Mansoura Veterinary Medical Journal

ANTI-ATHEROSCLEROTIC ROLE OF OLIVE OIL IN RATS

Bouzeed BouKhazeem*, El-Said El-Sherbini*, Gehad Ramadan*, Mosbah Abdel Gawad**

** Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine. Mansoura University*
***Department of Anatomy and embryology, Faculty of Veterinary Medicine. Mansoura University*

ABSTRACT

The increase in dietary cholesterol to perform a rat model for atherosclerotic diet would result in a significant increase in serum levels of total cholesterol and malondialdehyde and blood SOD activity in rats. Moreover, there was significant decrease in serum levels of LDL-C, triacylglycerol and GSH, In addition to blood catalase activity. Although, there was a non-significant change in in serum HDL-C and blood GPx activity. However, dietary supplementation with olive oil mixed with atherosclerotic diet ameliorates this undesirable effect.

INTRODUCTION

Atherosclerosis is an inflammatory disorder that involved the erythrocytes and leucocytes in the occurrence of such clinical manifestation which causes local thickness in the wall of blood vessels due to infiltration of cellular debris, inflammatory and immune cells to the site of injury **(Falk, 2006).**

Atherosclerosis is considered the most common cause of death in several cardiovascular disorders due to the reduction of blood flow to organs and tissues **(Campbell et al., 2012).**

Olive oil is used extensively in reduction of the risk of cardio vascular disorders. As a result, coronary artery diseases are limited to individual thrive on a diet rich in olive oil due to its content with polyphenols and mono unsaturated fatty acids **(Pelucchi et al., 2011).**

From this point this study aimed to study the protective effects of olive oil in atherosclerotic induced model in rats.

MATERIAL AND METHODS

I- Chemicals:

Cholesterol was purchased from Oxford Lab Chem., India and administrates orally in the diet at dose rate 1% for 30 days **(Park et al., 2005),** while Olive oil was purchased from famous market, Libya and administrated orally in the diet at rate of 10% for 30 days **(Mortensen et al., 1992).**

II- Animals:

Forty healthy male Sprague Dawley rats were used in this study. Rats were kept in metabolic cages in a controlled environment and maintained under a 12 hours' light: dark cycle, air condition at 25 ± 3^0 and 55-75% humidity and provided with standard basal diet consisted of yellow corn (74%), soya bean heal (15%), concentrates (9%), mineral and vitamin premix (0.25%), Calcium carbonate (0.5%) and water was offered *ad libitum* according to **National Research Council (1995)**.

III-Animal groups:

Group I (G_1), this group consisted of ten adult male rats received basal diet for 30 days.**Group II (G_2,** this group consisted of ten adult male rats received basal diet supplemented with olive oil at a dose of 10% of diet daily for 30 days **(Mortensen et al., 1992). Group III (G_3,** this group consisted of ten adult male rats received basal diet supplemented with cholesterol 1% of basal diet daily for 30 days (**Park et al., 2005**), and finally **Group IV (G_4): -** this group consisted of ten adult male rats received basal diet supplemented with olive oil at 10% and cholesterol 1% of diet daily for 30 days.

IV. Blood and tissue sampling

Blood samples were collected at the end of the experiment and each sample was divided into two parts. The first part was collected in heparinized tube used for determination of catalase activity (CAT), superoxide dismutase (SOD) activity and glutathione peroxidase (GPx) activity while the second part of blood sample was collected in vial without anticoagulant and lift to coagulant for 30 minutes. The coagulated blood samples were centrifuged at 3000 r.p.m for collection of clear serum sample used for biochemical analysis of serum malondialdelyde (MDA) level, total cholesterol, HDL-C, LDL-C and triacylglycerol. Tissue samples were collected from the heart and fixed in 10% neutral buffered formalin. Paraffin section of 5µ thick were prepared and stained with hematoxylin and Eosin and examined microscopically (**Fischer et al., 2008).**

V. Determination of serum lipid profile

Serum cholesterol was determined according to the method of **Allain et al., (1966),** Serum triacylglycerol was determined according to the method of **Fossati et al., (1982),** Serum HDL-C was determined according to the method of **Lopez-Virella et al., (1977) and** Serum LDL-C was determined according to the method of **Friedwald et al., (1972).**

