


Biochemical studies on olive leaves and oil against high sucrose diet in rabbits

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ABSTRACT

Background: Olive leaves and oil's beneficial effects are not only related to its high content of oleic acid, but also to the antioxidant potential of polyphenolics. **Objectives:** The present study was aimed to assess the effects of olive leaves and oil treatments on high sucrose induced oxidative damage in rabbits. **Methods:** Forty male rabbits were allocated into four groups; control group was kept on normal basal ration, sucrose fed group was received 30% sucrose containing ration, sucrose plus olive leaves group was administered olive leaves 5% in ration beside sucrose 30% and sucrose plus olive oil group was received olive oil 5% in basal ration together with sucrose 30% for 60 days. **Results:** Feeding high sucrose diet significantly increased total lipid, triacylglycerol, cholesterol and LDL-c levels while decreased HDL-c level with hyperglycemia. High sucrose induced hepatic damage assessed by increasing liver marker enzymes. Increased testosterone, copper levels with decreased serum zinc level were also observed. Furthermore, high sucrose diet caused an induction in oxidative stress biomarkers with subsequent depletion in antioxidant enzymes (glutathione-S-transferase and catalase) and reduced glutathione level in liver, heart and kidneys. On the other hand, treatment with olive leaves or oil improved serum lipid profile, liver functions, glucose, zinc and copper levels but decreased testosterone level. Moreover, olive leaves or oil attenuated lipid peroxidation with increase in the antioxidant enzymes. **Conclusion:** We concluded that olive leaves as well as oil had improved the biochemical alterations induced by feeding high sucrose diet for long time.

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INTRODUCTION

High-sucrose (HS) intake was shown to contribute to syndromes such as hyperlipidemia, glucose intolerance, hypertension, and atherosclerosis ⁽¹⁾. Numerous studies showed that a HS diet induces insulin resistance in rodents ⁽²⁾. The pathogenesis of insulin resistance caused by HS is unclear. It was reported that excess circulating free fatty acids (FFA) and glucose may contribute to insulin resistance ⁽³⁾.

On the other hand, it was reported that adipocytokines such as leptin, adiponectin, tumor necrosis factor (TNF)- α , plasminogen activator inhibitor (PAI)-, and IL-6 are secreted from adipose tissue. The alteration of lipid metabolism and the secretion of adipocytokines in adipose tissue may be critical for the development of lifestyle-related diseases such as obesity, hyperlipidemia, diabetes, hypertension, and atherosclerosis ⁽⁴⁾.

It is known that diet rich in sucrose increases the synthesis and secretion of VLDL by the liver and induces hypertriglyceridemia in humans and rats ⁽⁵⁾. Various in vivo studies in animals and humans had provided evidence that HSD induced hyperlipidemia and oxidative stress ⁽⁶⁾. Moreover, McDonald ⁽⁷⁾ reported that fructose obtained from hydrolysis of sucrose facilitate oxidative damage. High sucrose diet had been believed to affect the balance between production of free radicals and antioxidant defense mechanism and thus can cause oxidative stress. It also resulted in increment of plasma lipid peroxidation and greater susceptibility to peroxidation in various tissues ⁽⁸⁾. El-Hafidi et al. ⁽⁹⁾ mentioned that the activity of catalase is reduced in the sucrose fed rats liver homogenate which may be contributed to increased levels of lipid peroxidation and protein carbonylation in whole liver cells.

Olive oil is an integral ingredient in the Mediterranean diet. There is growing evidence that it may have great health benefits including the reduction in coronary heart disease risk, the prevention of some cancers and the modification of immune and inflammatory responses ⁽¹⁰⁾. Virgin olive oil appears to be a functional food with various components such as monounsaturated fatty acids that may have nutritional benefits. It is also a good source of phytochemicals, including polyphenolic compounds ⁽¹¹⁾. It is known that an increased consumption of monounsaturated fatty acids (MUFA) instead of polyunsaturated fatty acids (PUFA) reduced the risk of atherosclerosis because it decreased the circulating lipoprotein's sensitivity to peroxidation ⁽¹²⁾.

Furthermore, several studies showed a direct beneficial role of olive oil in improving plasma lipids in the treatment of metabolic syndrome ⁽¹³⁾. Unrefined or virgin olive oil had bioactive compounds with beneficial antioxidants action ⁽¹⁴⁾. In addition, the phenolics extract of olive leaves decreased the concentration of TG and LDL-cholesterol and improved the level of HDL-cholesterol that had been linked to a lower risk of coronary heart disease ⁽¹⁵⁾.

