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HEALTH SCIENCES

Effects of Emulsion Formulations of Oleuropein Isolated from Ethanol Extract of Olive Leaf in Diabetic Rats

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Abstract: This study was designed to investigate the effects of emulsion formulations of oleuropein isolated from ethanol extract of olive leaf in streptozotocin-diabetic rats. The rats were treated with the administration of the emulsion containing oleuropein at a low (150 mg/kg b.wt.) and high (225 mg/kg b.wt.) dose for 30 days. At the end of the study, blood samples were drawn from the heart of the rats to determine blood glucose, alanine transaminase, and aspartate transaminase levels. In addition, their liver tissues were dissected to determine the levels of glutathione and thiobarbituric acid-reactive substances, and superoxide dismutase activity. According to the results for both dose treatments, a statistically significant increase in superoxide dismutase activities and glutathione levels of the treated diabetic rats was observed when compared with those of the diabetic control rats. On the other hand, a statistically significant decrease in the levels of thiobarbituric acid-reactive substances, aspartate transaminase and alanine transaminase of the treated diabetic rats was determined. It should be highlighted that the administrations at the high dose were more effective compared to that of the low dose. Furthermore, a substantial decrease in the blood glucose levels of the diabetic rats exposed to the high dose was observed.

Key words: Antioxidant, diabetes, emulsion, oleuropein, olive leaf.

INTRODUCTION

Diabetes Mellitus (DM), a disease requiring continuous medical care for the patients, is a chronic metabolic disease that has high mortality and morbidity rates in both developed and developing countries. DM, a disease is characterized by hyperglycemia, occurs when the body fails to produce insulin or use it efficiently (American Diabetes 2014). In diabetes, uncontrolled increased blood glucose can lead to producing overwhelming free radicals. When an overload of free radicals cannot gradually be eliminated, they can accumulate in the body and generate a phenomenon called oxidative stress (Pham-Huy et al. 2008). Diabetes is generally accompanied by a chronic state of oxidative stress (Wu et al. 2018, Eidi et al. 2009, Jemai et al. 2009). Oxidative stress can cause diabetic complications such as neuropathy, nephropathy, and retinopathy in the long term (Giacco & Brownlee 2010). Furthermore, oxidative stress associated with diabetes has an increasing effect on lipid peroxidation (Davi et al. 2005) and hepatic enzyme levels (Eidi et al. 2009). On the other hand, it has a decreasing effect on antioxidant enzyme activities (Jemai et al. 2009, Al-Azzawie & Alhamdani 2006) and changes glutathione redox status in diabetic patients (Jemai et al. 2009, Lutchmansingh et

al. 2018). So far, although many scientists have conducted clinical and experimental studies on the treatment and prevention of diabetes, there is still no definitive treatment of diabetes. In recent years, many people with diabetes have turned to natural alternative therapies as a remedy for diabetes (Raskin et al. 2002).

Olive plant has long been accepted as one of the widely used medicinal plants (El & Karakaya 2009). Its leaves contain many potentially bioactive compounds such as oleuropein (OL), hydroxytyrosol, diosmetin, verbascoside, luteolin, rutin, and tyrosol. Among these natural compounds, OL is the main bioactive compound with the highest antioxidant activity (Benavente-Garcia et al. 2000). Several studies have reported cardio-protective (Omar 2010), neuroprotective (Omar 2010), hepatoprotective (Kim et al. 2010), antidiabetic (Jemai et al. 2009, Al-Azzawie & Alhamdani 2006) and anticancer (Hamdi & Castellon 2005) activities of OL. However, the downside of OL is that it has a very bitter taste and is therefore not directly consumed for health purposes (Benavente-Garcia et al. 2000). In addition, OL is sensitive to enzymatic oxidation during digestion and may be degraded during residence in the small intestine (Nikolaivits et al. 2017, Markopoulos et al. 2009). Therefore, emulsion systems are a satisfactory alternative (Souilem et al. 2014). Emulsions consist of at least two immiscible liquid phases, one of which is dispersed as globules in the other liquid phase. These systems are used as vehicles for the delivery of compounds and to mask their bitter taste (Khan et al. 2011).

Thus, the current study was designed to investigate the possible effects of the emulsion containing OL (EOL) in STZ-induced diabetic rats. As far as we know, the present study is the first study dealing with the antioxidant and hypoglycemic effects of emulsion formulations of OL in experimental models (*in vivo*).

MATERIALS AND METHODS

Animals and diets

The adult male Sprague-Dawley rats weighing 200-210 g were used for the experiment. The rats were provided by Ataturk University Medical Experimental Application and Research Center. The rats were kept in special cages under standard environmental conditions (22±2 °C, 12 hours on/off light cycle) throughout the experiment. The treatments were performed during the same period. The rats were fed (pellet diet and water) *ad libitum*. This study was carried out according to the Guide for the Care and Use of Laboratory Animals by the National Institute of Health (NIH). The Animal Experimentation Ethics Committee of Ataturk University approved the protocol (Permit number: 36643897-79).

