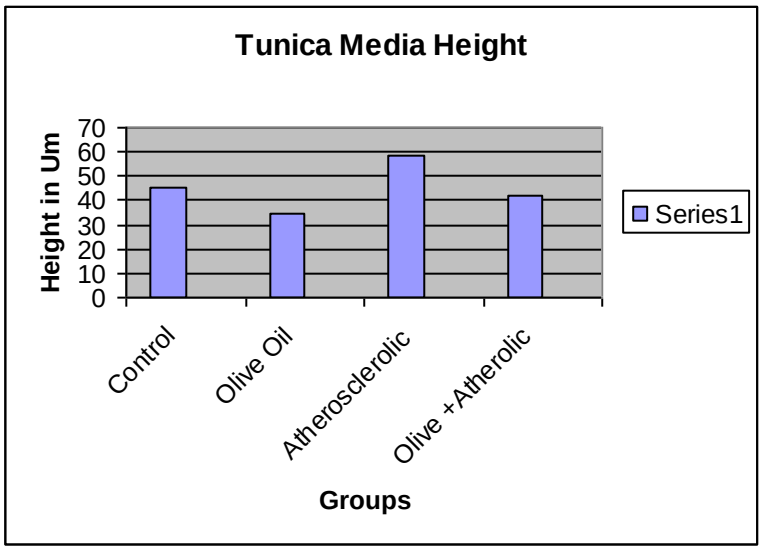

Fig.9: The height of tunica media of rats receiving a regular rat chow diet with (Olive oil, Atherosclerolic, Olive + Atherosclerolic) or without (control). Bars denote mean of 6 determinations.

DISCUSSION

The obtained results showed that, there is a relationship between average intake of dietary fat, its quality, and the incidence of atherosclerosis (Glagov *et al.*, 1987). The results of the current work indicated that (Atherosclerotic) group showed markedly thick endothelium and the intema of the aorta showed many vacuolation within the smooth muscle cells. Also, we noticed smooth muscle proliferation and migration from tunica media to intimae as indicated before (Wald and Law, 2003) who explained this as it was responding to cytokines secreted by damaged endothelial cells.

On the other hand, Huertas *et al.,* (1991) illustrated that there is a relationship between average intake of dietary fat and the endogenous lipid peroxidation with regard to coenzyme Q_9 and coenzyme Q_{10} concentrations in mitochondria and microsomes from rat liver. As, Coenzyme Q (Co Q) in food have a role in cellular bioenergetics and antioxidant-protection and this support the interest for a wide research concerning the relationship between dietary fats, Co Q content and biochemical behavior (Mataix *et al.*,1997). When virgin olive oil used in the dietary treatment of atherosclerosis, it appeared to be a valid alternative for maintaining adequate levels of Co Q_{10} and hydroperoxides in liver mitochondria.(Ramírez-Tortosa *et al.,* 1997).Also, Scaccini *et al*.,(1992) found that rats fed diets of olive oil had a decreased concentration of lipoproteins and thiobarbituric acid-reactive substances, end products of lipid peroxidation. And this can explain the positive effects of olive oil in this work, as co-administration of olive oil +mixture of (cholesterol 4% + Cholic acid 1% + thiouracil 05%) lessened most histopathological changes in aorta, as indicated before by (De La Cruz *et al.,* 2000 and Al Sewedy and Soliman 2006) and this may be du to the supplementation of olive oil which reduced vascular thrombogenicity and platelet (Wald and Law, 2003).Also, the intake of virgin olive oil increased Co Q mitochondrial contents (Glagov *et al.*,1987) and this led to reduce plasma triacylglycerols and cholesterol, which is desirable in many pathologic situations (Quiles *et al.,* 2003).

