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Research Article

Protective Effects of *Olea europaea* Fruit Extracts on Metabolic Disorders Associated With Sucrose-Induced Metabolic Syndrome in Rats

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Abstract

Background: Metabolic syndrome (MetS) increases the risk of diabetes. *Olea europaea* fruit exerts protective effects on metabolic disorders. Therefore, the aim of the present study was to investigate the effect of *O. europaea* fruit extracts on sucrose-induced MetS in rats.

Methods: Male adult Wistar rats (200±50 g, n=32) were randomly divided into four groups (n=8) consisting of control group, untreated sucrose group (sucrose 50% in drinking water for 10 weeks), sucrose plus aqueous extract of *O. europaea* fruit treated group (200 mg/kg) and sucrose plus hydroalcoholic extract of *O. europaea* fruit treated group (200 mg/kg) by gavage for 2 weeks. Body weight, serum glucose, insulin, leptin, lipid profile, homeostatic model assessment of insulin resistance (HOMA-IR) and hepatic enzymes were measured. Data were analyzed by one-way analysis of variance (ANOVA, SPSS, 16.0). $P<0.05$ was regarded as significance level.

Results: The aqueous extract exhibited higher protective effects on serum glucose, insulin and HOMA-IR than hydroalcoholic extract ($P<0.05$). Body weight, serum glucose, leptin ($P<0.01$), insulin, triglyceride, very-low-density lipoprotein cholesterol (VLDL-C), HOMA-IR, alkaline phosphatase (ALP), ($P<0.001$) and aspartate aminotransferase (AST) ($P<0.05$) significantly elevated but high-density lipoprotein cholesterol (HDL-C) ($P<0.05$) decreased in the sucrose group. Aqueous extract of *O. europaea* fruit significantly improved blood glucose, triglyceride, VLDL-C ($P<0.01$), insulin, HOMA-IR, ALP ($P<0.001$), body weight, AST and leptin ($P<0.05$) levels. Hydroalcoholic extract of *O. europaea* fruit significantly restored insulin, HOMA-IR ($P<0.01$), ALP ($P<0.001$), body weight, leptin, VLDL-C, triglyceride, blood glucose and AST ($P<0.05$).

Conclusion: Our results indicated *O. europaea* fruit extracts could improve metabolic disorders induced by MetS in the rats.

Keywords: Olea europaea, Sucrose, Metabolic syndrome, Hepatic enzymes, Leptin.

Background

Metabolic syndrome (MetS) is a group of symptoms including hyperglycemia, insulin resistance, hypertriglyceridemia and low plasma high-density lipoprotein cholesterol (HDL-C). It increses the risk of obesity, type 2 diabetes and coronary heart disease (1,2). Recently, a great interest in the understanding of mechanisms involved in MetS and its treatment has been observed due to increased prevalence of MetS worldwide (3). It has been estabelished that MetS is induced by high sucrose diet in animal models (4). After sucrose consumption, carbohydrates are converted to fats and insulin resistance is increased. Furthermore, previous reports demonstrated that high carbohydrate diet is associated with insulin resistance (5,6). Insulin resistance plays an important role in hyperlipidemia and triglyceride hydrolysis. In addition, hydrolysis of triglycerides increases free fatty acids in the blood and leads to metabolic disorders (7).

As a peptide hormone, leptin is encoded by the obese gene and is mainly secreted by adipose tissue. It decreases appetite and food intake via various signaling pathways in the hypothalamus (8). Leptin plays an important role in the control of vascular inflammation, oxidative stress, adiposity, insulin sensitivity, metabolism, reproductive function and the immune system (9). It has been revealed that secretion of adipokines including resistin, leptin and adiponectin and development of leptin resistance can increase MetS in rats with high sucrose diet (10).

