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Hypocholesterolemic Effects of Phenolic Extracts and Purified Hydroxytyrosol Recovered from Olive Mill Wastewater in Rats Fed a Cholesterol-Rich Diet

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In our previous studies, a phenolic-rich extract of olive mill wastewaters (OMW) was prepared under optimal conditions, using a continuous countercurrent extraction unit, and hydroxytyrosol was purified from the obtained OMW extract. The antioxidant activity of OMW extract and hydroxytyrosol was determined by a series of models in vitro. In this study, the hypocholesterolemic effects of hydroxytyrosol and OMW extract in rats fed a cholesterol-rich diet were tested. Wistar rats, fed a standard laboratory diet or a cholesterol-rich diet for 16 weeks, were used. Serum lipid levels, as well as thiobarbituric acid reactive substances (TBARS) and superoxide dismutase and catalase activities in liver were examined. Cholesterol-rich diet-induced hypercholesterolemia was manifested in the elevation of serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C). Administration of a low-dose (2.5 mg/kg of body weight) of hydroxytyrosol and a high-dose (10 mg/kg of body weight) of OMW extract significantly lowered the serum levels of TC and LDL-C while increasing the serum levels of high-density lipoprotein cholesterol (HDL-C). Furthermore, the TBARS contents in liver, heart, kidney, and aorta decreased significantly after oral administration of hydroxytyrosol and OMW extract as compared with those of rats fed a cholesterol-rich diet. In addition, OMW phenolics increased CAT and SOD activities in liver. These results suggested that the hypocholesterolemic effect of hydroxytyrosol and OMW extract might be due to their abilities to lower serum TC and LDL-C levels as well as slowing the lipid peroxidation process and enhancing antioxidant enzyme activity.

KEYWORDS: Antioxidant enzymes; cholesterol-fed rat; hydroxytyrosol; hypocholesterolemic; olive mill wastewaters extract; serum lipid levels

INTRODUCTION

Cardiovascular disease is the leading cause of death in both industrialized and developing nations. A recent survey, carried out by the World Health Organization (*1*), indicates that coronary heart disease (CHD) alone accounts for more than half of the total mortalities associated with cardiovascular diseases (*2*). Atherosclerosis is the focal point to pathogenesis of these diseases. It is a condition involving arterial damage and is associated with some other vascular pathogenic states such as angina pectoris, myocardial infarction, and cerebral thrombosis. Because atherosclerosis is considered to be a multicentric disease, it involves various factors and different cell types. However, the endothelial damages, which ultimately generate atheroma and plaque formation, are characterized by high cholesterol and lipid concentrations along with free radical oxidative stress (*3*). Perhaps the involvement of hydroxyl radicals ($OH^{\bullet}$) is the major causative factor for the peroxidative modifications in circulatory low-density lipoprotein (LDL) that is responsible for initiation and progression of atherosclerosis (*4*, *5*). The oxidation of LDL is a lipid peroxidation chain reaction that transforms the polyunsaturated fatty acid and cholesterol into lipid hydroperoxides and oxycholesterol, respectively, thereby causing damage of arterial endothelial cells and promoting the formation and deposition of atherosclerotic plaque (*6*). Hence, the hypothesis was formulated that antioxidants may at least in part prevent atherosclerosis and cardiovascular disease.

Dietary antioxidants, such as vitamins E and C, have received considerable attention in this regard (*7*). There are large population studies showing that natural foods rich in antioxidants are associated with a low incidence of cardiovascular diseases. For example, in the Mediterranean area, where the diet is rich in fruits and vegetables and olive oil is the principal source of fat, the incidence of heart disease is lowest (*8*). Olive oil is obtained by pressing the olive fruit, which contains a variety of phenolic compounds that give extra virgin olive oil its

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particular taste. During olive oil extraction, large volumes of water are generated and subsequently discarded. These olive mill wastewaters (OMW) contain notable amounts of polar phenolic compounds including tyrosol, hydroxytyrosol, cafeic acid, and *para*-coumaric acid, which are major contributors to the toxicity of OMW (*9*). However, these phenolic compounds, which are present in general in olives, olive oil, and olive byproducts (*10*), are endowed with several biological activities such as antioxidant properties (*11*). Indeed, experiments in vitro and in vivo on laboratory animals have demonstrated that low-density lipoprotein cholesterol (LDL-C) oxidation was inhibited by olive oil constituents (*11*, *12*). It was also observed that olive oil phenolics affect lipid peroxidation and antioxidant parameters and lead to favorable changes in the plasma lipid status (*12*). Numerous in vitro studies have shown that olive oil phenolics are able to inhibit platelet aggregation, lipoxygenases, and ecasnoid production (*13*, *14*). Recently, the good bioavailability of olive oil polyphenols has also been reported (*15*, *16*).

In our previous studies, a phenolic-rich extract of OMW was prepared under optimal conditions, using a continuous countercurrent extraction unit, and hydroxytyrosol was purified from the obtained OMW continuous extract (*17*). The antioxidant activity of OMW extract and hydroxytyrosol was determined by a series of models in vitro (*18*). Therefore, in the present work, the hypocholesterolemic effects of the phenolic extract and hydroxytyrosol recovered from OMW were studied by analyzing their effects on serum lipid levels and antioxidant enzymes activity in rats fed a cholesterol-rich diet (HCD).

