



Oleuropein ameliorates hyperlipidemia, oxidative stress, inflammatory and liver dysfunction biomarkers, in streptozotocin-induced diabetic rats

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ABSTRACT

Diabetes mellitus (DM) is a serious threat factor for chronic liver disorders and the development of nonalcoholic steatohepatitis into cirrhosis. However, numerous pharmacological actions of olive polyphenol have been discovered, with oleuropein being a primary phenolic substance found in olive leaves, olive oil, and olive fruit (*Olea europaea*). Our goal is to identify the impacts of oleuropein on lipid measurements, oxidative stress, serum leptin, adiponectin levels, inflammatory status, and hepatic dysfunctions in a male rat model with streptozotocin (STZ)-induced DM. Oleuropein was orally administered to diabetic rats at a 5 mg/kg bw/day dose for 15 days. Diabetic rats treated with oleuropein demonstrated improved hepatic function test results (ALT, AST, alkaline phosphatase, and bilirubin), while activity of the low liver glucose-6-phosphate dehydrogenase (G6PDH) increased. Moreover, serum triacylglycerol, very low-density lipoprotein, low-density lipoprotein, and total cholesterol (TC) were considerably reduced, while high-density lipoprotein increased markedly. In addition, serum antioxidative enzymes such as glutathione peroxidase, catalase, glutathione-S-transferase, and superoxide dismutase increased, as well as glutathione levels. Lipid peroxidation levels were lower than those in the STZ control group. Treating diabetic rats with oleuropein not only resulted in decreased serum leptin and increased adiponectin levels but also had a substantial inhibitory effect on the elevated COX-2 and TNF- α mRNA expression levels in the livers of the STZ group. In conclusion, oleuropein has shown promising results in mitigating hyperlipidemia, oxidative stress, inflammation, and hepatic dysfunction biomarkers in diabetic rats.

INTRODUCTION

Diabetes mellitus (DM) is a major international health emergency that affects 415 million persons aged 20–70 years, and its prevalence is increasing [1]. The most common forms of DM include type 2 DM (T2DM), gestational DM, and

type 1 DM (T1DM) [2]. T1DM is characterized by absolute insulin deficiency and results from autoimmune damage to pancreatic β -cells [3]. T2DM, which leads to at least 90% of DM occurrences, is characterized by long-term hyperglycemia, altered insulin secretion, and insulin resistance (IR) [4]. In addition to chronic hyperglycemia, lipid accumulation is also closely associated with IR [5].

The key organ in glucose, lipid, and protein metabolism and the primary target of insulin is the liver, which plays a substantial task in the improvement of IR in people with T2DM [6]. DM may produce hepatic injury and liver cirrhosis

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[7], this action may be due to inflammation that developed in fatty tissues and the liver through increases in mitochondrial oxidative stress and inflammatory chemical mediators such as adiponectin, leptin, interleukin (IL)-6, and TNF- α [8,9]. The formation of these chemical mediators in adipose tissues is substantially stimulated by IR. A deeper comprehension of the connection between these adipocytokines and DM may provide new insight regarding the physiopathology of diabetes [10].

Carbohydrate metabolism disorders in DM lead to high levels of free radicals that cause oxidative damage to biomolecules within the body, gradually resulting in premature aging and chronic illnesses such as hepatic disorder, atherosclerosis, and cancer. However, the use of antioxidant-rich plants and plant constituents may improve the antioxidant protection system, thereby defending against oxidative stress and damage produced by free radicals in DM [11].

Oleuropein, which is responsible for the distinctive bitter flavor of olive fruits, is the highest common phenolic element in the flesh of olives (*Olea europaea*). It is composed of 2%–3% (w/w) phenolic compounds [12]. Oleuropein was shown to have a maximum content of dry matter in olive fruit and 6%–9% dry material in olive leaves. However, some sources claim that olive leaves have an oleuropein concentration of up to 19% (w/w) [13]. Some preparations of olive oil, the consumption of which is associated with declined risks of DM, obesity, and cardiovascular disease [14,15] have been shown to contain up to 9.0 mg/l of oleuropein [16]. Following metabolic degradation, oleuropein can produce other bioactive constituents, namely hydroxytyrosol and tyrosol. Oleuropein and its degradation product, hydroxytyrosol, have been shown to possess anti-proliferative, anti-apoptotic, anti-inflammatory, and anti-obesity properties [17]. The present study will examine the roles of oleuropein, on hyperlipidemia, oxidative stress, inflammatory status, and liver dysfunction in diabetic male Wistar rats induced by streptozotocin (STZ).