Induction of diabetes mellitus

STZ, a cytotoxic glucose analog, is widely used in laboratory animals due to the ability of this compound to induce specific necrosis of the pancreatic beta cells (Eleazu et al. 2013). Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared STZ (50 mg/kg b.wt.) solution in 10 mM of cold citrate buffer with pH 4.5 after overnight fasting (12 hours). Approximately 4 to 6 hours after the injection, 5% doses of dextrose solution were given to the rats for 24 hours due to lethal hypoglycemia resulting from massive beta cell destruction. Seventy two hours following the STZ injection, the animals with non-fasting blood glucose levels (BGLs) above 300 mg/dL and symptoms of polyuria and polydipsia were considered to be diabetic (Chattopadhyay 1999, Gozen et al. 2017).

Preparation of the ethanol extract

Fresh olive leaves were collected from Yusufeli (Artvin, Turkey) and used as the experimental material for this study. After the leaves were

cleaned and dried at ambient temperature, the dried leaves were powdered by grinding in the presence of liquid nitrogen with a mortar and pestle. The powder was extracted successively with ethanol at a temperature of 45-50 °C with constant stirring for 72 hours. When the solvents became concentrated, the extracts were filtered through filter paper. The solvents were removed under vacuum using a rotary evaporator at a temperature of 40-45 °C and the crude extracts were obtained to yield OL. The extracts were then stored in the refrigerator at ± 4 °C for experimental use (Eidi et al. 2009).

Isolation of OL from the ethanol extract of olive leaf

The ethanol extract (30 g) was fractioned by silica gel column chromatography (300g, 700-230 mesh) using CH_2Cl_2:MeOH (90:10) solvent system. The fractions (50 mL) were checked by thin-layer chromatography (TLC) at the different solvent systems, and the same stains were combined. The oleuropein (7 g) compound was obtained in pure fractions that is 28th-53rd fractions (Kisa et al. 2018).

Structure characterization

The ^{1}H-NMR and ^{13}C-NMR spectroscopic methods were used for structure characterization of OL. NMR spectra was obtained by Bruker spectrometers (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR). Chemical shifts (δ) were stated as ppm using dissolvent as an internal standard and coupling constant (J) as hertz.

Preparation of the emulsions

EOLs at the doses of 150 and 225 mg/kg b.wt. were prepared using the formulation components as shown in Table I. The water phase containing OL was added dropwise to the oil phase while stirring on a magnetic stirrer at 600 rpm. Upon completion, the formulation was stirred for 1 hour. On the other hand, a blank emulsion was prepared using the above mention method.

Droplet size and image of the emulsions

The images of the emulsion formulations were obtained using an optical microscope. The droplet sizes were also determined using Mastersizer 2000 particle size analyzer (Malvern Ins. Ltd. UK) (Zalazar et al. 2016).

Experimental design

The study consisted of 72 adult male Sprague-Dawley rats in order to take into account the high risk of death in diabetes. The rats were divided into eight groups matched for body weight, with each group consisting of 9 rats. The groups were characterized as follows:

Group C consisted of untreated rats,

Group CD consisted of diabetic rats,

Group CE consisted of diabetic rats which received blank emulsion,

Group COL consisted of normal rats that received OL at a dose of 225 mg/kg b.wt./day,

Group EOL1 consisted of diabetic rats that received EOL at a dose of 150 mg/kg b.wt./day,

Group EOL2 consisted of diabetic rats that received EOL at a dose of 225 mg/kg b.wt./day,

Group OL1 consisted of diabetic rats that received OL at a dose of 150 mg/kg b.wt./day,

Group OL2 consisted of diabetic rats that received OL at a dose of 225 mg/kg b.wt./day.

Individual doses were calculated every six days based on the body weights of the rats. All doses were administered volumetrically at 2 mL. The groups C and DC received 2 mL of distilled water only. The group CE received 2 mL of blank emulsion only. Each animal was dosed by oral gavage. For all animals, dose administration was daily for 30 days. Dosing was at approximately the same time each day (± 2 hours).

Table I. The formulation components of the emulsions.

Component	Amount (mg)
Olive oil	180
Tween 80	648
PEG 400	72
Ultrapure water (pH 5)	17.4

Blood glucose measurement

The blood samples were collected from the tail vein of the animals. The BGLs were determined by Optium Xceed Glucometer (Abbott Laboratories, Inc., Australia) and On Call®Plus Glucometer (ACON Laboratories, Inc., USA) on 0, 6th, 12th, 18th, 24th and 30th day and the BGLs were expressed in term of mg/dL.