MATERIALS AND METHODS

Preparation of OMW Extract. OMW extracts were prepared from OMW as previously reported (*17*, *18*). Briefly, continuous countercurrent extractions of OMW were conducted at ambient temperature in a polyethylene mixer settler unit of Robatel design (mixer volume, 35 mL; settler volume, 200 mL). The total feed flow rate ranged from 2 to 5 L/h. For each run, the steady state was confirmed by phenolic monomer analysis in the organic stream and by verification of the mass flow rate balance. The maximum deviation of the latter was 2%. The organic extract was evaporated under vacuum at 40 °C in rotary evaporator. The residue was redissolved in a minimum volume of solvent and analyzed by high-performance liquid chromatography (HPLC).

Purification of Hydroxytyrosol. Hydroxytyrosol was purified from OMW as previously reported (*17*, *18*). Briefly, 1 g of OMW extract was chromatographed on a C-18 silica gel (liChroprep RP-18; 25−40 μm) column (2.5 mm × 70 mm) under medium pressure. Phenolic compound elution was carried out with the same gradient solvent as used in the HPLC. The flow rate was adjusted to 0.3 mL/min, and 4.5 mL fractions were collected. These fractions were measured by optical density at 280 nm, and the chromatogram (optical density versus fraction number) was represented (data not shown). The first separated peak corresponds to pure hydroxytyrosol.

Animals and Diets. Forty-eight male Wistar rats weighing between 180 and 200 g were purchased from the Pasteur Institute (Tunis). The animals were individually housed in stainless steel cages in a room at a 24 °C-controlled temperature and lighting alternating 12 h periods of light and darkness. All of the experimental procedures were carried out in accordance with internationally accepted guidelines for the care and use of laboratory animals. The rats were randomly divided into six groups of eight rats each. Group 1 was fed a normal diet (control diet, CD) (**Table 1**). Group 2 was fed a high cholesterol diet (HCD) (normal diet supplemented with 1% cholesterol and 0.25% bile salts). Groups 3 and 4 received HCD and hydroxytyrosol at 2.5 and 5 mg/kg of body weight, respectively. Groups 5 and 6 received HCD and OMW extract at 5 and 10 mg/kg of body weight, respectively. Phenolic compounds were administrated orally in drinking water. The duration of the treatment was 16 weeks. The animals were given food and water ad libitum during the experimental period. The body weight was measured daily. At the end of the experimental period, the rats were killed by decapitation. Blood samples were collected to determine the plasma lipid profile. The livers, hearts, and kidneys were removed and rinsed with physiological saline. All samples were stored at −80 °C until analyzed.

Table 1. Composition of the CD (g/kg)

diet ingredient	concentration
casein	200
DL-methionine	3
cornstarch	393
sucrose	154
cellulose	50
mineral mix[a]	35
vitamin mix[b]	10

[a] The mineral mixture contained (mg/kg of diet) the following: $CaHPO_4$, 17200; KCl, 4000; NaCl, 4000; MgO, 420; $MgSO_4$, 2000; Fe_2O_3, 120; $FeSO_47H_2O$, 200; and trace elements, 400 [$MnSO_4H_2O$, 98; $CuSO_45H_2O$, 20; $ZnSO_47H_2O$, 80; $CoSO_47H_2O$, 0.16; KI, 0.32; and sufficient starch to bring to 40 g (per kg of diet)]. [b] The vitamin mixture contained (mg/kg of diet) the following: retinol, 12; cholecalciferol, 0.125; thiamine, 40; riboflavin, 30; pantothenic acid, 140; pyridoxine, 20; inositol, 300; cyanocobalamin, 0.1; menadione, 80; nicotinic acid, 200; choline, 2720; folic acid, 10; *p*-aminobenzoic acid, 100; biotin, 0.6; and sufficient starch to bring to 20 g (per kg of diet).

Serum Lipids. Concentrations of TC, TG, and HDL-C in serum were determined by enzymatic colorimetric methods using commercial kits (Sigma). LDL-C was accomplished according to the procedures described by Friedwald and Levy (*19*). The atherosclerotic index (AI) was defined as the ratio of LDL-C, and HDL-C was also calculated for different groups.

Antioxidant Enzyme Activities. One gram of liver tissue was homogenized in 10 mL of 1.15% KCl and centrifuged at 7740*g* for 15 min. The supernatants were removed and stored at −80 °C for analysis. The amount of protein in supernatant was measured according to the method of Bradford (*20*) using bovine serum albumin as the standard.

The catalase (CAT) activity was measured using the method of Regoli and Principato (*21*). Twenty microliters of the supernatant was added to a cuvette containing 780 μL of a 50 M potassium phosphate buffer (pH 7.4), and then, the reaction was initiated by adding 200 μL of 500 mM H_2O_2 to make a final volume of 1.0 mL at 25 °C. The decomposition rate of H_2O_2 was measured at 240 nm for 1 min on a spectrophotometer. A molar extinction coefficient of 0.0041 mM^{-1} cm^{-1} was used to determine the CAT activity. The activity was defined as the μmol H_2O_2 decrease/mg protein/min.