MATERIALS AND METHODS

Chemicals

STZ and oleuropein were delivered from Sigma-Aldrich Co. in Germany. Entirety other chemicals were commercially available, and they were of ultragrade.

Experimental animals

Male albino rats (mean body weight, 180 ± 20 g) of the Wistar strain were gained from the College of Medicine, Alexandria University, Alexandria, Egypt. The environment in which the animals were kept was managed to have a 12-hour cycle of light and darkness as well as a constant temperature and humidity. The study protocol was approved by the (IACUC) College of Medicine, Alexandria University, Egypt with approval no. Alex/FS/2017/4 and the steps were followed by the committee's guidelines and standards for the management of animals.

Induction of DM

To induce DM, male Wistar rats were fasting overnight and given a single fresh preparation of 40 mg/kg BW STZ (pH 4.5) intraperitoneally. Using an Accu-Chek Sensor

Comfort glucometer, the glucose level of blood from the lateral tail vein was measured 1 week after the injection to check for hyperglycemia. The rats were judged diabetic and participated in the experimental study if their blood glucose concentrations at 2 hours postprandial were more than 200 mg/dl [18].

Experimental design

Following the accommodation period and induction of DM, the rats were allocated to the following groups (Fig. 1).

Normal negative control group: The animals were healthy normal rats that were supplied with the same volume of distilled water (vehicle in which oleuropein was dissolved) by oral gavage daily for 15 days. The animals in this group were considered as controls to those in the STZ animals group.

STZ animals positive control group: This group included STZ animals rats that were supplied with the corresponding amount of distilled water (vehicle in which oleuropein was dissolved) by oral gavage daily for 15 days. In this group, rats were considered as STZ animals' control over those in the STZ animals group that were treated with oleuropein.

STZ animals + oleuropein group: The STZ animal rats were supplied with oleuropein (5 mg/kg BW/day) for 15 consecutive days using oral gavage.

Blood sampling and tissue sampling

The rats were denied food and water, and blood sampling was assembled from the jugular vein into dry centrifuge tubes while being lightly sedated with inhalation anesthesia. For the blood to coagulate, room temperature (24°C–26°C) was used. The serum was then isolated from the clot by centrifuging for 10 minutes at 3,000 \times g. When not in use, the sera were placed in sterile, clean Eppendorf tubes and kept at -20°C .

Following the blood sampling, the rats were dissected, by decapitation, and livers were rapidly excised and perfused in sterile isotonic saline (0.9% NaCl). A half gram of liver was homogenized in 5 ml of phosphate-buffered saline at pH 7.4. Then, homogenates were centrifuged at 10,000 \times g for 15 minutes and cooled to 4°C. Liver homogenate supernatants were prepared to determine enzymatic and nonenzymatic antioxidants as well as lipid peroxidation (LPO).

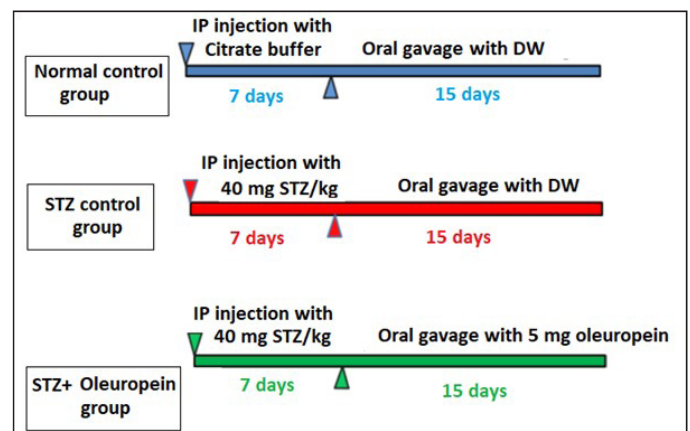


Figure 1. Scheme of animal grouping and schedule of doses.

