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Research Article

Studies on fertility of diabetic male rats treated with olive leaves extract

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ABSTRACT

Natural plant products have been consumed for centuries for health maintenance and treatment of a variety of diseases in various traditional approaches of medicine in Egypt and all over the world. Holy Quran, from more than 1400 years, has been directed the human attention toward certain herbs and fruits, including olive, which are definitely have physical and mental benefits to humans and known as prophetic medicine. Olive leaves extract (OLE) exhibit antioxidant and anti-inflammatory properties.

Aim of study: The present study was designed to investigate the antioxidant role of OLE in diabetes-induced reproductive damage in young male rats.

Material and method: In this experimental study 32 of young male albino rats were used, then divided into four groups, (n=8 in each group), control, olive leaves extract, diabetic and diabetic treated with olive leaves extract. Diabetes was induced by a single intraperitoneal injection of Streptozotocin (STZ) at a dose of 65 mg/ kg body weight in 1ml of freshly prepared 0.1M citrate buffer (pH 4.5), after 15 minutes of a single-dose administration of nicotinamide (NA) (230 mg/kg b. wt.; dissolved in normal saline). Olive leaves extract dose was 15 mg/kg b. w. for month; day after day by gastric- intubation.

Results: In diabetic animals treated with OLE, the testicular weight, sperm count, serum FSH, LH and testosterone levels were improved. Further, OLE administration improved the antioxidant status in the testicular tissues of the diabetic young rats, via improving the superoxide dismutase (SOD), catalase (Cat), glutathione peroxidase (GPX), glutathione S Transferase (GST) activities, and enhancement of the glutathione (GSH) content in parallel with declines the malondialdehyde (MDA) and nitric oxide (NO) levels.

ConclusionOLE improved the reproductive dysfunction via regulation of FSH, LH, Testosteron levels in the serum and the antioxidant status in the testicular tissuesof young diabetic rats. Therefore, regular administration of low doses of OLE is recommended to decrease the risk of infertility to regulate the different complications of diabetes in men.

Keywords: Olive leaves extract, anti-diabetic, testicular dysfunction, nicotinamide-streptozotocin.

INTRODUCTION:

Natural plant products have been consumed for centuries for health maintenance and treatment of a variety of diseases in various traditional approaches of medicine in Egypt and all over the world. In addition, during the past decades, the popularity of herbal and other natural products has been progressively increased in many western countries[1].

Holy Quran, the book of healing and mercy, from more than 1400 years, has been directed the human attention toward certain herbs and fruits such as grape, olive, pomegranate, date, and fig, which are definitely have physical and mental benefits to humans. Using herbs in medicinal purposes is referred as "prophetic medicine"[2-4].

The olive tree (*Oleaeuropaea* L., Family: Oleaceae) is native to the Mediterranean region, whereas it has been cultivated in the Mediterranean for more

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than a thousand years. The olive oil, fruits as well the leaves have been used for medical purposes and are known as a folk remedy for hypertension and diabetes[5]. Various parts of olive have wide pharmacological and biological activities, as antioxidant, anti-microbial, anti-viral, anti-malarial, antifungal, anti-diabetic, anti-cancer, anti-inflammatory,anti-atherogenic, hypolipidemic, cardioprotective, and hepatoprotective activities[5],due to the high polyphenolic, flavonoids, triterpenes and other biologically active constituents.Chemically, the active constituents of the olive leaves are oleic acid, phenolic constituents, and squalene. The main phenolic compounds, hydroxytyrosol and oleuropein. Triterpenes and flavonoids, namely rutin and glycosides of apigenin and luteolin, are also found[6]. Numerous studies have shown that oleuropein (up to 6%–9% of dry matter in the leaves), has been associated with improved glucose metabolism and responsible for the anti-hyperglycemic effect in diabetic rats[7].

One of the major health problems in life is infertility; male factors comprise approximately 30% of this problem[8]. Several factors can affect the spermatogenesis process and decrease sperm quality and quantity. Several factors affect spermatogenesis such as diabetes mellitus, liver and coronary heart diseases, air pollutants, chronic smoking and vitamin deficiency[9].

Diabetes mellitus is a rapid growing epidemic disease[10]. Type-2 diabetes mellitus developed resistance and malfunctioning of insulin, which induce hyperglycemia; increased glucose level in the bloodstream. Hyperglycemic condition is a causative connection between diabetes and its complications. The chronic hyperglycemia creates oxidative stress, resulting in an oxidative damage in different tissues, whereas, glucose auto-oxidation and protein glycosylation induce overproduction of reactive oxygen species (ROS)[11]. 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[12]. Further, oxidative stress can cause depolarization and calcium uptake in insulin producing pancreatic isletcells[13], a phenomenon that stimulates insulin secretion and resulting in hyper-insulinemia. Furthermore, oxidative stress can damage several biomolecules like proteins, lipids and DNA, leading to the inactivation of enzymes affecting DNA integrity and cellular membrane composition.

