Ameliorating Effect of Olive Leaf Extract on Testes of Diabetic Young Male Rats: Histopathological and Hematological Studies

Abir Khalil Mohamed, Samir Zaahkouk, Nehal Abo-Elnaga and Esraa Mousa

Department of Zoology, Faculty of Science (Girl's), Al-Azhar University, P.O 11754, Nasr City, Cairo, Egypt

Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt

Abstract: Plants with antioxidant properties have been consumed for centuries as supportive therapy in the treatment of diabetes. The present study aimed to investigate potential ameliorating effect of olive leaf extract (OLE) in a model of diabetes type-2, the nicotinamide-streptozotocin (NA-STZ) young rat. The effects on diabetes on hematological parameters and histoarchitectures of testes were investigated. The young rats were divided into three groups: control (C-Y), diabetic (D-Y) and diabetic treated with OLE (at a dose of 15 mg/kg body weight daily for a month) (D+O-Y). D-Y rats exhibited abnormalities in the testicular histoarchitectures, fibrosis inside and outside the seminiferous tubules and testicular atrophy. Collagen fibers and glycogen were greatly reduced. The hematological parameters of the D-Y rats demonstrated severe with a significant reduction in red blood corpuscles counts (RBCs), hemoglobin (Hb) concentrations, hematocrit (HCT) percentage, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentrations (MCHC), white blood cell counts (WBCs) and platelet (PLT) count. However, in D+O-Y rats, the testicular histoarchitectures and the hematological parameters were greatly improved. Our study suggests that OLE ameliorates the complications of NA-STZ diabetes, concerning the testes histoarchitecture and hematological parameters.

Key words: Olive leaves extract • Streptozotocin–nicotinamide • Fertility • Anti-diabetic • Anti-anemic • Histochemistry

INTRODUCTION

The use of natural plant products for health maintenance and treatment of a variety of diseases has been progressively increased in many western countries [1]. The olive tree (Olea europaea L., Family: Oleaceae) is native to Africa and the Mediterranean region. Olea europaea products have been consumed for centuries for health maintenance and treatment of a variety of diseases such as hypertension and diabetes [2]. The fruits as well as the leaves have been recognized for medical purposes because of their phenolic content [3]. Hydroxytyrosol and oleuropein are the most prominent phenolic content in the olives leaves [4]. Oleuropein is associated with improved glucose metabolism responsible for the antihyperglycemic effect in diabetic rats [5, 6].

Different parts of olive plant and its isolated components have shown a wide pharmacological and biological activities such as: antioxidant [3, 7], anti-inflammatory effect [8], anti-atherogenic effect [9, 10], anticancer effect [11, 12], antimicrobial effect [13], antiviral effect [14], skin protection [15], anti-aging [16], neuron-protective effect [17], anti-platelet aggregation [18], prevention of free radical formation [19] and immune-modulator and hepatoprotective activities [20] due to the high polyphenolic, flavonoids, triterpenes and other biologically active constituents.

Diabetes mellitus (DM) type-2 is a rapidly-growing epidemic disease, in which the body can't use the insulin it produces. Type-2 DM has severe complications due to the developing of hyperglycemic, an increase in the glucose level in the blood stream [21]. Hyperglycemia leads to glucose auto-oxidation and reactive oxygen species (ROS) overproduction and the resulting oxidative stress [21]. ROS have been shown to inactivate key enzymes of glucose metabolism in both the glycolytic
pathway and the electron transport chain coupled to oxidative phosphorylation [22]. *Diabetes mellitus* can damage different tissues especially the reproductive system [23, 24] or develop hypoxia which disturbs the physiological properties that lead to the occurrence of anemia [25]. The incidence and prevalence of type 2 diabetes in children and adolescents are increasing. Diabetes in children might lead to severe reproductive disorders and sexual disturbances [26, 27]. Diabetes in male reproduction is commonly characterized by pathological testicular changes, erectile dysfunction and related endocrine changes [24, 28]. Diabetes affects the testicular function due to the lack of insulin and subsequently the impairment of regulatory action of this hormone on both Leydig and sertoli cells [6, 29]. Also, diabetes induces changes in the histoarchitecture of the ventral prostate, a decrease in sperm count, along with low levels in plasma testosterone [30].

