Full Length Research Paper

Effect of *Nigella sativa* seeds on reproductive system of male diabetic rats

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This study aims to investigate the effects of *Nigella sativa* seeds (NSS) on fertility of male diabetic rats. Animals were divided into 3 groups, one group was kept as control and the two other were rendered diabetic by alloxan (120 mg/kg b.wt). One group was left as diabetic control (Diab) and the second were treated (Diab+N) with 2% of NSS in diet for 30 days. Blood samples were collected for glucose and testosterone levels. Testis, epididymis, prostates and seminal vesicles were removed for sperm parameters and oxidant/antioxidant status. NSS improve semen quantity and mobility, and testosterone levels and testis; they decrease blood glucose and lipid peroxidation product level (LPO) and improve antioxidant activities of glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), and aspartate and alanine aminotransferase (AST and ALT). NNS as a diet may be beneficial for diabetic fertility.

Key words: Diabetes, *Nigella sativa* seeds, oxidative stress, reproductive system.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disorder of the carbohydrate, lipid and protein metabolism that contribute to several kinds of complications including male infertility. Many reports indicate that diabetic complications are associated with overproduction of reactive oxygen species (ROS) and accumulation of lipid peroxidation by-products (Palanduz et al., 2001). The male reproductive damage expressed as rate of imbalance of the oxidative balance, increased levels of enzymatic glication products in testicular and epidydimal region, and resides on the seminal plasma (Amaral et al., 2008). Several studies have shown that antioxidant treatment reduces diabetic complications and protect cellular components from oxidative damage (Evans, 2007).

Nigella sativa L. (NS) is a plant of Ranunculaceae family that grows spontaneously and widely in several Southern Mediterranean and Middle Eastern countries (Tariq, 2008). NS seed (NSS) has over 100 different chemical constituents, including abundant sources of all the essential fatty acids. Although it is the oil that most often used medicinally, the seeds are a bit spicy and are often used whole in cooking curries, pastries and Mediterranean cheeses (Tariq, 2008). NSS are often used as a spice but are also used extensively in the traditional medicine of many countries (Meddah et al., 2009). NS has been used traditionally for the treatment of many diseases owing to the reported antiviral (Salem and Hossain, 2000), anti-schistosomiasis (El-Shenawy et al., 2008), anti-inflammatory (Hajhashemi et al., 2004) and immunomodulatory (Tekeoglu et al., 2007) activities. Furthermore, it was found that NS extract has anti-tumor properties (Ait-MbarekL et al., 2007) attenuating toxic side effects caused by several chemotherapeutic agents (Uz et al., 2008) and protects against gentamicin-induced nephrotoxicity (Yaman and Balikci, 2010). In addition, some pharmacological studies have demonstrated that thymoquinone (TQ), a main constituent of NSS, possesses important analgesic and anti- inflammatory properties (El Gazzar et al., 2006; Padhye et al., 2008), protects organs against oxidative damage induced by a variety of free radical generating agents (Chandra et al.,

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2009; El Gazzar et al., 2006; Gali-Muhtasib et al., 2006; Padhye et al., 2008;), inhibits ionofeicosanoid generation and membrane lipid peroxidation (Hosseinzadeh et al., 2007). The high lipid content of the reproductive organs makes it particularly susceptible to free radicals mediated insult. Antioxidant enzymes are involved in the defence system against free radical mediated tissue or cellular damage.

This research was therefore carried out in view of the paucity of literature on the action of *N. sativa* seeds on reproductive functions and antioxidant activities in male diabetic rats.

MATERIALS AND METHODS

Preparation of plants

N. sativa L. (Ranunculaceae), commonly called black cumin and "sinouj" in Tunisia, is an annual herbaceous plant cultivated in different parts of the world, mainly in countries bordering the Mediterranean Sea. *N. sativa* seeds (NSS) were collected at maturity from cultivated plants from the region of Menzel Temime (Northeastern Tunisia). The NSS were powdered mechanically into a fine powder and mixed with a diet to get 2% (200 mg/kg of diet) supplementation. These dietary seeds were used throughout in this study for treatment of diabetic rats.