The streptozotocin–nicotinamide (STZ–NA) rat model induce similar conditions to diabetes in humans, it is used for studying the drugs and natural products that can diminish the diabetic complications[14]**.** Streptozotocin (STZ) induced diabetes in male rats, resulting in atrophy of sex organ, changes in histoarchitecture of ventral prostate, decrease in sperm count , along with low levels in plasma testosterone[15]**.** Diabetic damage to male reproduction is commonly characterized by pathological testicular changes, diabetes-related erectile dysfunction and related endocrine changes[16]**.**

Accordingly, the present study aimed to investigate the effects of the OLE on the reproductive dysfunction in diabetes induced by nicotinamide- streptozotocin (NA-STZ) in young male rats.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Preparation of olive leaves (*Oleaeuropaea*) extract.

Extraction of dry olive leaves (*Oleaeuropaea*)was doneaccording to the method of **Samir *et al*.[17] and Alirezaei*et al*.[18].**Each 15 g of olive leaves were suspended in 70% ethanol and extracted using soxhlet apparatus for 10 hours (h), continuously. After then, ethanol was evaporated at 90 $^{\circ}$C in oven, for 24 h. The semisolid extract was left until dryness, weighed, then dissolved in deionized water (100 g/ 100 ml, wt./vol.) and was stored in refrigerator until using.

Induction of diabetes

Diabetes was induced to fasted rats (for 16 h, with free access to drinking water) by an intraperitoneal (ip) injection of streptozotocin (STZ) ,65 mg/kg body weight, freshly prepared in citrate buffer, pH (4.5), after 15 minutes of a single-dose administration of nicotinamide (NA) (230 mg/kg b. wt.; dissolved in normal saline)[19].Nicotinamide (NA) and streptozotocin (STZ) were obtained from

Sigma Chemicals (St. Louis, MO, USA), Nasr City, Cairo Egypt.

Progression of diabetes was confirmed in urine; using enzymatic test strips[20] and in blood by assessment of the blood glucose level using on call machine**,** after 48 h of the NA-STZ injection. Ultimately, rats with fasting blood glucose levels of more than 250 mg/dl were considered diabetic and used in the animal experiment as D group.

Animals and experimental design

In this experimental study 32 young rats were obtained from the Nile Pharmaceutical Co., Cairo, Egypt. All animals were housed at the animal facility at the Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt. The study was conducted in accordance with international guidelines for animal experiments and approved by the Ethical Committee of Genetic Engineering Center, Al-Azhar University, Nasr City, Cairo, Egypt. The experimental animals were weighting 70-100 g (3weeks old). Then, subdivided into four groups (8 animals/group) as follows: **First group:** normal control rats were administered orally with 1 ml of distilled water by gastric intubation day after day for month. **Second group**: normal rats supplemented with olive leaves extract (OLE), 15 mg/kg body weight (kg b. wt.) by gastric- intubation day after day for month.**Third group**: diabetic control rats were injected with (NA 230 mg/dl - STZ 65mg/dl) administered orally with 1 ml of distilled water by gastric intubation day after day for month.**Fourth group:** diabetic rats treated with olive leaves extract (OLE), 15 mg/kg body weight (kg b. wt.) by gastric- intubation day after day for month. All animal groups were weighed at the beginning and at the end of the experiment. At the end of the experimental period, the animals were fasted overnight, with free access to drinking water, after then, blood samples were collected and the sera were separated for fasting blood glucose and different hormones (FSH, LH and Testosterone).The testes of each animal were excised immediately, one testis was washed with ice cooled saline, and the wet weight was taken and then stored at -80^{o}C, for antioxidant, anti-inflammatory and other testicular biochemical analysis.

Epididymal sperms countwere determined according to **Pant and Srivastava**[21] method. The percentage of viability and total sperm abnormalities were assessed according to the method of **Wells and Awa**[22]**.**

Assessment of the serum hormonal activity

The follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (T) levels were determined in the serum using an ELISA kits for rat (MyBioSource,Inc.). The measurements were done according to the catalogue-instruction guidelines.