Biochemical analysis

Based on the technique developed by Bergmeyer *et al.* [19], the activity of ALT and AST were assayed. In accordance with the International Federation of Clinical Chemistry, alkaline phosphatase (ALP) activity was determined using kits provided by BioSystems S.A. Costa Brava 30, Barcelona, Spain. Liver glucose-6-phosphate dehydrogenase (G6PDH) was measured by rat G6PDH ELISA kit (E-EL-R0428) according to the instruction of the manufacturer (Elabscience Biotechnology Inc., USA). Serum leptin level was measured by using Abcam's rat leptin ELISA kit (ab100773) obtained from Abcam, Cambridge, United Kingdom, according to the manufacturer's instructions.

Serum adiponectin was measured by using a rat adiponectin ELISA kit (#JIM-K4903-100) attained from MBL International Corporation (Woburn, MA) following the manufacturer's instruction. Total serum bilirubin was measured as per [20]. The levels of TC, TG, LDL-C, and HDL-C were assayed by the enzymatic procedure in a Labmax Plenno[®] biochemical analyzer using Labtest[®] kits (Labtest Inc. Lagoa Santa, MG, Brazil). To calculate vLDL-C values, the formula $vLDL-C = TG/5$ was used [21].

Detection of LPO and antioxidant defense biomarkers

The hepatic thiobarbituric acid reactive substance (TBARS) as an indicator of LPO and reduced glutathione (GSH) level was assayed using a spectrophotometer (Chem-7 Semi-Auto Chemistry Analyzer, Erba Diagnostics Mannheim GmbH, Germany). The catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and Glutathione-S-transferase (GST), activities were estimated in the hepatic tissues by using kits.

Determination of the TNF- α and COX-2 by quantitative RT-PCR assay (qRT-PCR) in the liver

TNF- α and COX-2 genes mRNA expression levels were evaluated using qRT-PCR. As described in other studies, hepatic tissues' total RNAs were extracted by means of Biozol reagent using the GStract[™] RNA separation kit II guanidinium thiocyanate technique. The fold change was computed using the formulation $2^{-\Delta\Delta Ct}$ concerning β -actin as revealed by Livak and Schmittgen [22]. The primer sequences used for β -actin, TNF- α , and COX-2 [23] are described in the previous publications.

Statistical analysis

Data analysis was performed by SPSS version 22.0 (Chicago, IL). Multiple group comparisons were made using the post hoc Tukey's test, and the changes were judged significant at $p < 0.05$. Symbol "a" is used to refer to a comparison with the normal control and Symbol "b" is used to indicate a comparison with the STZ animal control.

Results

Effects of oleuropein on liver function biomarker

Hepatic function test, AST, ALT, ALP, and total bilirubin quantities in STZ animals' rats were significantly

($p < 0.05$) superior, whereas liver G6PDH activity was significantly ($p < 0.05$) lesser than those in the control group. Oral giving of oleuropein to STZ animals rats significantly ($p < 0.05$) ameliorated hepatic enzyme activities (ALT, AST, and ALP) and total bilirubin level, while oleuropein, induced an increase ($p < 0.05$) in liver G6PDH activity compared to the STZ animals control group (Table 1).

Effects of oleuropein on lipid profile, adiponectin, and leptin

The STZ animals group showed a significant elevation ($p < 0.05$) in cholesterol, LDL-C, VLDL-C, TG, and leptin, while a significant decrease ($p < 0.05$) in HDL-C and adiponectin when compared with the normal control group (Table 2).

Oleuropein administration in STZ animals rats caused a significant improvement in cholesterol, LDL-C, TG, VLDL-C, and leptin ($p < 0.05$) and enhanced HDL-C and adiponectin levels when compared with the STZ rats.

Effects of oleuropein on oxidative stress and antioxidant defense biomarkers

Table 3 reveals that hepatic CAT, SOD, GPx, GST activities, and GSH levels considerably reduced ($p < 0.05$), whereas TBARS significantly heightened ($p < 0.05$) in the

Table 1. Effects of oleuropein on serum AST, ALT, ALP, total bilirubin, and liver G6PDH levels in male diabetic rats.

