

Antidiabetic and Antioxidant Effects of Hydroxytyrosol and Oleuropein from Olive Leaves in Alloxan-Diabetic Rats

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This study was designed to test the antidiabetic and antioxidative activities of olive leaf oleuropein and hydroxytyrosol. Diabetes in Wistar rats was induced by intraperitoneal injections of alloxan. The serum glucose and cholesterol, hepatic glycogen, the thiobarbituric acid-reactive substances (TBARS), and the components of hepatic and serum antioxidant system were examined. Diabetic rats showed hyperglycemia, hypercholesterolemia, increased lipid peroxidation, and depletion in the antioxidant enzymes activities. The administration, for 4 weeks, of oleuropein and hydroxytyrosol rich extracts, leading to 8 and 16 mg/kg body weight of each compound, significantly decreased the serum glucose and cholesterol levels and restored the antioxidant perturbations. These results suggested that the antidiabetic effect of oleuropein and hydroxytyrosol might be due to their antioxidant activities restraining the oxidative stress which is widely associated with diabetes pathologies and complications.

KEYWORDS: Alloxan; diabetes; liver; oxidative stress; olive leaf polyphenol; hydroxytyrosol; oleuropein

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease with the highest rates of prevalence and mortality worldwide that is caused by an absolute or relative lack of insulin and or reduced insulin activity (1). It is characterized by hyperglycemia and long-term complications affecting the eyes, kidneys, nerves, and blood vessels, and is the most common endocrine disorder. Although the leading mechanism of diabetic complications remains unclear, much attention has been paid to the role of oxidative stress. It has been suggested that oxidative stress may contribute to the pathogenesis of different diabetic complications (2). Furthermore, with diabetes, several features appear including an increase in lipid peroxidation (3), alteration of the glutathione redox state, a decrease in the content of individual natural antioxidants, and finally a reduction in the antioxidant enzyme activities. These changes suggest an oxidative stress caused by hyperglycemia (4). Many defense mechanisms are involved in alloxan-induced oxidative damage. Among these mechanisms, antioxidants play the role of a free-radical scavenger (5). Nowadays, herbal drugs are gaining popularity in the treatment of diabetes and its complications. As a new strategy for alleviating the oxidative damage in diabetes, a growing interest has been noticed in the usage of natural antioxidants. It has been suggested that many of the negative effects of oxidative stress are diminished upon supplementation with certain dietary antioxidants such as vitamins and other non-nutrient antioxidants such as flavonoids (6).

Among natural antioxidants, the olive tree has been widely accepted as one of the species with the highest antioxidant activity

via its oil, fruits, and leaves. It is well known that the activity of the olive tree byproduct extracts in medicine and food industry is due to the presence of some important antioxidant and phenolic components to prevent oxidative degradations. The olive tree has long been recognized as having antioxidant molecules, such as oleuropein, hydroxytyrosol, oleuropein aglycone, and tyrosol (7, 8). Furthermore, olive leaves are considered as a cheap raw material which can be used as a useful source of high added value products (9). The main phenolic compounds in olive leaves are the glycosylated forms of oleuropein and ligstroside (10). The main active component in olive leaf extract is oleuropein, a natural product of the secoiridoid group. Several studies have shown that oleuropein possesses a wide range of pharmacologic and health promoting properties including antiarrhythmic, spasmolytic, immune-stimulant, cardioprotective, hypotensive, anti-inflammatory, antioxidant, and anti-thrombotic effects (11, 12). Many of these properties have been described as resulting from the antioxidant character of oleuropein (13). Previously, oleuropein was reported to have an antihyperglycaemic effect on diabetic rats (14). However, as regards the antioxidant properties of oleuropein, its mechanism in attenuating hyperglycaemia is still not well recognized. Upon hydrolysis, oleuropein can produce elenolic acid, hydroxytyrosol, tyrosol, and glucose (15).

However, particular attention has been paid to hydroxytyrosol (16), which occurs naturally in olive byproducts. This *o*-diphenol, like the majority of the olive phenols such as tyrosol, has been proven to be a potent scavenger of superoxide anion and hydroxyl radical (17, 18). It is endowed with significant antithrombotic, antiatherogenic, and anti-inflammatory activities (19).