VI. Determination of antioxidant and oxidative stress markers:

Reduced glutathione was determined according to **Beutler et al., (1963),** SOD activity was determined by **Nishikimi et al., (1972),** Catalase activity was determined according to **Aebi, (1984),** The concentration of MDA was determined by the method of **Drapper and Hadley, (1990) and** GPx activity was determined by the method of **Lawrence and Burk, (1976).**

VII. Statistical analysis:

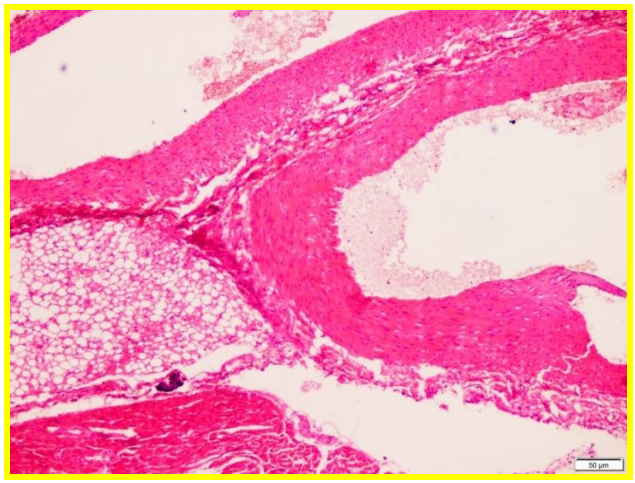
Data was analyzed with SPSS V.17 where means was expressed accompanied with its standard error. Means within groups were analyzed using analysis of variance method at 0.05 significant level .

Table (1): Effect of olive oil supplementation on serum lipid profile in rats

Groups	TC mg/dl	HDL-C mg/dl	LDL-C mg/dl	TAG mg/dl
Group 1	54.90±2.58^{c}	29.80±1.3 ^{a}	9.30±0.70^{a}	114.2±0.60^{b}
Group 2	54.90±2.01^{c}	29.96±0.25 ^{a}	8.04±0.65^{a}	92.82±2.93^{c}
Group 3	84.5±1.4^{a}	26.8±0.52 ^{a}	18.70±1.14^{c}	129.39±2.50^{a}
Group 4	76.8±1.46^{b}	27.90±1.32 ^{a}	15.97±0.89^{c}	97.00±7.49^{c}

Table (2): Effect of olive oil supplementation on antioxidant status and oxidative stress:

Groups	SOD U/ml	GPx U/ml	GSH mg/dl	CAT U/ml	MDA mg/dl
Group 1	274.20±4.02 ^{b}	24.61±.28^{b}	2.58±0.54^{c}	973.3±13.80^{a}	4.64±0.939^{b}
Group 2	124.50±10.69^{c}	18.78±0.64^{b}	2.01±0.57^{c}	852.5±24.00^{b}	2.68±0.312^{c}
Group 3	301.80±5.76^{a}	21.28±0.03^{b}	1.40±0.26^{a}	620.0±30.80^{c}	7.18±0.96^{a}
Group 4	262.2±13.61 ^{b}	46.33±0.59^{a}	1.46±0.99^{b}	902.8±40.19^{ab}	3.55±0.79^{bc}



Figure(1): showing normal arterial wall with normal vascular wall and large lumen .There are no abnormalities as atheroma, cholesterol clefcts or calcification; Control group H&E×40.

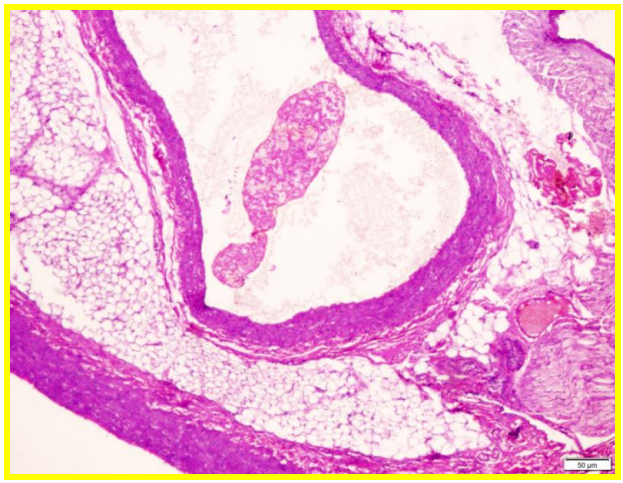


Figure (2): Showing normal arterial wall with normal vascular wall and large lumen. There are no abnormalities. Parts of the cardiac muscles appeared with normal features arrow; Control group H&E x40.