Many studies have reported that the Mediterranean diet is associated with health promotion. For example, *Olea*

europaea fruit consumption is associated with reduced cardiovascular risk factors (11). *O. europaea* or olive (from the family Oleaceae) is a globally well-known tree in traditional medicine and has high therapeutic value due to its special pharmacological benefits (12,13). *O. europaea* is rich in phenolic acid, flavonoids and stilbenoids and has antioxidant, hypolipidemic and anti-atherogenic activities due to the presence of phenolic compounds (14). Moreover, it is beneficial in treating gastrointestinal disorders because of its laxative properties (15). In addition, *O. europaea* has antidiabetic (16), anticancer (17), antimicrobial (18) and antifungal effects. It has been indicated that hydroxytyrosol, a phenolic compound of *O europaea* fruit, can exert antioxidant, anti-cancer and anti-atherogenic activites (19). In addition, the extract of *O. europaea* fruit inhibits pain and inflammation in rats (20).

Previous studies reported that the consumption of *Olea europaea* and oleuropein, which is the main compound of *O. europaea* fruit, can improve lipid profile and blood glucose in hypercholesterolemic and diabetic rabbits (21,22). One study has indicated olive fruit polyphenol-enriched yogurt decreases body weight, blood pressure, low-density lipoprotein cholesterol (LDL-C) and lipid peroxidation levels in individuals with unknown pathology (23). Another study has indicated that extra virgin olive oil exerted protective effects against hepatotoxicity induced by aluminum and acrylamide in adult rats (24). Recently, an experimental study has indicated that polyphenol-rich virgin olive oil can improve insulin resistance, liver inflammation, mitochondrial oxidative stress and nonalcoholic fatty liver disease in high-fat diet fed rats (25). In addition, Kang et al have reported that *O. europaea* fruit extract reduces the hepatic enzymes and lipid levels in carbon tetrachloride-treated mice (26). The study of Veciana-Galindo et al has demonstrated that leptin gene expression and adipogenesis are reduced by olive-seed extract polyphenols in mouse fibroblasts (27).

The present study was conducted to investigate protective effects of *O. europaea* fruit, as a main constituent of the Mediterranean diet, on metabolic and hepatic disorders associated with sucrose-induced MetS in rats. Since MetS, as an important health issue, increases the risk of diabetes and cardiovascular disorders and *O. europaea* fruit exerts protective effects on metabolic disorders, the aim of the present study was to investigate the effect of *O. europaea* fruit extracts on metabolic and hepatic disorders in rats with sucrose-induced MetS.

Methods

Plant Material

Plant material used in this study was collected from around the city of Ahvaz, Khuzestan, Iran. The voucher specimen (no.: 1114) of *O. europaea* fruit was deposited in the Herbarium of Department of Pharmacognosy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Preparation of Plant Extract

Aqueous extract (AE): The *O. europaea* fruits were separated from each other then the fruits were powdered by an electric blender, and the obtained powder (100 g) was mixed in 200 mL distilled water and boiled for 30 minutes. The mixture was filtered with Whatman No. 1 filter paper, and then was centrifuged at 3500 rpm for about 20 minutes. The solvent was evaporated at room temperature and the remaining powder was kept at 4°C until used. The extract was dissolved in saline at the requested concentration exactly just before use (4).

Hydroalcoholic extract (HE): The powder of the *O. europaea* fruit (50 g) was mixed in 200 mL of solution (60-40; distilled water-methanol), at room temperature for 72 hours, and then filtered with Whatman paper and centrifuged at 3500 rpm for 20 minutes. The supernatant was evaporated at room temperature and the obtained powder was kept at 4°C until used (4).

Experimental Animals

Male adult Wistar rats weighing (200 ± 50 g, n = 32) were purchased from the animal house of the University of Medical Sciences. Animals were housed in cages under the conditions of regulated temperature 25°C and 12/12-hour light/dark cycle for 10 weeks and freely received standard diet and water. The study protocol was performed after it was approved by Ahvaz Institutional Animals and under the current guidelines for the laboratory animals' care (IR.AJUMS.REC.1395.102) (http://veteditors.org/ethicsconsensusguidelines.html).

Experimental Design

After a week of habituation, the rats were randomly divided into 4 groups (of 8 each), consisting of control group), untreated sucrose group, sucrose plus aqueous extract treated group and sucrose plus hydro-alcoholic extract treated group. The control rats received saline solution, the sucrose group received sucrose 50% in drinking water and saline solution for 10 weeks, the aqueous extract treated rats received the aqueous extract of *O. europaea* fruit (200 mg/kg) and hydroalcoholic extract treated rats received the hydroalcoholic extract of *O. europaea* fruit (200 mg/kg) orally by gavage at the end of eighth week for two weeks (28). The animals in the aqueous and hydroalcoholic extract groups were given 3 mL/kg/d extract. The control and untreated sucrose groups were given 3 mL/kg/d normal saline. The dose was selected based on previous studies and a pilot study in which 200 mg/kg of extract was administered to Wistar rats and positive findings against metabolic disorders were reported (12,29).