Experimental design

Male Wistar rats weighing 230 to 250 g were obtained from the Central Pharmacy of Tunisia (SIPHAT, Tunisia). They were maintained under standard laboratory conditions (22 ± 3°C, 12-h light/dark cycle), with pelleted food (Industrial Company of Rodent Diet, Sfax, Tunisia) and tap water *ad libitum* during 30 days of experimental period. The general guidelines on the use of living animals in scientific investigations (Council of European Communities, 1986) and the guidelines for care and use of laboratory animals controlled by the Tunisian Research Ministry were followed. This experimental study was conducted on three 8-rats groups: control group (control), diabetic rats (Diab) and diabetic rats fed with diet supplemented with *N. sativa* seeds (NSS) at 2% (Diab + N).

Induction of diabetes

Experimental type 1 diabetes was induced in rats by a single intraperitoneal injection of freshly prepared alloxan solution in normal saline at a dose of 120 mg/kg b.wt. (Mansour et al., 2002; Sheweita et al., 2001). Because alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were orally treated with 20% glucose solution (5 to 10 ml) after 6 h. The rats were then kept for the next 24 h on 5% glucose water solution to prevent hypoglycemia. Rats with moderate diabetes that exhibited glycosuria and hyperglycemia (blood glucose concentration 200 to 300 mg dl^{-1}) were taken for the experimental tests.

Preparation of serum and tissue extract

After one month of treatment, the animals were sacrificed by decapitation. The trunk blood was collected and 2 ml of blood were distributed into tubes containing an anticoagulant agent, followed by centrifugation at 3000 rpm for determination of plasma glucose level. The remaining blood was placed in a dry tube, centrifuged and the serum was aliquoted into 1.5 ml vials, and frozen at -20°C for determination of testosterone level. Testis, epididymis, prostate and seminal vesicles were excised immediately, washed with ice-cold physiologic saline solution (0.9%, w/v), blotted and weighed. About 1 g of each organ was cut into small pieces, homogenized with an Ultra Turrax homogenizer in 2 ml ice-cold appropriate buffer (TBS, pH 7.4) and centrifuged at 9000 g for 15 min at 4°C. Supernatants were collected, aliquoted and stored at -80°C until use for enzyme assays.

Epididymis sperm count and motility

Epididymal contents were obtained after cutting the tail of epididymis, squeezing it gently on clean slide and the sperm progressive motility and cell count were determined according to the method described by Yokoi et al. (2003). Briefly, the cauda epididymis was minced with anatomical scissors in 2 ml of Earles buffer placed in a rocker for 10 min at 35°C. After dilution, the number of homogenization-resistant spermatozoon was counted in a haemocytometer and about 25 fields of view were examined under a light microscope at 40× magnification.

Biochemical assays

Blood glucose and testosterone levels

Plasmatic glucose was assayed by glucose-oxidase, using a commercial kit (Biomaghreb, Tunisia). Serum testosterone was assayed by radioimmunoassay (RIA). Testosterone levels were analyzed using RIA kits purchased from Immunotech (Marseille, France).

Testis enzyme activities

The activities of ALP, LDH, AST and ALT in testis were measured using commercial kits from Sigma Munich (Munich, Germany) and Boehringer Mannheim (Mannheim, Germany).

Measurement of malonaldialdehyde (MDA) in tissues

Concentrations of MDA, an index of lipid peroxidation and oxidative stress, in testis, epididymis, prostate and seminal vesicles, was determined spectrophotometrically by the method of Buege and Aust (1984).

Activities of antioxidant enzymes

Testis, epididymis, prostate and seminal vesicles catalase activities were measured according to Aebi (1984). Hydrogen peroxide (H_2O_2) disappearance was monitored kinetically at 240 nm for 1 min at 25°C. The enzyme activity was calculated using an extinction coefficient of 0.043 $mM^{-1}cm^{-1}$. One unit of activity is equal to the mmol of H_2O_2 destroyed/min/ mg protein.

Superoxide dismutase (SOD) activity in tissue extracts was assayed spectrophotometrically as described by Beyer and Fridovich (1987). This method is based on the capacity of SOD to inhibit the oxidation of nitroblue tetrazolium (NBT). One unit of SOD represents the amount of enzymes required to inhibit the rate of NBT oxidation by 50% at 25°C. The activity was expressed as units/mg protein.

Table 1. Effect of *N. sativa* seeds on the level of plasma glucose in male diabetic rats.