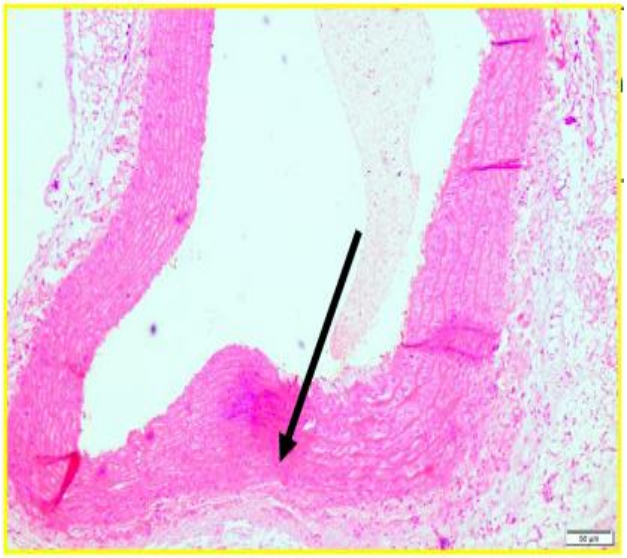


Figure (3): Showing segmental thickening arrow with narrowed lumen; K-3 group H&E x40.

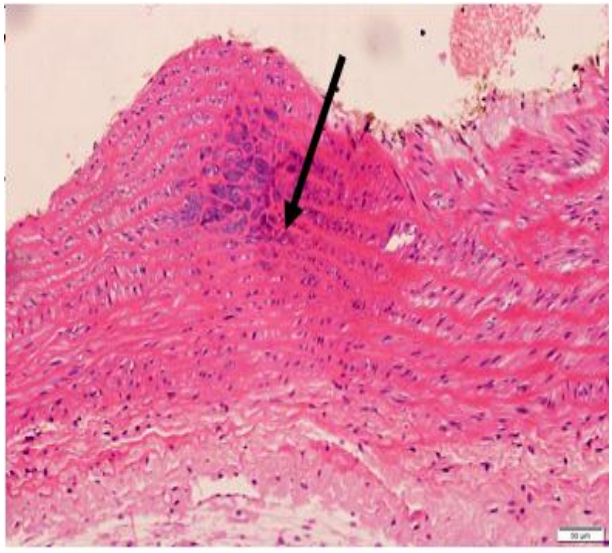


Figure (4): Showing segmental thickening with narrowed lumen. There is early atherosclerotic changes in the form of calcification arrow; K-3 group H&E x100.

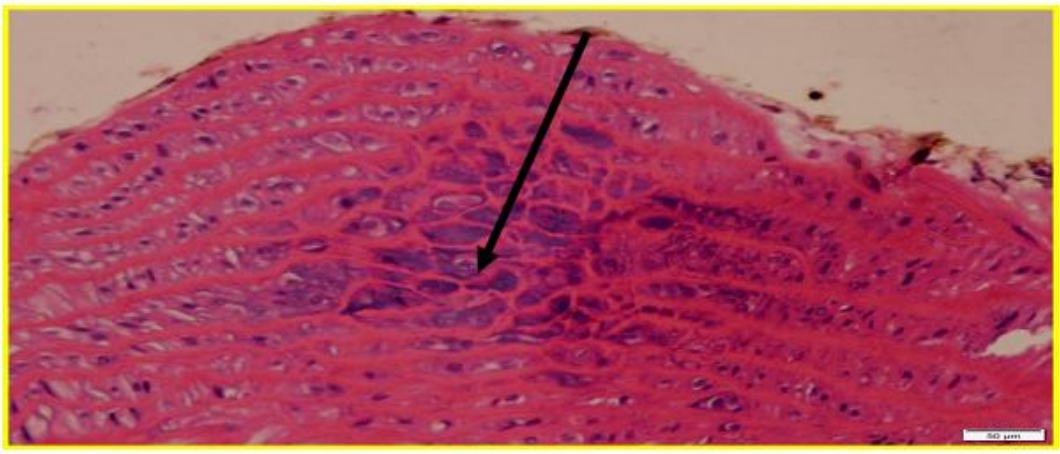


Figure (5): showing atherosclerotic changes in the form of segmental thickening with narrowed lumen; calcification arrow .K group H&E×100.