Alirezai et al. ⁽¹⁶⁾ reported that administration of purified oleuropein from olive leaves is able to reduce the oxidative damage caused by ethanol in rat's liver and suppresses oxidative stress as monitored by the elevation of the main anti-peroxidative enzyme activities; catalase and decreases lipid peroxidation products in the rat's liver. Also, polyphenols present in olive oil such as oleuropein, hydroxytyrosol, tyrosol and caffeic acid had an important antioxidant and anti-inflammatory effect ⁽¹⁷⁾.

Therefore, regular consumption of this oil in the diet provides a constant supply of potential antioxidants that could reduce oxidative stress through the inhibition of lipid peroxidation, a factor that is currently linked to a host of diseases and scavenging of free radicals ⁽¹⁸⁾. The present work was designed to study the efficacy of olive oil and leaves to alleviate the biochemical alteration resulted from feeding high sucrose ration in male rabbits with special reference to antioxidant enzymes and serum testosterone level.

Material and Methods

Chemicals, reagents and medicinal plants

Cummene hydroperoxide, 1-chloro-2, 4-dinitrobenzene (CDNB), 5-5-dithiobis-2-nitrobenzoic acid (DTNB) 3, 5- dichloro-2-hydroxybenzen-sulfuric acid (DHBS) and 4-aminophenazone (AAP) were obtained from (Sigma chemical Co. St., Louis, MO, USA). Thiobarbituric acid (TBA) and reduced glutathione (GSH) were obtained from Fluka

Chemical Co. Trichloroacetic acid (TCA) and tris base were obtained from Merck Chemical Co. All the reagents used were of analytical grade. Kits for cholesterol, triacylglycerol, ALT, AST, ALP, GGT, total proteins, albumin were obtained from Human Company, Germany. Kits for HDL-c, LDL-c, and Zinc provided from Dialab Production and Vertrieb von chemisch Company, Austria. Kits for urea and creatinine were obtained from Vitro Scient Company, Egypt. Kits of glucose was obtained from Spin React Company, Spain. Kits of copper was obtained from Centronic GmbH Company, Germany. Kits for total lipids was obtained from FAR Srl Company, Italy. Olive leaves were gathering from some regions of Borg El-Arab (Egypt), the leaves were washed by distilled water and dried. The leaves were then powdered and passed through mesh to increase their contact with powdered ration. Virgin olive oil was purchased from Wadi Food Company, Egypt and all were identified and authenticated by botanists in the department of Botany, Faculty of Agriculture, Alexandria University, Egypt.

Animals and experimental design

Forty adult male rabbits weighing 1300-1600 g (provided from Faculty of Veterinary Medicine, Alexandria University, Egypt) were used in this study. All rabbits were kept on the basal ration composed of 18% crude protein, 14% crude fiber, not less than 2.5% fat, 0.4% calcium, 0.32% phosphorus, 1.359% magnesium and of 2600 Kcal digestible energy/Kg ration. After acclimatization for 2 weeks, the animals were maintained in a strictly controlled temperature ($18 \pm 1^\circ\text{C}$). Humidity was kept at 50% and the lighting cycle was 14 h light and 10 h dark with adequate ventilation. Animals were handled with human care in accordance with the National Institutes of Health guidelines. The rabbits were randomly divided into four groups each consisting of ten animals as in the following design: the first group fed on basal ration and distilled and served as control, second group received 30% sucrose containing ration. Third group received 30% sucrose containing ration and olive leaves 5% mixed with basal ration; finally fourth group kept on 30% sucrose containing ration olive oil 5% mixed with basal ration. All treatment was continued for two month.

Blood sampling and preparation

At the end of experiment, blood samples were withdrawn from the ear vein of each rabbit and each sample was collected into clean tubes. The blood samples were allowed to coagulate and then centrifuged at 3000 rpm for 5 min. The separated sera were kept at -20°C until used for the estimation of serum activities of ALT, AST, ALP and GGT, total protein, albumin levels and lipid profile (total lipids, total cholesterol, triacylglycerol, HDL-c, LDL-c and VLDL-c). Also, the sera were used for determination of testosterone, zinc, copper, urea, creatinine and glucose levels.