Preparation of testis homogenates

Parts of the testis with known weight were homogenized in phosphate buffered saline (0.05 M, pH 7.4) in 1:10 w/v ratio; using Teflon homogenizer (Universal laboratory aid, Type MPW-309, Poland) and centrifuged at 1200 g for 15 min at 4 ^{o}C and stored at -80^{o}C. Clear supernatant of the testis homogenates were used for assessment of the antioxidant status and the pro-inflammatory cytokines.

Assessment of the antioxidant status in the testis tissues

Lipid peroxides (LPO), in terms of malondialdehyde (MDA) was measured according to the method of **Satoh**[23]**,** using 1, 1, 3, 3-tetraethoxypropane as a standard. MDA concentration was expressed as nmol/g wet tissue. Nitric oxide (NO) was determined as nitrite concentration. The method used depends on Griess reaction, which converts nitrite into a deep purple azo-compound which photometrically measured at 540 nm according to the method of **Montgomery and Dymock**[24]**.** No concentration was expressed as µmol/g wet tissue. Reduced Glutathione (GSH) concentration was measured according to **Beutler, *et al.***[25], using DTNB and was expressed as mg/g wet tissue. Superoxide dismutase (SOD) activity was determined according to **Nishikimi and Yogi**[26]**.** The assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium (NBT) dye, which was followed photometrically at 560 nm. The enzyme activity was expressed as U/g wet tissue. Catalase (CAT) activity was assessed according **to Aebi**[27]**.**Catalase reacts with a known quantity of hydrogen peroxide (H_2O_2) in the presence of horseradish peroxidase (HRP), remaining H_2O_2 reacts with 3,5-dichloro-2-hydroxybenzene

sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity measured at 510 nm, which is inversely proportional to the amount of catalase in the original sample. The enzyme activity was expressed as U/g wet tissue. Glutathione-peroxidase (GSH-Px) activity was measured according to the **Paglia and Valentine's** method[28], where the oxidation of NADPH by hydrogen peroxide was followed at 340 nm at 25 °C. The activity was expressed as the oxidized NADPH in U/g tissue. Glutathione-*S*-transferase (GST) activity was measured according to **Habig and Jakoby**[29], by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm. The enzyme activity was expressed as U/g wet tissue.

Assessment of total protein level in the testis tissues

Colorimetric estimation of total protein level in the testicular homogenate was evaluated according to **Lowry et al.**[30], using bovine serum albumin as standard. The total protein concentration was expressed as mg protein / g wet tissues.

Statistical analyses

Statistical analyses were performed using analysis of significances between treatment means of physiological data, using T-test Microsoft Excel 2007. Data were presented as mean + SD and p values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Effect of OLE on fasting blood glucose level, rats' body weight, testis weight, epididymis weight and sperm count.

Table (1) showed that the fasting blood glucose (FBG) level in young rats was significantly increased in diabetic group, as compared to the control group. The diabetic group supplemented with OLE showed significant decrease in fasting blood glucose.

Table 1: Fasting blood glucose level, Body weight, testis weights, epididymis weight and sperm count in young male albino rats:

Groups / Parameters		Young rats			
		c	**o**	**d**	**d+o**
FBG (mg/dl)	Mean±SD	73± 0.4	80± 0.7	260± 4.9	100±2.8
	Probability		≥ 0.5	≤ 0.001	≤ 0. 01
Body weight (g)	Mean±SD	74± 3.9	55±6.7	42± 4.6	60±2.1
	Probability		≤0.5	≤ 0.01	≤0.5
Testis weight (g)	Mean±SD	0.24±0.03	0.21±0.02	0.20±0.01	0.23±0.01
	Probability		≥ 0.5	≥ 0.5	≥ 0.5
Epididimis weight (g)	Mean±SD	0.08±0.02	0.068±0.02	0.06±0.05	0.07±0.03
	Probability		≤0. 5	≤0. 5	≥ 0.5
Sperm count (10^6/ml)	Mean±SD	74± 2.9	65± 7.7	42± 3.6	67± 12.1
	Probability		≥ 0.5	≤0.01	≥ 0.5

Each value represented the mean and ± standard deviation (SD).The values are considered non-significant at P ≥ 0.5 significant at p≤ 0.5and highly significant at p≤ 0.01 compared to the control.**c**, control, **o**, olive leaves extract, **d**, diabetic and **d+o**, diabetic & olive leaves extract in young.

As shown in Table (1) a significant decrease ($p<0.05$) in the body weight was detected in the mean values of group d. There was a non-significant change in the testis weight of all groups. There was also a significant decrease ($p<0.05$) in the mean values of epididymis weight of group d. The data in Table (1) also showed that a significant decrease ($p<0.05$) in the total count of sperms in the diabetic animals of young rats, as compared to control animals. However, the diabetic group

treated with OLE showed amelioration of these parameters.