	Normal	Diabetic	Diabetic + Oleuropein
AST (U/l)	39.33 \pm 2.18	85.33 \pm 4.43 ^a	46.14 \pm 2.55 ^{ab}
ALT (U/l)	28.30 \pm 2.18	63.60 \pm 2.37 ^a	36.54 \pm 0.50 ^{ab}
ALP (U/l)	163.29 \pm 10.66	601.85 \pm 40.76 ^a	206.44 \pm 7.00 ^{ab}
Total bilirubin (mg/dl)	0.399 \pm 0.05	2.28 \pm 0.19 ^a	0.81 \pm 0.053 ^{ab}
G6PDH (ng/mg protein)	258.43 \pm 5.37	77.57 \pm 2.39 ^a	126.57 \pm 5.47 ^{ab}

^aSignificant versus (vs.) the normal group at $p < 0.05$.

^bSignificant versus the diabetic group at $p < 0.05$.

The data are represented as mean \pm standard error of mean (SEM).

Table 2. Effects of oleuropein on serum lipid profile, leptin and diponectin in diabetic male rats.

	Normal	Diabetic	Diabetic + Oleuropein
TG (mg/dl)	67.77 \pm 4.05	111.00 \pm 4.72 ^a	83.60 \pm 4.11 ^{ab}
TC (mg/dl)	95.77 \pm 1.16	172.98 \pm 3.13 ^a	90.95 \pm 1.69 ^{ab}
LDL-C (mg/dl)	23.72 \pm 2.01	90.94 \pm 10.15 ^a	34.48 \pm 4.89 ^{ab}
vLDL-C (mg/dl)	12.80 \pm 0.30	22.20 \pm 0.94 ^a	15.82 \pm 0.41 ^{ab}
HDL-C (mg/dl)	58.50 \pm 9.55	29.08 \pm 0.98 ^a	44.25 \pm 0.83 ^{ab}
Leptin (ng/ml)	1.57 \pm 0.10	8.99 \pm 0.15 ^a	4.38 \pm 0.16 ^{ab}
Adiponectin (μ g/ml)	23.08 \pm 0.14	19.13 \pm 0.19 ^a	21.98 \pm 0.27 ^{ab}

^aSignificant versus the normal group at $p < 0.05$.

^bSignificant versus the diabetic group at $p < 0.05$.

The data are represented as mean \pm SEM.

Table 3. Effects of oleuropein on oxidative stress markers and antioxidant enzyme activities in the livers of male diabetic rats.

	Normal	Diabetic	Diabetic + Oleuropein
TBARS (nmole/g tissue)	27.45 ± 0.53	148.08 ± 11.58 ^a	26.59 ± 2.42 ^b
GSH (μmole/g tissue)	69.06 ± 4.49	18.19 ± 2.86 ^a	61.57 ± 3.18 ^{ab}
SOD (U/mg protein)	1.75 ± 0.01	0.86 ± 0.04 ^a	1.48 ± 0.05 ^{ab}
CAT (U/mg protein)	31.03 ± 0.52	16.30 ± 0.22 ^a	21.77 ± 0.52 ^{ab}
GPx (U/mg protein)	2.96 ± 0.09	1.38 ± 0.08 ^a	2.97 ± 0.09 ^b
GST (μmol/min/mg protein)	7.71 ± 0.35	5.55 ± 0.14 ^a	6.26 ± 0.19 ^{ab}

^aSignificant versus the normal group at $p < 0.05$.

^bSignificant versus the diabetic group at $p < 0.05$.

The data are represented as mean ± SEM.

Table 4. The effects of oleuropein on TNF- α and COX-2 mRNA expression in the liver of male diabetic rats.

	Normal	Diabetic	Diabetic + Oleuropein
TNF- α (fold change)	1.00 ± 0.00	2.32 ± 0.02 ^a	0.61 ± 0.01 ^{ab}
COX-2 (fold change)	1.00 ± 0.00	1.90 ± 0.02 ^a	0.15 ± 0.02 ^{ab}

^aSignificant versus the normal group at $p < 0.05$.

^bSignificant versus the diabetic group at $p < 0.05$.

The data are represented as mean ± SEM.

STZ animals in comparison with the normal group. The STZ animals' group that received oral oleuropein had considerably lower TBARS ($p < 0.05$), higher glutathione levels, and higher antioxidant enzyme activities when matched to the only STZ animals group.

Effects of oleuropein on liver TNF- α and COX-2 mRNA expression

Serum TNF- α and COX-2 were considerably higher ($p < 0.05$) in the diabetes group in comparison with the normal group. Oleuropein-treated STZ rats showed a substantial improvement ($p < 0.05$) in TNF- α and COX-2 levels when evaluated with the STZ animals' group (Table 4).

