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Hypoglycemic, Hypolipidemic, Antioxidant and Male Sexual Improvement Potentials of Olive Oil in Alloxan Treated Rats

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Abstract: *Diabetes mellitus* is a degenerative disease that has deleterious effects on male reproductive function, possibly through an increase in oxidative stress by free radicals. The protection against such deleterious effects can be offered by antioxidant supplementation. This study aimed to evaluate the significance of treatment of diabetic rats with olive oil in reducing oxidative stress, hyperglycemia, hyperlipidemia and testicular dysfunction induced by alloxan. The diabetic rats exhibited an increase in blood glucose, cholesterol, hydroperoxide levels and sperm abnormalities. Moreover, a significant decrease in the weights of sex organs, plasma testosterone, LH, sperm motility and sperm count was noticed in diabetic animals. Administration of olive oil to diabetic rats exhibited hypoglycemic and hypocholesterolemic effects associated with an improvement of sexual organ weights, hormone levels, sperm quality and sperm count. Furthermore, olive oil reduced the elevation of hydroperoxide level induced by alloxan. Administration of olive oil to normal rats showed hypocholesterolemic effect, a decrease in hydroperoxide level and increase in plasma testosterone level after eight weeks. On the other hand, olive oil has no significant influence on blood glucose, luteinizing hormone (LH) level, weight of sex organs, sperm quality and sperm count of normal rats. These results demonstrate that olive oil may be of advantage in lowering hyperglycemia, hypercholesterolemia, oxidative stress and deleterious effects on male reproductive functions induced by diabetes. It is suggested that the administration of olive oil may be helpful in alleviation of diabetic complications associated with oxidative stress and male reproductive dysfunction.

Key words: Diabetes, olive oil, Sperms, Testosterone, LH, hydroperoxide, hypercholesterolemia

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defective insulin secretion. Oxidative stress generated by hyperglycemia or by hyperlipidemia and decline of antioxidative defense mechanisms is regarded as important mediator for diabetic complications (Martin-Gallan *et al.*, 2003). Alloxan has been proposed to act as a diabetogenic agent due to its ability to destruct pancreatic β -islets cells, possibly by free radical mechanism. Diabetes represents a state of increased oxidative stress, which is mainly based on the evidence of increased lipid peroxidation (Palanivel *et al.*, 1998).

Diabetic patients have disturbances in sexual function, including a decreased in libido, impotency and infertility due to testicular dysfunction (Cameron *et al.*, 1990). Sperm concentration and motility

were decreased in diabetic rats (Amaral *et al.*, 2006). Diabetic rats were characterized by inhibition of spermatogenesis which can be detected as a decrease in testicular and seminiferous tubuli diameters (Altay *et al.*, 2003). Diabetes resulted in decreased body and reproductive organ weights, as well as diminished sperm counts in testis and epididymis that were associated with a decrease in the level of plasmatic testosterone (Scarano *et al.*, 2006).

In the Mediterranean region, olive oil is one of the main source of dietary fatty acids. Olive oil, in addition to oleic acid (Monounsaturated fatty acid), contains a range of micronutrients such as phenolic compounds, tocopherol, squalene, carotenoids and sterols (Visioli and Galli, 1995) which are associated with a lower incidence of coronary heart diseases. Phenolic compounds have shown to possess antioxidant (Moreno *et al.*, 2003), anti-inflammatory (Carluccio *et al.*, 2003) and antithrombotic activities (Singh *et al.*, 2007). Olive oil has proven to reduce the low density lipoprotein oxidation and increase the antioxidant capacity of plasma (Fito *et al.*, 2000).

The present study was undertaken to examine the antioxidative and alleviation effects of olive oil in an animal model of diabetes induced oxidative stress, hyperglycemia, hyperlipidemia and deleterious alterations in male reproductive system.

MATERIALS AND METHODS

Experimental Animals

Sixty four males albino rats weighting between 180 and 200 g were obtained from pharmacy College, King Saud University, Saudi Arabia. The animals were housed in a well ventilated 12 h light and dark cycle and allowed access to food and water *ad libitum* throughout the experiment.