In our previous studies, oleuropein and hydroxytyrosol-rich extracts from olive leaves were prepared, and their antioxidant

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activities were examined by a series of models *in vitro* (20, 21). Furthermore, we have studied their antioxidant activities *in vivo* as well as their hypocholesterolemic effects (7). No detailed study has been carried out on the efficacy of hydroxytyrosol purified from olive tree leaves, in moderating oxidative stress associated with diabetes mellitus in experimental animals. Hence, the present study was undertaken to investigate possible hypoglycemic and antioxidant effects of olive leaf extracts rich in hydroxytyrosol, compared with those rich in oleuropein in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Oleuropein-Rich Olive Leaf Extract Preparation. The extraction was carried out on Chemlali olive leaves dried and powdered. A mixture of methanol and water (200 mL, 4:1 v/v) was added to a sample of 50 g of olive leaf powder. The mixture was left to stand under agitation for 24 h and then was filtered. The extract was concentrated by evaporation to dryness at 40 °C, and the residue obtained was stored in glass vials, at 0 °C in the dark until HPLC analysis. A 4 g sample was dissolved in methanol (10 mL) and extracted three times with ethyl acetate (Prolabo, France) (40 mL) to prepare the oleuropein-rich extract.

Acid Hydrolysis. In order to obtain a hydroxytyrosol-rich extract, 1 g of the olive leaf extract was dissolved in 10 mL of a MeOH/H₂O (4:1) mixture in a sealed vial. The solution was hydrolyzed at 100 °C for 1 h using 5 mL of HCl (2 M) (Prolabo, France). After 1 h, the sample was cooled and diluted with water (10 mL), and the hydrophobic fraction was extracted by a separatory funnel three times with 25 mL of ethyl acetate (Prolabo, France), which was subsequently removed by evaporation.

HPLC Analysis. A reversed-phase high-performance liquid chromatographic (HPLC) technique was developed to identify and quantify the major phenolic compounds contained in the hydrolyzed extract. For this purpose, a standard mixture solution of phenolic compounds was analyzed. Sample concentrations were calculated on the basis of peak areas compared to those of each of the external standards. The HPLC chromatograph was a Shimadzu apparatus equipped with a (LC-10ATvp) pump and a (SPD-10Avp) detector. The column was 4.6 × 250 mm (Shim-pack, VP-ODS), and the temperature was maintained at 40 °C. The flow rate was 0.5 mL/min. The mobile phase used was 0.1% phosphoric acid in water (A) versus 70% acetonitrile in water (B) for a total running time of 40 min, and the gradient changed as follows: solvent B started at 20% and increased immediately to 50% in 30 min. After that, elution was conducted in the isocratic mode with 50% solvent B within 5 min. Finally, solvent B decreased to 20% until the end of running time.

Animals and Treatments. Adult male Wistar rats, weighing 180 ± 15 g, and obtained from the Central Pharmacy, Tunisia, were used in this study. The animals were kept in an environmentally controlled breeding room (temperature, 20 ± 2 °C; humidity, 60 ± 5%; 12 h dark/light cycle). All rats had access to a standard laboratory diet (SICO, Sfax, Tunisia) and were fasted overnight before blood and tissue collection. The handling of the animals was approved by the local Ethical Committee for the Care and Use of Laboratory Animals. Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared alloxan solution in normal saline at a dose of 180 mg/kg body weight (b.w.) (22). The feeding experiment was carried out for a period of 4 weeks after the induction of diabetes in 5 days (characterized by the presence of glucosuria). The rats were divided into 6 groups consisting of 10 rats each. Group C (normal control) consisted of normal rats. Group CD served as positive control (diabetic control). Groups OL1 and OL2 received oleuropein at 16 and 8 mg/kg of body weight respectively. Groups HY1 and HY2 rats received hydroxytyrosol at 16 and 8 mg/kg of body weight, respectively. The hydroxytyrosol- and oleuropein-rich extracts were dissolved in drinking water. The duration of the treatment was 4 weeks. The body weight was measured every day. At the end of the experimental period, the rats were killed by decapitation. Blood samples were collected to determine the plasma lipid profile. Pieces of livers were removed and rinsed with physiological saline solution. All samples were stored at -80 °C until analysis.

Serum Glucose and Hepatic Glycogen. Concentration of glucose in serum was measured using commercial kits from Sigma Munich (Munich, Germany). The concentration of hepatic glycogen was determined via the same kit after liver fragment hydrolysis.

Serum Cholesterol. Concentrations of total cholesterol (TC) in serum were determined by enzymatic colorimetric methods using commercial kits (Kyokuto Pharmaceuticals, Japan).