Body weight determination

The body weights of the rats were measured by means of a digital scale, and recorded every 6 days throughout the experiment.

Preparation of blood samples

After 30 days of the treatment, the animals were killed with an overdose of a general anesthetic (thiopental sodium, 50 mg/kg). The blood samples were drawn from the heart on the last day of the study. The samples were transferred to serum biochemical tubes. After standing for 5-10 minutes at 22 ± 2 °C, they were centrifuged at 3000 x g for 10 minutes in a cooled centrifuge. Serum fractions were transferred into a clean polypropylene tube using a pasteur pipette. The obtained serum samples were stored for one day at -20 °C for biochemical studies. The serum samples were used to determine AST and ALT levels of the rats.

Preparation of tissue homogenates

The liver tissue samples were obtained from each rat. The liver was removed from each rat and rinsed with physiological saline, blotted, and placed petri dishes, immediately. The liver tissues were then stored in the refrigerator for experimental use. The frozen liver tissues were powdered in the presence of liquid nitrogen using a mortar and pestle. The powdered liver tissue samples were homogenized to determine GSH, TBARS and protein levels, and SOD activities. Approximately 75 mg of each ground tissue was homogenized in 0.75 mL of its buffer. The tissue homogenization procedures were performed according to the kit instructions proposed by Cayman Chemical Company (Cayman chemical, MI, USA). For the SOD assay, the liver tissue samples were homogenized with 20 mM HEPES buffer (1 mM ethylene glycol tetraacetic acid, 210 mM mannitol, 70 mM sucrose, pH 7.2), and centrifuged at 1,500 x g for five min at 4 °C. The supernatant was removed and stored on ice. For the GSH assay, tissue samples were homogenized with 50 mM cold phosphate buffer (1 mM ethylenediaminetetraacetic acid, pH 6-7), and centrifuged at 10,000 x g for 15 min at 4 °C. The supernatant was removed and stored on ice. For the TBARS assay, 250 µl RIPA buffer was used to sonicate the tissue samples. The homogenate obtained from sonication was centrifuged at 1,600 x g for 10 min at 4 °C. The supernatant was removed and stored on ice.

Biochemical assays

The serum samples were used to determine AST and ALT levels of the rats. Serum AST and ALT were analyzed by Cobas c501 analyzer (Roche Ltd, Switzerland) at the Ataturk University Research Hospital, Department of Biochemistry. The liver tissue samples were used to determine GSH, TBARS and protein levels, and SOD activities. GSH, TBARS and protein levels, and SOD activities were measured by commercial enzyme-linked immunosorbent assay (ELISA) kits produced for rats (Item No. 703002, 10009055, 704002 and 706002, respectively, Cayman Chemicals, Ann Arbor, MI, USA).

Chemicals

STZ (C8H15N3O7; molecular weight 265.221 Da) was purchased from Sigma-Aldrich, USA. The other chemicals were purchased from Merck Chemicals, Germany. Distilled water was used for analytical procedures.

Statistical analysis

The data were expressed as means ± standard deviation (SD). All data were analyzed using SPSS ver. 20.0 software (SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) was performed, and a multiple range test of Duncan was used. Statistical significance was accepted at a level of $p \leq 0.05$ in 95% confidence of interval.

RESULTS

^{1}H and ^{13}C NMR characterization of OL

Oleuropein was characterized by ^{1}H and ^{13}C NMR experiments. The ^{1}H and ^{13}C NMR data corresponded to the literature, the analysis revealed that the chemical structure of the substance was OL. The structure of OL is in accordance with the one obtained by Al-Rimawi 2014, who studied the determination of OL in olive leaves (Al-Rimawi 2014). The chemical structure and numbering system used for NMR assignments of OL are shown in Figure 1.