Superoxide dismutase (SOD) was measured according to the method of Park et al. (*22*). A 100 μL amount of the supernatant was mixed with 1.5 mL of a Tris−EDTA−HCl buffer (pH 8.5) and 100 μL of 15 mM pyrogallol and then incubated at 25 °C for 10 min. The reaction was determined by adding 50 μL of 1 N HCl, and then, the activity was measured at 440 nm. One unit was determined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. The activity was expressed as units/mg protein.

Thiobarbituric Acid-Reactive Substances (TBARS) Concentration. As a marker of lipid peroxidation production, the TBARS(s) concentration was measured using the method of Park et al. (*22*). Briefly, 200 μL of a 10% (w/v) solution of the tissue homogenate was mixed with 600 μL of distilled H_2O and 200 μL of 8.1% (w/v) sodium dodecyl sulfate, vortexed, and then incubated at room temperature for 5 min. The reaction mixture was heated at 95 °C for 1 h after the addition of 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of 0.8 (w/v) TBA. After the mixture was cooled, 1.0 mL of distilled water and 5.0 mL of a butanol:pyridine (15:1) solution were added and vortexed. This solution was centrifuged at 1935*g* for 15 min, and the resulting colored layer was measured at 532 nm using malonaldialdehyde made by the hydrolysis of 1,1,3,3-tetramethoxypropane as the standard.

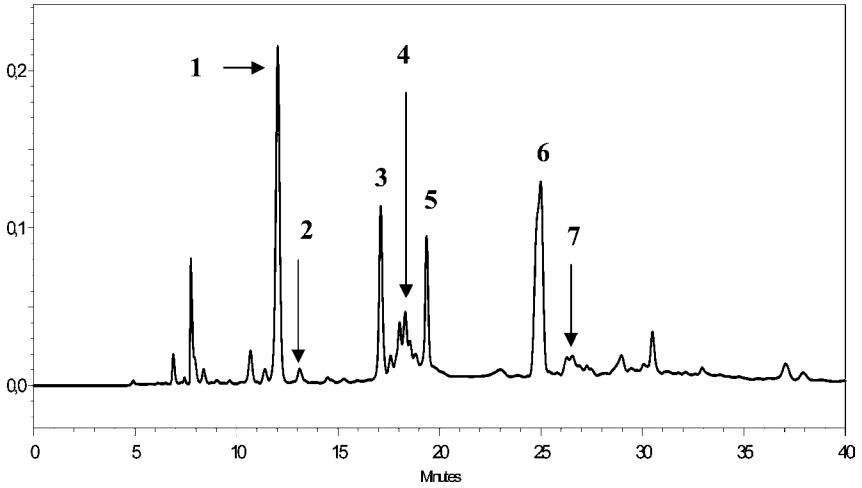

Figure 1. HPLC chromatogram of the liquid–liquid continuous ethyl acetate extract of OMV at 280 nm. Peaks: 1, hydroxytyrosol; 2, 3,4-dihydroxyphenyl acetic acid; 3, tyrosol; 4, *para*-hydroxyphenyl acetic acid; 5, cafeic acid; 6, *para*-coumaric acid; and 7, ferulic acid.

Table 2. HPLC Evaluation of Major Phenolic Compounds Identified in OMW Extract (g/L)

phenolic monomers	concentration in OMW extract (mg/L)
hydroxytyrosol	1225.6
tyrosol	345
3,4-dihydroxyphenyl acetic acid	70.2
para-hydroxyphenyl acetic acid	198
cafeic acid	256.7
para-coumaric acid	169
ferulic acid	70.2

Histopathological Analysis. At the time of sacrifice, the liver and aorta tissues were removed, cut into small pieces, and fixed in 10% formaldehyde solution. The washed tissues were dehydrated in the descending grades of ethanol and finally cleared in toluene. The tissues were then embedded in molten paraffin wax. Sections were cut at 5 μm thicknesses and stained with hemeatoxylin and eosin. The sections were then viewed under light microscope for histopathological changes.

Statistical Analysis. All data presented are the means ± standard errors (SEs). Statistical differences were calculated using a one-way analysis of variance, followed by Student's test. Differences were considered significant at $p < 0.05$.

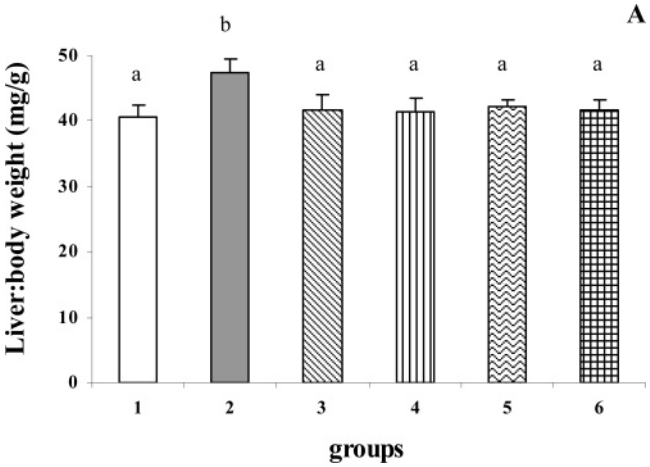

Figure 2. Effects of hydroxytyrosol and OMW extract on rat liver: body weight ratio. Group: 1, CD (standard diet); 2, HCD; 3, HCD + hydroxytyrosol (2.5 mg/kg); 4, HCD + hydroxytyrosol (5 mg/kg); 5, HCD + OMW extract (5 mg/kg); and 6, HCD + OMW extract (10 mg/kg). Each bar represents mean ± SE from eight rats. Bars with different letters differ; $p < 0.05$.