Antioxidant Enzyme Activities. The catalase (CAT) and superoxide dismutase (SOD) activities were evaluated in liver tissue. The preparation of the enzyme source fraction was as follows. One gram of liver tissue was homogenized in 10 mL of KCl (1.15%) and centrifuged at 7740g for 15 min. The supernatants were removed and stored at -80 °C for analysis. The protein content in the supernatant was measured according to the method of Bradford (23) using bovine serum albumin as standard. CAT activity was measured using the method of Regoli and Principato (24). Briefly, 20 μL 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 H₂O₂ to make a final volume of 1.0 mL at 25 °C. The decomposition rate of H₂O₂ 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 micromoles of H₂O₂ decrease per milligram of protein per minute. SOD activity was measured according to the method of Marklund and Marklund (25). This method is based on pyrogallol oxidation by the superoxide anion (O²⁻) and its dismutation by SOD. Briefly, 25 μL of the supernatant was mixed with 935 μL of a Tris-EDTA-HCl buffer (pH 8.5) and 40 μL of 15 mM pyrogallol. The activity was measured after 45 s 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 per milligram of protein.

ABTS Assay in the Serum Samples. The Trolox equivalent antioxidant capacity (TEAC) assay, measuring the reduction of the 2,2'-azinobis[3-ethylbenzothiazoline-6-sulfonate] (ABTS) radical cation by antioxidants, was derived from the method previously described (26) with minor modifications. Briefly, the ABTS radical cation (ABTS^{•+}) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For the study, ABTS^{•+} solution was diluted with phosphate buffered saline (PBS) at pH 7.4 to an absorbance of 0.70 (±0.02) at 734 nm. After the addition of 2 mL of diluted ABTS^{•+} solution to 50 μL of serum or Trolox standard, the reaction mixture was incubated for 6 min in a glass cuvette at 30 °C. The decrease in absorbance was recorded at 734 nm. All measurements were performed in triplicate. The free radical scavenging capacity of the biological sample, calculated as the inhibition percentage of ABTS^{•+}, was equated against a Trolox standard curve prepared with different concentrations (1.5–30 μmol/L). The results are expressed as micromolar Trolox equivalents.

Thiobarbituric Acid-Reactive Substances (TBARS) Assay. TBARS are the markers of lipid peroxidation. Their concentration was measured referring to assay by Park et al. (27). Briefly, 200 μL of a 10% (w/v) solution of the tissue homogenate was mixed with 600 μL of distilled H₂O and 200 μL of 8.1% (w/v) SDS, 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. Later, the mixture had cooled, and 1.0 mL of distilled water and 5.0 mL of a butanol/pyridine (15:1) solution were added under agitation using a vortex. This solution was centrifuged at 1935g for 15 min, and the resulting colored layer was measured at 532 nm. The concentrations were determined using a malondialdehyde (MDA) standard curve.

Histopathological Analysis. At the time of sacrifice, pieces of liver were removed and were fixed in a Bouin solution for 24 h and then embedded in paraffin. Sections were cut at 5 μm thicknesses and stained with hematoxylin and eosin. The sections were then viewed under a light microscope to detect eventual histopathological changes.

Statistical Analysis. Results were presented as the mean ± standard deviation (SD). The data follow a normal distribution. A two-way analysis of variance was performed using a Student's *t*-test on Microsoft Excel statistical software (Microsoft Corporation, Microsoft Office Excel 2003, Redmond, WA). The values were considered significantly different when the *p*-value was lower than 0.05.

RESULTS

Olive Leaf and Hydrolysate Extract Characterization. The phenolic composition of olive leaf extracts was analyzed via

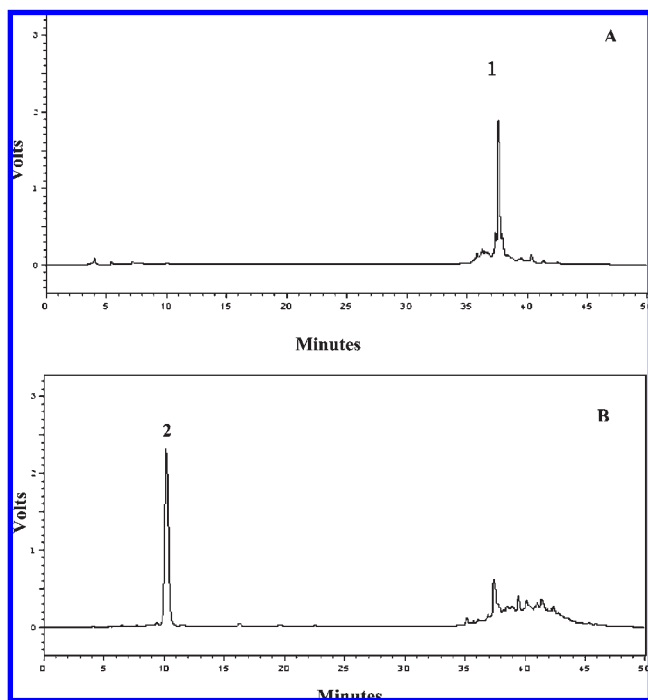


Figure 1. HPLC chromatograms at 280 nm of olive leaf extracts based on oleuropein (A) and olive leaf extract after acid hydrolysis based on hydroxytyrosol (B). (1) Oleuropein; (2) hydroxytyrosol.