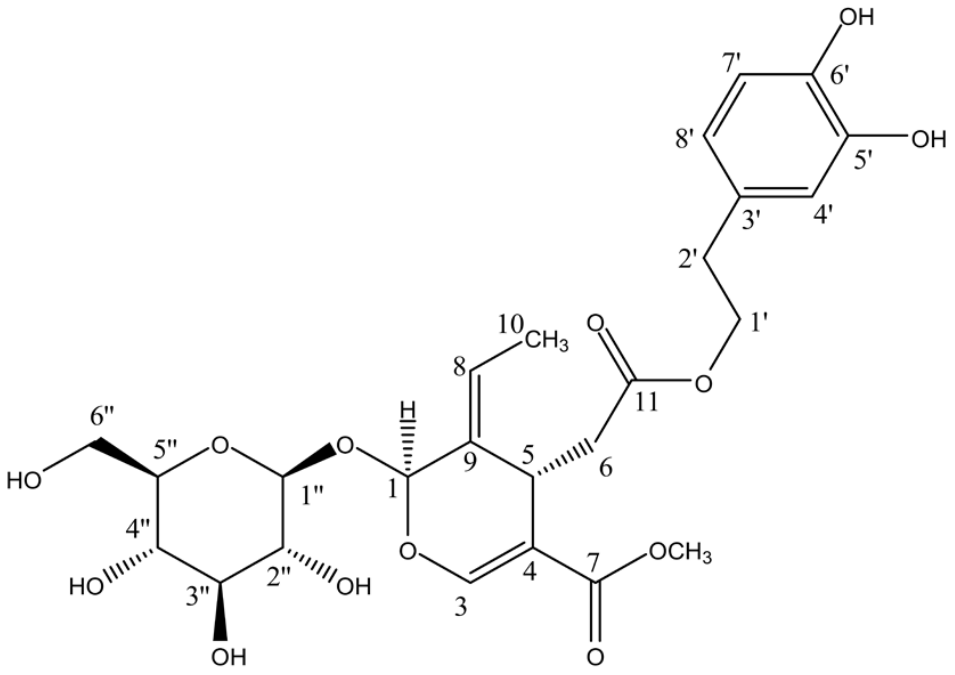

Figure 1. Chemical structure and numbering system used for NMR assignments of OL.

^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.66 (d, 3H, *J* = 6.24 Hz, H-10), 2.33 (d, 1H, *J* = 9.10 Hz, H-6b), 2.36 (d, 1H, *J* = 9.20 Hz, H-6a), 2.68 (t, 2H, H-2'), 3.65 (s, 3H, OMe), 3.79 (dd, *J* = 4.5 and 13.2 Hz, 1H, H-5), 4.13 (t, 2H, H-1'), 4.65 (d, *J* = 7.76 Hz, 1H, glc-1''), 5.82 (s, 1H, H-1), 5.92 (q, 1H, H-8), 6.46 (d, *J* = 8.00 Hz, 1H, H-8'), 6.59 (s, 1H, H-4'), 6.63 (d, *J* = 7.96 Hz, 1H, H-7'), 7.47 (s, 1H, H-3).

^{13}C-NMR (400 MHz, DMSO-d_6): δ 13.3 (C-10), 30.6 (C-5), 33.9 (C-2'), 40.11 (C-6), 51.8 (OMe), 61.1 (glc-6''), 65.6 (C-1'), 70.2 (glc-4''), 73.5 (glc-2''), 76.6 (glc-3''), 77.3 (glc-5''), 93.5 (C-1), 99.3 (glc-1''), 108.1 (C-4), 115.9 (C-7'), 116.5 (C-4'), 120.2 (C-8'), 123.7 (C-8), 129.27 (C-9), 129.3 (C-3'), 143.9 (C-6'), 145.2 (C-5'), 154.0 (C-3), 167.0 (C-11), 171.5 (C-7).

Ideal doses of OL

A preliminary study was carried out to determine the oral administration doses of OL before starting the experiment. Firstly, solutions were prepared by suspending OL (at six different doses of 20, 40, 60, 100, 150 and 225 mg/kg b.wt., respectively) in distilled water. Then, the STZ-induced diabetic rats were treated with the administrations of the solutions by oral gavage (once daily for 30 days). Based on our evaluation obtained the preliminary study, for the oral administrations of OL, the doses of 150 and 225 mg/kg b.wt. were considered to be the ideal doses (see Supplementary Material - Figure S1 and Table SI).

Droplet size and image of the emulsion formulations

The obtained images of the emulsion formulations are shown in Figure 2. The droplet sizes of the blank emulsion, EOLs at the low and high doses were in the range of 3.211-7.916 μm and the span values ranged from 3.269 to 4.647.