RESULTS

Extraction and Analysis of OMV Extract. The HPLC chromatogram of the final extract, obtained from OMW using the continuous countercurrent extractor under the optimal conditions, is shown in **Figure 1**. Hydroxytyrosol and tyrosol were the major compounds detected (**Table 2**). Their concentrations in the extract were 1225 and 345 mg/L, respectively. *para*-Hydroxyphenyl acetic acid, cafeic acid, and *p*-coumaric acid were present at lower concentrations. 3,4-Dihydroxyphenylacetic acid and ferulic acid were also detected at the same concentration, 70 mg/L (**Table 2**). Protocatechuic acid, vanillic acid, and synergic acids and other compounds were detected but not quantified.

Body and Organ Weights. The body weight increased in all groups throughout the treatment without significant differences between them (data not shown). The administration of cholesterol caused a significant increase in the liver:body weight ratio of HCD animals as compared with the control group (**Figure 2**). Simultaneous administration of hydroxytyrosol and OMW extract with dietary cholesterol significantly decreased the liver:body weight ratio. There were no differences in the heart and kidney:body weight ratios (data not shown).

Serum Lipids. Figure 3 shows the serum lipid levels at the end of the experiment. After 16 weeks of treatment, the TC and LDL-C concentrations of rats fed a HCD showed a significant increase as compared with the rats fed normal diet (CD). However, a decrease of HDL-C concentration of rats in the HCD group was observed. Rats that received an oral administration of hydroxytyrosol and OMW extract had lower concentrations of TC and LDL-C than those of rats that received an HCD. In particular, the administration of hydroxytyrosol at 2.5 and 5 mg/kg and OMW extract at 5 and 10 mg/kg reduced the TC and LDL-C levels by 30, 69%; 26, 48%; 19, 16%; and 27, 60%, respectively. Moreover, the concentration of HDL-C of rats treated with hydroxytyrosol and OMW extract increased significantly as compared with those of rats in the HCD group. The AI was significantly reduced by orally administrating phenolic compounds extracted from OMW. The AI and TC concentrations of animals treated with hydroxytyrosol at 2.5

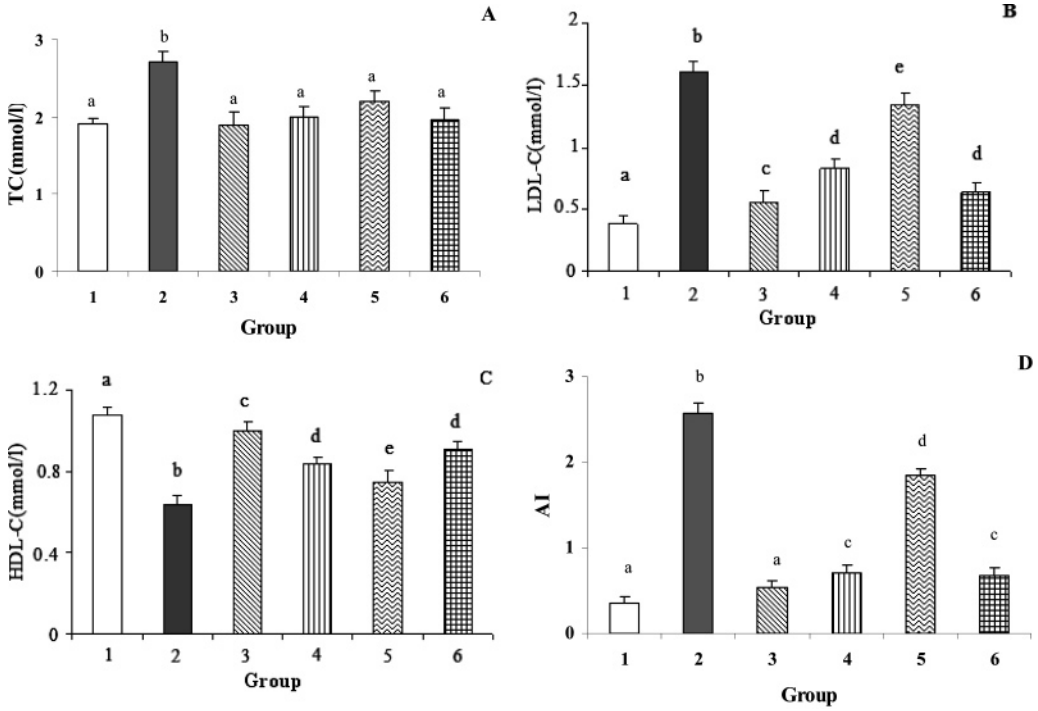

Figure 3. Effects of hydroxytyrosol and OMW extract on rat total cholesterol (TC) (**A**), low-density lipoprotein cholesterol (LDL-C) (**B**), high-density lipoprotein cholesterol (HDL-C) (**C**), and atherogenic index (AI) (**D**) levels. Groups: 1, CD (standard diet); 2, HCD; 3, HCD + hydroxytyrosol (2.5 mg/kg); 4, HCD + hydroxytyrosol (5 mg/kg); 5, HCD + OMW extract (5 mg/kg); and 6, HCD + OMW extract (10 mg/kg). Each bar represents mean ± SE from eight rats. Bars with different letters differ; $p < 0.05$.