The current study sought to determine the effect of OLE on reproductive function of diabetic male rats. The results showed that STZ induced diabetes in the young rats as demonstrated by elevation of the fasting blood glucose level. Similar results were observed in previous work[10,23]. Diabetic rats young, treated with OLE exhibited amelioration of the fasting blood glucose level, which is in agreement with the previous study [31]. It is well documented that the hyperglycemic condition induced different completions, including reproductive dysfunctions[11,32].

The results of the present study revealed a non-significant decrease in the total body weight as well in the testicular and epididymal weights of diabetic rats, which could be attributed to ingesting of the protein instead of the unavailable carbohydrates for energy production[33].This result is in agreement with the previous studies that concluded a decrease in the testicular and body weight of diabetic animals[34]. However, the diabetic rats treated with OLE exhibited an improvement in the body weight and the testicular weight. Further, the present study recognized a remarkable decrease in the sperm count, the earlier research works indicate that administration of STZ could trigger sperm depletion and abnormalities that could be attributed to the overproduction ROS. It was mentioned that, the oxidative stress has a critical role in the pathogenesis of diabetes-induced male reproductive dysfunction[14,34]. Overproductions ofROS lead to testicular oxidative injury, germ cell apoptosis, and sperm count and viability depletion. Also, the spermatozoa membrane, which contains high polyunsaturated fatty acid constituents, is very sensitive to oxidative stress[35]. The observed disturbance in the antioxidant status in the testicular tissues, which demonstrated by elevation of MDA and NO in parallel with decline in the antioxidant enzymes activities and GSH content in this study could lead to depletion in the adenosine tri-phosphate formation and sperm motility[34]. On the other hand, the data indicate that OLE treatment improved the total body weight, testis weight, and sperm count in diabetic animals. Diabetes decreased testicular weight and seminal vesicles, induced male reproductive dysfunctions, decreased serum testosterone levels and lowered semen quality and quantity. It is well-known that diabetes is positively associated with lowered male fertility and increased sexual disturbances.

Effect of OLE on the serum hormonal changes

Table (2) Showed that, the young diabetic rats displayed significant declines ($p<0.05$) in the serum level of FSH, LH and Testosterone as compared to the corresponding normal values. Conversely, the diabetic rats supplemented with OLE showed a significant improvement ($p<0.05$) in the level of these hormones towards normal values. However, the OLE treated group showed non-significant decrease ($p>0.05$) in these parameters.

On the other hand, the results of the current study displayed a significant hormonal decrease in LH, FSH and testosterone levels in diabetic rats. Diabetes is associated with reproductive disorders in men and women[14]. Testicular function is regulated by pituitary hormones. Male sexual dysfunction in STZ-induced diabetic rats results from the alterations of the pituitary–testicular tract axis[36]. The FSH and the LH regulate spermatogenesis and Leydig cell function, respectively[37]. Diabetes exhibited remarkable dimensioned levels of serum FSH and LH, which could be attributed to the physiological disturbance in insulin/glucose levels in the serum[38]. Previous studies have indicated that the neuropathy (nerve damage) and vascular insufficiency resulted from diabetes could be related to sexual dysfunction[39]. Healthy individuals tolerate stable blood glucose levels through basal insulin secretion[40]. But diabetes exhibit considerable increase in the plasma glucose level, accompanied by a significant decrease in plasma insulin level[41,42]. Hyper-glycaemia in diabetes is the main symptom induced by insulin deficiency and/or insulin resistance[11]. The declined insulin levels found in the current study is improved by OLE treatment. It was proposed that OLE is beneficial for reducing the blood glucose concentration, thereby promoting the regeneration of pancreatic islets and increasing insulin release, thus exhibit anti-diabetic effects, via direct scavenging of the lipid peroxides and indirectly enhancing the production of pancreatic antioxidants enzymes[31].

Table 2: Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Testosterone in young male albino rats.

Groups Parameters		Young rats			
		c	**o**	**d**	**d+o**
FSH (ng/ml)	Mean±SD	4.6±0.2	2.4±0.1	3.0± 0.20	4.2± 0.15
	Probability		≤0.01	≤0. 5	≥0.5
LH (ng /ml)	Mean±SD	3.5±0.08	2.2± 0.07	2.6± 0.01	3.1± 0.04
	Probability		≤0.01	≤0.01	≤0. 5
Testosterone (ng/dl)	Mean±SD	0.9± 0.06	0.34±0.02	0.42±0.02	0.65±0.03
	Probability		≤0.01	≤0.01	≤0.05

Each value represented the mean and ± standard deviation (SD).The values are considered non-significant at P ≥ 0.5 significant at p≤ 0.5and highly significant at p≤ 0.01 compared to the control.**c**, control, **o**, olive leaves extract, **d**, diabetic and **d+o**, diabetic & olive leaves extract in young.