Preparation of tissue sample

The rabbits were sacrificed by slaughtering and the liver, heart and kidneys were rapidly removed. 500 mg of each tissue was weighed and homogenized, using glass homogenizer with ice-cooled saline to prepare 25% w/v homogenate. The homogenate was divided into two aliquots. The first one was deproteinized with ice-cooled 12% trichloroacetic acid and the obtained supernatant, after centrifugation at $1000 \times g$ was used for the estimation of reduced glutathione (GSH) content. The second aliquot was centrifuged at $1000 \times g$ and the resultant supernatant was used for estimation of glutathione-S-transferase (GST) and catalase (CAT) activities, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels.

Biochemical analysis

Total serum testosterone was determined according to ⁽¹⁹⁾ using solid phase radioimmunoassay (RIA) kits purchased from Diagnostic Products Corporation, Los Angeles, USA. Zinc level was determined according to method described by ⁽²⁰⁾. Serum Zinc forms red

chelate complex with 2, 5-Bromo-2-pyridylzo-5-N-propyl-sulfo-propylamino-phenol. The increase of absorbance of this complex can be measured spectrophotometrically. Serum Copper level was determined according to method described by ⁽²¹⁾. Copper forms chelate complex with 4-/93, 5-Dibromo-2-pyridylazo-N-ethyl-N-sulfopropylaniline, the increase in absorbance of this complex can be measured and is proportional to the concentration of total copper in the sample. All other serum parameters were measured spectrophotometrically using commercial kits according to the manufacturer instructions.

Antioxidant status and oxidative stress indices

The determination of tissue GSH was determined according to method described by ⁽²²⁾. The method based on the reduction of 5, 5 dithiobis 2- nitrobenzoic acid with glutathione to produce a yellow compound. Tissue catalase activity was determined according to method described by ⁽²³⁾. Catalase reacted with a known quantity of H₂O₂; the reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase, remaining H₂O₂ reacted with 3, 5 Dichloro-2-hydroxybenzene sulfonic acid and 4-aminoantipyrine to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample. Glutathione-S-transferase activity was measured spectrophotometrically at room temperature as a rate of GSH conjugation of CDNB according to ⁽²⁴⁾. Lipid peroxide as Malondialdehyde was measured spectrophotometrically after the reaction with thiobarbituric acid according to ⁽²⁵⁾. Hydrogen peroxide was determined according to method described by ⁽²³⁾. In the presence of peroxidase, H₂O₂ reacts with 3, 5- dichloro-2-hydroxybenzen-sulfonic (DHBS) acid and 4-aminophenazone (AAP) to form a chromophore.

Statistical analysis

Statistical analysis was done by one way analysis of variance (ANOVA). Means and standard error were also calculated according to SAS ⁽²⁶⁾.

Results

Effect of high sucrose diet and olive leaves or oil on lipid profile

Administration of high sucrose diet induced significant increase in the levels of serum total lipid, total cholesterol, triacylglycerol, LDL-c and VLDL-c with significant decrease in serum HDL-c level as compared to control rabbits at P<0.05% (Table 1). Treatment with olive leaves or oil significantly reduced serum levels of total lipids, total cholesterol, triacylglycerol, VLDL-c and LDL-c (non-significant decreased with olive oil) with concurrent increased serum HDL-c level when compared to high sucrose administered rabbits.

Effect of high sucrose diet and olive leaves or oil on liver function

Compared to control rabbits, feeding rabbit with high sucrose diet exhibited significant increase in the activities of serum ALT, AST, ALP and GGT and globulins with significant reduction in serum total protein and albumin levels at P<0.05% (Table 2). However, on treatment with olive leaves, there were no significant difference in the activities of serum ALT and AST, total protein, albumin and globulins value but the activities of serum ALP and GGT tend to be significantly decreased as compared to high sucrose fed rabbits. Meanwhile, administration of olive oil statistically lowered the activities of serum ALT, AST, ALP and GGT without any significant changes in the levels of serum total protein, albumin and globulins when compared to high sucrose fed group (Table 2).

Effect of high sucrose diet and olive leaves or oil on serum testosterone, zinc, copper, urea, creatinine and glucose concentrations

Administration of high sucrose diet significantly increased serum levels of testosterone, copper and glucose with significant reduction in serum zinc level without any significant changes in serum urea and creatinine levels as compared to control rabbits at P<0.05% (Table

3). Treatment of high sucrose fed rabbit with olive leaves significantly decreased serum testosterone, copper and glucose levels with concurrent increase in the level of serum zinc level while, serum urea and creatinine values were not significantly changes when compared to high sucrose fed group. However, treatment with olive oil significantly reduced serum testosterone, copper, urea, creatinine and glucose contents with no significant change in serum zinc level as compared to high sucrose fed rabbit at $P < 0.05\%$ (Table 3).