HPLC (Figure 1). Figure 1A shows a concentration of oleuropein reaching 2.44 g/100 g dry weight. The identification of oleuropein was based on a comparison of the chromatographic retention time and UV absorbance spectra with those of an authentic standard. The analysis was confirmed using an LC-MS system operating in positive mode which showed a correspondence of the mass spectrum of the extract with the known fragmentation scheme for oleuropein (28).

However, the acid treatment of aqueous methanolic leaf extracts induced hydrolysis of the complex phenolic molecules. In fact, the HPLC profile of phenols in the hydrolysate extract showed that hydroxytyrosol was the major compound (Figure 1B) where its concentration reached 1.38 g/100 g dry weight. The identification of hydroxytyrosol was confirmed by LC-MS, which showed a fragmentation scheme consistent with the known hydroxytyrosol one previously reported (27).

Body and Organ Weights. There were no significant differences between the body and organ weight increases in all groups throughout the treatment (data not shown).

Serum Glucose and Hepatic Glycogen Concentration. The plasma glucose levels of control diabetic rats were significantly higher compared with those of the control group ($P < 0.05$) (Figure 2A). After oleuropein- and hydroxytyrosol-rich extract administration, a significant decrease in blood glucose was observed compared with that of the diabetic group ($P < 0.05$). Moreover, rats receiving oleuropein and hydroxytyrosol at 16 mg/kg b.w. showed a significantly pronounced hypoglycemic effect compared with those receiving these compounds at 8 mg/kg b.w. However, the hepatic glycogen levels show a significant decrease in diabetic rats compared with those in the controls (Figure 2B). However, all of the groups receiving the phenolic-rich extracts showed a significant increase in glycogen levels compared with those of the normal and to the diabetic controls. Rats receiving oleuropein at 16 mg/kg b.w. showed the significantly highest levels of hepatic glycogen concentration ($P < 0.05$).

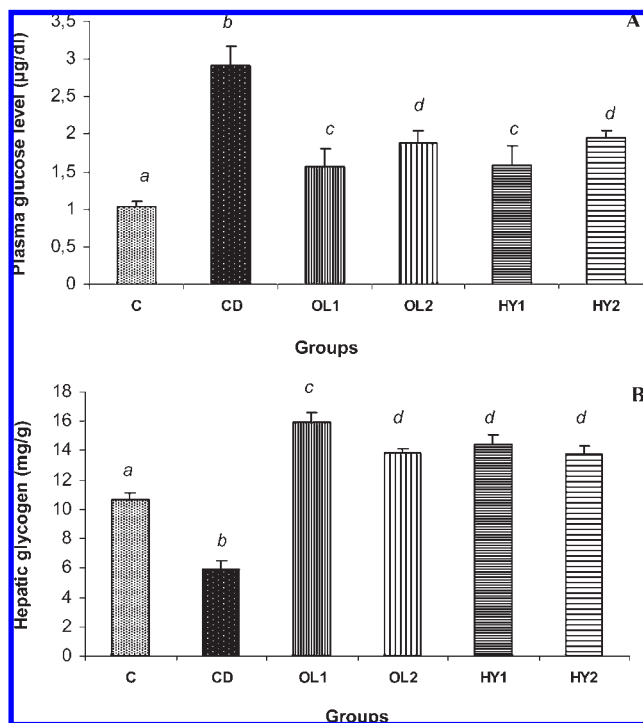


Figure 2. Effects of oleuropein and hydroxytyrosol rich extracts treatments on plasma glucose (mg/dL) (A) and hepatic glycogen (mg/g) (B) in diabetic rats. Group C, normal control; CD, diabetic control; OL1 and OL2, diabetic + oleuropein at 16 and 8 mg/kg b.w., respectively; HY1 and HY2, diabetic + hydroxytyrosol at 16 and 8 mg/kg b.w., respectively. Each bar represents mean \pm SD from 10 rats. Bars with different letters differ significantly; $P < 0.05$.