Induction of Diabetes

Thirty two animals were allowed to fast for 14 h and were injected intraperitoneally with 150 mg kg⁻¹ of freshly prepared aqueous solution of alloxan (Xie *et al.*, 2005) as a single dose. At 3 days after alloxan administration, the fasting blood glucose, cholesterol and hydroperoxide were estimated and their concentrations were considered the values of zero time. Alloxan was purchased from sigma chemical Co.

Oil Administration and Experimental Groups

The rats were divided into two equal main groups, the normal group and diabetic group. The two main groups were subdivided into two equal subgroups, control and other treated with olive oil which administrated orally (2.5 mL kg⁻¹) daily for 8 weeks.

Blood samples of 8 animals in each group were collected from retro orbital venous plexus at 4 and 8 weeks post oil administration. Plasma glucose (Pruden, 1995), cholesterol (Allain *et al.*, 1974), testosterone and LH levels were estimated using kits from Bio Merieux, France. Glucose and cholesterol were evaluated colorimetrically using spectrophotometer (U/V visible, Pharmacia Biotech., England), while testosterone and LH were assayed by enzyme immunoassay, ELISA (Labsystems Uniskan II, Finland). Blood hydroperoxide level was evaluated using free radical analytical system (Iram, Parma, Italy). The test is a colorimetric test that takes advantage of the ability of hydroperoxides to generate free radicals after reacting with some transitional metals. When buffered chromogenic substance is added, a colored complex appears.

At the end of each time interval, 8 animals from each group were sacrificed and then testes, vas deference, epididymis, seminal vesicle and prostate gland were removed and weighted. The epididymis was dissected in 10 mL of normal saline (0.9% NaCl), incubated at 37°C and sperm motility (%) was evaluated. Smears were prepared from the suspension stained with 1% eosin solution and examined for sperm abnormalities (Filler, 1993). We observed 300 spermatozoa for each sample. Classification of individual spermatozoa was (1) normal, (2) head abnormalities and (3) tail abnormalities.

The percentage of total sperm abnormalities was calculated. The head abnormalities include small head, amorphous head, no hook and two heads. The tail abnormalities include folded tail, short tail and two tails.

Statistical Analysis

Statistical difference was calculated by using one way analysis of variance and least significant difference range test (LSD). Data were expressed as means±SE.

RESULTS

Biochemical Parameters

Blood Glucose

As shown in Table 1, a significant increase ($p<0.01$) in plasma glucose levels of diabetic groups was observed at all time intervals. The glucose level of diabetic rats treated with olive oil was significantly lower than that of diabetic rats after 4 and 8 weeks ($p<0.05$, $p<0.01$). The plasma glucose level of diabetic control rats was 3.1 and 3.3 times that of control at the two intervals, while it was 2.8 and 2.4 times in case of diabetic rats treated with olive oil. Olive oil has no significant influence on plasma glucose level of normal rats.

Blood Cholesterol

Plasma cholesterol concentration (Table 1) elevated significantly ($p<0.01$) in diabetic rats at all time intervals. The cholesterol level decreased significantly in normal rats given olive oil after 4 and 8 weeks ($p<0.05$, $p<0.01$). Administrations of olive oil to diabetic rats lead to a decrease in cholesterol level at the experiment periods.

Blood Hydroperoxide Level

As shown in Table 2, the blood hydroperoxide level increased significantly ($p<0.01$) in diabetic rats as compared to normal ones.

Olive oil treatment significantly ($p<0.05$) lowered the hydroperoxide level in normal rats after 8 weeks. Moreover, the oil lowered the concentration of peroxide in diabetic rats ($p<0.01$) after 4 and 8 weeks as compared to diabetic control rats.