Effect of high sucrose diet and olive leaves or oil on antioxidant status and oxidative stress indices

As compared to control rabbits, high sucrose feeding significantly induced oxidative stress expressed in increased levels of MDA and H_2O_2 in liver, heart and kidneys at $P < 0.05\%$ (Table 5) with subsequent decrease in the activities of antioxidant enzymes; GST and CAT and level of GSH in the tissues at $P < 0.05\%$ (Table 4). Treatment with olive leaves or oil significantly counteract the oxidative stress as evident by the reduction in the contents of MDA and H_2O_2 in the examined tissues (Table 5) and subsequent increase in the activities of antioxidant enzymes; GST and CAT and the level of GSH in all tissues examined (Table 4) when compared to high sucrose feeding rabbits.

Discussion

Effect of high sucrose diet and olive leaves or oil on lipid profile

Feeding high sucrose caused significant elevation in serum lipid fractions with decreased level of HDL-c (Table 1). HSD causes an increase of serum TAG level and dyslipidemia as a consequence of sucrose hydrolysis into its constituents; glucose and fructose, and their conversion into TGs⁽⁶⁾. In this process, a few facts are important: (1) the conversion rate of fructose into lipids in the liver and the TAG secretion rate is greater than that of glucose⁽²⁷⁾, (2) fructose administration does not stimulate the lipoprotein lipase release, and (3) the inflow of fatty acids into the liver from adipose tissue is not depressed⁽²⁸⁾.

The obtained data was agree with Hollenbeck⁽²⁹⁾ who reported that the increase in total lipids level after high sucrose diet may be attributed to its content of fructose which had been shown to increase plasma concentrations of triacylglycerols and cholesterol. In this respect, fructose, as a component of sucrose, is a major ingredient of many processed foods and has been proposed to contribute to the development of obesity and dyslipidemia in humans⁽³⁰⁾. Also, short-term consumption of a high sucrose diet increased the triacylglycerol level in liver and in plasma⁽⁸⁾. Moreover, hypertriglyceridemia after simple carbohydrate feeding such as fructose and/or sucrose resulted from the induction of de-novo lipogenesis, the enhanced rate of hepatic VLDL-triacylglycerol synthesis and a decrease in peripheral triacylglycerol clearance⁽³¹⁾. In addition, Huang et al.⁽³²⁾ reported that hepatic steatosis and plasma dislipidemia induced by sucrose feeding.

Treatment with olive leaves and oil resulted in significant improvement of hyperlipidemia in high sucrose fed rabbits as observed in (Table 1). Daily supplementation on top of the normal diet, of at least 4 g of extra virgin olive oil in mildly hypocholesterolemic subjects was associated to favorable modifications of the plasmatic lipid profile⁽³³⁾. The olive oil phenolic content decreased LDL and increased HDL levels in a dose-dependent manner⁽³⁴⁾. Furthermore, Puiggros et al.⁽³⁵⁾ reported a significant decrease in total cholesterol with the olive oil-enriched diet. Olive oil consumption increased HDL, while decreasing LDL, LDL susceptibility to oxidation and lipid peroxidation⁽³⁶⁾. Also, Wang et al.⁽³⁷⁾ found that the olive leaf decreasing LDL-c and the anti-atherosclerotic effect of olive leaf extract was also demonstrated in rabbits fed a high lipid diet.

Effect of high sucrose diet and olive leaves or oil on liver function

The data represented in table (2) showed that high sucrose feeding caused a dramatic increase in liver enzyme markers with depletion of total protein and albumin levels. This

came in harmony with ⁽³⁸⁾ who reported that cellular and membrane phospholipids are the major targets of damaging free radicals and therefore depletion of phospholipids in liver of high fructose-fed rats could be attributed to oxidative stress and increased release of hepatic enzymes. The increased activities of ALT, AST, ALP and GGT in group fed high sucrose diet may be attributed to hepatic fatty changes and hydropic degeneration as high sucrose diet induced hepatic steatosis and increased plasma hyperlipidemia in rodents ⁽³⁹⁾.