Biochemical Analysis

At 24 hours after the last oral administration, rats were anaesthetized by intra-peritoneal injection of ketamine HCl and xylazine (50 and 5 mg/kg, respectively), and

the blood was collected from the heart and centrifuged at 3500 rmp for 20 minutes; then, the levels of glucose, insulin, lipid profile, hepatic enzymes, and leptin in serum were measured by commercial kits. Insulin and leptin were measured using IRMA (BioSource Europe S.A) and ELISA (Labor Diagnostika Nord GmbH, Germany), respectively. Intra- and inter-assay coefficients of variation were 5.8% and 4.3% for insulin and 6% and 6.1% for leptin, respectively. Low-end sensitivity for insulin and leptin were 1 µIU/mL and 0.5 ng/mL, respectively. Finally, very low-density lipoprotein cholesterol (VLDL-C) and insulin resistance were calculated using the following formulas: VLDL-C = Total serum triglycerides/5 (30) and homeostatic model assessment of insulin resistance (HOMA-IR) = fasting insulin (µU/mL) × fasting glucose (mg/dL)/25, respectively (31).

Statistical Analysis

The results were presented as mean ± standard error of mean (SEM). The normal distribution and homogeneity of the variances were investigated using Kolmogorov–Smirnov test and Levene's test, respectively. Results were analyzed by one-way analysis of variance (ANOVA), followed by the post-hoc test LSD. $P<0.05$ was regarded as significance level. Calculations were performed in the SPSS version 16.0.

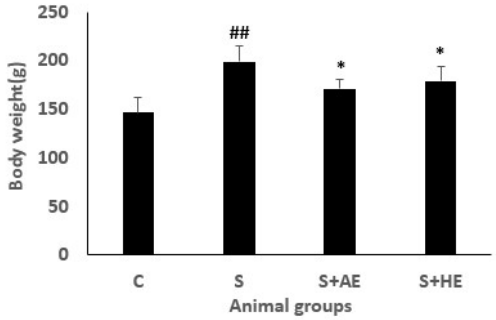

Figure 1. Effect of *Olea europaea* Fruit Extracts on Body Weight in Different Groups, (n=8). Data are presented as mean±SEM. ## $P<0.01$ versus control group; *$P<0.05$ versus untreated sucrose group. C, control group; S, untreated sucrose group; S+AE, sucrose treated with aqueous extract of *O. europaea* fruit group; S+HE, sucrose treated with hydroalcoholic extract of *O. europaea* fruit group.

Results

Effects of the *Olea* europaea Fruit Extracts on Body Weight, Serum Glucose, Insulin, Leptin and Fasting Insulin Resistance Index

Body weight, serum glucose, leptin ($P<0.01$), insulin and HOMA-IR ($P<0.001$) levels increased significantly in the untreated sucrose animals compared with the control rats. However, administration with aqueous extract of *O. europaea* fruit significantly decreased body weight, leptin ($P<0.05$), serum glucose ($P<0.01$), insulin and HOMA-IR ($P<0.001$) levels; and the hydroalcoholic extract of *O. europaea* fruit significantly decreased body weight, serum glucose, leptin ($P<0.05$), insulin and HOMA-IR ($P<0.01$) levels. There was no significant difference in body weight and leptin level between the two groups that received the extract of *O. europaea* fruit. Comparatively lower values of serum glucose, insulin and HOMA-IR ($P<0.05$) were observed in the rats treated with the aqueous extract of *O. europaea* fruit than in the rats treated with hydroalcoholic extract of *O. europaea* fruit (Figures 1-3).

