

Original Research Paper

## Biochemical Efficacy of *Nigella Sativa* Oil and Metformin on Induced Diabetic Male Rats

Yassmina K. Mahmoud, Sherif Y. Saleh, Abd El Rehim A. El Ghannam and Ibrahim A. Ibrahim

Department of Biochemistry, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

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Corresponding Author:  
Yassmina K. Mahmoud  
Department of Biochemistry,  
Faculty of Veterinary  
Medicine, Suez Canal  
University, Ismailia, Egypt  
Email: Yassmina\_kamal\_vet@hotmail.com

**Abstract:** This study was carried out to investigate possible beneficial effects of *Nigella sativa* oil supplementation to diabetic rats. Fifty male Sprague-Dawley rats were randomly divided into five groups (n= 10/group) as follows: Group I (normal control), group II (diabetic control), group III (diabetic rats treated with metformin), group IV (diabetic rats treated with metformin and *Nigella sativa* oil) and group V (diabetic rats treated with *Nigella sativa* oil). Insulin, testosterone, male gametogenic hormones, insulin resistance, serum lipid profile, testicular redox state, relative weight of testes, tail of epididymis, accessory sex glands (prostate and seminal gland), epididymal sperm count, alive sperm percent and abnormalities percent were estimated. Results revealed improvement in the studied parameters within group III, IV and V compared with non treated diabetic group with better improvement in group IV. Conclusion: Co-administration of metformin and *Nigella sativa* oil could result in better regulation of diabetes and diabetes induced subfertility in male rats due to hypoglycemic, hypolipidemic and antioxidant properties of *Nigella sativa*.

**Keywords:** Diabetes Mellitus, Fertility, Metformin, *Nigella Sativa*, Oxidative Stress

### Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia with disturbances in carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion, insulin action or both. Diabetic hyperglycemia is accompanied by damage, dysfunction of different organs especially the eyes, kidneys, nerves, heart and blood vessels (ADA, 2012).

Diabetes mellitus is classified into three types; type 1, type 2 and gestational diabetes (WHO, 2013). Type 1 is usually caused by an auto-immune reaction in insulin producing cells (IDF, 2013). Type 2 is ranging from insulin resistance to insulin deficiency which is the most common type of diabetes mellitus (ADA, 2014). Gestational diabetes (GDM) is hyperglycemia with onset or first recognition during pregnancy (WHO, 2013) and usually disappears after pregnancy (IDF, 2013).

Diabetic patients and experimental diabetic animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia which promotes free radical generation leading to depletion of the activity of the antioxidant defense system and development of oxidative stress (Baynes and Thorpe, 1996).

Reproductive dysfunction is recognized as a consequence of diabetes mellitus (Jamaludin *et al.*, 2012) represented in decrease in libido, impotence and infertility (Jiang, 1996). Moreover, poor semen quality has also been reported in diabetic men including decreased sperm motility, sperm concentration, increased abnormal sperm morphology (Ramalho-Santos *et al.*, 2008) and reduced levels of testicular hormone (Pitteloud *et al.*, 2005).

Consequently, effective management of diabetes requires sustained glycemic control to lower the risk of diabetic complications (Stratton *et al.*, 2000). There are different classes of anti-diabetic drugs and their preference depends on the nature of diabetes, age and situation of the person. Therefore treatment of Type 2 diabetes must include agent which lower blood glucose by increasing the amount of pancreatic insulin secretion, increase sensitivity of target organs to insulin or decrease the rate at which glucose is absorbed from the gastrointestinal tract (Rendell, 2004). Amongst diabetic drugs metformin, a biguanide, has become the most commonly used (Foretz *et al.*, 2010) and the first drug of choice for treatment of type 2 diabetes mellitus (Rotella *et al.*, 2006).