The total protein and albumin levels were decreased as a result of hyperlipidemia induced by high sucrose diet and diminished synthetic power of liver probably due to hepatocellular damage by fatty infiltration. Also, protein utilization in oxidative stress during hyperlipidemia as, protein and albumin are important components of the plasma antioxidant system (thiols used to reinforce the principal antioxidant system of the body which is glutathione) when this antioxidant system is overflowed by oxidative agents ⁽⁴⁰⁾. These obtained data were in agreement with Afolayan et al. ⁽⁴¹⁾ who found that total protein, albumin and albumin/globulin ratio were significantly lower in high cholesterol fed rats when compared to animals kept on basal diet.

Treatment with olive leaves or oil significantly improved liver function as shown in table (2). These obtained data agree with Alirezai et al. ⁽¹⁶⁾ who reported that the administration of oleuropein from olive oil resulted in reduction of transaminases activity in serum of the oleuropein group compared to ethanol-treated rats. Also, Nakbi et al. ⁽⁴²⁾ found that the extra virgin olive oil improved the elevated hepatic enzymes induced by 2, 4-Dichlorophenoxyacetic acid in rats. Furthermore, Hernández et al. ⁽⁴³⁾ reported that treatment with a balanced diet rich in olive oil contributed to the recovery of the liver from hepatic steatosis. This was achieved by decreasing activation of hepatic satellite cells by MUFAs, which are less susceptible to lipid peroxidation compared to PUFAs.

Moreover, previous studies carried out in fibrotic rats showed that olive oil, in contrast to polyunsaturated oils, could protect against the development of fibrosis ⁽⁴⁴⁾. Our obtained results also revealed that treatment with olive oil or leaves didn't ameliorate the decreasing effect of (total protein, albumin and A/G) resulted from high sucrose diet in treated animals. This result agrees with Rupić et al. ⁽⁴⁵⁾ who mentioned that no significant differences in the concentration of total proteins, albumin, alpha1, alpha2, beta and gamma globulin in the serum of rabbits in control group and in those received dried olive cake.

Effect of high sucrose diet and olive leaves or oil on testosterone, zinc, copper, urea, creatinine and glucose levels

Regarding testosterone concentration, high sucrose diet significantly increased serum testosterone level which may be attributed to increased caloric consumption which had been shown to elevate testosterone level, while decrease caloric intake depresses it ⁽⁴⁶⁾. The obtained data belonging to the effect of olive oil on testosterone concentration nearly agree with those obtained by Najafzadeh et al. ⁽⁴⁷⁾ who found that the hydro-alcoholic olive (*Olea europaea*) extract significantly decreased serum testosterone as well as sperm count, sperm motility, the weight of prostate, testis, epididymis, seminal vesicle. Also, Weber et al. ⁽⁴⁸⁾ reported that the olive is phytoestrogen which may have an inhibitory effect on the enzyme 17 β -hydroxysteroid hydrogenase therefore; the synthesis of testosterone in adrenal cortex is reduced. Another explanation for decreasing testosterone level may be attributed to hypocholesterolemic effect of olive leaves and oil according to Alirezai et al. ⁽¹⁶⁾ which may decrease the testicular testosterone synthesis.

Concerning zinc level, our study revealed that high sucrose diet significantly decreased serum zinc level which may be attributed to hyperlipidemia and oxidative stress and subsequently utilization of zinc as antioxidant. This result was in harmony with Ramalingam and Subrahmanyam ⁽⁴⁹⁾ who found that serum zinc level significantly reduced in high fat fed

rabbits than the control group. Increased zinc level after treatment with olive leaves may be attributed to potent antioxidant effect of olive leaves constituents (oleuropein) which prevent zinc depletion in oxidative process occurred in hyperlipidemic state and this was supported by Al-Azzawie and Alhamdani ⁽⁵⁰⁾ who reported that the positive impact of treatment with crude oleuropein on the antioxidant enzymes GPx, GR, CAT and SOD observed could be explained with two possible mechanisms. First, the antioxidative effect of oleuropein may prevent further glycosylation and peroxidation of proteins by interacting with free radicals and hence minimizing their noxious effects. Second, oleuropein may induce protein synthesis of these enzymes that explains the observed elevated activity after treatment.

Regarding the effect of high sucrose diet on serum copper level, our results were in agreement with Adekunle et al. ⁽⁵¹⁾ who reported that an increase in serum and tissue concentrations of lipids was accompanied with elevated serum copper concentration. Moreover, the increased concentration of lipid peroxides (malondialdehyde) which is an index of oxidative stress was found in rats given atherogenic diet. There is evidence that copper and zinc have pro-oxidant and antioxidant properties respectively, so that their imbalance may be expected in the oxidative stress associated conditions ⁽⁵²⁾. The significant decrease in serum copper level in groups kept on olive leaves or oil may be attributed to olive leaves and oil had hypolipidemic and antioxidant effect.