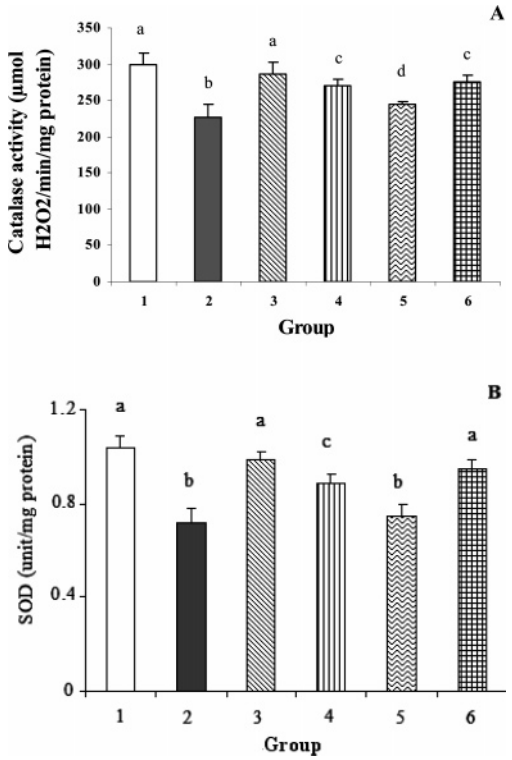

Figure 4. Effects of hydroxytyrosol and OMW extract on CAT (**A**) and SOD (**B**) activities in liver. Group: 1, CD (standard diet); 2, HCD; 3, HCD + hydroxytyrosol (2.5 mg/kg); 4, HCD + hydroxytyrosol (5 mg/kg); 5, HCD + OMW extract (5 mg/kg); and 6, HCD + OMW extract (10 mg/kg).

mg/kg were comparable to those of the control group ($p > 0.05$). However, AI values exceeded that of control group in rats administrated with hydroxytyrosol at 5 mg/kg and OMW at 5 and 10 mg/kg. The difference in the triglycerides (TGs) levels between the CD group and the HCD group of rats was not significant (data not shown).

Hepatic Antioxidant Enzyme Activities. The liver CAT and SOD activities of rats in HCD group were significantly lower ($p < 0.05$) than those of the CD group. The oral administration of phenolic compounds recovered from OMW in HCD animals significantly increased the SOD and CAT activities (**Figure 4**), excepted for SOD in group fed OMW extract at 5 mg/kg.

TBARS Levels. The TBARS levels showed a significant increase in liver, heart, kidney, and aorta of the animals fed on HCD when compared to the CD group. The administration of hydroxytyrosol at 2.5 mg/kg and OMW extract at 10 mg/kg in HCD animals significantly decreased the TBARS concentration (**Figure 5**). The simultaneous administration of hydroxytyrosol and OMW extract at 5 mg/kg with dietary cholesterol had no effect on the tissues TBARS (**Figure 5**).

Histopathologies of Liver and Aortic Wall. Figure 6 B showed the fatty changes of liver induced by the atherogenic diet in HCD animals. The photomicrograph pointed out vacuolated hepatocytes, with the nucleus being pushed to the periphery, and fatty cysts, an overall picture of fatty liver. **Figure 6C** depicted a more normal hepatic architecture, with the parenchymal structure preserved and occasional fat cells in the hydroxytyrosol (2.5 mg/kg) and OMW extract (10 mg/kg) treated group.

The histopathological examination of cholesterol-fed rats revealed the presence of lesion in the aortic wall when compared with the aorta of the control group (**Figure 7A**,**B**). However, these signs were significantly improved in the hydroxytyrosol (2.5 mg/kg) and OMW extract (10 mg/kg) treated group (**Figure 7C**).