Increased utilization of medicinal plants became a World Health Organization policy in 1970 (De Smet, 1997). *Nigella sativa* is an annual flowering plant belongs to the family Ranunculaceae. It is cultivated mainly in countries bordering the Mediterranean Sea, middle Europe and western Asia (Rchid *et al.*, 2004). It contains a complex mixture of more than 100 compounds (Salih *et al.*, 2009). Most of the therapeutic properties of *Nigella sativa* are due to the presence of the polyphenol Thymoquinone (TQ) (Ahmad *et al.*, 2013) which is the major component (28-57%) of *Nigella sativa* oil (Ali and Blunden, 2003). In addition, there are many fatty acids. The most important of which are linoleic acid (55.6%), oleic acid (23.4%) and palmitic acid (12.5%) (Nickavar *et al.*, 2003). *Nigella sativa* and TQ have been known by their hypoglycemic (Pari *et al.*, 2010), hypolipidemic (Al-Logmani and Zari, 2011) and radical scavenging activity (El-Dakhkhny *et al.*, 2002).

Consequently the aim of this study was to investigate the effect of *Nigella sativa* oil, metformin and their combination on fertility of streptozotocin induced diabetic male rats.

## Materials and Methods

### Animals and Housing

Fifty male Sprague-Dawley rats weighing 190-230 g were purchased from Laboratory Animal House, Faculty of Veterinary Medicine, Suez Canal University. Rats were maintained on standard laboratory diet and fresh water *ad libitum*.

### Experimental Design

Group I: n= 10, control non diabetic rats, received daily dose of distilled water. Group II: n= 10, control Diabetic rats, received daily dose of distilled water. Group III: n= 10, diabetic rats treated with metformin at a dose 350 mg/kg bw/day (Figuroa-Valverde *et al.*, 2012). Group IV: n= 10, diabetic rats treated with metformin at a dose 350 mg/kg bw/day (Figuroa-Valverde *et al.*, 2012) and *Nigella sativa* oil at a dose 1 mL/kg bw/day (Zaoui *et al.*, 2002). Group V: n= 10, diabetic rats treated with *Nigella sativa* oil at a dose 1 mL/kg bw/day (Zaoui *et al.*, 2002). Treatments were administered once daily by oral gavage for 64 days after induction of diabetes mellitus.

### Induction of Diabetes Mellitus

Diabetes was induced in overnight fasted experimental groups (II, III, IV and V) by a single intraperitoneal (i.p) injection of freshly prepared Streptozotocin (STZ) (CAS 18883-66-4, TUKU-E company, USA) in a dose of 45 mg kg<sup>-1</sup> b.w dissolved in 0.1 M citrate buffer (pH 4.5) according to (Davoud *et al.*, 2011). The animals were allowed to drink 20% glucose solution for 24 h (Chandramohan *et al.*, 2009) to avoid hypoglycemia

(Moreira *et al.*, 2005). Control rats were injected with the same volume of citrate buffer (Adeyemi *et al.*, 2010). Diabetes was confirmed 48 h after injection of STZ where plasma glucose was determined.

### Sampling

#### Blood Samples

At the end of the experimental period, blood samples were collected from 10 h fasted rats from retro orbital venous plexus under effect of light ether anesthesia. Blood was divided into 2 tubes; fluoride tubes for separation of plasma and plain tubes for serum separation. Both plasma and sera were stored at -20°C till estimation of biochemical parameters.

#### Tissue Specimens

After sacrifice, testes, tail of epididymis and accessory sex glands (prostate and seminal gland) were dissected quickly then weighed. Weights were expressed as a percent in relation to the body weight.

#### Serum Biochemical Analysis

##### Insulin, Testosterone and FSH ELISA

Insulin, testosterone and male gametogenic hormones (FSH) were determined by Enzyme-Linked Immunosorbent Assay (ELISA) using commercial rat specific ELISA kits (Cat. No. KA3811, Abnova, Germany), (Cat. No. KA3811, DSI, Italy) and (Cat. No. E0830r, EIAab, China) respectively according to manufacturer protocol.

##### Fasting Blood Glucose Level

Plasma glucose level was determined by enzymatic calorimetric method using glucose commercial kit (LOT No. 201940, Diamond Diagnostics, Egypt) according to manufacturer protocol.