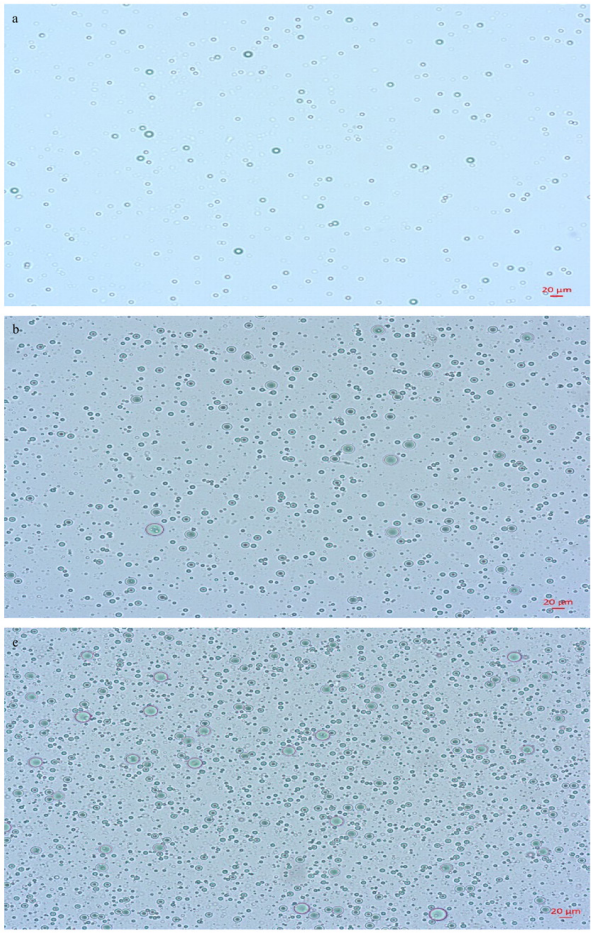

Figure 2. The microscopic images of the emulsion formulations. a: Blank emulsion; b: EOL at a dose of 150 mg/kg b.wt.; c: EOL at a dose of 225 mg/kg b.wt.

Blood glucose concentration

At the end of the study, the BGLs of the STZ-induced diabetic rats were significantly higher when compared with those of the rats in group C ($p<0.05$). After the oral administrations of OL and EOL at the high dose, a significant decrease in the BGLs of the diabetic rats was observed when compared with those of the group CD ($p<0.05$). Interestingly, the oral administrations at the low dose did not cause any decrease in BGLs of the diabetic rats. According to our results, the oral administration of EOL at the high dose produced significant hypoglycemic effects in STZ-induced diabetic rats. The variations in the

blood glucose between the groups for 30 days are shown in Figure 3.

Body weight

At the end of the study, the body weights of the groups CD and CE were significantly lower than their weights at the beginning of the experiment. After the administration of EOL at the low dose, a significant increase in the body weights of the rats in the groups OL1 and EOL1 throughout the experiment was not observed. On the contrary, the body weights of the rats in the groups OL2 and EOL2 were close to those of the rats in the group C at the end of 30 days. The administration of EOL at the high dose could prevent body weight loss in the diabetic rats. Figure 4 shows a comparison of the curves relating to the percent body weight gain of the rats throughout the experiment.

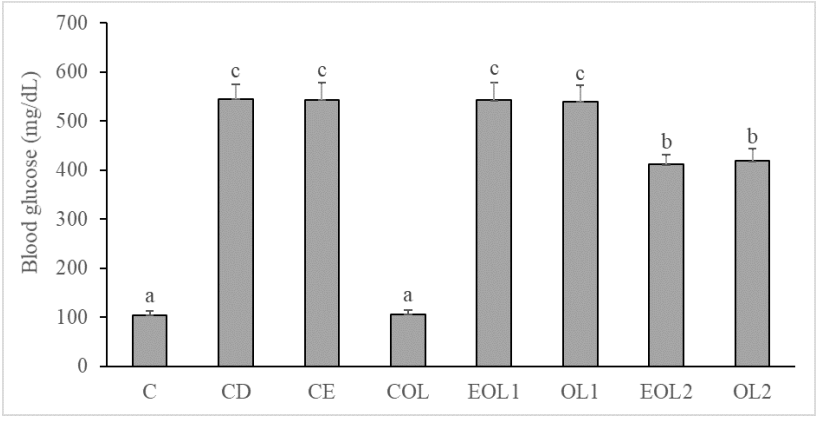

Figure 3. Effects of OL and EOL on BGLs in the diabetic rats at the end of 30 days.
Values are expressed as mean ± SD (n=5). Bars with different letters differ significantly; p<0.05. C: normal control; CD: diabetic control; CE: diabetic + blank emulsion; COL: non-diabetic + OL at a dose of 225 mg/kg b.wt.; EOL1 and EOL2: diabetic + EOL at a dose of 150 and 225 mg/kg b.wt., respectively; OL1 and OL2: diabetic + OL at a dose of 150 and 225 mg/kg b.wt., respectively.