The nicotinamide-streptozotocin (NA- STZ) rat model exhibit conditions similar to diabetes type 2 in humans and it is used for studying the drugs and natural products that could lessen the diabetic complications [23]. STZ is an antibiotic derived from *Streptomyces achromogenes* and it induces diabetes in rats by breaking DNA strands leading to apoptosis of β-cells in the pancreas and diabetic rat [31]. NA, pyridine-3-carboxamid, is a vitamin B2 derivate with antioxidant capacity that protects insulin-secreting cells against STZ cytotoxicity. This study was designed to develop young rat model of diabetes type 2 using NA- STZ and to investigate the effect of OLE on the amelioration of complications induced by (NA- STZ) diabetes, namely the testicular histoarchitecture and hematological parameters in both healthy and diabetes in young male rats.

**MATERIALS AND METHODS**

**Experimental Animals:** Twenty four young (3 week-old) (weighting 70-100 g) male Wistar rats were obtained from the Nile Pharmaceutical Co., Cairo, Egypt. Before the experiment, all rats were adapted for a week in metal cages under controlled conditions of light (12h: 12h light-dark cycle), temperature (25±20C). The animals were fed on a standard pellet diet and water was provided *ad libitum*. The study was conducted on rats in accordance with experimental animal ethics approved by Al-Azhar University- Cairo, Egypt.

**Preparation of Olive Leaf Extract:** Extraction of olive leaves (*Olea europaea*) was done according to the method of Zahkok *et al.* [6]. The leaves were dried and crushed to a moderately-coarse powder. A weight of 15 gm of powder was suspended in 70% ethanol and extracted using Soxhlet apparatus for 10 h. Then, ethanol was evaporated at 90 °C for 24 h. The dry semisolid extract was weighed, dissolved in de-ionized water (100 g/ 100 ml, wt/vol.) and stored in refrigerator until use.

**Induction of Type-2 Diabetes:** Young rats were fasted for 16 h, with free access to drinking water. Streptozotocin (STZ), freshly prepared in citrate buffer at pH (4.5), was intraperitoneally (ip) injected at a dose of 65 mg/kg body weight after 15 minutes administration of a single-dose of nicotinamide (NA) (230 mg/kg b. wt.; dissolved in normal saline) [32]. NA and STZ were obtained from Sigma Chemicals (St. Louis, MO, USA), Nasr City, Cairo Egypt. After 48 h of the NA-STZ injection, progression of diabetes was confirmed in urine using enzymatic test strips and in blood by assessment of the blood glucose level using an on call machine [6]. Ultimately, rats with fasting blood glucose levels of more than 250 mg/dl were considered diabetic and used in the experiment as D-Y group. The Haematology analyzer CELLCOUNTER System Sinothinker (LABOMED, inc-SK9000) was used to determine the hematological parameters.

**Experimental Design:** At the beginning of the experiment, young (3-4 week-old) male rats were divided into three groups (8 in each group) as follows:

**(C-Y):** Normal control rats. 1 ml of distilled water was administered orally with by gastric intubation daily for a month.

**(D-Y):** Rat model of diabetes type-2. Nicotinamide (230 mg/kg body weight) and Streptozotocin (at a dose of 65 mg/kg body weight) was injected intraperitoneally as recommended by Masiello *et al.* [32].

**(D+O-Y):** Diabetic rats treated with OLE. D-Y rats were fed OLE (15 mg/kg body weight) by carefully inserting the needle of the gastric- intubation tube into the oral cavity of rats daily for one month.

**Histopathological Study:** Testes were excised and immediately fixed in 10% neutral formalin. Paraffin sections (5µm in thickness) were prepared for histological and histochemical studies. For general histology, sections were stained with Harris’ haematoxylin and eosin [33]. Glycogen was detected by using periodic acid Schiff’s
(PAS) reagent [34]. Collagen fibers were stained by using Mallory's trichrome stain [35]. The optical transparency (in pixel) of glycogen was analyzed using the Bel Microimage Analyzer Program ver. 2.3 application software for microscopy.