##### Calculation of Homeostasis Model Assessment-Estimated Insulin Resistance (HOMA-IR)

HOMA-IR was calculated according to (Matthews *et al.*, 1985) formula:

$$\text{HOMA-IR} = \frac{\text{fasting insulin (U/L)} \times \text{fasting glucose (mg/dL)}}{405}$$

##### Serum Lipid Profile

Triacylglycerol (TAG), Total Cholesterol (TC) and High Density Lipoprotein-cholesterol (HDL-c). Their levels were determined by enzymatic calorimetric method, using commercial kits (LOT TRIG0203014, SPECTRUM, Egypt), (LOT CHOL0204014, SPECTRUM, Egypt) and (LOT HDLS0103014, SPECTRUM, Egypt) respectively according to manufacturer instructions.

Calculation of Low Density Lipoprotein-cholesterol (LDL-c). LDL-c was calculated according to

(Friedewald *et al.*, 1972) formula and described by (Davidson and Rosenson, 2009):

$$\text{Serum LDL-c (mg/dL)} = \text{TC} - \text{HDL-c} - \frac{\text{TAG}}{5}$$

### Testicular Redox State

Testicular level of Superoxide Dismutase (SOD) activity, catalase activity, glutathione reduced (GSH) concentration and Malondialdehyde (MDA) were determined by enzymatic calorimetric method, using commercial kits (Cat. No. SD 25 21, Biodiagnostic, Egypt), (Cat. No. GR 25 11, Biodiagnostic, Egypt), (Cat. No. GR 25 11, Biodiagnostic, Egypt) and (Cat. No. MD 25 29, Biodiagnostic, Egypt) respectively according to manufacturer instruction.

### Epididymal Sperm Evaluation

#### Epididymal Sperm Count

Sperms of the right side cauda of epididymis were counted by modified method of (Yokoi and Mayi, 2004).

#### Epididymal Alive Sperm Percent and Abnormalities Percent

A drop of epididymal contents from left epididymus of each rat was mixed with eosin and nigrosin stain. Two hundred sperms were randomly examined per slide at  $\times 400$  to detect alive sperm % (Bearden and Fuquay, 1980) and abnormalities % (Evans and Maxwell, 1987).

### Statistical Analysis

The obtained data were subjected to statistical analysis using SPSS program for analysis of data and COSTAT for determination of LSD. The level of statistical significance was taken as  $p < 0.05$ .

## Results

Table 1 showed that insulin hormone, testosterone hormone and FSH levels were markedly improved in groups III, IV and V than the diabetic group. However, their levels did not achieve that of the control group. Fasting blood glucose level, HOMA-IR, TAG, TC and LDL-c were significantly ( $p < 0.05$ ) decreased in groups III, IV and V compared with diabetic group but did not reach the control level except for group IV. On the other hand, HDL-c showed significant ( $p < 0.05$ ) elevation in groups III, IV and V compared with diabetic group. Moreover, its level in group IV reached that of the control.

The data revealed that testicular SOD activity, catalase activity and GSH concentration were significantly ( $p < 0.05$ ) increased in groups III, IV and V in comparison with the diabetic group. Their levels in group IV reached that of the control group whereas only GSH concentration in metformin treated group reached that of the control group. On the contrary, testicular MDA content was markedly declined in groups III, IV and V but none of them was able to achieve that of the control group (Table 2).

Table 3 revealed that treatment of rats with either metformin alone (III), *Nigella sativa* alone (V) or in combination (IV) resulted in significant ( $p < 0.05$ ) elevation of testes, epididymis tail, seminal and prostate glands relative weight. Also, sperm count and alive sperm % in tail of epididymis were markedly increased.

In addition, there was no significant difference in relative weight of testes in the different treated groups when compared with the control group. Moreover, group III and IV showed no significant difference in relative weight of epididymis tail in comparison with the control group.

