

# Olive Leaves Extract Effects on Sperm Quality Following Experimentally-Induced Diabetes in Rats

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## Abstract:

**BACKGROUND:** The experimental model of streptozotocine (STZ)-induced diabetes explains changes in the male reproductive system as part of the disease.

**OBJECTIVES:** The aim of the present study was to evaluate the olive leaf extract effects on STZ-induced diabetes and to examine its modulatory effects on sperm quality.

**METHODS:** Twenty adult male Sprague–Dawley rats were divided into four equal groups: the first group served as untreated control. Groups 2, 3 and 4 of rats were injected STZ (65 mg/kg). The animals which exhibited blood glucose levels higher than 250 mg/dl by days 4-6, were considered as diabetic rats. Groups 3 and 4 received olive leaf extract (100 and 150 mg/kg, orally) and vehicle to the control and diabetic rats (group 2) for 10 consecutive days.

**RESULTS:** Glycated haemoglobin percentage (%HbA1c) as a diabetic index significantly decreased in the animals that ingested 150 mg/kg of the extract compared to the diabetic group ( $P<0.001$ ). Olive leaf extract (150 mg/kg) could improve sperm quality of the treated rats against STZ deleterious effects in the diabetic rats, however, total sperm motility was significantly higher in the diabetic rats ( $P<0.001$ ). Cholesterol concentration significantly increased in the diabetic and the extract-treated groups compared to the controls ( $P<0.01$ ), and triglyceride level significantly decreased in the extract-treated animals (150 mg/kg) compared to the diabetic and the 100 mg/kg extract groups ( $P<0.01$ ).

**CONCLUSIONS:** Our data suggest that olive leaf extract possesses beneficial antidiabetic effects on STZ-induced diabetes in rats and may be a good candidate to reduce diabetes complications in men.

## Keywords:

Diabetes, Olive extract, Rat, Streptozotocine, Testis

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## How to Cite This Article

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## Introduction

Diabetes is a chronic and metabolic disease characterized by hyperglycemia that can result from defects in insulin secretion and/or insulin action (Temidayo and Stefan 2018; Alves et al., 2013; Sookhthezari et al., 2015). Moreover, there is a severe alteration in carbohydrate, lipid and protein metabolism (Alves et al., 2013) that causes several systemic complications such as nephropathy, retinopathy and male infertility (Temidayo and Stefan 2018; Vignera et al., 2012; Alves et al., 2013). Diabetes mellitus (DM) represents one of the greatest threats to modern global health. Its incidence is rising rapidly, and according to a report by the World Health Organization (WHO) by 2025 it will affect over 300 million people (American Diabetes Association 2016; Agbaje et al., 2007). A high prevalence (51%) of subfertility was reported among patients with DM (Vignera et al., 2012; Alves et al., 2013). This led to the perception that DM is responsible for inducing subtle but crucial changes in sperm quality and function (Vignera et al., 2012; Alves et al., 2013). It is well known that in men DM also is responsible for important sexual disorders such as decreased libido and impotence (Alves et al., 2013). Impotence, infertility and retrograde ejaculation have been described in diabetic men, but the etiology remains unclear. However, it is believed that pelvic autonomic neuropathy contributes to impotence and retrograde ejaculation in the male (Kerner and Brückel 2014; Turner and Lysiak 2008). DM may affect male reproductive function at multiple levels as a result of its effects on the endocrine control of spermatogenesis, spermatogenesis itself or by impairing penile erection and ejacu-

lation (Mangoli et al., 2013; Sookhthezari et al., 2015). In this regard, many studies have documented abnormalities in testicular function and spermatogenesis in diabetic animals (Vignera et al., 2012). Therefore, diabetes is a well-recognized cause of male sexual dysfunction, which in itself may contribute to subfertility.

Streptozotocine (STZ), a monofunctional nitrosourea derivative isolated from *Streptomyces achromogenes*, is a potent alkylating agent known to induce diabetes in animal models (Navarro-Casado et al., 2010). Several lines of evidence indicate that STZ-induced free radicals are involved in the damage of spermatogenesis and male reproductive function (Mangoli et al., 2013; Sookhthezari et al., 2015). It is generally accepted that streptozotocine-mediated diabetes can produce oxidative stress and induces testicular damage (Navarro-Casado et al., 2010). However, there are apparently contradictory results concerning sperm motility and other male reproductive parameters when studying male diabetic rats (Alves et al., 2013). The fatty acids are an essential requirement for the male germ cells to maintain sperm functions (Turner and Lysiak 2008). Therefore, oxidative stress in a tissue like testis, with high rates of metabolism and cell turn over, can be especially damaging. In addition, testicular cell membranes are rich in polyunsaturated fatty acids and thus susceptible to oxidative injury, which leads the spermatozoa to infertility due to defective sperm function (Alirezaei et al., 2012b). Olives, olive tree leaves and olive oil, in the Mediterranean area contain large amounts of oleuropein. This phenolic compound is responsible for their bitter taste and pungent aroma (Alirezaei et al., 2012a;

Al-Azzawie and Alhamdani 2006) and has been recognized as a powerful hypotensive, hypoglycemic and antioxidant agent (Alirezaei et al., 2012a; Tuck et al., 2001). In this regard, recent studies in several animal models have shown that olive leaf extract is a natural antioxidant source, which is naturally phenolic and completely non-toxic (Alirezaei et al., 2012a; Hamdi and Castellon 2005; Alirezaei et al., 2012b). Based on the previous reports, oleuropein and phenolic compounds of olive leaf extract scavenge superoxide anions and hydroxyl radicals and inhibit the respiratory burst of neutrophils and hypochlorous acid-derived radicals, subsequently inhibiting oxidative stress (Alirezaei et al., 2012a).