Our results regarding blood glucose level, high sucrose diet increased serum glucose level. In animal model of experimental diabetic diet containing higher level of fructose had been reported to contribute to a metabolic disturbance in animal models and result in hyperglycemia and hyperlipidemia ⁽⁵³⁾. There are many studies demonstrated the association between high fructose diet with elevation of plasma glucose, insulin and triglycerides in animal models. A 10% (w/v) fructose solution given for 12 weeks period increased blood glucose and TG level in Wistar rats ⁽⁵⁴⁾. In another report, a 10% (w/v) of HFD supplementation for four weeks had been shown to induce insulin resistance in male Sprague-dawley rats ⁽⁵⁵⁾.

Furthermore, Yadav et al. ⁽⁵⁶⁾ showed that a 21% (w/v) concentration of fructose in diet conferred a significant increase in blood glucose and TG after eight weeks on the diet regimen. In another study, a high fructose diet (10% w/v) for eight weeks had been shown to induce diabetes mellitus type 2 in two-month old male Sprague-dawley rats ⁽⁵⁷⁾.

Treatment with olive leaves or oil significantly reduced serum glucose level. Jamal and Ibrahim ⁽⁵⁸⁾ found that four weeks of consumption of olive oil significantly lowered the levels of fasting blood glucose, triglycerides, total cholesterol, and low density lipoproteins in diabetic patients. Also, Eidi et al. ⁽⁵⁹⁾ mentioned that in streptozotocin (STZ)-induced diabetic rats, olive leaf extract decreased serum concentration of glucose implying that olive leaf extract is more effective than glibenclamide and may be of use as an antidiabetic agent.

Effect of high sucrose diet and olive leaves or oil on oxidative stress markers and antioxidant status

The data represented in table (4, 5) showed that high sucrose diet significantly increased MDA and H₂O₂ with concurrent decrease in the antioxidant status in liver, heart and kidneys. These results came in harmony with Nandhini et al. ⁽⁶⁰⁾ who mentioned that male Wistar rats received fructose enriched diet resulted in increased lipid peroxidation and impaired antioxidant status. Fructose feeding can induce free radical formation by a number of mechanisms. It causes down regulation of the key enzymes of the hexose monophosphate pathway, namely glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase that generate a reduced environment in the form of NADPH and NADH, impaired regeneration of NADPH could result in an increased oxidative state of the cell ⁽⁶¹⁾. Moreover, Srividhya and Anuradha ⁽⁶²⁾ reported that increases in the levels of the

thiobarbituric acid-reactive substances (TBARS) and hydroperoxides were observed in the liver of fructose-fed rats.

Treatment with olive leaves or oil significantly decreased lipid peroxidation with enhancement of antioxidants. These results came with Masella et al. ⁽⁶³⁾ who found that oleuropein increased the expression of glutathione related enzymes at the transcriptional level. Also, olive leaves extract was shown to have a modulatory effect on the expression of the enzyme SOD in response to oxidative stress in vitro ⁽⁶⁴⁾. Treatment with crude oleuropein to diabetic rabbits showed a significant restoration in the levels of glutathione, β carotene and vitamin E and C. Moreover, multiple mechanisms had been proposed to explain beneficial effects of Mediterranean olive oil diet. These mechanisms include: decrease in LDL cholesterol, increase HDL-cholesterol, and reduction of oxidative stress due to polyphenols and flavonoids, which may act as scavengers and protect heart tissue and LDL from free radical damage ⁽⁶⁵⁾.

Virgin olive oil provides a rich variety of natural antioxidants including carotenoids, tocopherols, and polyphenols. These minor compounds may act by different mechanisms to confer an effective defense system against free radical attack. They seem to have a high antioxidant activity and provide greater stability to olive oil. Antioxidant capacity of olive oil can be attributed to the increase in tissue sensitivity to the lipid antioxidant effect of vitamin E ⁽⁶⁶⁾. Another possibility comes from some evidence that β -sitosterol and the polyphenols in olive oil (oleuropein, tyrosol, hydroxytyrosol, and caffeic acid) inhibit the formation of oxygen reactive species ⁽⁶⁷⁾, reduce the susceptibility of low density lipoprotein oxidation, erythrocyte membranes to lipid peroxidation ⁽⁶⁸⁾. Moreover, Kasdallah-Grissa et al. ⁽⁶⁹⁾ showed that the oral supplementation of olive oil to rats administered ethanol chronically restored damage caused to the liver by inhibiting lipid peroxidation and improving enzymatic activities. The mechanism proposed to explain the positive effects of olive oil may be attributed to its richness in MUFA, mainly oleic acid which has different effects on lipid profiles and peroxidation in rabbit hepatic mitochondria ⁽⁷⁰⁾.