Table 1. Serum insulin, fasting blood glucose level, serum lipid profile, testosterone and FSH at the end of the experimental period

Item	Insulin (pmol/L)	Fasting blood glucose level		TAG (mg/dL)	TC (mg/dL)	HDL-c (mg/dL)	LDL-c (mg/dL)	Testosterone (ng/mL)	FSH (IU/L)
		(mg/dL)	HOMA-IR						
Group I	6.30 <sup>a</sup> ±0.29	101.33 <sup>d</sup> ±4.32	0.2286 <sup>c</sup> ±0.019	65.83 <sup>d</sup> ±3.11	117.89 <sup>c</sup> ±4.92	43.99 <sup>a</sup> ±1.96	60.73 <sup>c</sup> ±3.35	3.37 <sup>a</sup> ±0.13	0.53 <sup>d</sup> ±0.04
Group II	3.39 <sup>d</sup> ±0.15	353.33 <sup>a</sup> ±14.43	0.4257 <sup>a</sup> ±0.026	100.59 <sup>a</sup> ±4.58	166.77 <sup>a</sup> ±6.67	29.73 <sup>c</sup> ±1.23	116.92 <sup>a</sup> ±6.23	0.48 <sup>c</sup> ±0.03	2.00 <sup>a</sup> ±0.08
Group III	4.84 <sup>bc</sup> ±0.28	146.50 <sup>c</sup> ±6.63	0.2517 <sup>bc</sup> ±0.023	78.02 <sup>b</sup> ±3.79	129.18 <sup>b</sup> ±6.12	35.70 <sup>b</sup> ±1.66	77.85 <sup>b</sup> ±4.97	1.98 <sup>c</sup> ±0.14	1.08 <sup>c</sup> ±0.05
Group IV	5.47 <sup>b</sup> ±0.21	118.00 <sup>d</sup> ±6.10	0.2291 <sup>c</sup> ±0.013	70.43 <sup>cd</sup> ±2.68	125.48 <sup>b</sup> ±5.38	40.85 <sup>ab</sup> ±1.73	70.54 <sup>bc</sup> ±5.07	2.72 <sup>b</sup> ±0.12	0.89 <sup>c</sup> ±0.06
Group V	4.69 <sup>c</sup> ±0.20	178.67 <sup>b</sup> ±7.15	0.2268 <sup>b</sup> ±0.014	85.76 <sup>b</sup> ±3.81	141.20 <sup>b</sup> ±7.78	38.06 <sup>b</sup> ±2.03	85.99 <sup>b</sup> ±6.95	1.04 <sup>d</sup> ±0.06	1.50 <sup>b</sup> ±0.12

Values represent mean  $\pm$  SE

Means with different letter in the same column are significant at  $p < 0.05$  while means with the same letter in the same column are non significant at  $p < 0.05$

Table 2. Testicular SOD activity, catalase activity, GSH concentration and MDA content at the end of the experimental period

Item	SOD activity (U/g testes)	Catalase activity (U/g testes)	GSH concentration (mg/g testes)	MDA content (nmol/g testes)
Group I	1221.72 <sup>a</sup> ±52.24	90.94 <sup>a</sup> ±4.63	46.96 <sup>a</sup> ±2.1	108.12 <sup>d</sup> ±4.23
Group II	677.16 <sup>c</sup> ±35.34	42.95 <sup>c</sup> ±2.27	8.30 <sup>c</sup> ±0.43	537.76 <sup>a</sup> ±28.28
Group III	940.36 <sup>b</sup> ±39.56	85.04 <sup>ab</sup> ±3.93	34.45 <sup>b</sup> ±1.93	177.44 <sup>bc</sup> ±9.36
Group IV	1135.29 <sup>b</sup> ±49.74	88.77 <sup>ab</sup> ±5.06	42.03 <sup>a</sup> ±2.28	152.49 <sup>c</sup> ±7.19
Group V	877.69 <sup>b</sup> ±35.12	77.09 <sup>b</sup> ±4.03	31.34 <sup>b</sup> ±1.39	199.28 <sup>b</sup> ±8.08

Values represent mean  $\pm$  SE

Means with different letter in the same column are significant at  $p < 0.05$  while means with the same letter in the same column are non significant at  $p < 0.05$

Table 3. Relative weight of testes, epididymis, seminal gland and prostate gland, epididymal sperm count, alive sperm % and sperm abnormalities % at the end of the experimental period