As mentioned above, there is an urgent need for more studies focused on the antioxidant agents in a diabetic model of rats. Therefore, the aim of the present study was to evaluate olive leaf extract effects on conventional parameters of sperm fertility following STZ-induced diabetes in rats. We also investigated how cholesterol and triglyceride levels as indices of lipid metabolism varied in a short-term period of hyperglycemia in diabetic rats.

## Materials and Methods

The olive leaves extract was isolated from *Olea europaea* leaves according to the method described previously (Alirezaei et al., 2012b). Streptozotocine (STZ) was purchased from Sigma Chemical Company (St Louis, Missouri, USA). HbA1c ion-exchange chromatography kit was obtained by BioSystem S.A. Company (Costa Brava, Barcelona, Spain) and cholesterol and triglycerides kits were supplied by Zeist Chemistry Company (Tehran, Iran). Twenty adult male Sprague–Dawley rats (weighing

220–250 gram) were housed in temperature-controlled conditions under a 12:12-h light/dark photocycle with food and tap water supplied ad libitum. All rats were treated humanely and in compliance with the recommendations of Animal Care Committee for the Lorestan University (Khorramabad, Iran) with approval number: LU.ECRA. 2017.3. All of the experimental procedures were carried out between 08:00 -11:00 am, weight gain and food consumption were also determined at weekly intervals.

**Experimental design:** The rats were divided into four equal groups (control, diabetic, olive leaf extract 100 mg/kg and olive leaf extract 150 mg/kg) and treated in the following order: control group received 0.25 ml normal saline subcutaneously at the first day. Diabetic, and both olive leaf extract groups were injected subcutaneous with STZ 65 mg/kg (dissolved in 0.1 M citrate buffer, pH 5) at the first day. All animals were housed in standard cages in groups of 5 rats. Four days after STZ injection, animal glucose metabolism was measured using enzymatic oxidation method by a glucometer (ACCU Check, Germany). Those animals that had blood glucose levels higher than 250 mg/dl were considered diabetic (Navarro-Casado et al., 2010); they also manifested typical polyuria, polyphagia, and polydipsia. All of the STZ-injected animals that exhibited blood glucose levels higher than 250 mg/dl were considered diabetic. The second metabolic evaluation was performed at day 6 to confirm the diabetic status and to measure food-water intake. Two different doses of olive leaf extract were administered (100 and 150 mg/kg body weight) after diabetic confirmation in two groups and vehicle (normal saline) to diabetic group orally by gavage in a 10

consecutive day period. Dose of STZ was determined according to the previous studies (Alves et al., 2013; Navarro-Casado et al., 2010) and olive leaf extract on the average concentration of its oleuropein to obtain 10 and 15 mg/kg of oleuropein in each dose (Alirezaei et al., 2012b). One day after the last gavage, the rats were sacrificed using diethyl ether anesthesia (Dagenham, UK) by cardiac puncture to assess whole blood and plasma by heparinized syringe. Immediately after rat killing, the right testis was removed and carefully cleaned of fat and adhering, and the epididymis in all groups was separated for sperm quality evaluations.

**HbA1c measurement and plasma analysis**

Glycated haemoglobin was measured with BioSystems (Barcelona, Spain) HbA1c detection kit according to the manufacturer's instructions using ion-exchange chromatography approach, spectrophotometrically (S2000 UV model; WPA, Cambridge, UK), as described previously (Sookhthezari et al., 2015). HbA1c concentration was calculated on the basis percentage of total haemoglobin as %HbA1c. Cholesterol and triglyceride levels were also measured according to the manufacturer's instruction kits (Zeist Chemistry Company, Tehran, Iran), spectrophotometrically and the results were expressed as mg/dl of plasma (mg/dl).

**Sperm evaluation:** Rat spermatozoa were obtained by the method described previously (Cancel et al., 2000). In brief, 5 mm of right cauda epididymis was minced in 2ml of physiological saline and incubated at 37 °C for 45 min to allow dispersion of spermatozoa. The obtained spermatozoa from the four groups were assessed for total sperm motility (TSM), forward progressive movement (FPM), plasma membrane integrity by hypoosmotic swelling test (HOS),