Effects of the *Olea europaea* Fruit Extracts on Lipid Profile

As shown in Table 1, serum triglyceride and VLDL-C ($P<0.001$) levels significantly increased, but HDL-C ($P<0.05$) significantly decreased in the high sucrose animals compared with the control group. Moreover, treatment with the aqueous extract of *O. europaea* fruit at 200 mg/kg significantly decreased serum triglyceride and VLDL-C ($P<0.01$) levels, and also the hydroalcoholic extract of *O. europaea* fruit significantly reduced serum triglyceride and VLDL-C ($P<0.05$) levels. There was no significant difference in the lipid profile between the *O. europaea* aqueous extract and *O. europaea* hydroalcoholic extract groups.

Effects of *Olea europaea* Fruit Extracts on Hepatic Enzymes

As indicated in Table 2, serum alkaline phosphatase (ALP) ($P<0.001$) and aspartate aminotransferase (AST) levels ($P<0.05$) significantly increased in the sucrose administrated rats compared with the control animals. Besides, both the aqueous and hydroalcoholic extracts

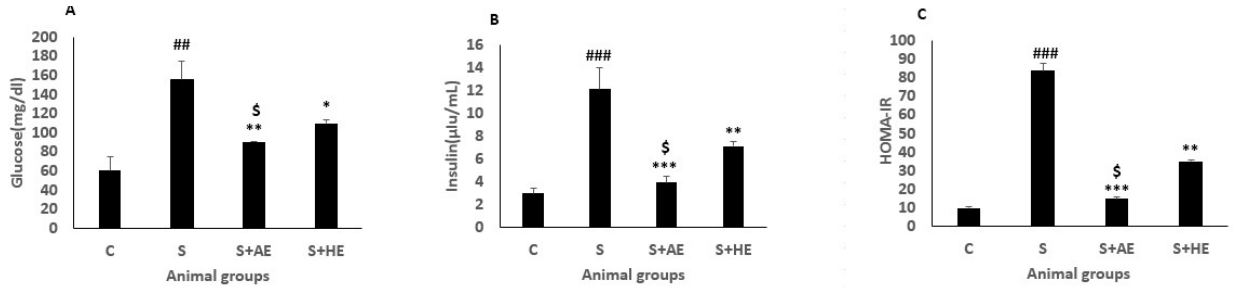

Figure 2. Effect of *Olea europaea* Fruit Extracts on the Levels of Blood Glucose (A), İnsulin (B) and HMOA-IR (C) in Different Groups, (n=8). Data are presented as mean±SEM. ## $P<0.01$; ### $P<0.001$ versus control group; * $P<0.05$; **$P<0.01$; *** $P<0.001$ versus untreated sucrose group, $ $P<0.05$ versus S+HE group. C, control group; S, untreated sucrose group; S+AE, sucrose treated with aqueous extract of *O. europaea* fruit group; S+HE, sucrose treated with hydro-alcoholic extract of *O. europaea* fruit group. HOMA-IR, homeostatic model assessment of insulin resistance.

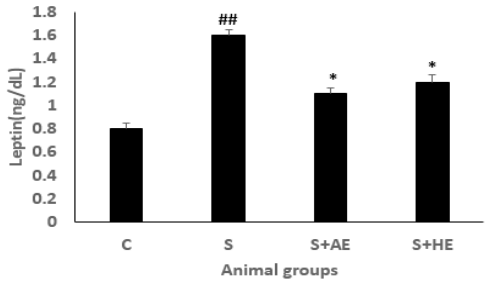

Figure 3. Effect of Olea europaea Fruit Extracts on Leptin Level in Animal Groups, (n=8). Data are presented as mean±SEM. ## $P<0.01$ versus control group, * $P<0.05$ versus untreated sucrose group. C, control group; S, untreated sucrose group; S+AE, sucrose treated with aqueous extract of O. europaea fruit group; S+HE, sucrose treated with hydroalcoholic extract of O. europaea fruit group.

of *O. europaea* fruit significantly reduced serum ALP ($P<0.001$) and AST ($P<0.05$) levels compared with the untreated sucrose animals. Furthermore, there was no significant difference in hepatic enzymes between aqueous and hydroalcoholic extracts of *Olea europaea* fruit groups.