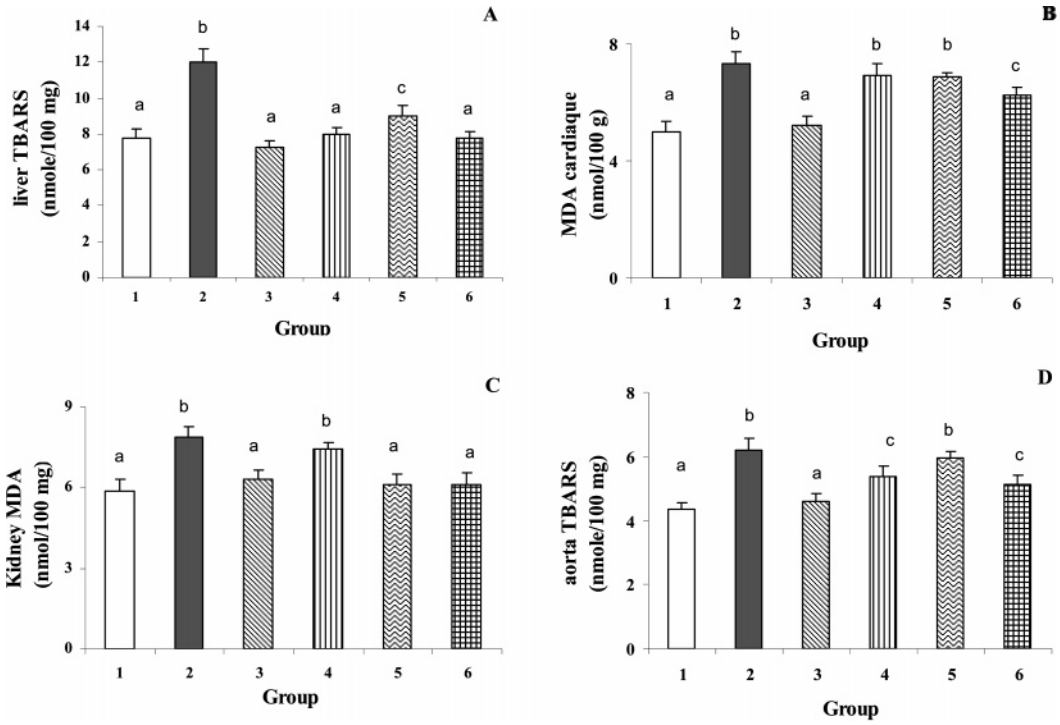

Figure 5. Effects of hydroxytyrosol and OMW extract on rat liver (**A**), heart (**B**), kidney (**C**), and aorta (**D**) TBARS levels. Group: 1, CD (standard diet); 2, HCD; 3, HCD + hydroxytyrosol (2.5 mg/kg); 4, HCD + hydroxytyrosol (5 mg/kg); 5, HCD + OMW extract (5 mg/kg); and 6, HCD + OMW extract (10 mg/kg). Each bar represents mean ± SE from eight rats. Bars with different letters differ; $p < 0.05$.

DISCUSSION

Phenolic compounds are now widely accepted as antioxidants that have a significant potential to protect against many degenerative diseases linked to free radical-related tissue damage (*23*). The health benefits of polyphenols would appear to arise from their antioxidant activity and capacity to protect critical macromolecules such as chromosomal DNA, structural proteins and enzymes, LDL, and membrane lipids from damage resulting from exposure to reactive oxygen species (*24*, *25*).

The present study investigated the effect of dietary phenolic compounds recovered from OMW to determine their possible role in a high-cholesterol-fed state. The results suggested that the serum lipid-lowering and antioxidative effect of these phenolic supplements were very potent in high-cholesterol-fed rats.

In this study, the administration of purified hydroxytyrosol and OMW extract induced a protective effect against atherosclerosis. Indeed, the concentration of hydroxytyrosol in OMW is 100−500-fold higher than that in olive oil; their recovery from OMW could be addressed with high priority. A simple, relatively low cost continuous extraction technique is adopted in this study in order to recover these high-value products. Optimal operational conditions were developed to extract the phenolic compounds present in OMW using a continuous countercurrent extraction unit. The HPLC analysis showed a predominant presence of hydroxytyrosol (1.225 g/L). In the current study, the hypocholesterolemic and antioxidative effects of OMW extract could be related to its content of hydroxytyrosol. Indeed, previous reports showed that this phenolic is the main antioxidant components in olives, olive oil, and olive byproducts (*10*, *26*). Other phenolic compounds with and without antioxidant activity, such as tyrosol and cafeic acid, were detected in OMW extract. Several studies have brought evidence that 3,4-dihydrocynamate possesses hypocholesterolemic activity and antioxidant properties when they are administrated at high doses. For instance, the oral administration of cafeic acid at 100 mg/kg was previously found to lower the plasma cholesterol in cholesterol-fed rats (*27*). In our study, the mean daily dose of this compound contained in the orally administered extract in the treated rats was lower than 1 mg/kg body weight, which is a lower dose than those previously reported.

Our finding demonstrated that rats fed a cholesterol-rich diet showed a higher concentration of TC and LDL-C and a reduced level of HDL-C as compared with rats fed a standard diet. Most cholesterol present in the body serves as a structural element in the walls of cells, whereas much of the rest is in transit through the blood or functions as the starting material for the synthesis of bile acids in the liver, steroid hormones in endocrine cells, or vitamin D in the skin (*28*). However, an increased concentration of TC and LDL-C raises the risk of developing CHD (*5*). In our study, the high concentrations of TC and LDL-C induced by the diet were significantly reduced by orally administrating hydroxytyrosol and OMW extracts. Lowering levels of TC and LDL-C has been linked to a lower risk of CHD (*5*). Therefore, the results of the present study indicate that phenolics recovered from OMW might be capable of reducing the incidence of CHD and agree with the results of previous study by Gorinstein et al. (*12*) who reported that polyphenols from olive oil decrease plasma LDL-C levels and prevent their oxidation in vivo. The hypocholesterolemic activity of olive oil polyphenols has been attributed to an increase in biliary cholesterol and bile acids concentrations and subsequent increases in the fecal excretion of these compounds (*29*). In our knowledge, no mechanisms independent from olive oil have been described for hydroxytyrosol with regard to reduction of blood lipids. Phenolic compounds from fruits and vegetables have been shown to have hypolipidemic properties in animal