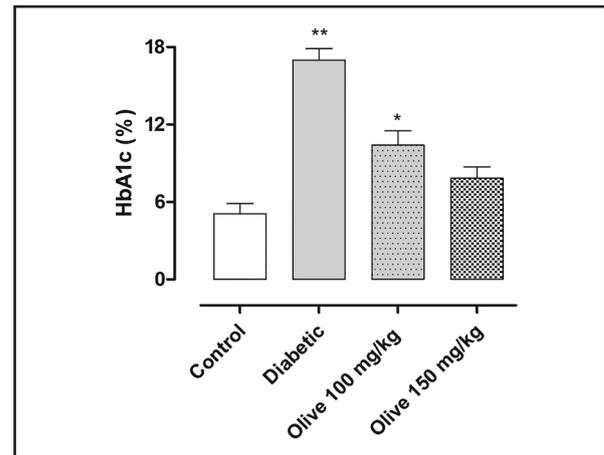
and concentration, The TSM (cells showing any kind of movement) and FPM percentage (of the motile spermatozoa showing progressive movement) were assessed according to the method as described previously (Sonmez et al., 2005). The fluid obtained from cauda epididymis was diluted to 2 ml with PBS and an aliquot of this suspension was placed on the microscope slide covered with a cover slip and examined visually under a light microscope (Olympus, CX-31, Philippine) at magnification of  $\times 400$ . Motility estimations were performed from four different fields in each sample and the mean of the four estimations was used as the final motility score. Samples for motility evaluation were kept at 37 °C (Kheradmand et al., 2013). To evaluate the plasma membrane integrity, hypoosmotic swelling (HOS) test was applied. Assessment of functional integrity of sperm membrane was determined by HOS-water test according to the method as described previously (Sliwa and Macura 2005, Kheradmand et al., 2013). In short, 10  $\mu$ l of sperm was added into 0.4 ml of distilled water and incubated for 5 min at 37 °C. The swelling reaction was measured by counting of spermatozoa with curled tail using a light microscope at magnification of  $\times 400$ . The concentration of spermatozoa was determined after adding of 50  $\mu$ l of sperm into the 1 ml of formalin-saline to achieve the dilution rate of 1:20. Approximately 10  $\mu$ l of the diluted sperm suspension was transferred to each counting chamber of the haemocytometer and the total number of spermatozoa per ml was counted using light microscope (Kheradmand et al., 2009; Kheradmand et al., 2013). All of the above examinations were performed by the same person with counting of at least 200 spermatozoa.

**Statistical analysis:** All results are presented as mean  $\pm$  S.E.M. All data were tested for normality followed by Levene's static test for homogeneity of variances. When the variances were homogenous, data were compared by One-way analysis of variance (ANOVA) with Tukey's post hoc analysis. A calculated p value of less than 0.01 was considered to be statistically significant. Statistical analysis was performed using the statistical package SPSS version 19 (SPSS Inc., Chicago, IL, USA).

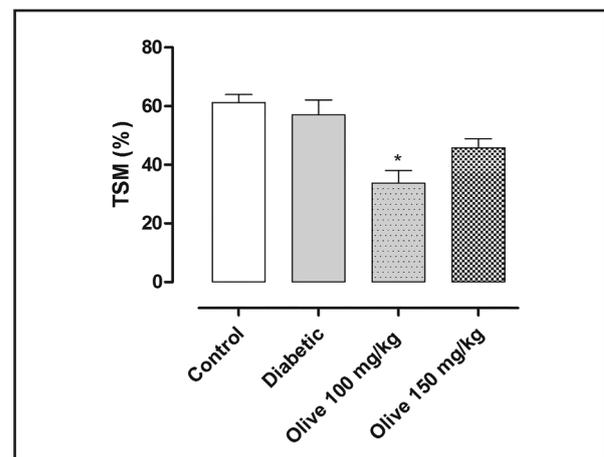
## Results

Regarding diabetes confirmation in the diabetic and olive leaf extract-treated groups (on blood sugar, Data not shown), HbA1c results were indicated in Fig. 1. Administration of STZ induced significant increase of HbA1c in diabetic group when compared to the control and both olive leaf extract-treated (100 and 150 mg/kg) groups ( $P < 0.001$ ). However, this parameter increased significantly in olive leaf extract (100 mg/kg) in comparison with control group ( $P < 0.01$ , Fig. 1).

The mean values ( $\pm$  SEM) of the total sperm motility (TSM), forward progressive movement (FPM), plasma membrane integrity (HOS test) percentage and sperm concentration of the four groups of rats are presented in Figs. 2-5. Interestingly, total sperm motility was significantly higher in the diabetic group compared to the olive leaf extract 100 mg/kg treated rats ( $P < 0.01$ ). Forward progressive movement (FPM) and sperm concentration parameters (Fig. 3 and 5) in olive leaf extract 150 mg/kg treated rats were significantly higher than those of the diabetic group ( $P < 0.01$ ), however these enhancements were not statistically significant for olive leaf extract 100 mg/kg treated



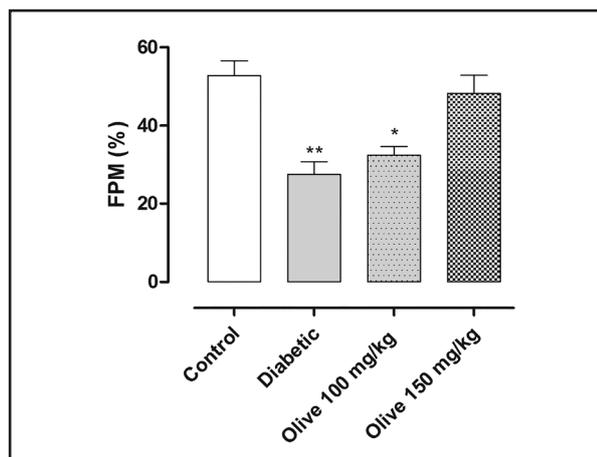
**Figure 1.** Comparison of glycosylated haemoglobin percentage (%HbA1c) among the control and treated groups. Values represent mean  $\pm$  S.E.M. of HbA1c. \*\* indicates the statistical difference among diabetic group with control and both olive leaf extract treated groups ( $P < 0.001$ ) and \* demonstrates the statistical difference between controls with olive leaf extract (100 mg/kg) treated group ( $P < 0.01$ ).