**Statistical Analyses:** Analyses of significance between treatment means of physiological data and histochemical data (glycogen content for all groups) were determined using T-test Microsoft Excel 2007. Data were presented as mean ± SD and \( p = 0.05 \) was considered statistically significant.

**RESULTS**

**Behavioral and Hematological Observations:** D-Y rats were decreased in their daily activity, food and water consumption and body weight gain, whereas D+O-Y, showed normal activity and feeding desire similar to the control rats.

In the hematological study, D-Y rats had significantly reduced (at \( p < 0.05 \)) RBC numbers, Hb, HCT, MCV and MCH when compared to C-Y group (Table 1). The WBCs and PLT were also markedly reduced (at \( p < 0.01 \)) in D-Y rats. However, the mean MCHC was not significantly different. The percentage reduction was 55% in WBCs count, 73% in RBCs count, 82% in Hb concentration, 83% in HCT percentage, 67% in MCV and 67% in PLT.

D+O-Y rats were not-significantly different (\( p = 0.05 \)) from C-Y rats in the mean values of WBCs and RBCs count, Hb concentrations, HCT percentage, MCV, MCHC and PLT count (Table 1). The percentage of improvement in the physiological values of the treated rats over the diabetic rats was 82% in WBCs count, 88% in RBCs count, 94% in Hb concentration, 95% in HCT percentage, 93% in MCV, 105% in MCH, 91% in MCHC and 95% in PLT as compared to the normal values.

**Histopathological Observation:** In C-Y rats (8 weeks old), sections of the testes had normal histological structure and most of the seminiferous tubules were at the spermatid stage (Fig. 1A). The lumen of most tubules appeared almost empty, but a few sperm were present in other tubules (Fig. 1A). However in D-Y rats, the seminiferous tubules exhibited atrophy and spermatogenic layers were hardly detectable (1B). The tunica albuginea was abnormally highly thickened with fibrotic tissue (Fig. 1B). Also, debris of degenerated spermatogenic cells with highly thickened basement membranes was observed (Fig. 1C). Numerous vacuoles appeared in between the spermatogenic cells and some cells with pyknotic nucleus were observed (1C). Sections of the testes of D+O-Y rats showed that most of the seminiferous tubules regained their normal histoarchitecture similar to the control rats (Fig. 1D).

Sections of the testes stained with Mallory's trichrome showed that C-Y rats had a normal distribution of collagen fibers in the basement membrane of seminiferous tubules (Fig. 2A). However, the distribution of collagen fibers in D-Y rats was highly reduced in the basement membrane of most tubules and the connective tissue surrounding the tubules (Figs. 2B). The red coloration indicating fibrosis of both spermatogenic and interstitial cells of D-Y rats was observed (Fig. 2B). Sections of the testes of D+O-Y rats showed that the seminiferous tubules restore their normal content of collagen fibers similar to the control group (Fig. 2C). Areas of edema showed scattered collagen fibers.

Testes of C-Y rats stained with PAS showed normal distribution of glycogen (Fig. 3A). However, testes of D-Y rats stained with PAS showed changes in the distribution of glycogen especially in the degenerated spermatogenic cells and boundaries of the tubules (Fig. 3B). The mean optical transparency (MOT) of the stained glycogen was significantly reduced when compared to C-Y rats (\( p = 0.05 \)) (Fig. 3D).

In D+O-Y rats, glycogen content appeared normally distributed in the seminiferous tubules (Fig. 3C) and the value of MOT of glycogen stain affinity was significantly increased when compared to C-Y rats (\( p = 0.05 \)) (Fig. 3D).