Group	Glucose (g/L)
Control	1.2 ± 0.17
Diab	3.02 ± 0.319**
Diab + N	1.9 ± 0.82* ##

Values are mean ± SD (n = 8). Diab: diabetic, Diab + N: diabetic treated with *N. sativa* seeds. Values are statistically significant; **, $p < 0.01$ vs controls rats. ##, $p < 0.01$ vs diabetic rats.

Glutathione peroxidase (GPx) activity in tissue extracts was assayed according to the method of Flohe and Gunzler (1984). The activity was expressed as mmol of GSH oxidized/min/mg of protein at 25°C.

Protein content

The level of total protein was determined by the method of Lowry et al. (1955).

Statistical analysis

The results were expressed as means ± standard deviation (SD). The significance of differences among groups was analyzed statistically by One-way ANOVA (Analysis of Variance), followed by Student's unpaired t-test. The differences were considered statistically significant if $p < 0.05$.

RESULTS

Blood glucose

The blood glucose level increased significantly ($p < 0.01$) in diabetic rats by 150% as compared to controls. The treatment of diabetic rats with NSS reduced significantly ($p < 0.01$) the plasma glucose level by 37% compared to the diabetic group (Table 1).

Body and reproductive organs absolute weight

The body and reproductive organs weights (testis, epididymis, prostate and seminal vesicles) decreased significantly ($p < 0.01$) in alloxan-diabetic rats by 15, 32, 32, 45, and 48%, respectively compared to control. However, the treatment of alloxan-diabetic rats with NSS caused a significant increase (P < 0.05) in body, testis, epididymis, prostate and seminal vesicles weights by 14, 26, 25, 34 and 35%, respectively compared to diabetic rats (Table 2).

Semen parameters and serum testosterone level

The count and mobile sperm showed a significant

reduction ($p < 0.001$) in alloxan-diabetic rats by 37 and 42%, respectively compared to control rats. Treatment of alloxan-diabetic rats with NSS caused a significant increase ($p < 0.05$) in count sperm by 35% and in mobile sperm by 38%, compared to diabetic rats (Table 3).

In alloxan-diabetic rats, the serum testosterone level decreased by 85% compared to the control rats. However, a significant improvement ($p < 0.05$) was observed in the rats treated with NSS by 53% compared to the diabetic rats.

Lipid peroxidation of reproductive organs

The MDA level in testis, epididymis, prostate and seminal vesicles increased significantly (p<0.05) in diabetic rats (0.741 ± 0.1652; 1.281 ± 0.19; 2.66 ± 0.182; 1.47 ± 0.192 nmol/mg proteins, respectively), compared to the control group (0.45 ± 0.03; 0.73 ± 0.17; 1.53 ± 0.53; 0.59 ± 0.078 nmol/mg proteins, respectively). The administration of NSS decreased significantly ($p < 0.05$) the level of MDA in testis (0.52 ± 0.095 nmol/mg proteins), epididymis (0.932 ± 0.05 nmol/mg proteins), prostate (1.81 ± 0.18 nmol/mg proteins) and seminal vesicles (0.98 ± 0.31 nmol/mg proteins) of the treated group, as compared to the diabetic group (0.741 ± 0.16; 1.281 ± 0.19; 2.66 ± 0.182 and 1.47 ± 0.19 nmol/mg proteins, respectively) (Figure 1).

Activities of antioxidant enzymes in different reproductive organs

The activity of SOD was significantly lower ($p < 0.01$) in testis, epididymis, prostate and seminal vesicles of the diabetic rats (0.72 ± 0.06; 1 ± 0.13; 2.3 ± 0.78 and 2.52 ± 0.25 U/mg proteins, respectively) compared to the control group (1.46 ± 0.2; 1.86 ± 0.29; 3.97 ± 0.7 and 4.02 ± 0.59 U/mg proteins, respectively). Similarly, the activities of CAT and GPx were significantly decreased in diabetic rats as compared to the control group. The treatment with NSS resulted in a significant restoration ($p < 0.05$) of all these enzymes (Figures 2 to 4).