Item	Relative weight of testes (g%)	Relative weight of epididymis tail (g%)	Relative weight of seminal gland (g%)	Relative weight of prostate gland (g%)	Epididymal sperm count (125×10000/mL)	Epididymal alive sperm %	Epididymal sperm abnormalities %
Group I	0.63 <sup>a</sup> ±0.037	0.067 <sup>a</sup> ±0.003	0.353 <sup>a</sup> ±0.031	0.242 <sup>a</sup> ±0.010	157.14 <sup>a</sup> ±6.85	65.48 <sup>a</sup> ±2.98	10.67 <sup>a</sup> ±0.95
Group II	0.29 <sup>b</sup> ±0.021	0.027 <sup>c</sup> ±0.001	0.128 <sup>c</sup> ±0.011	0.048 <sup>c</sup> ±0.004	17.58 <sup>c</sup> ±0.87	17.07 <sup>c</sup> ±0.95	69.45 <sup>a</sup> ±3.61
Group III	0.55 <sup>a</sup> ±0.045	0.058 <sup>b</sup> ±0.002	0.296 <sup>b</sup> ±0.027	0.192 <sup>b</sup> ±0.009	119.50 <sup>b</sup> ±7.42	47.17 <sup>b</sup> ±1.97	26.26 <sup>b</sup> ±1.60
Group IV	0.60 <sup>a</sup> ±0.035	0.061 <sup>ab</sup> ±0.003	0.347 <sup>a</sup> ±0.030	0.204 <sup>b</sup> ±0.008	149.00 <sup>b</sup> ±6.03	51.17 <sup>b</sup> ±3.03	25.85 <sup>b</sup> ±1.45
Group V	0.53 <sup>a</sup> ±0.033	0.058 <sup>b</sup> ±0.002	0.289 <sup>b</sup> ±0.026	0.185 <sup>b</sup> ±0.007	126.17 <sup>b</sup> ±8.59	44.67 <sup>b</sup> ±1.96	30.31 <sup>b</sup> ±2.13

Values represent mean ± SE

Means with different letter in the same column are significant at  $p < 0.05$  while means with the same letter in the same column are non significant at  $p < 0.05$

Furthermore, group V showed no significant difference in relative weight of seminal gland and epididymal sperm count compared with the control group. In contrast, epididymal sperm abnormalities % was markedly decreased in groups III, IV and V when compared with the diabetic group. Only group IV could reached the percent displayed by the control group.

## Discussion

Infertility is a major health problem, both types of DM are well recognized to cause sexual dysfunction, which in turn also contributes to infertility (Agbaje *et al.*, 2007). Hyperglycemia causes structural and functional changes in target organs of diabetic patients (Cai *et al.*, 2000). Besides, there are detrimental effects of diabetic oxidative stress on testicular cellular integrity (Adewole *et al.*, 2007). In this study a medicinal and herbal protective strategy to alleviate the deleterious effect of diabetes on male fertility was examined.

Results revealed improvement in insulin hormone and fasting blood glucose levels in group III, IV and V than diabetic group (Table 1). The decrease in fasting glucose levels in group III and IV could be attributed to the ability of metformin to decrease hepatic glucose production, mainly by inhibiting gluconeogenesis (Hundal *et al.*, 2000). Also, metformin can reduce the overall rate of glycogenolysis and decrease the activity of hepatic glucose-6-phosphatase (G6Pas) (Wiernsperger and Bailey, 1999) which is a key enzyme in glucose homeostasis (Nordlie and Sukalski, 1985). Besides, it increase peripheral tissue insulin sensitivity (Kirpichnikov *et al.*, 2002). Moreover, metformin could restore normal secretory pattern in rat pancreatic islets whose function has been impaired (Piro *et al.*, 2012).