DISCUSSION

Serums ALT, AST, and the total bilirubin levels were the precise bioindicators applied to monitor hepatic disorders [24]. The elevation in these parameters in the STZ rats may be due to hepatic cell membrane damage or necrosis, which releases these enzymes and bilirubin into the circulatory system [25]. According to Swamy *et al.* [26], these increased values indicate cellular leaking and a loss of the functional integrity of the cell membranes. In addition, people with DM are more likely than those without DM to have abnormal liver function tests [27]. The development of NASH to cirrhosis and chronic liver disease have also been linked to DM. While the elevation of serum ALP in conjunction with an increase in serum bilirubin levels indicates hepatobiliary illness, the rise in the activity of serums ALT and AST in diabetic rats

suggests damage to hepatocytes caused by STZ-induced DM [28].

Oleuropein's ability to stabilize membranes may explain why it can reduce the rise in serum liver enzyme levels by preventing the release of membrane-bonding enzymes as well as leakages of intracellular enzymes. Furthermore, the protective effect of oleuropein against hepatic disorders may be due to its ability to maintain liver cell integrity. Moreover, olive oil's phenolic hydroxytyrosol and tyrosol compounds (the breakdown products of oleuropein) ameliorate hepatotoxicity in rats by repressing oxidative stress and programmed cell death [29].

The current results demonstrate that oleuropein therapy significantly reduced the elevated serum bilirubin levels. This finding agrees with Karakoç and Sekkin [30], who found that the administration of oleuropein to cyclophosphamide and epirubicin-injected rats significantly improved the elevated total bilirubin level in conjunction with the decrease in the elevated serum transaminases' activities. However, these results are in discordance with Domitrović *et al.* [31] who demonstrated that oleuropein, *in vivo*, induced the liver heme oxygenase, which stimulates the breakdown of heme to iron, bilirubin, and carbon monoxide.

According to the current investigation, insulin insufficiency in the STZ rats that was left untreated may be the cause of the decreased liver G6PDH activity, which is important as a rate-limiting enzyme of the pentose phosphate pathway for nicotinamide adenine dinucleotide phosphate (NADPH) synthesis. This contributes significantly to maintaining the antioxidant defense mechanism [32]. It was hypothesized that the decrease in G6PDH activity in DM is the main reason for the redox imbalance [33].

Moreover, Choukem *et al.* [34] stated that G6PDH deficiency may be related to oxidative stress in T2DM owing to the insufficient or limited production of reduced NADPH that recovers GSH (a physiologic antioxidant) to eradicate glucose-generated free radicals. It is worth noting that oleuropein treatment upregulated the expression of hepatic G6PDH by enhancing the synthesis of insulin. Oleuropein administration had a positive effect on glucose metabolism and the consecutive metabolic correlations between elevated glycolysis and lowered gluconeogenesis, elucidating the biochemical mechanisms by which regulation of glucose homeostasis is achieved [35,36].

Serum lipids levels in diabetic rats increased [37], leading to a rise in the mobilizations of free fatty acids (FFAs) from the peripheral depot fat which is primarily responsible for the abnormally high concentration of plasma lipids and lipoproteins in DM [38]. A deficiency of insulin diminishes lipoprotein lipase activity, resulting in disturbances in the metabolism of lipoprotein in DM. The increase in LDL levels in STZ animals might be because of the overproduction of LDL by the hepatic tissues as a result of the stimulation of hepatic triglyceride synthesis by the free fatty acid influx [39].

Decreases in plasma HDL-C in a rat model of DM and patients with DM were due to defects in reverse cholesterol transport. Oleuropein exhibited a hypolipidemic effect that may be due to decreased cholesterol formation and fatty acid

synthesis [40]. Oleuropein also has an anti-diabetic impact by improving glucose absorption and utilization, insulin secretory response, and antioxidant activities [41]. Mice supplied with a high-sugar food, with *O. europaea* fruit resulted in lower levels of triglycerides and VLDL-C; this effect is presumably caused by the reduced IR and an anti-diabetic action [42]. In addition, studies have shown that oleuropein decreases triglyceride formation inside cells and both the quantity and size of lipid drops treated with FFAs [43].