Table 3: Antioxidant status and total protein level of the testis tissues of different experimental groups.

Groups Parameters		Young rats			
		c	**o**	**d**	**d+o**
MAD (nmol/g tissue)	Mean ±SD	28±0.4	35±1.3	40±1.6	33.6±0.5
	Probability		≤0.5	≤0.01	≥0.5
NO (µmol/g tissue)	Mean±SD	7.23±0.2	5.6± 0.27	15.4± 0.19	6.23±0.22
	Probability		>0.5	<0.001	>0.5
GSH (mol / g tissue)	Mean±SD	17±1.5	15±0.5	12±0.9	16±1.3
	Probability		≥0.5	≤0.5	≥0.5
GST (U/g tissue)	Mean±SD	6.3±0.3	4.9±0.9	4.4±0.7	5.9±0.6
	Probability		≥0.5	≤0.5	≥0.5
CAT (U/g tissue)	Mean±SD	10±1.3	8.4±0.39	6.3±0.75	8.1±0.49
	Probability		≤0. 5	≤0.01	≥0. 5
GSH-PX (U/g tissue)	Mean±SD	9.5±0.29	9.5±0.19	6.7±0.25	8.2 ±0.15
	Probability		≤0. 5	≤0.01	≥0. 5
SOD (U/g tissue)	Mean±SD	11±0.6	7.3±0.8	4.7±0.3	9±0.6
	Probability		≤0.5	≤0.01	≥0.5
Total Protein (mg/g tissue)	Mean±SD	0.9±0.06	0.7±0.09	0.5±0.07	0.8±0.1
	Probability		≥ 0.5	≤0.05	≥ 0.5

MDA: malondialdehyde, NO: nitric oxide, GSH: reduced glutathione, SOD: super oxide dismutase, Cat: catalase, GSH-Px: glutathione peroxidase, and GST: glutathione-S- transferase. Each value represented the mean and ± standard deviation (SD).The values are considered non-significant at P ≥ 0.5 significant at p≤ 0.5and highly significant at p≤ 0.01 compared to the control.**c**, control, **o**, olive leaves extract, **d**, diabetic and **d+o**, diabetic & olive leaves extract in young.

Effect of OLE on the oxidative stress and antioxidant status in the testes

The data illustrated in Table (3) showed that MDA and NO levels were significantly increased ($p<0.05$), but the GSH content and the antioxidant enzymes, SOD, CAT, GSH-Px, GST activities were significantly ($p<0.05$) declined in the testis tissue of the diabetic group, as compared to the corresponding normal values. However, the diabetic animals supplemented with OLE showed amelioration of these parameters towards normal values. While, the o group showed non-significant decrease in this enzymes, but in case of MAD there was a significant increase ($p<0.05$) in o group.

Under physiological conditions of hyperglycemia and insulin deficiency, testicular cells could be undergoing metabolic adaptations and use alternative substrates, mainly via lactate pathway, which is expected to induce overproduction of reactive oxygen species (ROS) and induce oxidative stress[42]. Oxidative stress induced in diabetes, resulting in the development of testicular dysfunction that could be attributed to the insulin deficiency[38,43]. Another study using STZ showed that homeobox-containing gene (Arx) inactivation in α-cells results in pancreatic islet hypertrophy and increased number of cells that are phenotypically β-cells[44]. They suggested that when α-cells are subjected to Arx inactivation, they undergo transdifferentiation to β-cells. Therefore, strategies aiming at inhibiting the expression of Arx may offer new avenues for diabetes treatment. Over production of free radicals or ROS cause attenuation of the endogenous antioxidant enzymes, induction of inflammatory responses, the decline of the testicular function, and hence lead to apoptosis and cell death[42]. SOD protects the cells against oxidative stress via the dismutation of the highly reactive superoxide radical $O_2^{\bullet-}$ to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2)[45]. After then, CAT catalyzed the removal of H_2O_2 via its reduction to water and O2. GSH-Px removed hydrogen peroxide by converting reduced glutathione into oxidized glutathione[46].Thus, the decrease in the activities of SOD and CAT could be attributed to the feedback inhibition or oxidative inactivation of the enzymes' proteins because of the excess ROS generation. However, GST catalyzes the conjugation of GSH with a wide spectrum of electrophiles and is considered to be an important component of the detoxification system. It also, catalyzes a peroxidative reaction with the production of GSSG. The ability of GST to alter the level of intracellular GSH in the testes in response to generation of ROS has been implicated in protection of cells against free-radicals inducting agents[46,47]. The observed depletion of GSH could be attributed to the excess utilization during the detoxification process demanding the glutathione enzymes system, which evoked enhancement of the lipid peroxidation and inactivate GSH-Px in the testicular tissues[48]. The data of this study revealed alterations in antioxidant defense system established by increase of MDA, NO levels, and decrease in GSH content and the activity of the antioxidant enzymes; SOD, CAT, GSH-Px and GST. The results of the present study are in agreeing with the previous study[49]. However, OLE improved the antioxidant status in the diabetic rats by enhancing the antioxidant enzymes activity and GSH content accompanied by significant decreases in MDA and NO levels in the testicular tissues. It has been demonstrated that oral administration of OLE improved the physiological reproductive status in diabetic rats, which was attributed to its potent antioxidant and estrogenic activities. OLE contains vitamins E which have been reported to enhance the antioxidant status in adult male rats[50].