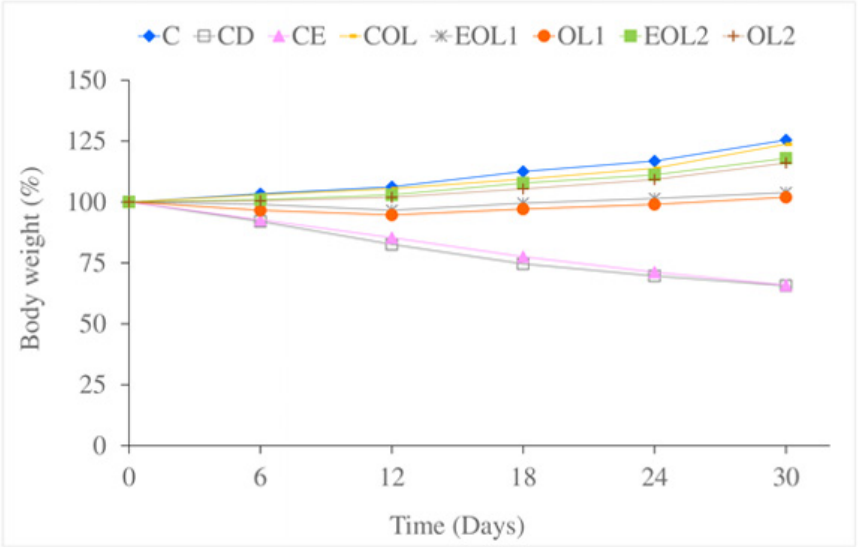

Figure 4. Effects of OL and EOL on percent body weight in the rats throughout the experiment.
Weights are plotted as percentiles with the starting, weights all standardized to 100%. C: normal control; CD: diabetic control; CE: diabetic + blank emulsion; COL: non-diabetic + OL at a dose of 225 mg/kg b.wt.; EOL1 and EOL2: diabetic + EOL at a dose of 150 and 225 mg/kg b.wt., respectively; OL1 and OL2: diabetic + OL at a dose of 150 and 225 mg/kg b.wt., respectively.

Hepatic enzymes

The various enzyme levels of all the groups in the study were measured after 30 days. From the data obtained, it was observed that the serum AST and ALT levels were significantly elevated in the CD group compared with the C group. The increased AST and ALT levels were dramatically decreased in the presence of OL and EOL at the high dose ($p<0.05$). The oral administrations of OL and EOL at the low and high doses significantly decreased serum AST and ALT levels of the treated diabetic rats compared with the diabetic rats ($p<0.05$, at all groups). Moreover, the oral administrations of OL and EOL at the high dose were more effective than those at the low dose. Also, serum AST levels of the rats receiving the oral administration of EOL at the high dose were lower than those of the rats receiving the oral administration of OL at the high dose ($p<0.05$). Although the difference between the groups was small, the difference was statistically significant. Serum AST and ALT levels of all the groups are shown in Table II.

Antioxidant status

SOD activities and GSH levels of all the groups are shown in Table III. SOD activities and GSH levels were significantly decreased in the STZ-induced diabetic rats compared with the normal rats ($p<0.05$). The decreased SOD and GSH values were restored in the presence of OL and EOL. OL and EOL at two different doses significantly elevated SOD activities and GSH levels in diabetic rats treated for 30 days ($p<0.05$). Moreover, the administrations at the high dose were more effective than the administrations at the low dose ($p<0.05$). Although SOD activity of the group treated with the high dose of EOL was higher than that of the group treated with the high dose of OL, insignificant differences between SOD activities of these two groups were observed. Also, GSH levels of the rats receiving the oral administrations of EOL and OL at the high dose were close to each other.

Table II. Effects of OL and EOL on serum AST and ALT levels in all the groups.

Groups	AST (U/L)	ALT (U/L)
C	113.60 ± 10.5^{a}	42.40 ± 5.94^{a}
CD	975.20 ± 91.55^{e}	426.00 ± 44.32^{d}
CE	947.80 ± 80.16^{e}	432.80 ± 40.26^{d}
COL	114.00 ± 10.79^{a}	43.20 ± 4.32^{a}
OL1	643.40 ± 70.82^{d}	281.00 ± 37.29^{c}
OL2	330.80 ± 43.53^{c}	147.00 ± 16.32^{b}
EOL1	617.00 ± 58.43^{d}	272.60 ± 32.94^{c}
EOL2	247.20 ± 35.88^{b}	134.00 ± 13.10^{b}

Values are expressed as mean ± SD (n=5). The groups in the same column with different superscript letters are statistically significant ($p<0.05$). C: normal control; CD: diabetic control; CE: diabetic + blank emulsion; COL: non-diabetic + OL at a dose of 225 mg/kg b.wt.; EOL1 and EOL2: diabetic + EOL at a dose of 150 and 225 mg/kg b.wt., respectively; OL1 and OL2: diabetic + OL at a dose of 150 and 225 mg/kg b.wt., respectively.

Table III. Effects of OL and EOL on SOD activities, GSH and TBARS levels in all the groups.