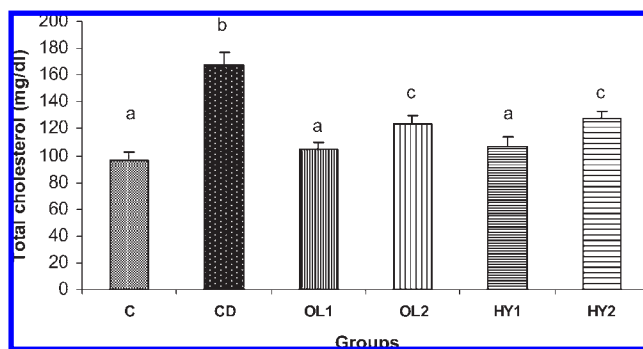


Figure 3. Effects of olive leaf extracts on rat total cholesterol levels. Group C, normal control; CD, diabetic control; OL1 and OL2, diabetic + oleuropein at 16 and 8 mg/kg b.w., respectively; HY1 and HY2, diabetic + hydroxytyrosol at 16 and 8 mg/kg b.w., respectively. Each bar represents mean \pm SD from 10 rats. Bars with different letters differ significantly; $P < 0.05$.

Total Cholesterol (TC). Serum lipid levels were measured at the end of the experiment (Figure 3). The total cholesterol concentrations of diabetic rats showed a significant increase compared with those of the control rats. However, rats having received an oral administration of oleuropein and hydroxytyrosol, at two different doses, had significantly lower concentrations of TC compared with those in the diabetic group. The administration of the phenolic-rich extracts was able to restore the lipid profile, mainly in groups receiving hydroxytyrosol and oleuropein at 16 mg/kg b.w., which had no significant differences compared to the normal levels of the control group ($P < 0.05$). Hydroxytyrosol and oleuropein significantly correct the hypercholesterolemia coupled with hyperglycemia.

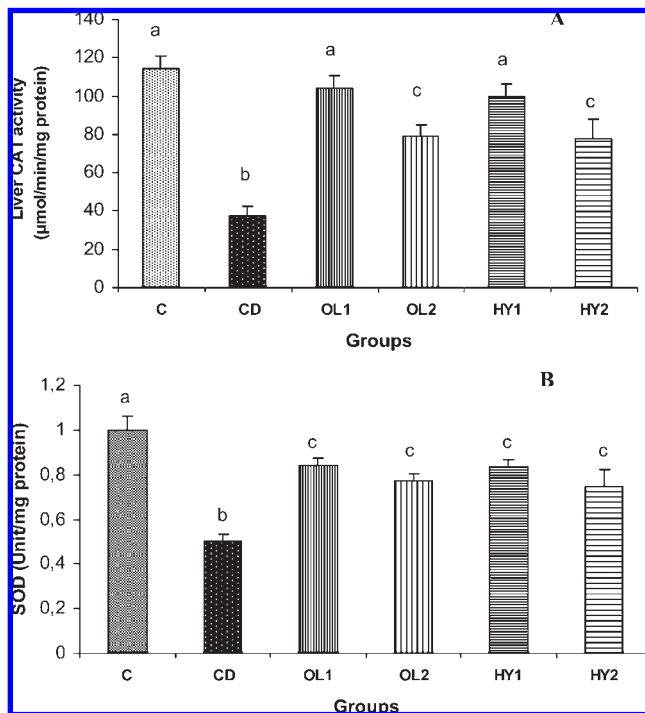


Figure 4. Effects of oleuropein and hydroxytyrosol rich olive leaves extracts on CAT (A) and SOD (B) activities in liver. Group C, normal control; CD, diabetic control; OL1 and OL2, diabetic + oleuropein at 16 and 8 mg/kg b.w., respectively; HY1 and HY2, diabetic + hydroxytyrosol at 16 and 8 mg/kg b.w., respectively. Each bar represents mean \pm SD from 10 rats. Bars with different letters differ significantly; $P < 0.05$.

Antioxidant Enzymes Activities. The hepatic antioxidant enzyme activities, superoxide dismutase (SOD) and catalase (CAT), significantly decreased in diabetic rats compared with those fed a control diet (Figure 4). The decrease was significantly restored ($P < 0.05$) in the presence of oleuropein and hydroxytyrosol. Olive leaf extracts based on oleuropein and hydroxytyrosol at two different doses significantly elevated the enzyme activity in diabetic animals treated for 4 weeks. There are no significant differences in SOD activities in the rats treated with phenolic extract, whereas the groups OL1 and HY1 showed significantly higher CAT activities compared with those of rats in groups OL2 and HY2 ($P < 0.05$).