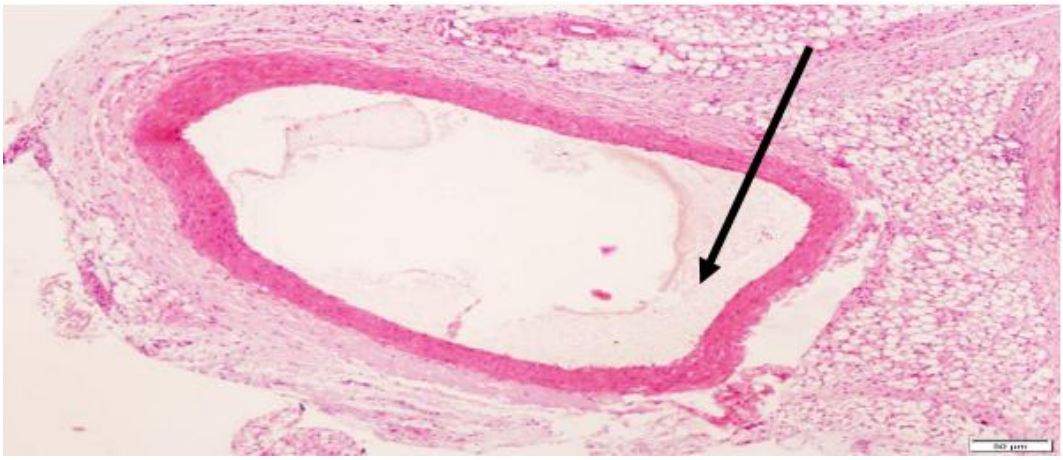


Figure (6): showing segmental atherosclerotic changes in the form of segmental thickening arrow of the arterial wall with narrowed lumen; O+K-group H&E×40.

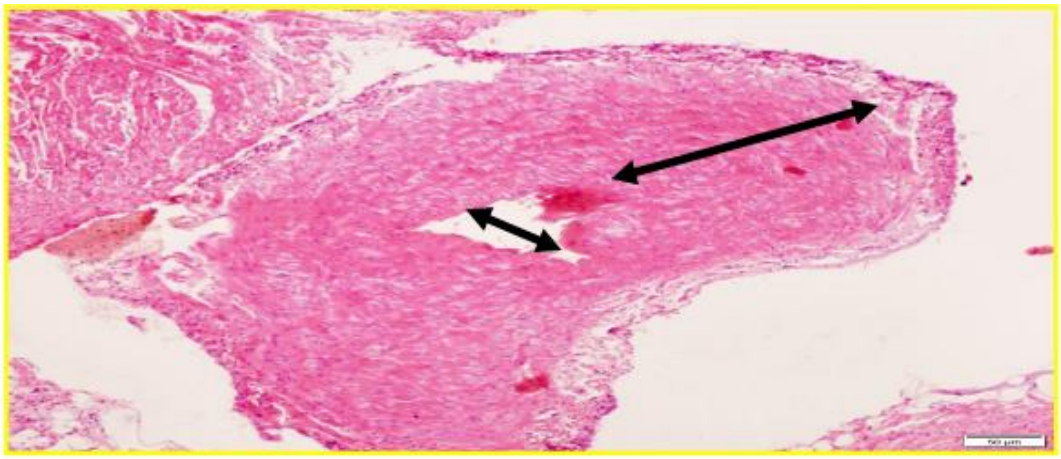


Figure (7): Showing markedly atherosclerotic changes in the form of thickened arterial wall long arrow with narrowed lumen short arrow. K-3 group H&E x40