Fertility Parameters

Weight of Sex Organs

A significant decrease in vas deferens ($p<0.05$), seminal vesicle and prostate gland weights ($p<0.01$) of diabetic control rats was observed after 4 weeks (Table 3). Moreover, the weights of testis and accessory sex organs (Vas deferens, epididymis, seminal vesicle and prostate gland) decreased

Table 1: Effect of olive oil on plasma glucose and cholesterol levels (mg/100 mL) in normal and diabetic rats

Time (week)	Treatments			
	Normal		Diabetic	
	Control	Olive oil	Control	Olive oil
Glucose				
0	93.15±5.03	81.41±6.14	290.00±9.41**	314.00±15.08**
4	98.35±6.91	93.14±2.63	312.36±12.61**	275.93±9.51**◆
8	90.00±2.53	85.46±3.10	299.40±11.00**	220.20±9.83**◆◆
Cholesterol				
0	92.11±2.85	84.81±3.01	170.53±7.31**	166.44±5.93**
4	96.24±5.38	73.14±2.80**	200.46±10.00**	136.16±9.10**◆◆
8	89.40±5.31	65.80±2.00**	215.91±8.50**	130.88±2.90**◆◆

Each value is the mean±SE (n =8); *, ** Significant difference compared to normal control $p<0.01$.◆,◆◆ Significant difference compared to diabetic control $p<0.05$, $p<0.01$

Table 2: Effect of olive oil on blood hydroperoxide level (mg/100 mL) in normal and diabetic rats

Time (week)	Treatments			
	Normal		Diabetic	
	Control	Olive oil	Control	Olive oil
0	25.84±2.32	24.50±1.22	34.41±0.90**	33.23±1.37**
4	24.17±1.23	22.46±1.08	39.67±1.05**	34.44±0.96**
8	24.08±1.26	20.01±1.06*	43.01±0.80**	30.00±1.30**◆◆

Each value is the mean±SE (n = 8); *, ** Significant difference compared to normal control, p<0.05, p<0.01; ◆◆ Significant difference compared to diabetic control, p<0.01

Table 3: Effect of olive oil on weights (g) of testis and accessory sex organs relative to body weight in normal and diabetic rats

Organs	Treatments			
	Normal		Diabetic	
	Control	Olive oil	Control	Olive oil
4 Weeks				
Testis	1.23±0.01	1.25±0.02	1.190±0.03	1.25±0.01
Vas deferens	0.07±0.003	0.06±0.002	0.046±0.002*	0.06±0.003◆
Epididymis	0.25±0.003	0.27±0.01	0.220±0.01	0.26±0.02
Seminal vesicle	0.52±0.01	0.46±0.03	0.300±0.02**	0.48±0.03◆◆
Prostate gland	0.31±0.02	0.32±0.01	0.190±0.007**	0.28±0.02◆◆
8 weeks				
Testis	1.26±0.02	1.23±0.04	1.060±0.04**	1.28±0.05◆◆
Vas deferens	0.06±0.005	0.05±0.003	0.037±0.002**	0.05±0.006◆
Epididymis	0.23±0.007	0.24±0.007	0.200±0.01*	0.25±0.01◆◆
Seminal vesicle	0.46±0.01	0.42±0.02	0.210±0.01**	0.39±0.02**◆◆
Prostate gland	0.33±0.01	0.30±0.02	0.160±0.005**	0.22±0.01**◆◆

Each value is the mean±SE (n = 8), *, ** Significant difference compared to normal control p<0.05, p<0.01; ◆, ◆◆ Significant difference compared to diabetic control p<0.05, p<0.01

significantly (p<0.01) after 8 weeks as compared to normal control. The weights of seminal vesicle and prostate gland of diabetic rats given olive oil lowered significantly (p<0.01) after 8 weeks, while weights of other sex organs did not change significantly. Alloxan injection has no significant influence on weights of sex organs of olive oil given rats after 4 weeks.

The weights of sex organs of rats treated with alloxan and olive oil were significantly higher than those of diabetic control. Olive oil has no significant effect on sex organ weights of normal rats.

Testosterone Level

The values of plasma testosterone level (Table 4) in diabetic groups were significantly less than those of control group. The hormone level of diabetic rats given olive oil was significantly (p<0.01) higher than that of diabetic control.

The hormone level increased significantly (p<0.05) in normal rats given olive oil 8 weeks post-treatment.

LH Level

Plasma LH level (Table 5) lowered significantly (p<0.01) in diabetic groups at all time intervals. The hormone level of diabetic rats treated with olive oil was significantly (p<0.01) higher than that of diabetic control. The olive oil had no significant influence on the hormone level of normal rats.