ABTS Assay in the Serum Samples. ABTS radical cation scavenging ability (Figure 5) in the serum of diabetic rats was significantly low in comparison to that of the normal control rats. Oral administration of olive leaf extract allowed for the repairing of the impairment between both groups. In fact, there is a significant increase of the TEAC values in the rats receiving the phenolic extracts compared with those of the diabetic controls. The correction was significantly pronounced in the groups OL1 and HY1 receiving the higher concentration of oleuropein and hydroxytyrosol, respectively ($P < 0.05$).

Hepatic Oxidative Damages. The thiobarbituric acid-reactive substance (TBARS) levels were significantly increased ($P < 0.05$) in the livers of diabetic rats compared to those in the normal control group. The administration of oleuropein and hydroxytyrosol in two different doses significantly reduced the TBARS concentrations ($P < 0.05$) (Figure 6). This effect was significantly more pronounced in the group of rats treated with hydroxytyrosol and oleuropein at 16 mg/kg b.w. ($P < 0.05$).

Histological Results. In the diabetic rats, the photomicrographs pointed out vacuolated hepatocytes with the nucleus being pushed to the periphery and fatty cyst. This structure is different

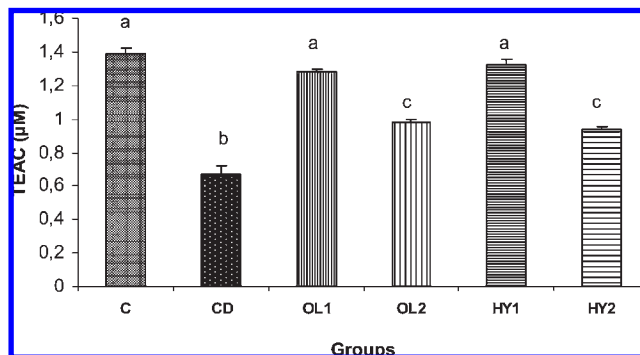


Figure 5. Effects of oleuropein and hydroxytyrosol rich olive leaves extracts on the serum antioxidant activity. Group C, normal control; CD, diabetic control; OL1 and OL2, diabetic + oleuropein at 16 and 8 mg/kg b.w., respectively; HY1 and HY2, diabetic + hydroxytyrosol at 16 and 8 mg/kg b.w., respectively. Each bar represents mean \pm SD from 10 rats. Bars with different letters differ significantly; $P < 0.05$.

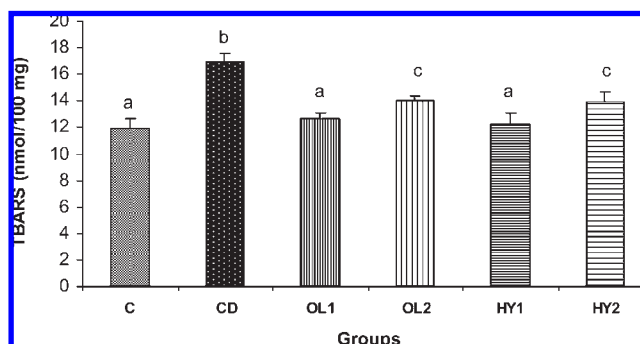


Figure 6. Effects of oleuropein and hydroxytyrosol rich olive leaves extracts on the TBARS levels. Group C, normal control; CD, diabetic control; OL1 and OL2, diabetic + oleuropein at 16 and 8 mg/kg b.w., respectively; HY1 and HY2, diabetic + hydroxytyrosol at 16 and 8 mg/kg b.w., respectively. Each bar represents mean \pm SD from 10 rats. Bars with different letters differ significantly; $P < 0.05$.

compared with the livers of normal control rats. Oleuropein- and hydroxytyrosol-rich extracts allowed to a certain degree to clearly prevail the hepatic architecture aberrations with the preservation of parenchymal structure and an occasional lipid droplet (Figure 7).

DISCUSSION

Recently, much attention has been focused on antioxidants in food that are potential compounds for preventing diseases caused by oxidative stress including diabetes because of their distinctive biological activity and low toxicity. In our previous study on olive leaf extracts rich in oleuropein and hydroxytyrosol, we found that they were endowed with important antioxidant activities. These confirmed antioxidant properties allow oleuropein and hydroxytyrosol to be efficient in the protection against some metabolic diseases related with oxidative stress such as diabetes. In fact, several studies reported that scavengers of oxygen radicals are effective in preventing diabetes in experimental animal models (29).