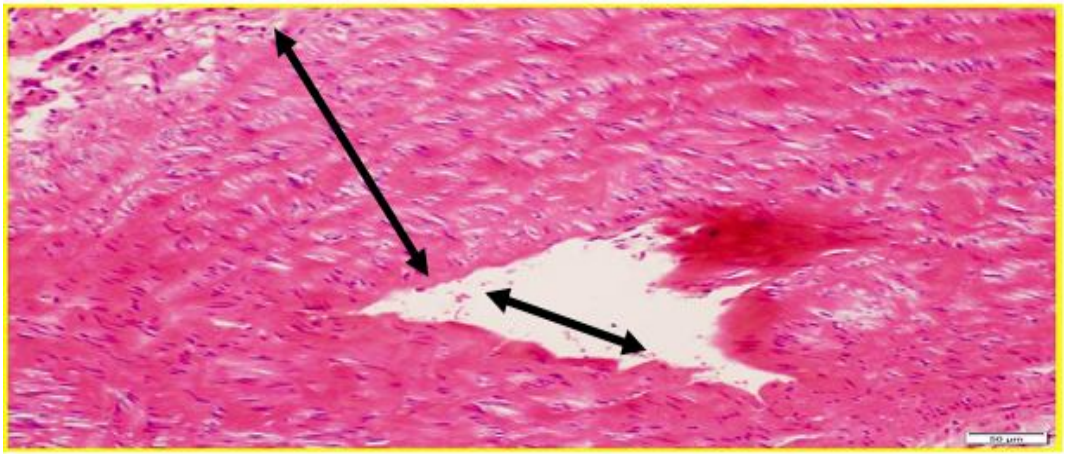


Figure (8): Showing markedly atherosclerotic changes in the form of thickened arterial wall (long arrow) with narrowed lumen (short arrow). K- group H&E x100.

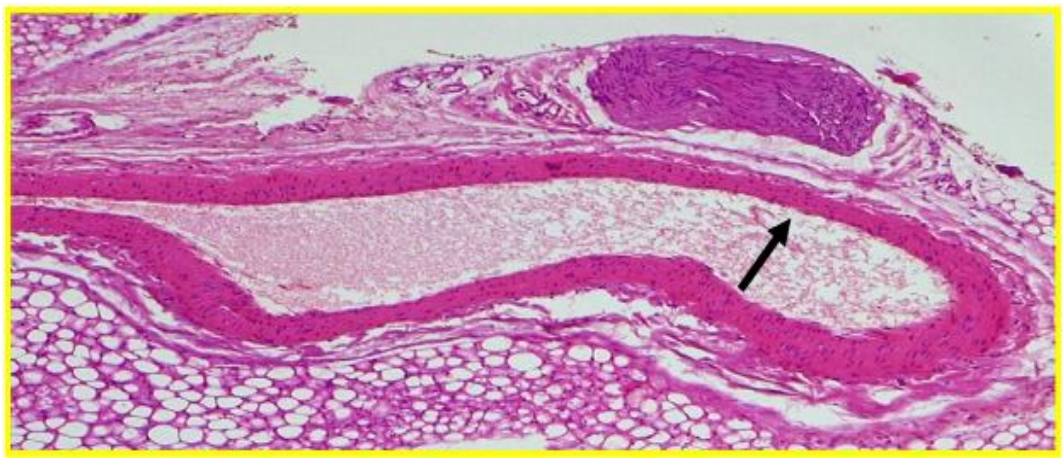


Figure (9): Showing normal arterial wall with normal vascular wall and large lumen. There are no abnormalities as atheroma, cholesterol clefts or calcification; O-2 group H&E x100.

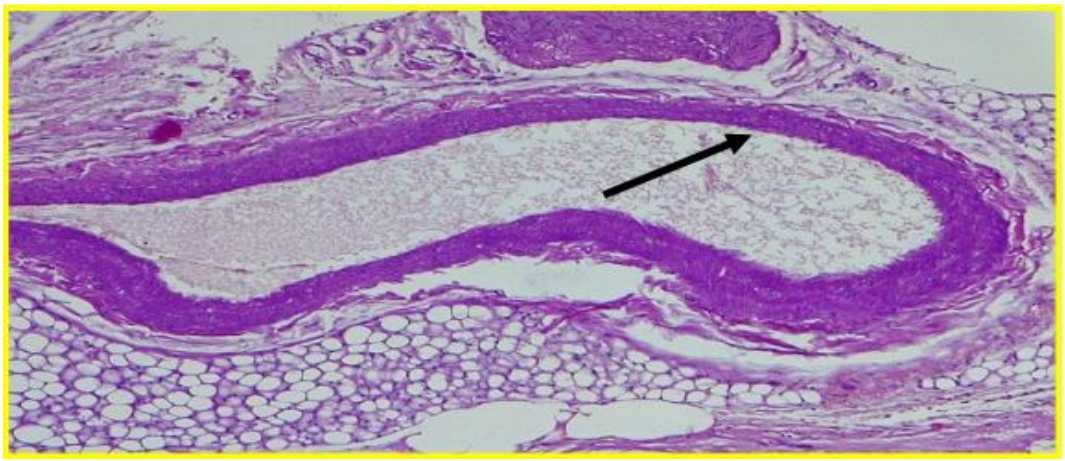


Figure (10): Showing normal arterial wall with normal vascular wall and large lumen. There are no abnormalities as atheroma, cholesterol clefts or calcification; O-2 group PAS x100.