Further, the oleuropein has both the ability to scavenge nitric oxide and to cause an increase in the inducible nitric oxidesyntheses expression in the cell[51]. The structures of oleuropein is characterized by the presence of two hydroxyl groups, , which are believed to play a critical role in its biologic function .Thus, oleuropein can be considered as effective quenchers of singlet oxygen and related reactive oxygen species . In addition to quenching ROS directly, oleuropein was reported to effectively prevent protein, lipid or DNA from oxidative damage by regulating other cellular antioxidant systems[15]. A number of studies have reported that intake of antioxidants can increase stability of the testicular blood barrier and protect sperm DNA from oxidative stress caused by active free radicals, oleuropein, a phenolic anti-oxidant, can reduce the oxidative stress that results from diabetes[52].

Conclusion

The young diabetic rats treated with olive leaves extract (OLE) showed improvement in the testicular weight, sperm count, serum FSH, LH and testosterone level. Further, OLE administration improved the antioxidant status in the testicular tissues of the young diabetic rats, by improving the superoxide dismutase (SOD), catalase (Cat), glutathione peroxidase (GPX), glutathione-S-Transferase (GST) activities, and glutathione (GSH) content in parallel with decline the malondialdehyde (MDA), and nitric oxide (NO) levels. Consequently, OLE administration could improve the reproductive parameters and testicular tissue antioxidant in young diabetic rats. Therefore, regular administration of low doses of OLE is recommended to decrease the risk of infertility to regulate the different complications of diabetes in men.

REFERENCES

1. **MoudgilD, Khali A.** (2016): The 1st Euro-Mediterranean Workshop: Natural Products in Health and Diseases Asian J. Pharma. Sci., 11(2): 292–296.
2. **Akhu-ZaheyaL,Alkhasawneh M.** Complementary alternative medicine use among a sample of Muslim Jordanian oncology patients. Comp. Therap.in Clinic. Pract.18 (2012), 2012, 121-126.
3. **EskandariH,Jalali A.** Agriculture Landscape in the Holy Quran. Int. J. of Agri. and Crop Sci. (IJACS),5(3), 2013, 232-235.
4. **Nikmoeen J, Akbarian A, Mohammadi M**. Evaluating Therapeutic Properties of Quranic Fruits, and Their Effects on Health Promotion. Quran Med. 3(1), 2014, e11147.
5. **Omar S,** Oleuropein in olive and its pharmacological effects. J. Sci.Pharm.,78(2), 2010,133–154.
6. **Bolzan AD, Bianchi MS. Bruneton J. (1999):**Pharmacognosy, Phytochemistry, Medicinal Plants.2nd ed., Genotoxicity of streptozotocin. Mutat. Res., 512, 2002, 121-134.
7. **Barbaro B, Toietta G, Maggio R , Arciello M, Tarocchi M, Galli A, Balsano C.** Effects of the Olive-Derived Polyphenol Oleuropein on Human Health.Int. J. Mol.Sci., 15(10), 2014,18508-18524.
8. **Andrew J, Jason AS, Kelly J, Andrew J, Philip R.***In* vivo efficacy of acyl CoA: diacylglycerolacyltransferase (DGAT) inhibition in rodent models of postprandial hyperlipidemia. Europe. J. Pharmaco., 2010, 10: 1-5.
9. **Antolin G,Pajares F, Vallecillo M.):**Fibroscan evaluation of liver fibrosis in liver transplantation.Valladolid, Spain, 41**,** 2009, 1044-1046.
10. **Arya A, Al-Obaidi M, Shahid N.** Synergistic effect of OLE and quinic acid by alleviating structural degeneration in the liver, kidney and pancreas tissues of STZ-induced diabetic rats: A mechanistic study. Food and Chem. Toxicol., 71,2014,183–196.
11. **AlamM, Meerza D, Naseem I.** protective effect of quercetin on hyperglycemia, oxidative stress and DNA damage in alloxan induced type 2 diabetic mice. Life Sci.,109**,** 2014, 8–14.
12. **Janero D,Hreniuk D, Sharif H .**Hydroperoxide induced oxidative stress impairs heart muscle cell carbohydrate metabolism. Am. J. Physiol., 266, 1994, C179–88.
13. **Wahl M, Koopman I. and Ammon H.** Oxidative stress causes depolarization and calcium uptake in the rat insulinoma cell RINm5F. Exp. Clin. Endocrinol. Diab.,106, 1998, 173–7.
14. **Ahangarpour A, Oroojan A, Heidari H, Ghaedi E, Nooshabadi M.** Effects of Hydro-Alcoholic Extract of *Rhuscoriaria*(Sumac) Seeds on Reproductive Complications of Nicotinamide-Streptozotocin Induced Type-2 Diabetes in Male Mice. World J. Men's Health, 32(3), 2014, 151-158
15. **FataniAJ, Al-RejaieSS, AbuohashishHM, Al-AssafA, ParmarMY, Ahmed MM**. Lutein Dietary Supplementation Attenuates Streptozotocin-induced testicular damage and oxidative stress in diabetic rats.Altern. Med. 15, 2015, 204.
16. **Cheng-Jun S,Zhen-Jun Y,Qi-Feng T, Zhi-Hong CH.** Effects of sericin on the testicular growth hormone/insulin-like growth factor-1 axis in a rat model of type 2 diabetes Int. J. Clin. Exp. Med., 8(7), 2015, 10411–10419
17. **Samir AMZ,Eman GE, Somaia MA.** Effect of the extract of *Cleome droserifolis* (Samwah) on some physiological parameters in streptozotocin diabetic rats." Al-AzharBull.Sci.,12(2), 2001, 93 -107.