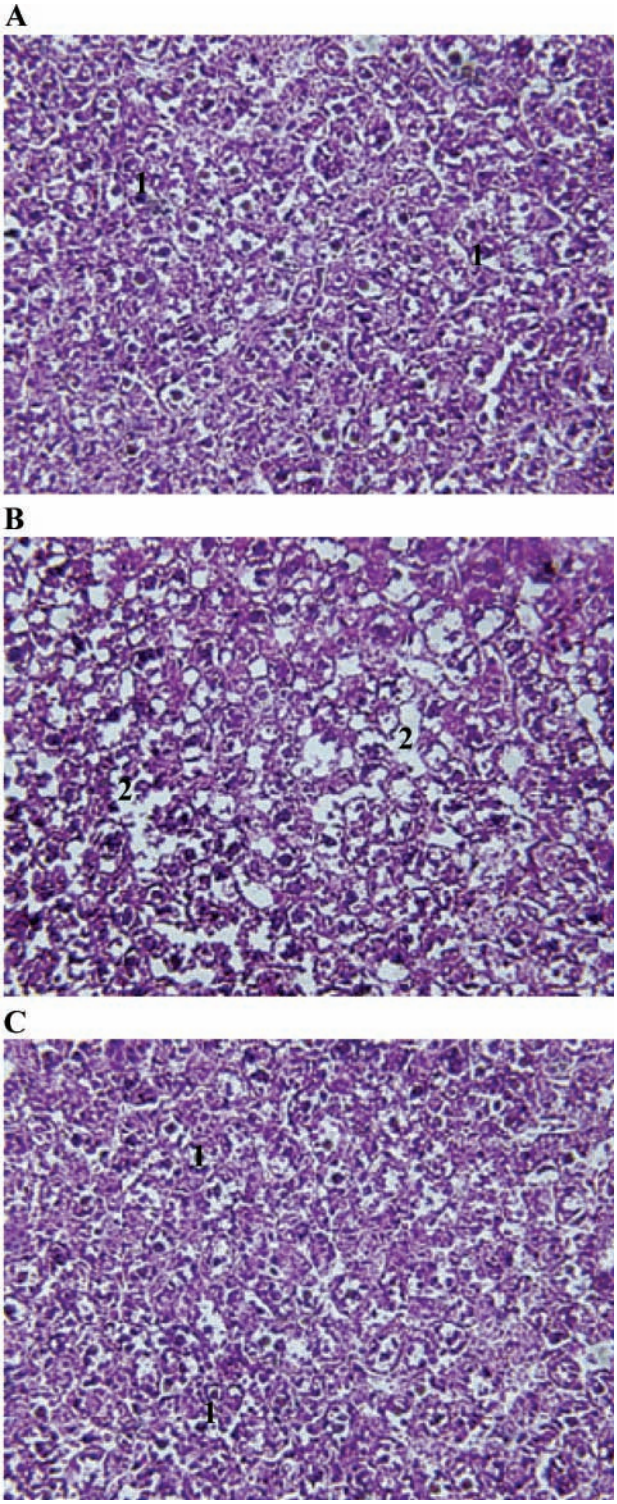

Figure 6. Microscopic views of transverse sections of liver in normal (**A**), high cholesterol-fed (**B**), and hydroxytyrosol or OMW extract supplements rats (**C**). Hematoxylin-Eosin (H-E) staining, 400×. Key: 1, normal hepatocyte; 2, hepatic lipid droplet.