---

**Table 1:** Mean and Standard Deviation (SD) of Hematological Parameters in Young Male Albino Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C-Y</th>
<th>D-Y</th>
<th>D+O-Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (10³/m³)</td>
<td>6.1±0.12</td>
<td>4.8±0.07*</td>
<td>5.7±0.12</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.9±6</td>
<td>11.2±0.21*</td>
<td>12.7±0.12</td>
</tr>
<tr>
<td>HCT%</td>
<td>40.6±0.27</td>
<td>34±0.04*</td>
<td>8.7±0.44</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>4.9±0.3</td>
<td>3.3±0.9*</td>
<td>4.1±0.6</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>22.6±0.72</td>
<td>22.2±1.0*</td>
<td>3.9±1.4</td>
</tr>
<tr>
<td>MCHC (g/dl red cells)</td>
<td>36.6±1.5</td>
<td>32.6±1.3*</td>
<td>33.9±1.5</td>
</tr>
<tr>
<td>WBC (10³/mm³)</td>
<td>8.5±0.5</td>
<td>4.7±0.10**</td>
<td>7.0±2.5</td>
</tr>
<tr>
<td>PLT / million</td>
<td>201±14.6</td>
<td>135±7.7**</td>
<td>192±4.6*</td>
</tr>
</tbody>
</table>


- The values are considered significant at \( p \leq 0.05 \) and highly significant at \( **p \leq 0.01 \) compared to the control group.
Fig. 1: A, C-Y rats shows seminiferous tubules (ST) with spermatogenesis process mostly at the spermatid stage, but few mature sperms was developed. B, D-Y rats shows hardly detected spermatogenic layers (head arrows) and highly thickened fibrotic tunica albuginea (TA). C, D-Y rats shows debris of degenerated spermatogenic cells (Star) with highly thickened basement membrane (BM). The seminiferous tubules exhibited pyknotic (p) and signs of vaculation (V). D, D+O rats shows well developed spermatogenic layers. The lumen (Lu) is filled with spermatid cells (St). (Hx&E).

Fig. 2: A, C-Y rats shows normal distribution of collagen fibers in the testes. B, D-Y rats shows the testes with highly decreased in collagen fibers. Fibrin appeared in red color increased inside and outside the seminiferous tubules. Insert: shows atrophy in the seminiferous tubules (head arrows) and fibrin inside the tubules (arrows). C, D+O-Y rats restored their normal content of collagen fibers, but scattered collagen appeared in the area of edema (arrow). (Mallory Trichrome Stain).
Fig. 3: A, C-Y rats showing moderate stain affinity for glycogen in the spermatogenic cells, but the basement membrane (head arrow) and the interstitial cells (arrow) between the seminiferous tubules showed an increase in staining affinity. B, D-Y group showing the testes with highly reduced in glycogen especially in the spermatogenic cells (arrow). C, D+O-Y rats shows testes with slightly reduced glycogen inside the seminiferous tubules, however, glycogen increased in the interstitial area between the seminiferous tubules (star) and arterial walls (A) inside the interstitial spaces. D. Mean of optical transparency (MOT) value of stained glycogen. (PAS stain).

**DISCUSSION**

The NA-STZ young rat model of type-2 diabetes (D-Y rats) exhibited significant morphological alterations in the spermatogenesis process, thickened basement membranes and testicular atrophy. High reduction in collagen fibers and glycogen were also observed with common fibrosis inside and outside the seminiferous tubules of diabetic group. Also, the D-Y animals exhibited severe anemic conditions: a reduction in RBCs, Hb, HCT, MCH, MCHC, WBC and PLT values. In contrast, NA-STZ young rats treated with a small dose of OLE (a dose of 15mg/kg.b.w for a month, daily), the D+O-Y rats, had the same morphological status as the control group.

The significant reduction in the physiological parameters observed in the present study in D-Y rats is consistent with previous studies which indicated that diabetes mellitus is accompanied by the development of hypoxia that disturbs the physiochemical properties of the erythrocyte membrane and leads to anemia [25]. The body then activates compensatory reactions directed at a renewal of the red blood cell pool and an increase in tissue oxygenation. This leads to the development of oxidative-nitrative stress and results in various cytotoxic effects and ultimately to increase cell death by apoptosis of immune-competent blood cells [36].

The present study revealed in addition abnormalities in the spermatogenesis process and testicular atrophy in D-Y group. The oxidative-nitrative stress produced under the diabetic condition is likely involved in the progression of spermatogenesis process dysfunction as previously reported [6, 29 & 37]. Our previous study [6] showed that NA-STZ diabetes in young rats caused a significant increase in the formation of ROS such as malondialdehyde (MDA) and nitric oxide (NO) and a significant reduction in the antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in the testis. Elevated levels of ROS, due to insufficiency of the antioxidant defense system, may lead to disruption of cellular function and oxidative damages to membranes [38, 39].