Conclusion

Our results concluded that, high sucrose diet increase the serum lipid profile and glucose level. Also, HSD increased hepatic injuries and the levels of testosterone and zinc. Furthermore, the oxidative processes will be increased with depletion of the antioxidant enzymes in tissues of heart, kidney and liver. On the other hand olive leaves and oil improved the lipid profile, liver function, oxidative processes and increased antioxidant enzymes.

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Table 1: Mean \pm SE of total lipids, triacylglycerol, total cholesterol, HDL-c, LDL-c and VLDL-c (mg/dl) levels in rabbit's serum kept on high sucrose diet, olive leaves and oil

	Control group	High sucrose group	High sucrose + Olive leaves group	High sucrose + Olive oil group
T.lipids	255.60 \pm 4.98 ^c	407.60 \pm 7.50 ^a	306.20 \pm 13.46 ^b	334.60 \pm 18.78 ^b
TAG	53.50 \pm 2.01 ^d	242.86 \pm 15.43 ^a	135.00 \pm 5.00 ^c	171.38 \pm 4.38 ^b
TCH	60.00 \pm 4.30 ^b	72.14 \pm 3.60 ^a	55.00 \pm 3.02 ^b	60.57 \pm 3.15 ^b
HDL-c	24.80 \pm 1.62 ^b	15.00 \pm 1.00 ^c	23.40 \pm 1.66 ^b	30.40 \pm 1.54 ^a
LDL-c	15.80 \pm 2.82 ^b	23.80 \pm 2.42 ^a	16.00 \pm 1.30 ^b	20.25 \pm 1.11 ^a
VLDL-c	10.70 \pm 0.40 ^d	48.57 \pm 3.09 ^a	24.08 \pm 0.87 ^c	34.28 \pm 0.88 ^b

The means within the same row with different superscript letters are significantly differed at ($P < 0.05$)

Table 2: Mean \pm SE of ALT, AST, ALP and GGT (U/l) activities and total protein, albumin and globulins (g/dl) levels in rabbit's serum kept on high sucrose diet, olive leaves and oil

	Control group	High sucrose group	High sucrose + Olive leaves group	High sucrose + Olive oil group
ALT	38.30 \pm 0.94 ^b	48.75 \pm 2.47 ^a	46.33 \pm 0.61 ^a	25.38 \pm 2.57 ^c
AST	30.06 \pm 0.41 ^b	36.25 \pm 3.30 ^a	35.00 \pm 3.03 ^a	26.50 \pm 1.44 ^c
ALP	629.50 \pm 89.88 ^b	883.00 \pm 10.66 ^a	502.50 \pm 81.69 ^b	654.25 \pm 12.53 ^b
GGT	4.15 \pm 0.30 ^c	25.23 \pm 1.60 ^a	11.10 \pm 1.38 ^b	8.14 \pm 1.53 ^{bc}
T.protein	4.95 \pm 0.06 ^a	4.55 \pm 0.13 ^b	4.40 \pm 0.15 ^b	4.53 \pm 0.15 ^b
Albumin	3.97 \pm 0.09 ^a	3.03 \pm 0.09 ^b	2.90 \pm 0.11 ^b	3.13 \pm 0.06 ^b
Globulins	1.00 \pm 0.06 ^b	1.43 \pm 0.09 ^a	1.50 \pm 0.14 ^a	1.41 \pm 0.14 ^a

The means within the same row with different superscript letters are significantly differed at ($P < 0.05$)

Table 3: Mean \pm SE of testosterone (ng/ml), zinc and copper (ug/dl), urea, creatinine and glucose (mg/dl) levels in rabbit's serum kept on high sucrose diet, olive leaves and oil