Testis LDH, ALP, AST and ALT activities

In alloxan-diabetic rats, the activities of testis LDH, ALP, AST and ALT were significantly ($p < 0.01$) increased by 84, 41, 160 and 145%, respectively compared to the control group. When diabetic rats were treated with NSS, a significant ($p < 0.05$) decrease of these enzyme activities was observed by 33, 19, 25 and 43%, respectively in the rats treated with NSS in comparison with untreated diabetic rats (Table 4).

DISCUSSION

In the present study, oral administration of *N. sativa*

Table 2. Effect of *N. sativa* seeds on body and reproductive organs weights in male diabetic rats.

Group	Body weight (g)	Testis weight (g)	Epididymis weight (g)	Seminal vesicles weight (g)	Prostate weight (g)
Control	240.59 ± 4.27	1.81 ± 0.26	0.4 ± 0.05	0.86 ± 0.25	0.5 ± 0.07
Diab	203.61 ± 15.1**	1.242 ± 0.22**	0.27 ± 0.03**	0.42 ± 0.1**	0.27 ± 0.07**
Diab + N	231.26 ± 9.96[#]	1.56 ± 0.22[#]	0.34 ± 0.04[#]	0.57 ± 0.07[#]	0.42 ± 0.03[#]

Values are mean ± SD (n = 8). Diab: diabetic rats, Diab + N: diabetic treated rats with *N. sativa* seeds. Values are statistically ** $p < 0.01$ vs. controls rats. [#], $p < 0.05$ vs. diabetic rats.

Table 3. Effect of *N. sativa* seeds on serum testosterone level and epididymal sperm characters in male diabetic rats.

Group	Sperm count (10^6/epididyme)	Sperm motility (10^6/epididyme)	Testosterone level (ng/ml)
Control	28.5 ± 1	24.75 ± 1.7	2.65 ± 0.61
Diab	19 ± 1***	14.33 ± 2.08***	0.48 ± 0.11**
Diab + N	25.25 ± 3.5[#]	21.25 ± 3.86[#]	1.85 ± 0.46[#]

Values are mean ± SD (n = 8). Diab: diabetic rats, Diab + N: diabetic treated rats with *N. sativa* seeds. Values are statistically ** $p < 0.01$; *** $p < 0.001$ vs. controls rats; [#] $p < 0.05$ vs. diabetic rats.

Table 4. Variation of AST, ALT, LDH and ALP activities in testis of control, diabetic and diabetic treated rats with *N. sativa* seeds.

Group	LDH (U/mg protein)	ALP (U/mg protein)	AST(U/mg protein)	ALT(U/mg protein)
Control	0.83 ± 0.08	0.58 ± 0.2	0.31 ± 0.03	0.24 ± 0.01
Diab	1.53 ± 0.1**	0.82 ± 0.11*	0.82 ± 0.09**	0.59 ± 0.07**
Diab + N	1.01 ± 0.27[#]	0.66 ± 0.06[#]	0.61 ± 0.02[##]	0.34 ± 0.06[#]

Values are mean ± SD (n = 8). Diab: diabetic rats, Diab + N: diabetic treated rats with *N. sativa* seeds. Values are statistically * $p < 0.05$; ** $p < 0.01$ vs. controls rats; [#], $p < 0.05$; [##] $p < 0.01$ vs. diabetic rats.

seeds (NSS) in diet of alloxan diabetic male rats at the dose of 2% for 30 days caused the increase of body weight and weight of testis, epididymis, prostate and seminal vesicles tissues, the improve of semen quantity and mobility, other than the decrease of blood glucose level and the increase of serum testosterone level as compared with the diabetic group.

The improvement of male reproductive function in diabetic rats appears to be due to the protective effect of NNS. These findings agree with those reported by Al-Sa'aidi et al. (2009) and Mohammad et al. (2009) who demonstrate that black seed produce increase effects on fertility and reproductive system in adult male rat. The improvement of reproductive functions of male rats by NNS is probably related to its constituents of proteins, vitamins like A, B and C, in addition to the presence of important minerals like zinc, copper and magnesium which increase reproductive organs weight (Ahlobom et al., 2001; Al–Okbi et al., 2000; Kanter et al., 2005) and to the fact that black seeds contain alkaloids and phenols which stimulate the secretion of testosterone (Al–Dejyli, 2001).