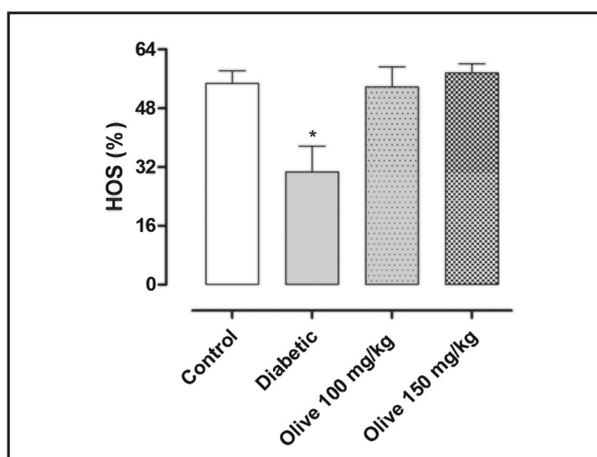
**Figure 2.** Comparison of total sperm motility percentage (%TSM) among the control and treated groups. Values represent mean  $\pm$  S.E.M. of TSM. \* indicates the statistical difference among olive leaf extract 100 mg/kg group with control and diabetic groups ( $P < 0.01$ ).

rats ( $P > 0.05$ ). HOS test was significantly lower in the diabetic group compared to the control and olive leaf extract 150 mg/kg groups ( $P < 0.01$ ). In contrast, when olive leaf extract 100 and 150 mg/kg was administered in diabetic rats, it could increase the level of this parameter near to that of the control group.

Regarding lipid metabolism, adminis-



**Figure 3.** Comparison of forward progressive movement percentage (%FPM) among the control and treated groups. Values represent mean  $\pm$  S.E.M. of FPM (%). \*\* indicates the statistical difference among diabetic group with control and olive leaf extract (150 mg/kg) treated rats ( $P < 0.001$ ) and \* demonstrates the statistical difference among olive leaf extract (100 mg/kg) treated group with controls and olive leaf extract (150 mg/kg) treated group ( $P < 0.01$ ).



**Figure 4.** Comparison of plasma membrane integrity percentage (%HOS) among the control and treated groups. Values represent mean  $\pm$  S.E.M. of HOS (%). \* indicates the statistical difference among diabetic group with control and olive leaf extract (150 mg/kg) treated rats ( $P < 0.01$ ).

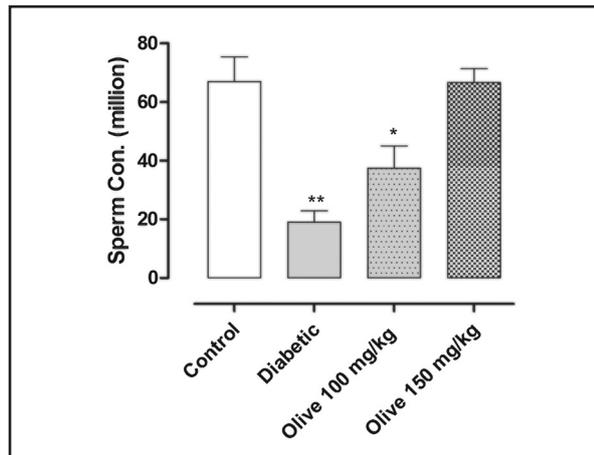
tration of rats with STZ significantly increased cholesterol and triglyceride levels in diabetic group when compared to the controls ( $P < 0.001$ ). Administration of olive leaf extract in dose of 150 mg/kg could prevent increase of triglyceride concentration ( $P < 0.01$ ), while it was unable to suppress cholesterol elevation when compared

to controls ( $P > 0.05$ ). Indeed, cholesterol concentrations in diabetic and both olive leaf extract-treated groups were remarkably higher compared to the control rats ( $P < 0.01$ ) (Figs. 6 and 7).

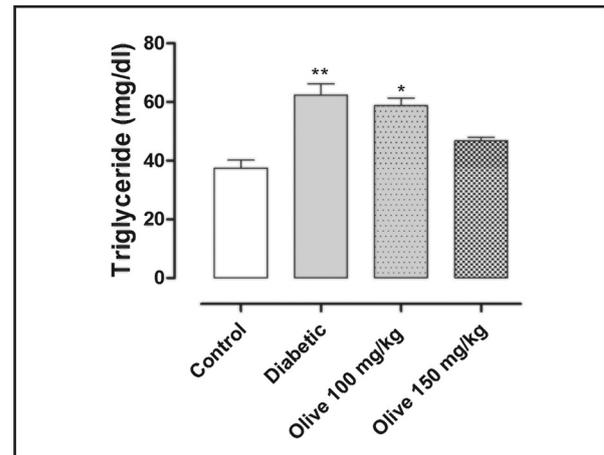
## Discussion

The purpose of the present study was to evaluate the effects of olive leaf extract in a short-term streptozotocine-induced hyperglycaemia on the sperm quality parameters including total motility, forward progressive movement, plasma membrane integrity and concentration of cauda epididymal sperm. We found higher serum glucose levels in the diabetic and both olive leaf extract-treated groups because the hyperglycemia was confirmed in these animals and diabetic animals showed further elevation of HbA1c, another characteristic sign of the disease (George et al., 2013). The results of the present study demonstrated that the diabetic state results in decreased sperm quality and these alterations were prevented by olive leaf extract with oral dosage of 150 mg/kg for 10 consecutive days. To the best of our knowledge, this study is the first in vivo experiment that investigated the effect of olive leaf extract on rat sperm kinematic parameters in an animal diabetic model.