	Control group	High sucrose group	High sucrose + Olive leaves group	High sucrose + Olive oil group
Testosterone	1.86 \pm 0.19 ^b	2.20 \pm 0.28 ^a	0.41 \pm 0.07 ^c	0.35 \pm 0.06 ^c
Zinc	294.75 \pm 6.13 ^a	190.75 \pm 4.50 ^c	206.00 \pm 2.80 ^b	193.75 \pm 2.87 ^{bc}
Copper	39.68 \pm 1.08 ^c	48.23 \pm 1.37 ^a	38.50 \pm 0.76 ^c	44.80 \pm 0.60 ^b
Urea	30.00 \pm 1.08 ^b	34.00 \pm 1.47 ^{ab}	26.00 \pm 1.47 ^{bc}	22.75 \pm 2.02 ^c
Creatinine	0.60 \pm 0.04 ^a	0.68 \pm 0.06 ^a	0.61 \pm 0.06 ^{ab}	0.50 \pm 0.04 ^b
Glucose	94.05 \pm 3.04 ^b	120.17 \pm 2.08 ^a	83.06 \pm 4.27 ^c	92.20 \pm 2.43 ^b

The means within the same row with different superscript letters are significantly differed at ($P < 0.05$)

Table 4: Mean \pm SE of GST, CAT (U/g tissue) activities and GSH (umole/g tissue) levels in rabbit's liver, heart and kidney kept on high sucrose diet, olive leaves and oil

	Control group	High sucrose group	High sucrose + Olive leaves group	High sucrose + Olive oil group
<i>Liver</i>				
GST	288.25 \pm 4.52 ^a	230.00 \pm 2.48 ^b	329.25 \pm 10.23 ^a	302.75 \pm 23.60 ^a
CAT	327.00 \pm 3.34 ^a	289.00 \pm 2.89 ^c	296.50 \pm 3.12 ^b	314.75 \pm 2.06 ^a
GSH	744.75 \pm 52.72 ^a	598.25 \pm 12.22 ^b	822.75 \pm 26.51 ^a	843.50 \pm 40.23 ^a
<i>Heart</i>				
GST	149.50 \pm 4.37 ^a	121.25 \pm 4.27 ^b	150.50 \pm 3.80 ^a	131.00 \pm 4.10 ^b
CAT	80.00 \pm 3.19 ^a	60.75 \pm 1.65 ^b	70.25 \pm 0.85 ^a	72.00 \pm 4.81 ^a
GSH	36.25 \pm 1.31 ^b	29.75 \pm 0.85 ^c	46.50 \pm 1.32 ^a	43.00 \pm 1.47 ^a
<i>Kidneys</i>				
GST	281.75 \pm 10.27 ^a	151.00 \pm 12.63 ^c	235.25 \pm 2.14 ^b	222.00 \pm 12.50 ^b
CAT	280.75 \pm 3.90 ^a	240.25 \pm 8.62 ^c	252.25 \pm 4.61 ^b	266.00 \pm 5.85 ^{ab}
GSH	75.50 \pm 4.52 ^a	29.50 \pm 2.22 ^c	47.50 \pm 2.78 ^b	50.25 \pm 4.48 ^b

The means within the same row with different superscript letters are significantly differed at (P<0.05)

Table 5: Mean \pm SE of MDA (nmole/g tissue) and H₂O₂ (mM/g tissue) levels in rabbit's liver, heart and kidney kept on high sucrose diet, olive leaves and oil

	Control group	High sucrose group	High sucrose + Olive leaves group	High sucrose + Olive oil group
<i>Liver</i>				
MDA	15.25 \pm 2.50 ^c	34.50 \pm 2.53 ^a	27.00 \pm 1.08 ^{ab}	25.50 \pm 3.38 ^b
H ₂ O ₂	13.25 \pm 0.80 ^b	45.73 \pm 1.05 ^a	12.30 \pm 2.41 ^b	13.53 \pm 0.65 ^b
<i>Heart</i>				
MDA	691.75 \pm 20.38 ^b	795.00 \pm 6.96 ^a	595.25 \pm 14.64 ^c	557.50 \pm 8.54 ^c
H ₂ O ₂	0.78 \pm 0.06 ^b	1.05 \pm 0.06 ^a	0.83 \pm 0.05 ^b	0.80 \pm 0.06 ^b
<i>Kidneys</i>				
MDA	29.00 \pm 3.08 ^c	85.50 \pm 2.47 ^a	30.00 \pm 2.04 ^c	39.50 \pm 2.10 ^b
H ₂ O ₂	0.74 \pm 0.11 ^b	5.20 \pm 0.40 ^a	0.41 \pm 0.07 ^b	0.73 \pm 0.04 ^b

The means within the same row with different superscript letters are significantly differed at (P<0.05)