Discussion

The present study indicated that sucrose administration enhanced body weight, blood glucose, insulin, HOMA-IR, leptin, triglyceride, VLDL-C and hepatic enzymes in the animals fet with high sucrose diet. MetS is associated with increased levels of these parameters. Furthermore, previous investigations have demonstrated that high sucrose diets induce insulin resistance in animal models (32,33). It has been indicated that high sucrose diet may increase triglyceride level through various pathways, including overproduction of VLDL-C in the liver, its release into the bloodstream (34) and reduction in the lipolysis of triglyceride rich lipoproteins (35). In addition, blood VLDL-C elevation causes an increase in LDL-C level; hence, high sucrose diet increases LDL-C levels (36). However, in our investigation, there was no alteration in LDL-C level in the rats fed with high sucrose diet, which is consistent with the study of Mousavi et al (37).

Both extracts of *O. europaea* fruit attenuated the sucrose-elevated body weight, insulin resistance and levels of glucose, insulin, leptin, lipid profile and hepatic enzymes in serum. It has been indicated that oleanolic acid, a triterpenoid component of *O. europaea* fruit exerts Takeda-G-protein-receptor-5 (TGR5) agonist effect (38). TGR5 agonists regulate mitochondria energy homeostasis in the brown adipose tissue and muscle, and reduce body weight gain resulting from high fat diet (38). In addition, oleuropein exhibits antidiabetic effect by increasing glucose consumption, insulin secretion and antioxidant activity (39). Therefore, in the present study, the antidiabetic effect of *O. europaea* fruit could be partially attributed to oleanolic acid and oleuropein.

Olea europaea fruit treatment for 14 days resulted in the reduction of triglyceride and VLDL-C levels in the animals under high sucrose diet, which is probably due to insulin resistance reduction and antidiabetic effects. In addition, researchers have demonstrated that oleuropein reduces the size and number of lipid droplets in cells treated with free fatty acid, and the accumulation of triglyceride within the cells (40,41).

A study has indicated that insulin indirectly increases leptin release by increasing the metabolism of nutrients, specially glucose, in adipocytes (42). Consistently, our

Table 1. Effect of *Olea europaea* Fruit Extracts on Lipid Profile

	Groups			
	C	S	S+AE	S+HE
Total cholesterol (mg/dL)	100.8±5.4	83.8±10.4	78.25±6.7	85.8±11.7
Triglyceride (mg/dL)	73.1±4.06	141±19$^{\#\#\#}$	83 ±8.3**	102.2±14.3*
HDL-C (mg/dL)	60±1.29	40.6±4.2$^{\#}$	41.2±4.3	44.4±6.3
LDL-C (mg/dL)	24.9±3.1	26.2±1.5	20.3±1.9	20.9±2.2
VLDL-C (mg/dL)	14.63±0.8	28.2±3.81$^{\#\#\#}$	16.6±1.6**	20.4±2.8 *

Data are presented as mean ± SEM, n=8. C, control group; S, untreated sucrose group; S+AE, sucrose treated with aqueous extract of *O.europaea* fruit group; S+HE, sucrose treated with hydroalcoholic extract of *O. europaea* fruit group. $^{\#}$ $P<0.05$, $^{\#\#\#}$ $P<0.001$ versus control group; * $P<0.05$, ** $P<0.01$ versus untreated sucrose group.

Table 2. Effect of *Olea europaea* Fruit Extracts on Hepatic Enzymes

	Groups			
	C	S	S+AE	S+HE
ALP (U/dL)	297.4±28.26	381±49.32$^{\#\#\#}$	261.33±6.35***	255±10.83***
ALT (U/dL)	129.4±39.85	136.14±23.33	125±43.55	120.75±9.7
AST (U/dL)	261±39.04	306.1±21.22$^{\#}$	249±5.85 *	240.25±13.33*

Data are presented as mean±SEM, n=8. C, control group; S, untreated sucrose group; S+AE, sucrose treated with aqueous extract of *O. europaea* fruit group; S+HE, sucrose treated with hydroalcoholic extract of O. europaea fruit group. # $P<0.05$, ### $P<0.001$ versus control group; * $P<0.05$, *** $P<0.001$ versus untreated sucrose group.

results also showed that increased leptin release is associated with increased insulin release in the animals fed with high sucrose diet (43). An experimental study has shown that the olive seed extract reduces the expression of the adipogenic peroxisome proliferator-activated receptor-g (PPARg) and leptin genes in mouse fibroblasts (27). Similarly, our results indicated that the consumption of *O. europaea* fruit reduced leptin level in the animals under high sucrose diet.