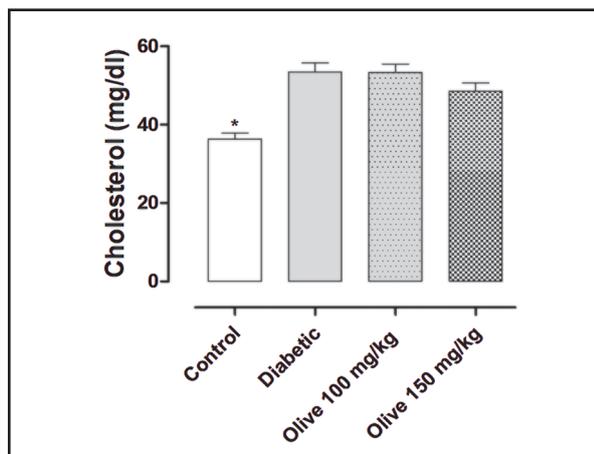
The rising incidence of DM worldwide will inevitably result in an increased prevalence in men of reproductive age (Kerner and Brückel 2014; Sookhthezari et al., 2015). Infertility is already a major health problem in both the developed and developing world with up to one in six couples requiring specialist investigation or treatment in order to conceive (Kerner and Brückel 2014; Alves et al., 2013; Mangoli et al., 2013). The three parameters, glucose, HbA1c and insulin play fundamental roles in the pathogenesis



**Figure 5.** Comparison of sperm concentration (million) among the control and treated groups. Values represent mean  $\pm$  S.E.M. of sperm concentration (million). \*\* indicates the statistical difference among diabetic group with control and olive leaf extract (150 mg/kg) treated rats ( $P < 0.001$ ) and \* demonstrates the statistical difference among olive leaf extract (100 mg/kg) treated group with controls and olive leaf extract (150 mg/kg) treated group ( $P < 0.01$ ).



**Figure 6.** Comparison of triglyceride concentration (Triglyceride mg/dl) among the control and treated groups. Values represent mean  $\pm$  S.E.M. of triglyceride (mg/dl). \*\* indicates the statistical difference among diabetic group with control and olive leaf extract (150 mg/kg) treated groups ( $P < 0.001$ ) and \* demonstrates the statistical difference among olive leaf extract (100 mg/kg) treated rats with controls and olive leaf extract (150 mg/kg) treated group ( $P < 0.01$ ).



**Figure 7.** Comparison of cholesterol concentration (Cholesterol mg/dl) among the control and treated groups. Values represent mean  $\pm$  S.E.M. of cholesterol (mg/dl). \* indicates the statistical difference among controls with diabetic and both olive leaf extract treated groups ( $P < 0.01$ ).

of diabetes depending on their levels in circulation (George et al., 2013). Glucose toxicity which could be direct or indirect may be as a result of direct interaction with proteins and membranes or indirect as a result of the production of reactive sugars acting on membranes and enhancing diabetic complications. Increased glucose concentration

as evidenced in hyperglycemia is known to be a major predisposing factor in production of reactive glucose by-products, in particular  $\alpha$ -deoxyglycosone and glyoxal which is more than 10,000x more chemically reactive than glucose (Aydin 2010; George et al., 2013). The use of glycated hemoglobin as a screening test for diabetes mellitus has become relevant. Its advantage has vitiated the use of other tests like oral glucose tolerance (OGTT), 2-hours postprandial, random or fasting blood glucose (George et al., 2012). HbA1c provides an accurate and reliable method to assess the glycemic control in patients with diabetes (George et al., 2013). Measurement of glycated hemoglobin in patients with diabetes is accepted as a standard for assessment of recent glycemic control and is a critical element in clinical practice (American Diabetes Association 2016; George et al., 2012). Diabetes studies using animal models of STZ-induced DM have demonstrated an elevation in %HbA1c

(George et al., 2013). In assessing the relationship between %HbA1c and diabetes, we observed a positive linear correlation in the previous report (Sookhthezari et al., 2015). In the current study, there was also a positive relationship between diabetic state and %HbA1c in diabetic group. There was however a slight elevation of HbA1c after treatment with olive leaf extracts. The results suggest a greater amount of the extract in treated rats with dosage of 150 mg/kg reduced %HbA1c in comparison with the diabetic rats. The effects of flavonoids, quercetin and ferulic acid on pancreatic  $\beta$ -cells which leads to their proliferation and secretion of more insulin have been previously reported (Ceriello 2006; George et al., 2013). The previous study also suggested  $\beta$ -cells recovery as the mechanism by which hyperglycemia caused by streptozotocine reduces glucose (George et al., 2013). In this regard, olive leaf extract is a phenolic compound which has been shown to possess diverse healing properties for its vasodilatory, hypotensive, anti-inflammatory, anti-rheumatic, diuretic, anti-atherogenic, antipyretic and antioxidant effects (Alirezaei et al., 2012a; Al-Azzawie and Alhamdani 2006). It seems that many of these pharmacologic features of the extract are due to its potent antioxidant element, oleuropein (Alirezaei et al., 2012a). In the present study, oleuropein acted as a natural suppressor of oxidative stress which resulted in decreased HbA1c. It seems that olive leaf extract enhanced insulin secretion and mimicked the effect of insulin on glucose metabolism, although we did not detect insulin level in the blood circulation. This dual pancreatic and extra pancreatic action of oleuropein would prove to be an important advance on existing therapies used to

manage diabetes mellitus.