RESULTS & DISCUSSION

The obtained results (table 1 and graph 1) revealed that Dietary supplementation of cholesterol 2% produced a significant increase in total cholesterol in serum of rats in comparison with that of control one and the addition of olive oil to diet result in a significant decrease in the level of total cholesterol but still a significantly higher than that of the control group. This result is supported by that of **Shrapnel et al, (1992)** who cited that Dietary cholesterol increased serum cholesterol levels in some people and may increase risk of coronary heart disease.

Moreover, in agreement with that of **Widmer et al., (2013)** Diets rich in plant-derived polyphenols such as olive oil (OO) and/or catechins such as epigallocatechin 3-gallate (EGCG) have been improving endothelial function, an important surrogate for atherosclerosis. The possible shown to reduce the incidence of cardiovascular diseases, potentially by augmentation of endothelial function with the combined efforts of OO and EGCG is intriguing, yet unknown. In the aspect, **Moreno Luna et al., (2012)** found that the consumption of a diet containing polyphenol-rich olive oil can decrease BP and improve endothelial function in young women with high-normal BP or stage 1 essential hypertension. Furthermore, our findings demonstrate that there is a strong relationship between plasma and aortic arterial wall levels of cholesterol oxides and suggest that in addition to exogenous sources, formation of cholesterol oxides proceeds via free radical oxidation acting upon elevated cholesterol levels resulting in the accumulation of these potentially cytotoxic and atherogenic products

The present results (tables 2, 3 and 4 Figures, 2, 3 and 4) revealed a significant increase in serum TAG of cholesterol group and the addition of olive oil resulted in a significant decrease in TAG level in comparison with the serum level of control group. Furthermore, the addition of cholesterol or olive oil has a non-significant effect on serum HDL-c of rats. Although, the addition of cholesterol 2% to rat's diet produced a significant increase in serum LDL-c level and the addition olive oil resulted in a significant decrease in serum level when compared with that of control group. The obtained results agree with that of **Packard et al., (1983)** who examine the effects of increased dietary cholesterol (6 eggs/d) on the metabolism of low density lipoproteins in a group of seven healthy volunteers. Egg supplementation raised high density and low density lipoprotein cholesterol levels by 18 and 40%, respective .

The reduction in the level of LDL-c by olive oil feeding is due to the primary source of fat in the Mediterranean diet which is associated with a low mortality for cardiovascular disease. In spite of this, data concerning olive oil consumption and primary end points for cardiovascular disease are scarce. However, a large body of knowledge exists providing evidence of the benefits of olive oil consumption on secondary end points for cardiovascular disease. The benefits of olive oil consumption are beyond a mere reduction of the low-density lipoprotein cholesterol. Here, we review the state of the art concerning the knowledge of the most important biological and clinical effects related to the intake of olive oil rich diets on lipoprotein metabolism, oxidative damage, inflammation, endothelial dysfunction, blood pressure, thrombosis, and carbohydrate metabolism. The extent to which we possess

evidence of the health benefits of olive oil minor components is also assessed. The wide range of anti-atherogenic effects associated with olive oil consumption could contribute to explain the low rate of cardiovascular mortality found in Southern European Mediterranean countries, in comparison with other western countries, despite a high prevalence of coronary heart disease risk factors **(Covas, 2007).**

The obtained results are in agreement with those **of Vessby et al., (1982)** who found that both HDL and LD cholesterol are reduced in subjects on diets enriched in polyunsaturated fatty acids (PUFA diets). The only exceptions to this rule are hyper triglyceridemic patients with low HDL or LDL concentrations. The changes in HDL and LDL cholesterol are inversely related to the respective HDL and LDL concentrations before dietary treatment. These results are supported by histopathological examination (Graph 1-10).