Supplemental diet of extra virgin olive oil was found to decrease LDL oxidation in rabbits with experimentally induced arteriosclerosis (Ramirez-Tortosa *et al.,* 1998) and led to lower atherosclerotic lesions in all aortic fragments isolated from the rabbits (Aguilera *et al.,* 2002).In atherosclerosis, oxidative stress generates free radicals, which has harmful effects on every organ (Pandya *et al*., 2006) and this may explain the negative effects on liver of (Atherosclerotic) group which revealed drastic alteration in histo-architecture where the hepatocytes were disrupted, vacuolated and lost their polyhedral shape as indicated before in liver treated with carbon tetrachloride (Omara *et al.,* 2006).

The liver of rat treated with olive oil +mixture of (cholesterol 4% + Cholic acid 1% + thiouracil 05%) showed good recovery as was evident from the well defined hepatic cords and polyhedral hepatocytes with round nuclei, so we considered olive oil as a hepatoprotective factor. Many substances have hepatoprotective effect on hepatotoxicity induced by CCI_4 in rats like artichocke, curcumin, ginger and rutin (Ahmed *et al*., 2006) and nutraceutical compound from carotenoid (Omara *et al*., 2006).

CONCLUSION

Results from the present study supported the idea that, increased visceral fat is associated with further deposition of fat in the liver and muscle ,the deposition of lipoproteins (plasma proteins that carry cholesterol and triglycerides) lead to inflammatory response in the liver tissue .Animals of (G4) showed, compared with the (G3), less severe histological lesions of the endothelium and vascular wall so, these results may be helpful in determining the effect of olive oil in the human thrombogenic system. This indicated that olive oil showed improvement in the structure of the aorta and liver of rat .So the usage of olive oil was recommended for healthy life.

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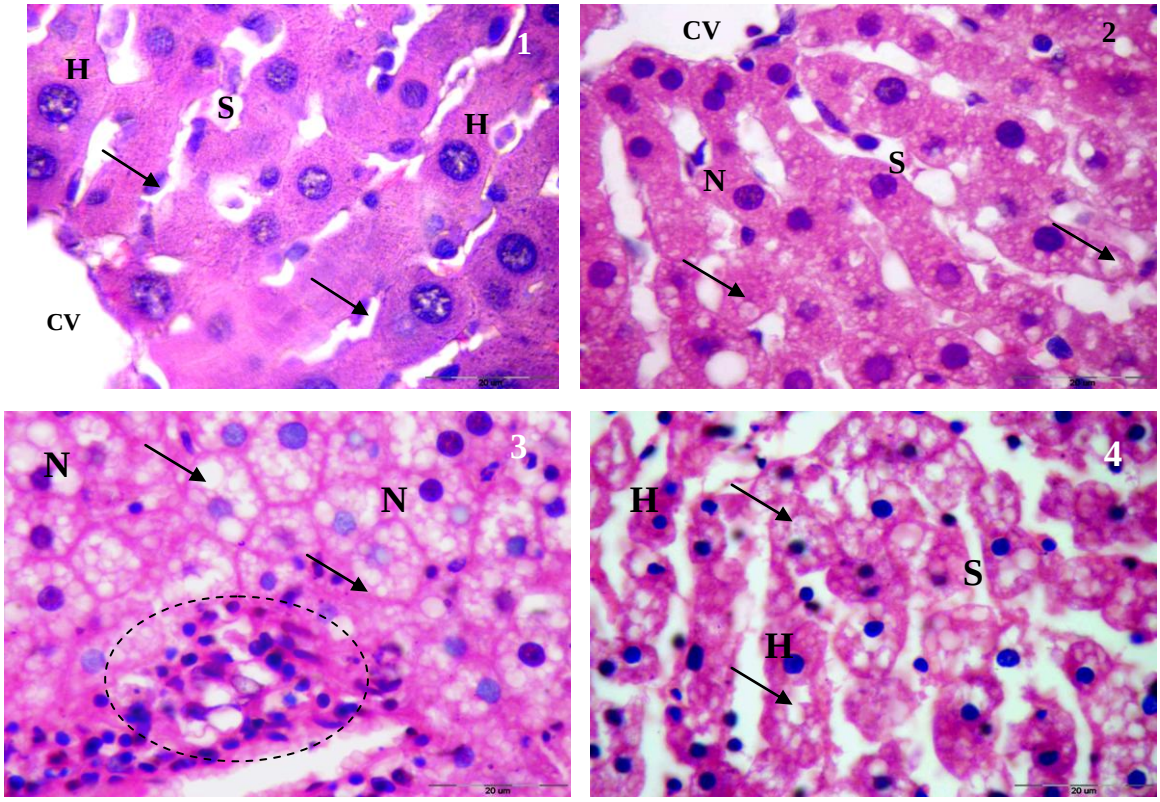