Leptin is a peptide hormone, produced from adipose tissues and encoded by the obese gene [44]. Increased IR and vascular damage caused by leptin may have a role in the development of DM and cardiovascular disorders [45]. Leptin has been shown to have antisteatotic properties, but in some situations, such as hyperleptinemia, this hormone may also promote the aggravation of liver steatosis.

Leptin also promotes nonalcoholic fatty liver disease (NAFLD) which contributes to the development of nonalcoholic steatohepatitis and liver fibrosis [46]. Leptin appears to have a dual impact on NAFLD in experimental animals, having anti-steatotic, pro-inflammatory, pro-fibrogenic, and perhaps carcinogenic properties [47]. In the current study, the serum leptin level was significantly raised in STZ-induced diabetic rats, which may be due to decreased pancreatic insulin secretion, that has been destroyed by STZ, leading to impaired negative feedback control [48].

Local and circulating levels of adiponectin decrease during obesity, IR, T2DM, and atherosclerosis. This action is because it suppresses glucose-6-phosphatase transcription, which reduces gluconeogenesis. The high-molecular-weight adiponectin complex causes a more pronounced reduction in glucose levels in mice [49]. Adiponectin is an efficient preventive agent against several types of liver damage, according to animal-based research, and some data point to adiponectin's direct opposition to TNF- α 's necrotic and destructive effects on liver tissues [50] where it binds to its receptor in hepatocytes, which increases aldehyde oxidase-1 activity and lowers intracellular ROS levels via increasing PPAR α activation [51].

However, in DM oxidative stress is detrimental to adiponectin action; specifically, oxidative stress in adipose tissue suppresses adiponectin secretion [52], resulting in a decline in adiponectin levels and losing its ability to reduce the risky effects of TNF- α within the liver tissue, as demonstrated in the current study. Particularly, an inflammatory state raises the cytokines as TNF- α and leptin, predisposing tissues to hepatic illnesses and causing a concurrent downregulation of protective adipocytokines like adiponectin [53]. In the current study, serum adiponectin significantly diminished in STZ-induced diabetic rats [48]. The increased intracellular ROS production following mitochondrial dysfunction, STZ-induced hyperglycemia, and exacerbated fatty acid oxidation may disturb adipocyte functions and suppress adiponectin secretion [54,55].

Leptin concentrations were significantly reduced in the oleuropein while the opposite results were observed in adiponectin. These results are in concurrence with Fki *et al.* [56] who reported a significant decrease in plasma leptin due to treatment of high-fat supplemented rats with oleuropein. In

addition, adiponectin in patients who received oral ascorbic acid supplementation improves insulin sensitivity which could be associated with increased FFAs' oxidation as well as decreased glucose synthesis in the liver [57].

Oxidative stress is a principal factor in the etiology of DM, so antioxidants may help treat this condition (Fig. 2). Free radical levels rise as a result of the antioxidant defense mechanism being insufficient. Increased LPO, oxidative damage to membranes, and disturbances in essential cell activities may result from elevated free radical levels [58]. The current investigation demonstrated that higher LPO (TBARS) and reduced antioxidant enzymes were related to diabetes-induced liver damage (GSH, SOD, GPx, and CAT). In the current study, the antioxidant enzymes' decreased activity resulted in higher ROS generation in diabetic rats not receiving treatment, which exacerbated oxidative stress.

The progress of diabetes problems may be related to the elevated TBARS concentration in STZ rats. Hazardous free radicals are scavenged by various enzymatic antioxidants involving SOD and CAT [59]. Hyperglycemia enhances the production of reactive oxygen species by increasing glucose auto-oxidation. As a result, the antioxidant defense mechanism is less active, which might damage the liver cells. Since various tissues are more susceptible to oxidative damage, which may cause several complications in chronic DM, the enhancement of antioxidant condition is a vital component to consider when evaluating the advantages of antidiabetic medicines [60,61].

Several bioactive ingredients in olive oil are associated with antioxidative and anti-inflammatory preventive functions, particularly those from biophenols, such as oleuropein and its degradation product, hydroxytyrosol. Oleuropein incubated with the cells displayed a significant decrease in cytokine-induced ROS generation and alleviated the attenuated antioxidant protection system [62]. The antioxidant ability of oleuropein is to remove ROS. Oleuropein-treated rats showed a significant reduction in LPO and oleuropein has beneficial antioxidant properties against gastric damage and reduces hepatic oxidative stress in rats [63].