The present results showed a significant increase in malondialdehyde level in rats after feeding of cholesterol 2% and the addition of olive oil produced a significant reduction in malondialdehyde level. On the other hand, cholesterol feeding in rats caused a significant decrease in glutathione peroxidase (GPX) and catalase activities in addition to GSH level. While olive oil feeding produced an opposite effect when compared to those of control group. These results are supported by histopathological examination (Groph 1-10(

These results are nearly similar to those of **Mohamedain et al., (2000)** who concluded that Erythrocyte glutathione peroxidase (GPx) activity significantly decreased whereas catalase activity significantly increased in HC rabbits. In rats' cholesterol feeding increased the plasma cholesterol only twofold and had no effect on plasma or liver lipid peroxidation. Only 7α- and 7β-hydroxycholesterol increased and no change was observed in any of the antioxidant enzymes activity in the erythrocytes. Although cholesterol feeding caused a 10-fold increase of liver cholesterol as ester in both rats and rabbits, the antioxidant enzyme GPx and catalase activities in the liver significantly increased in rats but significantly decreased in rabbits. The increase of GPx and catalase activities in the liver of cholesterol fed rats could have a protective role against oxidation, thus preventing the formation of lipid peroxidation and oxysterols.

The obtained result supported by that **of Deniz et al., (2007)** Hypercholesterolemia diet induced significant increases in GSH ($p < 0.001$) and Cu Zn SOD ($p < 0.001$) levels, whereas a significant decrease in GPx activity ($0.05 > p > 0.02$) was observed in aged rats. In young rats hyperchlesterolemic diet caused a significant increase in both GSH and CuZnSOD levels. Our results indicate an imbalance between radical production and destruction in favour of pro-oxidant conditions in the young rats and the induction by hypercholesterolemic diet of the ant oxidative response in erythrocytes. Furthermore, **Guasch-Ferré et al., (2014)** Olive oil consumption, specifically the extra-virgin variety, is associated with reduced risks of cardiovascular disease and mortality in individuals at high cardiovascular risk.

On the opposite side, the obtained results disagree with those of **Mohammed et al.; (2014)** who demonstrated that feeding oils rich in polyunsaturated fatty acids (PUFA) increases lipid peroxidation significantly and may raise the susceptibility of tissues to free radical oxidative damage.

REFERENCES

Falk, E. (2006): Pathogenesis of atherosclerosis. Journal of the American College of Cardiology, 47(8s1), C7-C12.

Campbell, K. A., Lipinski, M. J., Doran, A. C., Skaflen, M. D., Fuster, V. and McNamara, C. A. (2012): Lymphocytes and the adventitial immune response in atherosclerosis. Circulation research. 110(6): 889-900.

Pelucchi, C., Bosetti, C., Negri, E., Lipworth, L. and La Vecchia, C. (2011): Olive oil and cancer risk: an update of epidemiological findings through Current pharmaceutical design. 17(8): 805-812.

National Research Council, (1995): Committee on Animal Nutrition Nutrient requirements of the laboratory rat. In: Nutrient Requirements of Laboratory Animals. National Academy Press, Washington, DC. 27-38.

Mortensen, A., Espensen, P. L., Hansen, B. F. and Ibsen, P. (1992): The influence of dietary olive oil and margarine on aortic cholesterol accumulation in cholesterol-fed rabbits maintained at similar plasma cholesterol level. Atherosclerosis. 96(2): 159-170.

Park, K., et al. (2005):"Initial validation of a novel rat model of vasculogenic erectile dysfunction with generalized atherosclerosis." International journal of impotence research. 424-430.

Fischer, A. H., Jacobson, K. A., Rose, J. and Zeller, R. (2008): Hematoxylin and eosin staining of tissue and cell sections. Cold Spring Harbor Protocols. (5): pdb-prot. 4986.

Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W. and Fu, P.C. (1966): Enzymatic determination of total serum cholesterol. Clin. Chem. 20: 470-475.

Fossati, P. and Prencipe, L. (1982): Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem. 28(10): 2077-2080.

Lopez-Virella, M. F. P., Stone, S. and Ellis, J. A. (1977): Colwell Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem. 23(5): 882-884.

Friedwald, W. T., levy, R. E. and Frederickson, D. S. (1972): Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of the ultracentrifuge. Clin. Chem. 18: 499 – 502.

Beutler, E., Duron O. and Kelly, M. B. (1963): Improved methods for the determination of reduced glutathione. J. Lab Clin. Med. 61: 882-888.