Sperm Motility

The sperm motility (Fig. 1) decreased significantly (p<0.01) in diabetic control by 21 and 41% at 4 and 8 weeks post-treatment. Also, the motility decreased significantly (p<0.05) in diabetic rats

Table 4: Effect of olive oil on plasma testosterone level (ng/100 mL) in normal and diabetic rats

Time (week)	Treatments			
	Normal		Diabetic	
	Control	Olive oil	Control	Olive oil
4	2.98±0.12	3.10±0.22	1.39±0.14**	2.39±0.20**◆◆
8	3.16±0.15	3.70±0.12*	0.84±0.09**	2.21±0.16**◆◆

Each value is the mean±SE (n = 8), *, ** Significant difference compared to normal control p<0.05, p<0.01; ◆◆ Significant different compared to diabetic control, p<0.01

Table 5: Effect of olive oil on plasma LH level (m L U/m L) in normal and diabetic rats

Time (week)	Treatments			
	Normal		Diabetic	
	Control	Olive oil	Control	Olive oil
4	3.25±0.16	3.10±0.21	1.49±0.15**	2.31±0.13**◆◆
8	3.41±0.15	3.30±0.18	0.84±0.05**	2.50±0.17**◆◆

Each value is the mean±SE (n = 8), ** Significant difference compared to normal control, p<0.01; ◆◆ Significant difference compared to diabetic control p<0.01

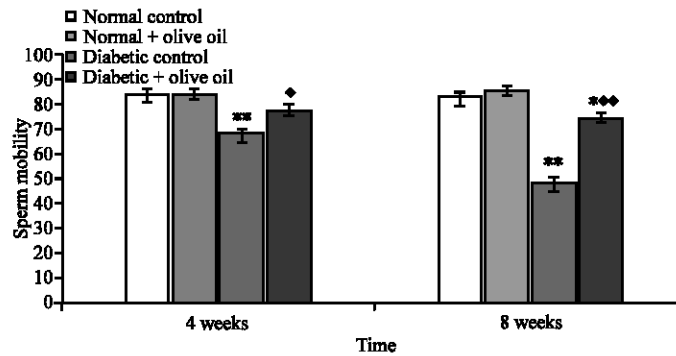


Fig. 1: Effect of olive on sperm motility (%) in normal and diabetic rats. Each value is the mean±SE, n = 8 *p<0.05 and **p<0.01 significant difference compared to normal control, *p<0.05, **p<0.01 significant difference compared to diabetic control

+ olive oil group by 11% 8 weeks post treatment. The motility of diabetic rats + olive oil group was significantly (p<0.05, p<0.01) higher than that of diabetic control at the two time intervals. The sperm motility did not change significantly in normal rats given olive oil.

Sperm Count

A significant decrease (p<0.01) of sperm count was observed in diabetic control at all time intervals (Fig. 2). The administration of olive oil to diabetic rats keep the sperm count in normal range.

The sperm count of normal rats treated with olive oil did not change significantly.

Sperm Abnormalities

The sperm abnormalities (Fig. 3) elevated significantly (p<0.01) in the diabetic control rats during experiment periods. Similar results were observed in the diabetic rats given olive oil after 4 and 8 weeks (p<0.05 and p<0.01). The sperm abnormalities of diabetic control were 2.09 and 2.73 times that of control throughout the two intervals, while they were 1.26 and 1.88 times that of control in case of diabetic rats administered olive oil. However, the sperm abnormalities of diabetic + olive oil group were significantly less than those of diabetic control. The olive oil had no significant influence on sperm abnormalities of normal rats.

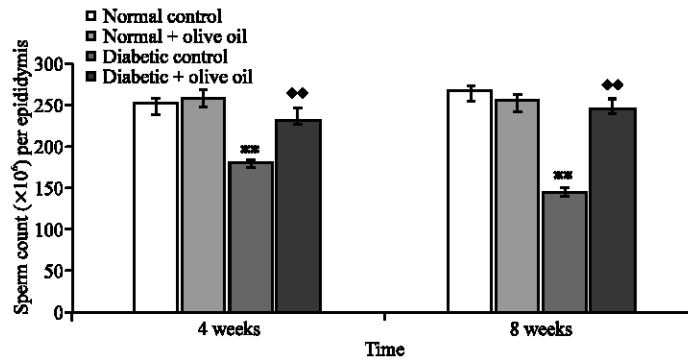


Fig. 2: Effect of olive oil on sperm count ($\times 10^6$) per epididymis in normal and diabetic rats. Each value is the mean + SE, n =8, **p<0.01 significant difference compared to normal control, ♦♦p<0.01 significant difference compared to diabetic control