18. **AlirezaeiM,KheradmandA,Heydari R, Tanideh N,NeamatiSH**. Oleuropein protects against ethanol-induced oxidative stress and modulates sperm quality in the rat testis. J. Nutr. Metab.,5(3) **,** 2012, 205-211.
19. **Syed IR, Kshama D, Salma k.** Effect of rosiglitazone on streptozotocin and nicotinamide induced type-2 Diabetes mellitus mediated defect in sperm abnormalities and oxidative defense system in male Waster rats." Acta. Pharma.Scienc., 52, 2010, 121-128.
20. **Singab AN, El-Beshbishy HA , Yonekawa M , Nomura T, Fukai T.** Hypoglycemic effect of Egyptian Morus alba root bark extract: effect on diabetes and lipid peroxidation of streptozotocin-induced diabetic rats." J. Ethnopharmacol. , 100, 2005,333-338.
21. **Pant N, Srivastava S.** Testicular and spermatotoxic effects of quinalphos in rats. J. Appl.Toxicol., 23, 2003, 271–4.
22. **Wells M, Awa O**. New technique for assessing acrosomal characteristics of spermatozoa J. Dairy. Sci.,53,1970, 227.
23. **Satoh K,** Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. J.Clinica. Chimica. Acta., 90**, 1978**, 37-43**.**
24. **Montgomery H,Dymock J. 1961** The determination of nitrite in water. Analyst., 86,1961, 414–416.
25. **Beutler E, Duron O, Kelly MB.**Improved method for determination of blood glutathione. J. Lab. Clin. Med., 61,1963**,** 882-888.
26. **Nishikimi M,Roa NA, Yogi K.**The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Bioph. Res. Common., 46,1972, 849 – 854.
27. **AebiH**. Catalase. In: Methods in Enzymatic Analysis. Bergmeyer HU (ed); Chemic Academic Press; Inc. Verlag. 1984, 2, 673-678.
28. **Paglia D,ValentaineW.**Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70, 1967,158-69.
29. **Habig WH, Pabst MJ, JakobyWB**, Glutathione-S-transferase. J Biol Chem.,249, 1974, 7130–7139.
30. **Lowry OH, Rosebrough NJ, Farr AL, Randall RJ,** Protein measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
31. **Wainstein J , Ganz T, Boaz M , Dayan YB , Dolev E, Kerem Z Madar Z** Olive Leaf Extract as a Hypoglycemic Agent in Both Human Diabetic Subjects and in Rats J. Med. Food, 15 (7), 2012, 1–6.
32. **Salem A, Eassawy M, Ismail A.** Therapeutic effect of quercetin on the complications of nicotinamide-streptozotocin induced type-2 diabetes in male rats. J. Biomed. Pharma. Res., 5(2), 2016, 14-28.
33. **SouvikR, Noorjaman R, Faiqa A, Satyajit M, Santanu S.**Naringenin attenuates testicular damage, germ cell death and oxidative stress in streptozotocin induced diabetic rats: naringenin prevents diabetic rat testicular damage. J. Appl. Biomed., 11, 2013, 195-208.
34. **El-ShafeyaM,Abd-Allaha G, Mohamadina A, Harisaa G, Marieea A.** Quercetin protects against acetaminophen-induced hepatorenal toxicity byreducing reactive oxygen and nitrogen species. Pathophysio., 22, 2015,49–55.
35. **Aitken R, Roma S.** Antioxidant systems and oxidative stress in the testes. Adv.Exp. Med. Biol. , 636,2008, 154 -71.
36. **Horn T, Jung J, ChristoffersenP.**Alcoholic liver injury: early changes of the Disse space in acinar zone. Liver, 6, 1985,301-310.
37. **Ward D, Bousfield G, Moore K, InCupps P.** Reproduction in domestic animals. San Diego, Calif: Academic Press,1991, 25-67.
38. **Adewole S, Caxton-Martins E, Salako A, Doherty O,Naicker T.** Effects of oxidative stress induced by streptozotocin on the morphology and trace minerals of the testes of diabetic Wistar rats. Pharmacology, 2**,** 2007, 478-497.
39. **Holvoet P.** The relationship between oxidized LDL and other cardiovascular risk factors and subclinical CVD in different ethnic groups: The Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis,194(1), 2006,245-252.
40. **Brezar V, Carel J, BoitardC ,Mallone R.** Beyond the hormone: insulin as an autoimmunetarget in type 1 diabetes. Endocr. Rev., 32, 2011**,** 623–669.