As discussed earlier, conventional semen analysis remains core to the evaluation of male fertility in the clinical setting (Kheradmand et al., 2013; Alirezaei et al., 2012b). Data provided by this study indicate that diabetes affects mainly the epididymal reserve of sperm and promotes a negative effect on FPM, HOS with increase in total motility of sperms. Although we have observed a significant elevation in total sperm motility in diabetic group, it still remains unexplained. The significant total sperm motility in diabetic group was seen in agreement with our result in diabetic mice (Sookhthezari et al., 2015). It is our contention that the significant differences lie at a 'molecular' and not a 'cellular' level. The differences could be one; due to the spermatozoa being stored in extra-testicular ducts in an immotile state and their motility activated by dilution once released from the ducts (Navarro-Casado et al., 2010). In this regard, a previous study found only semen volume and total sperm output to be significantly lower, while sperm total motility was higher in diabetic mice (Mangoli et al., 2013). Another reason is particularly due to the presence of the blood–testis barrier (BTB). This barrier not only physically divides the seminiferous epithelium in two compartments but also is responsible for the maintenance of different levels of substances and metabolites between rete testis fluid and the lymph or plasma (Alves et al., 2013). It is well known that Sertoli cells (SCs) have such an important role on the male reproductive system (Sharpe et al., 2003). These SCs are responsible for the conversion of glucose, a non-metabolized substrate, by developing germ cells in lactate which is the preferential substrate for those cells (Alves et al., 2013). As

previously mentioned, the epididymis was ruptured to provide semen in our laboratory, at this time SCs had the ability to produce greater lactate from stored glucose as a fuel for released spermatids (Sookhthezari et al., 2015). Therefore, higher sperm motility was seen in diabetic rats in comparison with other groups. However, FPM, HOS and concentration of sperm were significantly lower in diabetic rats when compared to controls and olive leaf extract 150 mg/kg group.

Oxidative stress is recognized to be an important factor in the pathogenesis of many of the chronic complications of diabetes. Indeed, oxidative damage in the diabetic vasculature is an important stimulus for the initiation of mechanisms resulting in sperm infertility (Kheradmand et al., 2013). In the present study, we hypothesized that use of olive leaf extract is able to trap free radicals and prevent oxidative stress upon our previous work (Alirezaei et al., 2012a; Alirezaei et al., 2012b). Herein, it is possible that oleuropein attenuates STZ-induced oxidative stress by two pathways; First, by rapid conversion of  $H_2O_2$  to  $H_2O$  and preventing  $H_2O_2$  accumulation and second, by quenching the hydroxyl radicals through which trapping of  $HO^\circ$  leads to oxidative breakdown of the phenolic compounds. Indeed, both olive leaf extract treated groups have been shown to be HOS test positively rather than diabetic group. Therefore, we concluded that oleuropein protects the membrane of testicular cells against diabetes-induced oxidative damage and appears to be a good candidate in the prevention of diabetes-induced injuries in the testis. Oxidative stress is one of the factors associated with decline in fertility of spermatozoa (Kheradmand et al., 2013; Turner and Lysiak 2008; Alirezaei et al., 2012). The sperm plasma membrane

contains a high amount of unsaturated fatty acids and therefore is particularly susceptible to peroxidative damages with subsequent loss of membrane integrity, impaired cell function and decreased motility of spermatozoa (Kheradmand et al., 2013). The results of our study showed that oleuropein ameliorated the plasma membrane integrity (PMI) of spermatozoa and this enhancement is possibly due to the antioxidant effects of oleuropein which caused higher HOS score in the spermatozoa. These findings confirm a fact that oxidative stress is a consistent feature of testicular physiology (Kheradmand et al., 2013; Turner and Lysiak 2008). Our data are in accordance with previously described detrimental effects of STZ on sperm characteristics in the treated rats.

Regarding glucose metabolism in the testes under glucose deprivation and/or insulin deregulation, testicular cells are expected to suffer metabolic adaptations and use alternative substrates (Alves et al., 2013). In fact, the role of these alternative substrates is underestimated when analyzing some of the available data. SCs have an enormous metabolic plasticity and can use several metabolic substrates such as palmitate and ketone bodies as well as glutamine, alanine, leucine, glycine and valine (Kaiser et al., 2005; Alves et al., 2013). Besides, these cells can produce ATP via the  $\beta$ -oxidation of free fatty acids or even through recycled lipids from apoptotic spermatogenic cells and residual bodies (Xiong et al., 2009; Alves et al., 2013). DM is known to induce severe alterations in lipid metabolism and studies in mice with inactivated genes of lipid metabolism proved that lipid metabolism is essential for a normal spermatogenesis (La Vignera et al., 2009; Alves et al., 2013). Herein, elevations in the plas-

ma levels of cholesterol and triglyceride are thought to be important steps both in the alteration of a variety of glucose metabolism and in the initiation of lipid metabolism changes (Alirezaei et al., 2012a). As results indicated, the plasma levels of cholesterol and triglyceride were higher in diabetic rats, but were decreased by the olive leaf extract with dosage of 150 mg/kg. The above results suggest that hypercholesterolemia and triglyceride elevation seen in diabetes may be suppressed with olive leaf extract.