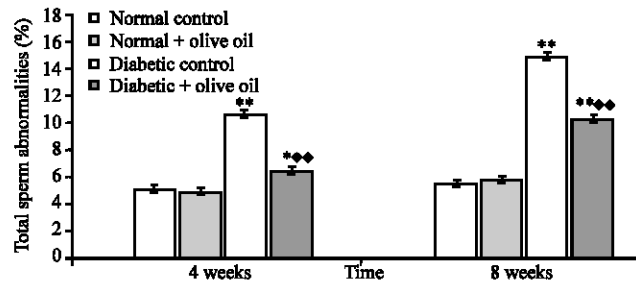


Fig. 3: Effect of olive oil on total sperm abnormalities (%) in normal and diabetic rats, Each value is the mean +SE, n =8, *p<0.05, **p<0.01 significant difference compared to normal control, ♦♦p<0.01 significant difference compared to diabetic control

DISCUSSION

Oxidative stress is increased in the diabetic condition due to overproduction of reactive oxygen species and decreased efficiency of antioxidant defenses (Martin-Gallan *et al.*, 2003). Moreover, oxidative stress can generate by hyperglycemia (Ceriello and Motz, 2004) and hyperlipidemia (Ohara *et al.*, 1993). In the present investigation, blood glucose, cholesterol and hydroperoxide levels elevated significantly in diabetic control rats. The increased hydroperoxide level in diabetic animals can be attributed to hyperglycemia and hypercholesterolemia. Lipid hydroperoxides are free radical mediated reactions (Wolff, 1994). The increase of lipid peroxide level in diabetic rats suggests that increased generation of free radicals by hyperglycemia related glucose auto-oxidation. Free radicals have a direct toxic effect on the tissues (Sener *et al.*, 2005). The observed increase in lipid hydroperoxide level may be due to the increase of peroxidative damage of lipids. The alloxan administration produced marked oxidative stress as evidenced by a significant increase in testicular lipid peroxidation as well as a significant decrease in testicular antioxidants (El-Missiry, 1999).

Studies on the oxidative impairments of male reproductive system in experimental animals under diabetic conditions are limited. It is reported that disturbances in reproductive system functions of diabetic laboratory animals are associated with destructive changes in the gonads and dysfunction of the hypothalamo-hypophyseal complex (Babichev *et al.*, 1998). The present investigation showed a

significant decrease in testis, vas deferens, epididymis, seminal vesicle and prostate gland weights in diabetic control rats associated with low levels of plasma testosterone and LH. The decrease in the weight of sex organs is likely due to the decrease of testosterone and LH levels. Altered steroidogenesis as a consequence of oxidative stress has been shown (Peltola *et al.*, 1996). It was postulated that the reduction in serum testosterone and LH levels of diabetic rats may be due to accumulation of lipids in Leydig cells (Murray *et al.*, 1983) or decrease of their number (Ballester *et al.*, 2004).

The current study was conducted in order to clarify the relationship between oxidative stress originated by diabetic condition and parameters related to spermatogenesis and sperm function. In the present investigation, sperm motility and count of diabetic control decrease, while sperm abnormalities increase. The decrease in sperm concentration is likely due to a decrease in testosterone and LH levels observed in the present diabetic rats, since the two hormones affect spermatogenesis (Braunstein, 1991). In addition, the increased hydroperoxide level can affect the spermatogenic process, since germ cells are more susceptible to peroxidative damage (Hemachand and Shaba, 2003). The decrease in sperm count can be attributed to the influence of hyperglycemia on late stages of spermatogenesis, possibly through an increase of reactive oxygen species. The consequences of such oxidative damage could include loss of motility due to lipid peroxidation (Sikka, 2001). The increase of free radicals mediated toxicity is well documented in clinical diabetes (Niskanen *et al.*, 1995). Free radicals induced lipid peroxidation result in morphological changes in sperm (Sanchez *et al.*, 2006). The increase of sperm abnormalities in diabetic control may be due to elevation of hydroperoxide level. Diabetes with sustained hyperglycemia may result in testicular dysfunction associated with decreased fertility potential. These findings suggest that oxidative stress is likely to contribute significantly in the development of pathophysiological dysfunctions in the testis of alloxan diabetic rats. Evidence in favor of our hypothesis was obtained in term of enhanced hydroperoxide level.