Fig. 1. Photomicrograph of the liver of (G1), illustrating central vein (CV), hepatocytes (H) and sinusoidal spaces (S), with Kupffer cells (arrow) (H&E) (X1000).

Fig. 2. Photomicrograph of the liver of rat (G 2), illustrating few small vacuoles (arrows), dark stained nuclei (N) and sinusoids (S).Central vein (CV). (H&E) (X1000).

Fig. 3. Photomicrograph of the liver of rat (G3), revealing loss of the normal radiating pattern, foamy cells (arrows), cellular infiltration (detached-line) and pyknotic nuclei (N) (H&E) (X1000).

Fig. 4. Photomicrograph of the liver of rat (G4), revealing liver with radiating pattern in between sinusoid (S), variable sized-microvacuolation (arrows), regenerated hepatocytes (H) (H&E) (X1000).

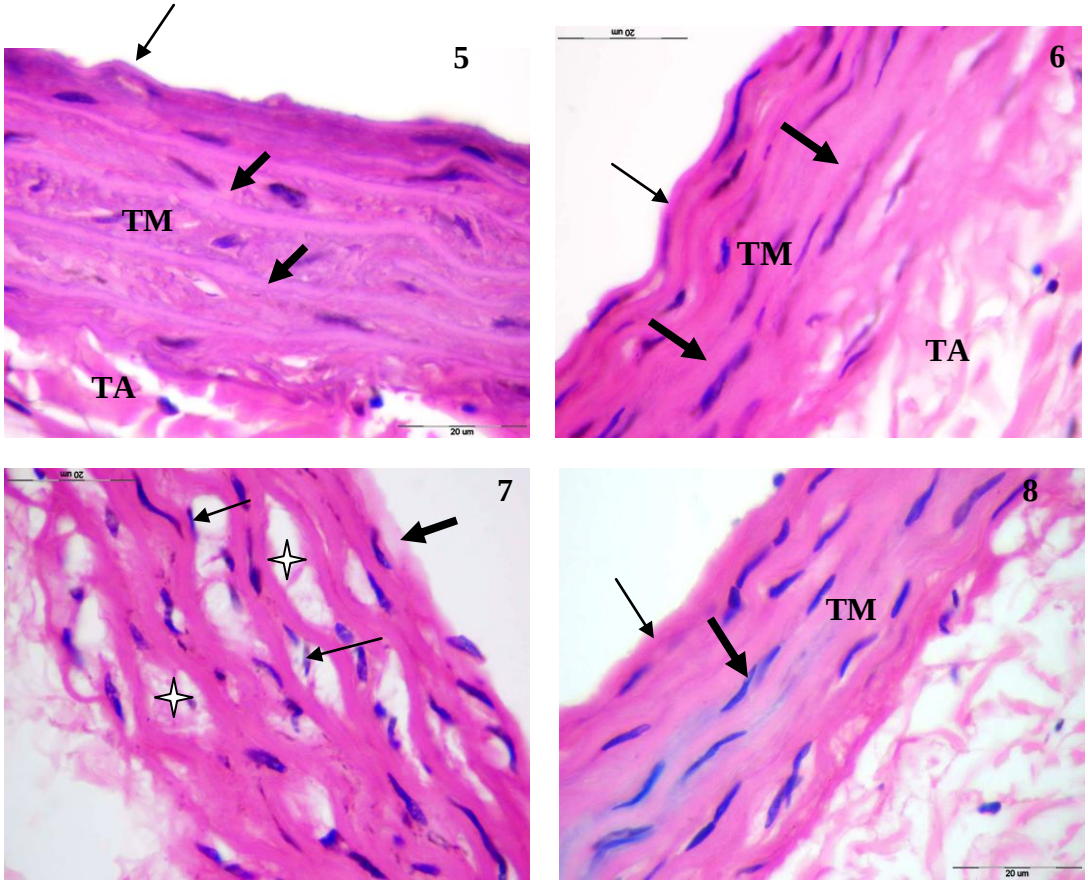

Fig.5. Photomicrograph of the aorta of rat (G1), illustrating irregular tunica intimae (thin-arrow), tunica media (TM) with elastic fibers (thick-arrows) and tunica adventitia (TA) (H&E) (X1000).

Fig.6. Photomicrograph of the aorta of rat (G2), 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&E) (X1000).

Fig.7. Photomicrograph of the aorta of rat (G3), revealing straight tunica intimae (thick-arrow), disorganized tunica media with large lipid deposits (stars) and dense irregular nuclei (thin-arrows) (H&E) (X1000).

Fig.8. Photomicrograph of the aorta of rat (G4) showing, less severe morphological lesions of the vascular wall, tunica intimae (thin-arrow), nearly normal tunica media (TM) with elongated nuclei (thick-arrows) (H&E) (X1000).

ARABIC SUMMARY

فعالية التغذية بزيت الزيتون على التغيرات النسيجية المرضية المحدثة في الجرذان مرتفعة الكوليسترول