41. **Friedman K, Young D.** "Effects of Disease on Clinical Laboratory Tests". 3rd ed. Washington: AACC Press **1997**.
42. **Alves M, Martins A, Rato L , Moreira P , Socorro S , Oliveira P.** Molecular mechanisms beyond glucose transport in diabetes-related male infertility. Biochim. Biophy. Acta. Molec. Basis of Disease.1832, 2013, 626-635.
43. **Sexton W,JarowJ**. Effect of diabetes mellitus upon male reproductive function. J. Urology., 49, 1997, 508 – 13.
44. **Courtney M, Gjernes E, and Druelle N.** The inactivation of Arx in pancreatic α-cells triggers their neogenesis and conversion into functional β-like cells. P.L.O. Genetics, 9, 2013,1003934-1003944.
45. **White C, Brock T, Chang L, Crapo J, Briscoe P, Ku D, Bradley W,Gianturco S, Gore J, Freeman B.** Superoxide and peroxynitrite in atherosclerosis. Proc. Natl. Acad. Sci.,91,1994, 1044–1048.
46. **TewK, and RonaiZ.** GST function in drug and stress response. Drug Resist. Updat., 2, 1999, 143-147.
47. **Tsuchida S, Sato K.** Glutathione transferases and cancer. Crit. Rev. Biochem. Mol. Biol., 27,1992,337-384.
48. **McCord J, Omar B.** Sources of free radicals. Antioxidants: Chemical; Physiological; Nutritional and Toxicological Aspects; Princeton Scientific Publishing Co. Inc; New Jersey, 1993, pp. 23–37.
49. **Arya A, Cheah S, Looi C, Taha H, Mustafa M, Mohd M.** The methanolic fraction of *Centratherumanthelminticum*seed down-regulates pro-inflammatory cytokines, oxidative stress, and hyperglycemia in STZ nicotinamide- induced type 2 diabetic rats. Food Chem. Toxicol.,50**,** 2012,4209–4220.
50. **JohnsonM .**Hyperlipidemia disorders in dogs. Compendium on Continuing Education for the Practicing Veterinarian, 27,2005, 361-364.
51. **Andreadou I, IliodromitisEK, MikrosE, SkaltsounisA, KremastinosDTh.** The Use of Oleuropein on Myocardium, In:Olives and Olive Oil in Health and Disease Prevention. 2010, 1313–1320.
52. **El-KholyThA, Al-AbbadiHA, QahwajiD, Al-GhamdiAK, ShelatVG,SobhyHM, Abu HilalM .** Ameliorating effect of olive oil on fertility of male rats fed on genetically modified soya bean. J.FoodNutr. Res., 59, 2015, 27758.