**Conclusion:** The data we have presented herein provide compelling evidence that the reduction in fertility potential after a short period of hyperglycaemia is likely to be the result of alterations in sperm quality and deleterious effects of oxidative stress in testis of rats, Indeed we did this experiment during a part of spermatogenesis cycle to detect short-term effects of oxidative stress on cauda epididymis but not on spermatogenesis. Therefore, restoration of HbA1c, sperm quality and lipid metabolism changes following treatment of rats with the extract may be attributable to antioxidant properties of oleuropein as a good candidate to reduce diabetes complications in men.

### Acknowledgments

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### Conflicts of interest

The author declared no conflict of interest.

### References

- Agbaje, I.M., Rogers, D.A., McVicar, C.M., McClure, N., Atkinson, A.B., Mallidis, C., Lewis, S.E.M. (2007) Insulin dependent diabetes mellitus: implications for male reproductive function. *Hum Reprod.* 22(7): 1871-1877.
- Al-Azzawie, H.F., Alhamdani, M.S.S. (2006) Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. *Life Sci.* 78(12): 1371-1377.
- Alirezaei, M., Dezfoulian, O., Kheradmand, A., Neamati, SH., Khonsari A., Pirzadeh, A. (2012a) Hepatoprotective effects of purified oleuropein from olive leaf extract against ethanol-induced damages in the rat. *Iran J Vet Res.* 13(3): 218-226.
- Alirezaei, M., Kheradmand, A., Heydari, R., Tanideh, N., Neamati, S., Rashidipour, M. (2012b) Oleuropein protects against ethanol-induced oxidative stress and modulates sperm quality in the rat testis. *Mediterr J Nutr Metab.* 5(3): 205-211.
- Alves, M.G., Martins, A.D., Rato, L., Moreira, P.I., Socorro, S., Oliveira, P.F. (2013) Molecular mechanisms beyond glucose transport in diabetes-related male infertility. *BBA-Mol Bas Dis.* 1832(5): 626-635.
- American Diabetes A (2016) 2. Classification and diagnosis of diabetes. *Diabetes care* 39(Supplement 1): S13-S22.
- Aydin S. (2010) The presence of the peptides apelin, ghrelin and nesfatin-1 in the human breast milk, and the lowering of their levels in patients with gestational diabetes mellitus. *Peptides.* 31(12): 2236-2240.
- Cancel, A.M., Lobdell, D., Mendola, P., Perreault, S.D. (2000) Objective evaluation of hyperactivated motility in rat spermatozoa using computer-assisted sperm analysis. *Hum Reprod.* 15(6): 1322-27.
- Ceriello, P.A. (2006) Oxidative stress and diabetes-associated complications. *Endocr Pract*