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يعتبر زيت ا لزيتون مصدر رئيسي للأحماض الدهنية غير المشبعة بمنطقة البحر المتوسط و بديل غذائي أكتسب اهتمام كبير لأثره المفيد على حالات الالتهابات و أمراض القلب الوعائية و السرطان . ويهدف البحث الحالي إلى دراسة تأثير زيت الزيتون على التغيرات المرضية التي يسببها خليط م ن الدهون المشبعة في كل من الشريان الأبهر و كبد الحرذان.

في البحث الحالي تم تغذية مجموعة من ذكور الجرذان بخليط من (كولسترول + حامض الكوليك + ثيويوراسيل) و مجموعة أخرى بنفس الخليط السابق مضافا إليه زيت الزيتون، كما استخدمت مجموعتان ضابطاتان، أحداهما تم تغذيتها بزيت الزيتون فقط و الأخرى مجموعة ضابطة سالبة غير معاملة واستمرت التجربة 16 أسبوعا بعدها تم قتل الحيوانات و الحصول على الشريان الأبهر و الكبد وتم أعداد قطاعات شمعية منها وتم الفحص بالميكروسكوب الضوئي .ولقد أوضحت نتائج البحث أن الكبد فقد النمط المفصص ا لطبيعي وظهر الالتهاب به و تجمع عدد كبير من الفجوات السيتوبلازمية و كذلك أخذت الطبقة الداخلية للشريان الأبهر الشكل المستوى وظهر تشتت و تدمير للألياف العضلية و ظهرت أنويتها داكنة وغير منتظمة وزاد طول الطبقة الوسطى حتى 58,8 ميكروميتر في المجموعة التي أعطيت الخليط المكون من الدهون المشبعة (كوليسترول + حامض الكوليك + ثيويوراسيل) .أما المجموعة التي غذيت بالخليط مضافا إليه زيت الزيتون فقد أظهرت تحسنا كبير في تلك التغيرات حيث أستعاد الكبد النمط المفصص و نقصت أحجام الفجوات السيتوبلازمية و ظهرت بعض الخلايا المتجددة، أيضا ظهر الجدار الوعائي متعرج من الداخل و الطبقة الوسطى أقل ارتفاعا (41,7 ميكروميتر).إلا أنه لم يكن هناك أي تغيرات نسيجية واضحة في الحيوانات التي غذيت بزيت الزيتون فقط و هي تشبه إلى حد كبير المجموعة الضابطة السالبة.و تدل تلك النتائج على أن زيت الزيتون يحسن من تركيب الشريان الأبهر و الكبد للجرذان لذلك نوصى باستخدام زيت الزيتون لصحة أفضل.