Hyperglycemia can stimulate stress signaling as well as pro-inflammatory pathways. Nuclear factor-kappaB (NF- κ B) signaling to form NF- κ B p65 and NF- κ B p50 is the main

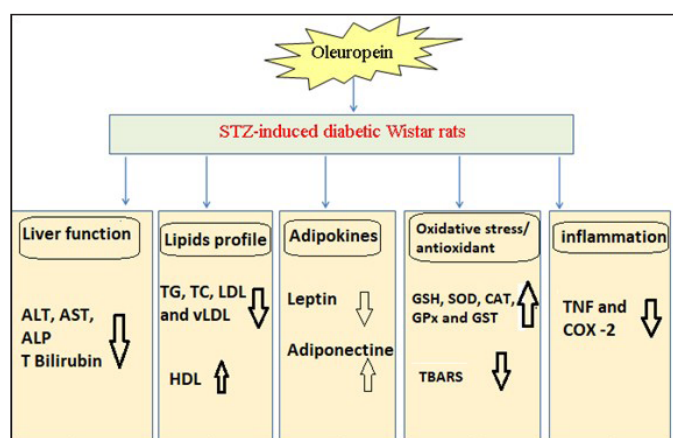


Figure 2. Schematic figure depicting the effects and modes of action of oleuropein in STZ-induced rats.

signal transduction pathway, which is connected with the gene control and activation of pro-inflammatory cytokines, including COX-2, inducible nitric oxide synthase (iNOS), TNF- α and IL-1 β , [64]. Increased synthesis of chemokines and cytokines from activated Kupffer cells recruit the neutrophils and other inflammatory cells to inflamed liver and activate endothelial cells, resulting in more production of ROS and the progress of liver necrosis and damage [65].

Oleuropein oral administration to STZ rats reduced COX-2 and TNF- α expression in the diabetic rats' livers (Fig. 2). According to Wardyn *et al.* [66], oleuropein decreased NF- κ B p65, phospho-p65, COX-2, and TNF- α , production in the kidneys as a result of cisplatin therapy. The degradative metabolic product of oleuropein, hydroxytyrosol, also showed anti-inflammatory characteristics, decreasing iNOS, COX-2, TNF- α , and nitric oxide release in the lipopolysaccharide-activated human monocytic cell line [67]. Oleuropein, a polyphenolic substance, allegedly reduced NF- κ B phosphorylation in models of spinal cord injury and ileum ischemia/reperfusion in mice [68]. Oleuropein specifically reduced the expression of IL-1 β and IL-6 in the colon, and in diabetic rats [69].

Overall, oleuropein has potent ameliorative effects on liver function, lipid profile, leptin and adiponectin, inflammation, and oxidative stress (Fig. 2). In our opinion, the improvement in lipid profile and adipocytokines (leptin and adiponectin) levels as well as the inhibition of oxidative stress and improvement of antioxidant defense system may show a vital effect in the prevention of liver disorders in DM.

In conclusion, the oral administration of oleuropein could prevent liver dysfunctions in diabetic rats through its anti-hyperlipidemic, antioxidant, and anti-inflammatory effects (Fig. 2).

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AUTHOR CONTRIBUTIONS

Conceptualization, NAM, OMA, and HMA; project administration, NAM, OMA, SSA, HMA, KAA, and HMA; supervision, NAM and SSA; funding acquisition, MMH, SSA, HMA, and KAA; methodology, NAM, MMH, and HMA; data curation, NAM, MMH, and HMA; statistical analysis, NAM, MMH, OMA, and HMA; software, MMH; validation and visualization, NAM, MMH, OMA, SSA, HMA, KAA, and HMA; formal analysis, NAM, MMH, and HMA; writing the original draft, NAM, MMH, OMA, and HMA; revising and editing, NAM, MMH, OMA, SSA, HMA, KAA, and HMA. All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

The study protocol was approved by the Institutional Animal Care and Use Committee of College of Medicine, Alexandria University, Egypt with approval number Alex/FS/2017/4 and date: 7/2/2024.

DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

PUBLISHER'S NOTE

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