Another explanation of the improvement in reproductive functions is the reduced level of MDA and the restoration of antioxidant enzymes in reproductive organs. LPO leads also to the failure of the antioxidant mechanisms in preventing the formation of excessive reactive oxygen species (ROS), including oxygen radicals and their reaction products are known to react with biological molecules, leading to cell and tissue damage. The increase in antioxidant status and the decrease in MDA concentration in reproductive system in NSS treated diabetic rats, underlines its anti-lipid peroxidative and antioxidative effects. The latter activities were previously demonstrated by Salem (2005) who found that the oil of black cumin and its active ingredients, in particular thymoquinine (TQ), possess anti-oxidant effects by enhancing the scavenger system leading to an antitoxic effects derived by several insults. Equally, Kanter (2008) have reported that NSS decreased the LPO and liver enzymes, and increased the antioxidant defense system activity. Same authors also showed that NSS treatment decreased tissue MDA and protein carbonyl levels and prevented inhibition of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) enzyme activities following experimental spinal cord
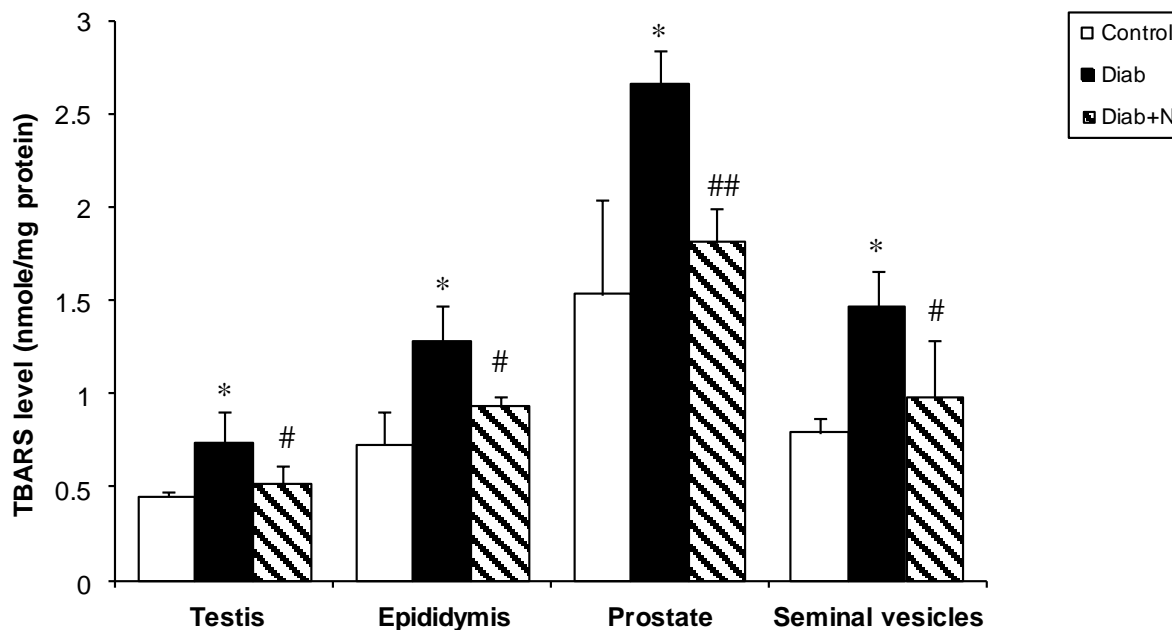
Figure 1. Variation of TBARS levels in testis, epididymis, prostate and seminal vesicles of control, diabetic and diabetic treated rats with *N. sativa* seeds. Values are mean ± SD (n = 8). Diab: diabetic rats, Diab + N: diabetic treated rats with *N. sativa* seeds. Values are statistically * $p < 0.05$ vs. controls rats; # $p < 0.05$; ## $p < 0.01$ vs. diabetic rats.
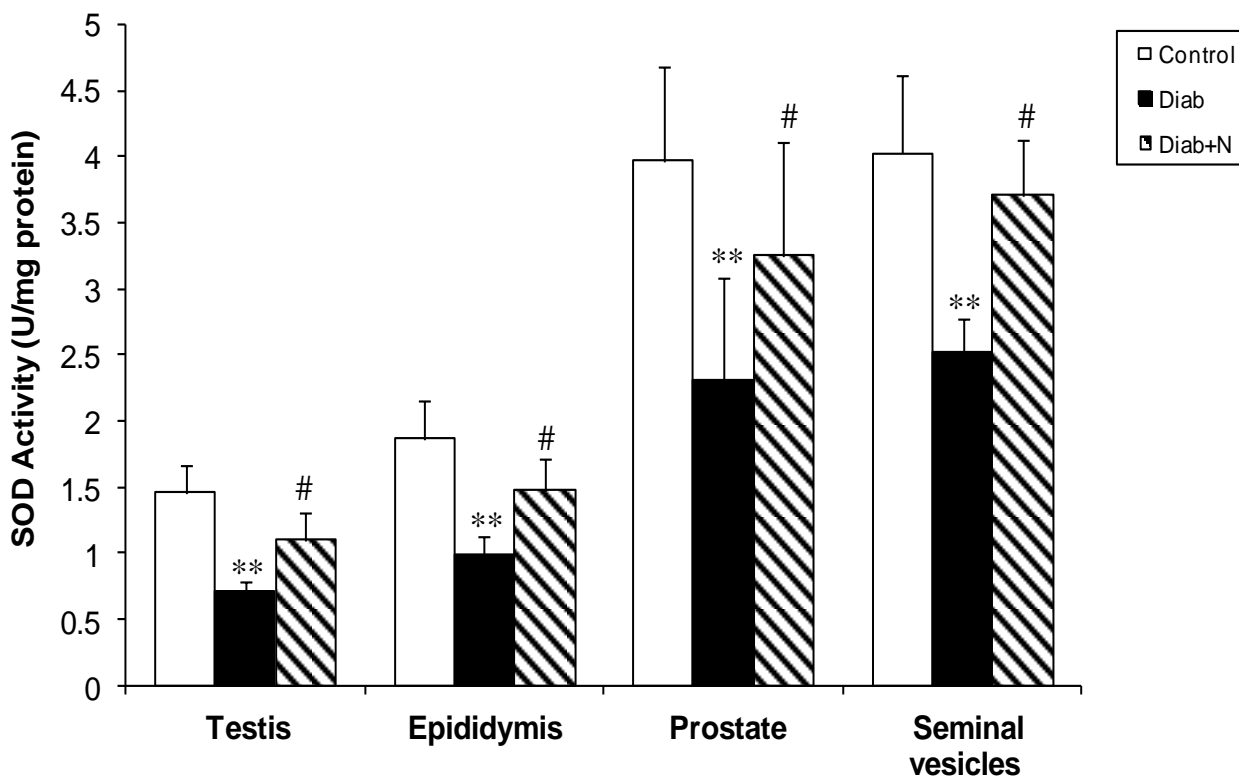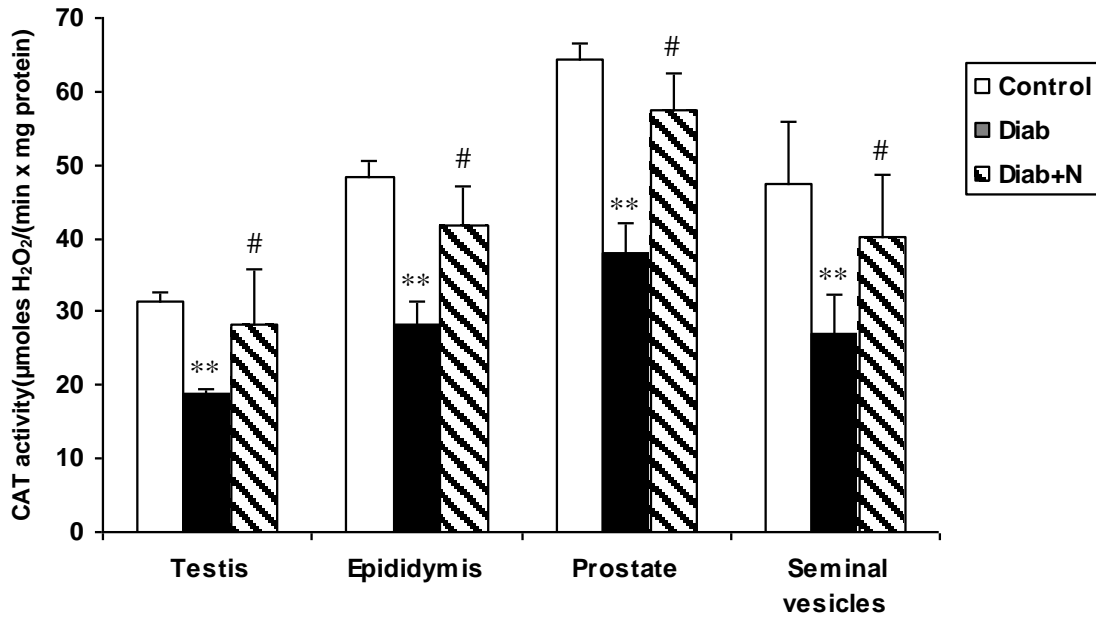