- 12(Supplement 1): 60-62.
- George, G.E., Uwakwe, A.A., Ibeh, G.O. (2012) Relationship of glycated haemoglobin (HbA1c) and glucose in streptozotocine induced Wistar rats is determined by linear regression. *Asian J Med Sci.* 3(3): 1-5.
- George, G.S., Uwakwe, A.A., Ibeh, G.O. (2013) Glycated haemoglobin, glucose and insulin levels in diabetic treated rats. *Can J Pure Appl Sci.* 7(1): 2223-2226.
- Hamdi, H.K., Castellon, R. (2005) Oleuropein, a non-toxic olive iridoid, is an anti-tumor agent and cytoskeleton disruptor. *BBRC.* 334(3): 769-778.
- Kaiser, Gr.R.R.F., Monteiro, S.C., Gelain, D.P., Souza, L.F., Perry, M.L.S., Bernard, E.A. (2005) Metabolism of amino acids by cultured rat Sertoli cells. *Metabolism.* 54(4): 515-521.
- Kerner, W., Brückel, J. (2014) Definition, classification and diagnosis of diabetes mellitus. *Exp Clin Endocrinol Dia.* 122(07): 384-386.
- Kheradmand, A., Alirezaei, M., Dezfoulian, O. (2013) Cadmium-induced oxidative stress in the rat testes: protective effects of betaine. *Int J Pept Res Therap.* 19(4): 337-344.
- Kheradmand, A., Taati, M., Babaei, H. (2009) The effects of chronic administration of ghrelin on rat sperm quality and membrane integrity. *Anim Biol.* 59(2): 159-168.
- La Vignera, S., Calogero, A.E., Condorelli, R., Lanzafame, F., Giammusso, B., Vicari, E. (2009) Andrological characterization of the patient with diabetes mellitus. *Min Endocrinol.* 34: 1-9.
- Mangoli, E., Talebi, A.R., Anvari, M., Pourehtezari, M. (2013) Effects of experimentally-induced diabetes on sperm parameters and chromatin quality in mice. *Int J Reprod Biomed.* 11(1): 53-60.
- Navarro, Casado, L., Juncos, Tobarra, M.A., Chaffer, Rudilla, M., Onzono, L.A.I., Blazquez, Cabrera, J.A., Miralles, Garcia, J.M. (2010) Effect of experimental diabetes and STZ on male fertility capacity. Study in rats. *J Androl.* 31(6): 584-592.
- Sharpe, R.M., McKinnell, C., Kivlin, C., Fisher, J.S. (2003) Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. *Reproduction.* 125(6): 769-784.
- Sliwa, L., Macura, B. (2005) Evaluation of cell membrane integrity of spermatozoa by hypoosmotic swelling test (water test) in mice after intraperitoneal Daidzein administration. *Sys Biol Reprod Med.* 51(6): 443-448.
- Sonmez, M., Türk, G., Yüce, A. (2005) The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. *Theriogenology.* 63(7): 2063-2072.
- Sookhthezari, A., Alirezaei, M., Kheradmand, A., Dezfoulian, O. (2015) Streptozotocin-induced Diabetic Effects on the Sperm Fertility Parameters, Glycated Hemoglobin and Total Cholesterol in Mice. *SSU J.* 23(10): 980-988.
- Temidayo, S., du Plessis Stefan, S. (2018) Diabetes mellitus and male infertility. *Asian Pac J Reprod.* 7(1): 6-14.
- Tuck, K.L., Freeman, M.P., Hayball, P.J., Stretch, G.L., Stupans, I. (2001) The in vivo fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds to rats. *J Nutr.* 131(7): 1993-1996.
- Turner, T.T., Lysiak, J.J. (2008) Oxidative stress: a common factor in testicular dysfunction. *J Androl.* 29(5): 488-498.
- Vignera, S., Condorelli, R., Vicari, E., D'Agata, R., Calogero, AE. (2012) Diabetes mellitus and sperm parameters. *J Androl.* 33(2): 145-153.
- Xiong, W., Wang, H., Wu, H., Chen, Y., Han, D. (2009) Apoptotic spermatogenic cells can be energy sources for Sertoli cells. *Reproduction.* 137(3): 469-479.

## اثرات عصاره برگ زیتون بر کیفیت اسپرم بدنبال دیابت القا شده تجربی در موش‌های صحرایی

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### چکیده

زمینه مطالعه: مدل تجربی القا شده توسط استرپتوزوتوسین بیانگر تغییرات ایجاد شده در سیستم تولید مثل نر بعنوان بخشی از بیماری می باشد.

هدف: هدف از انجام مطالعه حاضر بررسی اثرات عصاره برگ زیتون بر دیابت القا شده توسط استرپتوزوتوسین و هم چنین بررسی اثرات آن بر کیفیت اسپرم بود.

روش کار: ۲۰ سر موش صحرایی نر از نژاد اسپراگ-داولی به چهار گروه مساوی تقسیم شدند: گروه اول بعنوان گروه کنترل بدون درمان و گروه‌های ۲، ۳ و ۴ از موش‌ها با استرپتوزوتوسین دوز ۶۵ mg/kg تزریق شدند. حیواناتی که در روزهای ۶-۴ قند خون بالای ۲۵۰ mg/dL نشان دادند بعنوان موش‌های دیابتیک در نظر گرفته شدند. گروه‌های ۳ و ۴ عصاره برگ زیتون ۱۰۰ و ۱۵۰ mg/kg بصورت خوراکی و حلال آن به گروه‌های کنترل و دیابتیک (گروه ۲) برای ۱۰ روز پیوسته خوراندند.

نتایج: درصد هموگلوبین گلیکوزیله (HbA1c%) به عنوان شاخص دیابت در حیواناتی که عصاره با دوز ۱۵۰ mg/kg دریافت کردند در مقایسه با گروه دیابتیک بطور معنی داری کاهش یافت ( $P < 0/001$ ). در موش‌های درمان شده با عصاره برگ زیتون ۱۵۰ mg/kg عصاره توانست باعث بهبود کیفیت اسپرم در مقابل اثرات خطرناک استرپتوزوتوسین در گروه موش‌های دیابتیک گردد ( $P < 0/001$ )، اما حرکت کلی اسپرم بطور معنی داری در گروه دیابتیک بالاتر بود ( $P < 0/001$ ). غلظت کلسترول در گروه دیابتیک و گروه‌های درمان شده با عصاره نسبت به گروه کنترل بطور معنی داری افزایش یافت ( $P < 0/001$ ) و سطح تری گلیسرید در گروه دریافت کننده عصاره ۱۵۰ mg/kg در مقایسه با گروه دیابتیک و عصاره ۱۰۰ mg/kg بطور معنی داری کاهش یافت ( $P < 0/001$ ). نتیجه گیری نهایی: نتایج ما پیشنهاد می کنند که عصاره برگ زیتون دارای اثرات آنتی دیابتیک مفیدی در دیابت ناشی از استرپتوزوتوسین است و ممکن است کاندیدای مناسبی جهت کاهش پیچیدگی‌های دیابت در مردان باشد.

واژه‌های کلیدی:

دیابت، عصاره برگ زیتون، موش صحرایی، استرپتوزوتوسین، بیضه