Furthermore, diabetes can be produced in animals by intraperitoneal injection of alloxan, which is toxic to β -cells and is widely used for such purposes. This induction produces active oxygen species responsible for diabetes complications (30). Therefore, in this work, we have employed such a diabetic animal model system to examine the hypoglycemic effect of olive leaf extracts

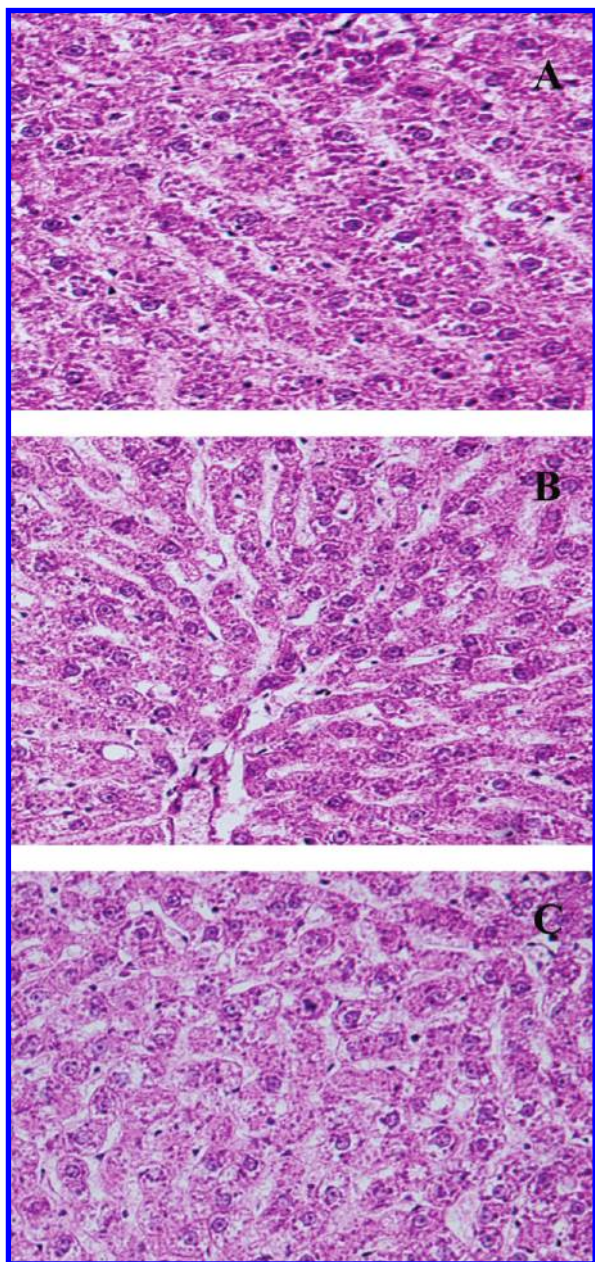


Figure 7. Microscopic views of transverse sections of liver in normal rats (A), diabetic rats (B), and oleuropein or hydroxytyrosol rich extract supplemented rats (C) (hematoxylin–eosin, H&E staining, 400 \times).

rich in oleuropein and hydroxytyrosol known to be efficient antioxidants *in vivo* (7, 8) as well as *in vitro* (21). In this study, hydroxytyrosol was obtained in a short time by a simple hydrolysis reaction of oleuropein-rich *Olea europaea* leaf extract. By the way, hydroxytyrosol could be recovered from olive mill wastewaters (31) or by chemical (21), biochemical (32), or biotechnological (33) synthesis starting from a synthetic precursor. Although some research had studied the hypoglycemic activity of olive leaf extracts (14, 34), this is the first time that extracts with identified phenolic composition have been tested. Furthermore, this data presents the first study dealing with the hypoglycemic activity of hydroxytyrosol, a potential olive antioxidant, compared with oleuropein. The latter was studied by Al-Azzawie et al. for its hypoglycemic activity in diabetic rabbits receiving 20 mg/kg b.w. during 16 weeks (6). This study showed that oleuropein presents significant hypoglycemic activity which is essentially due to its antioxidant potential. In fact, oleuropein as well as