The Mediterranean diet with its high content of olive oil represents a healthy and disease preventive diet and reduces mortality from heart disease (Visioli and Galli, 1998). In the present study, we observe a significant decrease in glucose level (12% after 4 weeks and 30% after 8 weeks) and in cholesterol level (18 and 21% at the two intervals) of diabetic rats treated with olive oil. Al-Azzawie and Alhamdani (2006) found that oleuropein reduced the oxidative stress and hyperglycemia in alloxan-induced diabetic rabbits. Picinato *et al.* (1998) reported that olive oil may increase the response of insulin secretion to glucose stimulus in pancreatic islets *in vitro*. Polyunsaturated fatty acids from olive oil have hypocholesterolemic activity (Reaven *et al.*, 1993). The observed decrease in blood hydroperoxide level of diabetic rats given olive oil can be attributed to hypocholesterolemic and hypoglycemic activities of olive oil, since hypercholesterolemia and hyperglycemia can induce oxidative stress and increase the free radicals production (Ohara *et al.*, 1993; Ceriello and Motz, 2004). Moreover, Olive oil has antioxidant effect (Reaven *et al.*, 1993). The present results showed that olive oil exerted antioxidant, antihyperglycemic and antihypercholesterolemic effects and consequently may alleviate deleterious effects of alloxan on testosterone and LH levels, weight of sex organs and quality of sperms.

It has been postulated that supplementation with dietary antioxidant compounds such as ascorbic acid and vitamin E may offer some protection against diabetes complications through their roles as inhibitors of glycation and as free radical scavengers (Sinclair *et al.*, 1992). Non-vitamin antioxidant, polyphenols reduced the negative effect of oxidative stress and free radicals in diabetic patients (Asgary *et al.*, 2002). Several mechanisms have been proposed to explain the antioxidant capacity of olive oil. One possible mechanism is the increase of tissue sensitivity to the lipid antioxidants of vitamin E (Scaccini *et al.*, 1992). The diet rich in olive oil reduces tissue oxidative stress not only by decreasing lipid peroxidation but also by enhancing the glutathione antioxidant defense system. Olive oil elevates glutathione, glutathione peroxidase and glutathione transferase activities in different tissues

(De La Cruz *et al.*, 2000). It was showed that the oleic acid, the major component of olive oil has antioxidant effect (Reaven *et al.*, 1993). The polyphenols in olive oil (Oleuropein, tyrosol, hydroxytyrosol and caffeic acid) inhibit the formation of oxygen reactive species in the cells (De La Puerta *et al.*, 1999). Oleuropein, a phenolic antioxidant can reduce the oxidative stress that result from doxorubicin (Andreadou *et al.*, 2007). Furthermore, hydroxytyrosol can scavenge hydrogen peroxide (O'Dowd *et al.*, 2004) and exhibited a protection against ultraviolet radiation induced lipid peroxidation (D'Angelo *et al.*, 2005). The administration of diets enriched in oils rich in unsaturated fatty acids was shown to reduce the tissues to produce lipid peroxides (L'Abbe *et al.*, 1991).

Sever effects on hydroperoxide, testosterone and LH levels, weight of sex organs and sperm parameters (motility, count, abnormalities) were seen in rats treated with alloxan only, while diabetic rats treated with olive oil showed milder effects, possibly due to moderate hypercholesterolemia and hyperglycemia or antioxidant activity of the olive oil. In conclusion, the administration of olive oil rich in phenolic compounds seems to alleviate male reproductive dysfunction associated with diabetes. A reduction in hydroperoxide level, cholesterol, glucose and sperm abnormalities and improvement of sperm motility, sperm count and hormone levels of diabetic rats treated with olive oil could explain these reported findings.

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