Groups	SOD (U/mg protein)	GSH (nmol/mg protein)	TBARS (nmol/mg protein)
C	24.37 ± 1.12^{c}	4.61 ± 0.18^{c}	1.62 ± 0.07^{a}
CD	12.89 ± 0.56^{a}	1.96 ± 0.10^{a}	4.17 ± 0.16^{c}
CE	12.83 ± 0.67^{a}	1.98 ± 0.09^{a}	4.11 ± 0.15^{c}
COL	23.98 ± 0.89^{c}	4.61 ± 0.16^{c}	1.63 ± 0.09^{a}
OL1	19.40 ± 0.79^{b}	3.49 ± 0.16^{b}	3.05 ± 0.13^{b}
OL2	23.62 ± 0.97^{c}	4.43 ± 0.17^{c}	1.76 ± 0.10^{a}
EOL1	19.71 ± 0.73^{b}	3.64 ± 0.14^{b}	2.98 ± 0.09^{b}
EOL2	23.76 ± 0.80^{c}	4.46 ± 0.17^{c}	1.71 ± 0.10^{a}

Values are expressed as mean ± SD (n=5). The groups in the same column with different superscript letters are statistically significant (p<0.05). C: normal control; CD: diabetic control; CE: diabetic + blank emulsion; COL: non-diabetic + OL at a dose of 225 mg/kg b.wt.; EOL1 and EOL2: diabetic + EOL at a dose of 150 and 225 mg/kg b.wt., respectively; OL1 and OL2: diabetic + OL at a dose of 150 and 225 mg/kg b.wt., respectively.

Lipid peroxidation

TBARS levels of all the groups are shown in Table III. TBARS levels were assessed at the end of the study. The results demonstrated that the injection of STZ significantly increased TBARS levels in the liver tissues of the diabetic rats. TBARS levels were significantly increased in the group CD compared with the group C ($p<0.05$). The administrations of OL and EOL, at two different doses, significantly reduced TBARS levels in the treated diabetic rats. Moreover, the administrations at the high dose are significantly more efficient compared with those at the lower dose ($p<0.05$). TBARS levels of the rats receiving the oral administrations of EOL and OL at the high dose were close to each other. An insignificant difference between TBARS levels of the rats in the groups CE and CD was observed. In addition, TBARS levels of the rats in the groups C and COL were close to each other.

DISCUSSION

In recent years, much attention has been focused on natural antioxidant usage for preventing oxidative damage caused by diabetes because of their distinctive biological activity and low toxicity. Reports of the experimental studies of OL, a natural antioxidant compound, have been published in 2006 (Al-Azzawie & Alhamdani 2006) and 2009 (Jemai et al. 2009). These confirmed antioxidant properties allow OL to be efficient in the protection against some metabolic diseases related to oxidative stress such as diabetes. Moreover, a previous study showed that OL is an effective antioxidant in different *in vitro* assays including hydrogen peroxide scavenging, superoxide anion radical scavenging, $ABTS^{\cdot+}$ scavenging, and $DPPH^{\cdot}$ scavenging when compared to natural antioxidant compounds, such as a-tocopherol and trolox (Gulcin et al. 2009). Also, it has been reported that OL has an antioxidant activity higher than vitamin C and E (Benavente-Garcia et al. 2000). The long list of biological activities of OL makes it a good target

for further studies, however, its bitter taste and its ability to be degraded by digestive enzymes discourages its use (Souilem et al. 2014). For this reason, emulsion systems are considered. In our study, the emulsion formulation of OL was developed for its potential use as a functional food. To our knowledge, this is the first study to demonstrate the beneficial effects of emulsion formulation of OL in an experimental animal model.

There is no available data showing the oral median lethal dose (LD50) and a toxic dose of OL in the literature (Yu et al. 2016, Hamdi & Castellon 2005). The LD50 value of OL in rats is estimated to be more than 1000 mg/kg b.wt. Thus, OL is considered a harmless substance (Talapatra & Sarkar 2015). Moreover, some researchers administered OL at different doses orally to experimental animals in their studies. For example, Jemai et al. 2009 investigated the antidiabetic and antioxidant effects of OL-rich olive leaf extract (at the doses of 8 and 16 mg/kg b.wt.) in diabetic rats for 4 weeks. They noted that the administration of the high dose is significantly more efficient when compared with that of the low dose (Jemai et al. 2009). Al-Azzawie & Alhamdani 2006 demonstrated the hypoglycemic and antioxidant effects of OL at a dose of 20 mg/kg b.wt. in diabetic rabbits for 16 weeks (Al-Azzawie & Alhamdani 2006). Nekooeian et al. 2014 investigated the effects of OL at a dose of 60 mg/kg b.wt. in rats with simultaneous type 2 diabetes and renal hypertension (Nekooeian et al. 2014). In the current study, the rats were treated with the oral administrations of the emulsions containing OL (at the doses of 150 and 225 mg/kg b.wt., respectively) for 30 days. The reason for the difference between the studies on OL may be due to the differences in rat species, the high BGLs in the animals, the administration mode of OL, and its purity degree.