hydroxytyrosol has been shown to be scavengers of superoxide anions and inhibitors of the respiratory burst of neutrophils and hypochlorous acid-derived radicals (13). Both compounds also scavenged hydroxyl radicals with oleuropein showing greater activity (35). Our results showed that these two phenolic compounds at two different doses, each one at 8 and 16 mg/kg b.w., had significant hypoglycemic, hypolipidemic, and antioxidant effects for all of the test rats. First, the serum glucose data obtained clearly indicate that the oral administration of oleuropein and hydroxytyrosol in the *Olea europaea* extracts produced significant hypoglycemic effects in alloxan-induced diabetic rats, mainly at 16 mg/kg b.w. In agreement with the present results, a few reports are available on the hypoglycemic effects of the leaves of *Olea europaea* (36). The eventual mechanism responsible of the hypoglycemic activity of oleuropein and hydroxytyrosol may result from a potentiation of glucose-induced insulin release or increased peripheral uptake of glucose (14). In our case and based on the observed glycogen levels in the livers of normal and tested rats, we can conclude that these two phenolic compounds at two different doses act as hypoglycemic agents via the enhancement of the peripheral uptake of glucose which is transported to the liver and polymerized to synthesize glycogen. In fact, the hepatic glycogen levels in diabetic rats receiving oleuropein and hydroxytyrosol were significantly higher than those of the normal as well as diabetic rats.

However, our study also indicated that oleuropein and hydroxytyrosol can decrease the total cholesterol levels in diabetic rats, which is important in preventing or treating the complications of diabetes. Moreover, studies in our laboratory have previously confirmed the hypocholesterolemic effects of olive tree byproducts such as phenolics (37). This has clinical implications since these two phenolic compounds present in the olive leaves extract, if used as hypoglycemic agents, may also reverse hypercholesterolemia associated with diabetes and prevent the cardiovascular complications which are very prevalent in diabetics. Furthermore, the levels of plasma lipids are usually raised in diabetes, and such an elevation represents a risk factor for cardiovascular disease (38). Consistently, in agreement with our results, other studies have reported that *Olea europaea* has hypolipidemic effects in diabetic rats (39).

It is well known that hyperglycemia leads to the overproduction of free radicals and the nonenzymatic glycation of proteins which exert deleterious effects on different organs acting in the glycemia regulation such as livers (29). In our study, we have observed decreased activities of hepatic antioxidant enzymes SOD and CAT and increased TBARS level in the livers of diabetic rats. Moreover, the serum antioxidant potential presented a significant depletion in the diabetic rats compared with that in the normal controls. Our results are in agreement with other findings showing that hyperglycemia is accompanied with an increase in marked oxidative impact as evidenced by the significant increase in hepatic lipid peroxidation resulting in the formation of TBARS and a significant decrease in hepatic antioxidants including SOD and CAT activities (40). All of these found perturbations in the antioxidant system were restored by the administration of oleuropein and hydroxytyrosol mainly at a 16 mg/kg b.w. dose. Subsequently, the hypoglycemic action of hydroxytyrosol and oleuropein in diabetic animals might be explained by the increase of antioxidant enzyme expressions and/or activities. In fact, several studies show (41) that polyphenolic substances increased the expression of SOD and CAT enzymes at the transcriptional level. However, these antioxidants could inactivate the circulating free radicals that quench NO before it reaches pancreatic β -cells, where they induced their damage and/or death (41).

This study demonstrated a potential and beneficial effect of oleuropein and hydroxytyrosol in attenuating oxidative stress and enhancing the antioxidant defenses in diabetic rats with established oxidative stress and may add another explanation of the hypoglycemic effect of phenolic compounds in olive leaves through their action as antioxidants. We have noted that these biological effects present dose dependent features. In fact, oleuropein and hydroxytyrosol are significantly more efficient at 16 mg/kg b.w. compared with the lower dose at 8 mg/kg b.w. Our results could be useful to elucidate one of the polyphenolic mechanisms in glucose metabolism regulation. We can conclude that oleuropein and hydroxytyrosol act as hypoglycemic components by the stimulation of the liver glycogen synthesis and the restoration of the antioxidant defense system. Furthermore, the beneficial effects of the used extracts seem to be due to the major compound, which is hydroxytyrosol, present in the hydrolysate and the olive leaf extract. Moreover, oleuropein could be converted in vivo by esterases into hydroxytyrosol (7, 42).

In conclusion, we demonstrate that polyphenols recovered from olive leaf extracts, oleuropein and hydroxytyrosol, at two different doses exhibited a pronounced hypoglycemic and hypolipidemic effects, reduced the lipid peroxidation process, and enhanced the antioxidant defense system in an experimental diabetic model. These effects highlighted once again the olive tree byproduct as a source of antioxidants able to reduce the frequency of oxidative stress-related metabolic diseases such as diabetes.

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