The alteration in the testicular structure in D-Y animals might be responsible also for the interruption in the testosterone production during diabetes. Zahkok *et al.* [6] showed that NA-STZ diabetes in young rats caused a significant decrease in serum FSH and LH levels.
that lead to the reduction in testosterone secretion, sperm count and testicular weights. Previous studies reported the effect of diabetes on the sex hormones [27, 40].

Further, D-Y animals had reduced the collagen content and glycogen in numerous seminiferous tubules. Collagen in testes regulates the adhesion of spermatogonia to the basement membrane and the detachment of late spermatids to the tubule lumen [41]. The exposure to ROS produced in diabetes cause changes in collagen amino acids leading to change in the mechanical strength of extra-cellular matrix and blood vessels in testes [40]. The reduced glycogen in the testes of D-Y rats could be due to the lack of insulin in diabetes. This would affect the performance of glycogenolysis and glycogen enzymes [29] and prevent the synthesis of glycogen or an increase in its breakdown in the liver, testis and the organism as a whole [42]. Glycogen breakdown in diabetes would facilitate the release of a greater number of polysaccharides to the blood, increasing the hyperglycemia and causing even more damage to the testis and the organism as a whole [42]. To conclude, there is an agreement between the development of anemia and alteration in the testicular structure. NA-STZ diabetes has a potential cause of hematological complication leading to fertility malformation.

Additionally, our results revealed that extract of olive leaves administrated orally to the NA-STZ rats prevented the development of the complication of diabetes. OLE ameliorated the testicular histochemical defects including reduction of collagen fibers and glycogen of D-Y animals. Also, the hematological values including RBCs, Hb, HCT, MCH, MCHC, WBC and PLT of D-Y animals were highly improved toward the normal value. The mechanism of action of OLE in diabetes is still unknown and needs more research. However, olives bear potent antioxidants [3]. The leaf constituents scavenge free radicals and exert a protective effect against oxidative damage to cellular macromolecules, inhibiting or preventing the deleterious consequences of oxidative stress. The constituents are free radical scavengers like polyphenols, flavonoids and phenolic compounds. A number of scientific reports indicate phenolic compounds such as hydroxytyrosol and oleuropein in the olives leaves have antioxidant properties [4, 5]. Oleuropein has two hydroxyl groups which are believed to play a critical role in quenching reactive oxygen species. Oleuropein in the OLE has hypoglycemic effect that improves glucose-induced insulin release and retards of carbohydrate absorption from the gut, resulting in a reduction of plasma-glucose concentration [43]. The antihyperglycemic effect of OLE might reduce the free radical formation and ameliorate the anaemic condition and changes in testes histoarchitecture. A number of studies have reported that the intake of antioxidants can stabilize the testicular histoarchitecture and protect sperm DNA from oxidative stress caused by free radicals [30, 38 & 39]. OLE also counteracts the oxidative stress induced in the testes by the exposure to the hyperglycemic condition induced of diabetes [6]. The improvement of the carbohydrate profile in the testes of D+O-Y animals might be through the inhibition of carbohydrate-hydrolyzing enzymes such as alpha amylase and alpha glucosidase [44].

CONCLUSIONS

Oral administration of OLE to young diabetic rats decreased the anemic condition of diabetic animals and improved their hematological parameters. Also, the histopathology and histochemistry of testis tissues was improved. Therefore, this study suggests that OLE may be helpful for diabetic patients who suffer from anemia and sexual impotency, due to its anti-diabetic activity, immunity and fertility enhancing properties.

ACKNOWLEDGEMENT

Authors thank the technicians in the Genetic Engineering Center, Al- Azhar University, Egypt, for their efforts and help in animals care and using facilities. The authors thank technicians at the Zoology department at Faculty of Science, Al- Azhar University, Egypt for help in slides preparation. The authors thank Dr. Jay A. Burr, Department of Biological Sciences, SFU, Canada, for helpful suggestions on the manuscript and English corrections.

REFERENCES