Nishikimi, M., Roa, N. A. and Yogi, k. (1972): Biochem. Bioph. Res. Common. 46: 849- 854

Aebi H Catalase in vitro (1984): Methods Enzymol. 105: 121–126.

Draper, H. H. and Hadley, M. (1990): Malondialdehyde determination as index of lipid Peroxidation. Methods in enzymology.186: 421-31.

Lawrence, R. A. and Burk, R. F. (1976): Glutathione peroxidase activity in selenium-deficient rat liver.

Biochemical and biophysical research communications. 71(4): 952-958.

Shrapnel, W. S., Calvert, G. D., Nestel, P. J . and Truswell, A. S. (1992): Diet and coronary heart disease. The National Heart Foundation of Australia. Med J Aust. 4;156 Suppl: S9-16.

Widmer, R. J., Freund, M. A., Flammer, A. J., Sexton, J., Lennon, R., Romani, A., Mulinacci, N., Vinceri,, F. F., Lerman, L. O. and Lerman, A. (2013): Beneficial effects of polyphenol-rich olive oil in patients with early atherosclerosis.

Moreno-Luna, R., Munoz-Hernandez, R., Miranda, M. L., Costa, A. F., Jimenez, L., Vallejo., et al., (2012): Olive oil polyphenols decrease blood pressure and improve endothelial function in young women with mild hypertension. American J. of hypertension. 25(12): 1299- 1304.

Packard, C. J., McKinney, L., Carr, K. and Shepherd, J. (1983): Cholesterol feeding increases low density lipoprotein synthesis. Hum Nutr Clin Nutr. 72(1): 45–51.

Covas, M. I. (2007): Olive oil and the cardiovascular system. Pharmacological Research. 55(3): 175-186.

Vessby, B., Lithell, H. and Boberg, J. (1982): Reduction of low density and high density lipoprotein cholesterol by fat-modified diets. A survey of recent findings. Hum Nutr Clin Nutr. 36(3):203-11.

Mohamedain, M., Mahfouz, Fred, A. and Kummerow. (2000): Cholesterol-rich diets have different effects on lipid peroxidation, cholesterol oxides, and antioxidant enzymes in rats and rabbits Journal of Nutritional Biochemistry. 11(5): 293-302.

Deniz, O., Gumus, S., Yaman, H., Ciftci, F., Ors, F., Cakir, E., Tozkoparan, E., Bilgic, H. and Ekiz, K., (2007): Serum total cholesterol, HDL-C and LDL-C concentrations significantly correlate with the radiological extent of disease and the degree of smear positivity in patients with pulmonary tuberculosis. Clinical biochemistry, 40(3), pp.162-166

Mohammad El-Sayed, Yassin El-Sayed, Haggag, Rafaat, Mohamed Elsanhoty and Mohamed Fawzy Ramadan (2014): 12:78. doi: 10.1186/1741-7015-12-78.

الملخص العربى
دور زيت الزيتون فى تصلب الشرايين فى الجرذان

بوزيد بوخزيم* ، السعيد الشربينى*، جهاد رمضان*، مصباح عبد الجواد**

* قسم الكيمياء الحيوية و كيمياء التغذية ، كلية الطب البيطرى، جامعه المنصورة
** قسم التشريح و الأجنه ، كلية الطب البيطرى، جامعه المنصورة

من هدة الدراسة نستنتج ان تناول الغداء ذات الكوليسترول العالي يؤدى الى زيادة في مستوى الكوليسترول الكلى ومعدل المالون داى الدهيد في مصل الدم وكدا نشاط انزيم السوبر اوكسيد دبسميوتيز في دم الفئران كما تؤدى الى نقص معنوي في معدل الكوليسترول منخفض الكثافة ومعدل الجلوتاثيون المختزل وكدا نشاط انزيم الكتاليز. كما حدث تغير غير معنوي في معدل الكوليسترول عالي الكثافة وكدا نشاط انزيم نشاط انزيم الجلوتاثيون بروكسيديز في الفئران. وقد تم تأكيد هذة النتائج بنتائج الهستوباثولوجى. ووجد ان تناول زيت الزيتون يقلل من خطر امراض القلب والكوليسترول.

من هده الدراسة ننصح بأن تناول زيت الزيتون الطازج يقلل من خطر امراض القلب وتصلب الشرايين.