Several studies have investigated the hypoglycemic effect of OL in experimental models of diabetes. For example, Al-Azzawie & Alhamdani 2006 and Jemai et al. 2009 have demonstrated that OL can decrease BGLs in diabetic animals (Al-Azzawie & Alhamdani 2006, Jemai et al. 2009). In the current study, it was found that OL, the main phenolic compound of olive leaf, has a hypoglycemic effect. According to our results, the oral administrations of OL and EOL produced significant hypoglycemic effects in STZ-induced diabetic rats, mainly at a dose of 225 mg/kg b.wt. BGLs of the rats receiving the oral administrations of EOL and OL at the high dose were similar to each other. Also, BGLs of the rats in these two groups were higher when compared with those of the rats in the normal control group. The hypoglycemic effect of OL can be due to its antioxidant potential (Jemai et al. 2009). Moreover, the mechanism responsible for the hypoglycemic activity of OL may result from the increased peripheral uptake of glucose (Gonzalez et al. 1992).

Recently, there are shreds of experimental evidence supporting the possible role of free radicals in the pathogenesis of diabetes (Asmat et al. 2016, Kurup & Mini 2017). In the long term, uncontrolled high blood glucose levels can induce free radicals (Jemai et al. 2009, Ito et al. 2019), and impair the endogenous antioxidant defense system in patients with diabetes (Mendes-Braz & Martins 2018). Also, it is believed that reactive species in diabetes may increase due to a drastic change in SOD activities and GSH levels (Sugumar et al. 2016). SOD and GSH are a crucial part of the antioxidant defense mechanism (Gozen et al. 2017). In the present study, the SOD and GSH values of the diabetic rats are much lower when compared to those of the untreated rats. The decreased SOD and GSH values could be due to the injection of STZ. Our results were consistent with the findings

showing that the decrease in SOD and GSH values could be due to STZ injection (Sugumar et al. 2016). After the treatment, a statistically significant increase in SOD activities and GSH levels of the diabetic rats was observed. The increased SOD and GSH values show that OL can stimulate the antioxidant defense system. Thus, it can help protect the body from oxidative stress. The antioxidant potential of OL can mainly be related to its ability to improve radical stability (Barbaro et al. 2014).

Lipid peroxidation is a well-established mechanism of cellular injury in rats. It is used as an indicator of oxidative stress in tissues and cells. TBARS is a standard marker for screening and monitoring lipid peroxidation (Gozen et al. 2017, Jemai et al. 2009). In the current study, after the injection of STZ, TBARS levels of the diabetic rats were significantly increased. The increased TBARS levels are in agreement with reported findings, indicating that hyperglycemia is accompanied by an increase in oxidative impact as shown by a substantial increase in hepatic lipid peroxidation resulting in the formation of TBARS (Jemai et al. 2009). After the treatment, a statistically significant decrease in TBARS levels of the diabetic rats was observed. Moreover, TBARS levels were restored in the presence of OL and EOL at the high dose. Thus, the results obtained from the current study showed that OL could prevent lipid peroxidation and attenuate oxidative stress associated with diabetes.

Hepatic cell injury is manifested by elevated serum transaminase activity before the appearance of clinical symptoms and signs. AST and ALT used in the diagnosis of hepatic cell injury are aminotransferases in mitochondria of rats. Comparable elevations of both enzymes often reflect liver damage (Kurup & Mini 2017). Usually, the liver enzyme levels in the blood are low. When the liver is damaged, it will release more AST and ALT into the blood, and the enzyme levels will rise (Eidi et al. 2009, Kurup & Mini 2017). The focus of our study was on the most common liver enzymes, AST and ALT. The enzyme levels of all the groups in the current study were measured at the end of 30 days. According to our results, the serum AST and ALT levels were significantly elevated in the diabetic rats when compared with the untreated rats. The increase in these enzyme values may be due to the injection of STZ that has a significant role in the change of liver functions. The increased AST and ALT levels were dramatically decreased in the presence of OL and EOL.

CONCLUSIONS

In conclusion, based on results obtained from our study, the emulsion formulation of OL exhibited a pronounced hypoglycemic effect, lowered the lipid peroxidation process, and improved the antioxidant defense system in a rat model experiment. These effects emphasize the importance of OL as a source of antioxidant, which has the effect to reduce the frequency of oxidative stress-related metabolic diseases such as diabetes. According to the results, both OL and EOL administrations gave similar experimental results. These results convey that both formulations are equally effective, however, the emulsion formulation has an advantage over OL in that it is able to mask the bitter flavor of the compound.

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SUPPLEMENTARY MATERIAL

Figure S1.
Table SI.

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