It has been indicated that insulin increases leptin release from adipocytes (44). Therefore, the effects of *O. europaea* fruit on blood insulin level may play a significant role in leptin reduction. Leptin increases insulin resistance and vascular damage that may be involved in the pathogenesis of diabetes and cardiovascular diseases (45). Thus, the effect of *O. europaea* fruit on leptin can improve these disorders. Furthermore, Lama et al have reported that polyphenol-rich virgin olive oil decreases insulin resistance and mitochondrial dysfunction in rats fed with high-fat diet (25). Furthermore, a study has indicated that polyphenols of *O. europea* decreases cytokine-induced β-cell damage by controling redox homeostasis in cell culture (46).

Liver damage increases the release of hepatic enzymes into the circulation, resulting in increased levels of them in the blood. In agreement with our results, it has been reported that normal or near normal serum ALT level is found in male rats with fructose-induced insulin resistance (4). In several studies, it has been demonstrated that hydroxytyrosol and polyphenol-rich olive oil reduce oxidative stress and nitrosative stress in the liver, and ameliorate nonalcoholic fatty liver disease in rats receiving high fat diet (25,47). Similarly, in our study, the administration of *O. europaea* fruit extract decreased serum ALP and AST levels in the animals under high sucrose diet.

It has been indicated that flavonoides may decrease NO production in the liver and have hepatoprotective effects (48). A possible mechanism of hepatic enzymes reduction can be attributed to the presence of flavonoids in the *O. europaea* fruit. The hepatocytes are involved in glucose metabolism; therefore, the beneficial effect of the *O. europaea* on the liver may be attributed to its antihyperglycemic activity.

Bioactive ingredients found in *O. europaea* fruit extracts, including polyphenols, flavonoids, and hydrotyrosols, may contribute to protective effects on metabolic disorders induced by MetS. Jiang et al (49) reported that flavonoids in fenugreek exhibited potent anti-diabetic effects by reducing islet cells damage, decreasing insulin resistance and enhancing gluconeogenesis in streptozotocin-induced rats. Polyphenols in *Morus nigra* leaf extracts have been observed to improve hyperlipidemic condition in hyperlipidemic rats (50). The polyphenols in green tea (51) produce protective effects against non-alcoholic fatty liver disease, probably due to decrease in lipogenesis of the liver through increasing AMP-activated protein kinase activation in high fat fed Zucker fatty rats. In addition, Maalej et al have reported that phenolic compounds obtained from olive fruit extract has a protective effect on deltamethrin-induced hepatotoxicity by reducing apoptosis and inflammation (52). Hydroxytyrosol decreases lipid levels (43) and blood cholesterol (54) in rats fed with cholesterol diet and streptozotocin-induced diabetic rats.

The findings of the present study showed that hydroalcoholic and aqueous extracts of *O. europaea* fruit similarly reduced body weight, blood glucose, insulin, HOMA-IR, leptin, lipid profile and hepatic enzymes in the animals with MetS. The aqueous extract of *O. europaea* fruit indicated better effects on blood glucose, insulin and HOMA-IR compared to the hydroalcoholic extract, which may be due to the higher phenolic compoundsof the aqueous extract. Previous studies demonstrated that the highest phenolic compound concentrations were found for aqueous extraction (under hot conditions) rather than for hydroalcoholic extraction (at room temperature) (55-57).

No side effects have been reported during the use of *O. europaea* fruit and its side effects are rare, especially in the absence of a history of food allergy. However, high and chronic consumption of *O. europaea* fruit can cause stomach irritation or diarrhea. In conclusion, our results indicate that *O. europaea* fruit consumption may improve metabolic and hepatic disorders induced by MetS. Therefore, it, as a beneficial agent, can help protect against MetS and its complications. However, further studies are necessary to figure out the precise molecular mechanisms and important components that are involved in producing such effects.

Authors' Contribution

AA drafted manuscript, interpreted the data, planned the study, prepared the manuscript and approved the final version of the manuscript; and FRA did animal handling and biochemical analysis, and drafted the manuscript.

Conflict of Interest Disclosures

None.

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