Figure 2. Variation of SOD activities in testis, epididymis, prostate and seminal vesicles of control, diabetic and diabetic treated rats with *Nigella sativa* seeds. Values are mean ± SD (n = 8). Diab: diabetic rats, Diab + N: diabetic t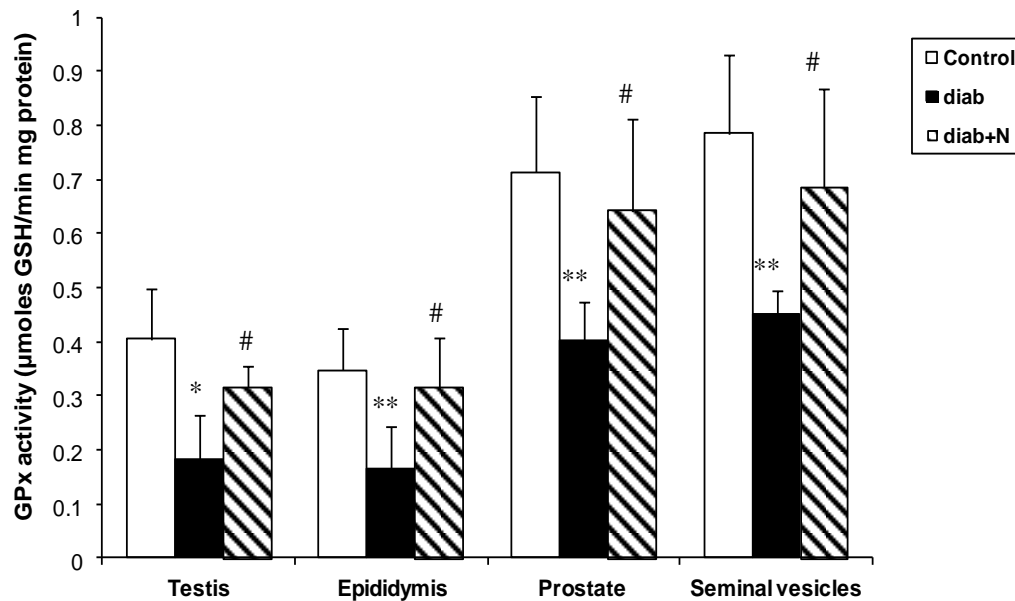reated rats with *Nigella sativa* seeds. Values are statistically ** $P < 0.01$ vs. control rats. # $P < 0.05$ vs. diabetic rats. Values are statistically ** $P < 0.01$ vs. control rats. # $P < 0.05$ vs. diabetic rats.

Figure 3. Variation of CAT activities in testis, epididymis, prostate and seminal vesicles of control, diabetic and diabetic treated rats with *Nigella sativa* seeds. Values are mean ± SD (n = 8). Diab: diabetic rats, Diab + N: diabetic treated rats with *Nigella sativa* seeds.

Figure 4. Variation of GPx activities in testis, epididymis, prostate and seminal vesicles of control, diabetic and diabetic treated rats with *Ni. sativa* seeds. Values are mean ± SD (n = 8). Diab: Diabetic rats; Diab + N: diabetic treated rats with *Nigella sativa* seeds. Values are statistically * p < 0.05; ** p < 0.01 vs. control rats. # p < 0.05 vs diabetic rats.

injury in rats (Kanter et al., 2006).

In conclusion, dietary supplementation of NNS induces favorable effects on fertility and the reproductive system in diabetic rats, and that the beneficial are attributable to its anti–oxidative and androgenic effects.

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