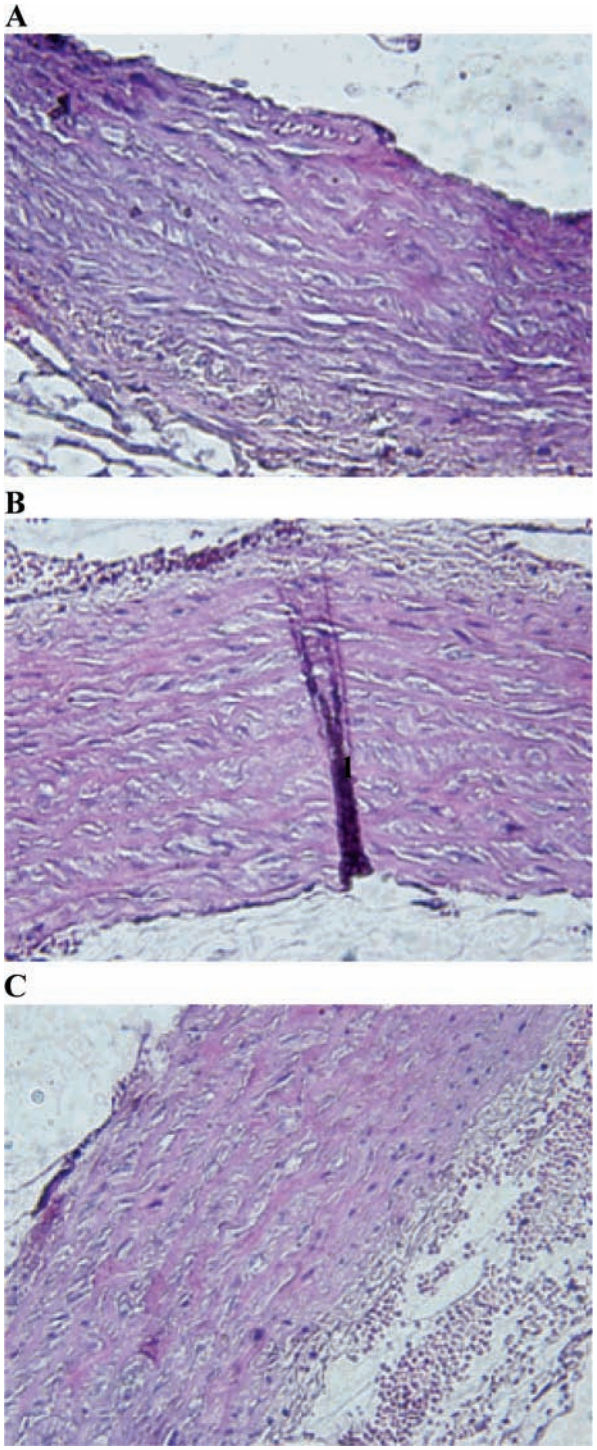

Figure 7. Microscopic views of transverse sections of aorta in normal (**A**), high cholesterol-fed (**B**), and hydroxytyrosol or OMW extract supplements rats (**C**). Hematoxylin-Eosin (H-E) staining, 400×. Key: 1, lesion of aortic wall.

models fed with a high-fat and/or a HCD (*30*, *31*). Studies performed on liver and intestinal cells have attributed these effects to a decrease in the assembly and/or secretion of apoB-containing lipoproteins (*32*, *33*). Green tea polyphenols have been shown to reduce blood lipids by decreasing micellar solubilization of cholesterol in the digestive tract and increasing fecal bile acids and cholesterol excretions (*34*). An antioxidant mechanism cannot be ruled out as liver antioxidant status has also been demonstrated to influence lipid synthesis and metabolism (*35*).

It is widely accepted that reduction in plasma HDL is a risk factor for developing atherosclerosis. HDL-C is considered to be "good" cholesterol in the circulation. It carries the cholesterol or cholesterol esters from peripheral tissues or cells to the liver where cholesterol is metabolized into bile acids (*36*). This pathway plays a very important role in reducing the cholesterol level in blood and peripheral tissues and in inhibiting the atherosclerotic plaque formation in the aorta (*37*). Our results

showed that OMW phenolics-supplemented diet increased the concentration of serum HDL-C when compared with the HCD. AI, believed to be an important risk factor of atherosclerosis, was significantly decreased in HCD animals supplemented with hydroxytyrosol and OMW extract. This decrease in AI can be said to be another positive change after treatment with phenolics recovered from OMW. Moreover, the histopathological analyses of the liver and aorta supported the biochemical data and indicated a retarding effect of hydroxytyrosol and OMW extract on the development of atherosclerosis in rats fed a cholesterol-enriched diet.

It is well-documented that an overabundance of free radicals in the cell leads to uncontrolled chain reactions and lipid peroxidation, resulting in various pathological conditions that may include atherogenesis and cancer (*38*). Prasad (*39*) has reported that hypercholesterolemic atherosclerosis is associated with an increase in the level of the lipid peroxidation product (TBARS), which is an index of the level of oxygen free radicals. Hence, a decrease in lipid peroxidation leads to the reduction of atherosclerosis caused by hypercholesterolemia (*40*). In the present study, oral administration of hydroxytyrosol and OMW extract prevented the cholesterol-rich diet-induced TBARS elevation and resulted in a significantly decreased of liver, heart, kidney, and aorta TBARS. The ability of phenolic compounds extracted from OMW to suppress the lipid peroxidation may partially be attributed to their antiradical activities acting by free radical scavenging or chain-breaking mechanisms (*41*−*43*). The obtained data suggest that hydroxytyrosol and OMW extract might be capable of lowering or slowing down oxidative stress-related lipid peroxidation.

It has been reported that during oxidative stress tissues respond by the induction of antioxidant mechanisms (*44*). However, enhanced oxidative stress may depress the antioxidant defenses. In the present study, we have observed decreased activities of antioxidant enzymes SOD and CAT in the liver of rats fed on HCD as compared to those on a normal diet. Our results are in agreement with reports of other workers, which suggest that feeding a HCD to experimental animals depresses their antioxidant system due to increased lipid peroxidation and formation of free radicals (*45*). The present study showed that the simultaneous treatment of cholesterol-fed rats with phenolic compounds recovered from OMW increased the activities of SOD and CAT. These effects could be due to a protective antioxidant effect of these phenolics along with the significant lipid-lowering effect (*46*, *47*).

In conclusion, the present study demonstrated that phenolic extract and hydroxytyrosol recovered from OMW had very pronounced hypocholesterolemic effects. They could significantly lower the concentrations of serum TC and LDL-C and elevate the serum HDL-C level. They decreased the content of TBARS in liver, heart, and kidney, while they also significantly increased the activities of CAT and SOD in liver. These results suggest that the hypocholesterolemic effect of hydroxytyrosol and OMW extract might be due to their abilities to lower serum TC and LDL-C levels as well as to slow the lipid peroxidation process and to enhance the antioxidant enzyme activity.

ABBREVIATIONS USED

OMW, olive mill wastewaters; CHD, coronary heart disease; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AI, atherosclerotic index; TGs, triglycerides; CAT, catalase; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; HPLC, high-performance liquid chromatography; HCD, cholesterol-rich diet; CD, control diet; ACAT, acyl-CoA, cholesterol acyltransferase; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase.

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