Improvement in serum insulin and decline in blood glucose levels in *Nigella sativa* treated groups could be explained by the effect of *Nigella sativa* itself. Where *Nigella sativa* could cause partial regeneration and proliferation of pancreatic  $\beta$ -cells leading to increase in insulin secretion (Mansi, 2005). Thus indicating the insulinotropic properties of *Nigella sativa* oil (Fararh *et al.*, 2002) which helps in glucose utilization by cells in glycolysis, tricarboxylic acid cycle, hexose monophosphate

shunt and glycogenesis pathways (Krishnamurthy *et al.*, 2012). Beside its effect in decreasing liver glucose production via gluconeogenesis (Fararh *et al.*, 2004) and decreasing intestinal glucose absorption (Meddah *et al.*, 2009). These results are augmented by the decrement in insulin resistance (expressed by HOMA-IR) in group III, IV and V.

Data showed significant ( $p < 0.05$ ) improvement in serum lipid profile in group III, IV and V compared with diabetic group (Table 1). This could be attributed to correction of abnormal glucose metabolism in metformin treated groups (DeFronzo and Goodman, 1995) as well as reduction in triglyceride levels caused by decreased hepatic synthesis of very low-density lipoprotein (Chehade and Mooradian, 2000). Moreover, Clinical studies suggest that promotion of insulin sensitivity by metformin is accompanied by reduction in plasma free fatty acid level in diabetic patients (Hundal *et al.*, 2000; Zhang *et al.*, 2009).

The amelioration of diabetic dyslipidemia associated with *Nigella sativa* supplementation is caused by its ability to stimulate insulin secretion and action (Fararh *et al.*, 2002). Also, flavanoids are thought to enhance the efficiency of liver cells to remove LDL from the blood circulation by increasing LDL receptor densities in the liver and binding to apolipoprotein (El-Beshbishy *et al.*, 2006). Moreover, *Nigella sativa* is able to regulate cholesterol synthesis through regulation of HMG-CoA reductase, Apo-A1, Apo-B100 and LDL-receptor genes, (Ismail *et al.*, 2010).

Data of the present study demonstrated significant ( $p < 0.05$ ) improvement in testicular Redox state and testosterone hormone in group III, IV and V compared with the diabetic group, whereas male gametogenic hormone (FSH) was markedly decreased (Table 2 and 3).

This could be attributed to reduction of ROS formation by metformin therapy suggesting diminishing effect of oxidative stress (Chakraborty *et al.*, 2011). Metformin improved testicular redox state by possessing hypoglycemic effect thus decrease the production of superoxide radicals and other reactive oxygen species. Consequently, Leydig cell structure and function is preserved. Moreover, elevated insulin level and improved insulin sensitivity may be also involved in

restoring structure and function of Leydig cells. This leads to elevation of testosterone hormone with subsequent decrease in FSH where testosterone has negative feedback effect on FSH (Dickey and Swanson, 1998; Shimon *et al.*, 2006).

*Nigella sativa* oil has the ability to protect testis against oxidative stress possibly through antioxidant effects of its bioactive compounds (Danladi *et al.*, 2013). Antioxidant property of TQ is attributed to the quinone structure of TQ molecule (Gokce *et al.*, 2011) and the easy access to sub cellular compartments thus facilitating the ROS scavenging effect (Badary *et al.*, 2000). Also, TQ was shown to inhibit non-enzymatic lipid peroxidation (Ismail *et al.*, 2010). This leads to decreased oxidative stress and protection of the antioxidant enzymes of testis (Danladi *et al.*, 2013). Moreover, the hypoglycemic effect of *Nigella sativa* oil adds more to its antioxidant effect by decreasing ROS production due to lowering glucose circulating in the blood stream. In addition, *Nigella sativa* oil increase the number of Leydig cells in rat testes (Mukhallad *et al.*, 2009) beside the presence of unsaturated fatty acids in *Nigella sativa* oil that stimulate 17  $\beta$ -hydroxysteroid dehydrogenase activity (Gromadzka-Ostrowska *et al.*, 2002), thus increasing testosterone level.

Results of the present work revealed significant ( $p < 0.05$ ) improvement in relative weight of testes, epididymis tail, seminal and prostate glands, epididymal sperm count, alive sperm % and sperm abnormalities % in group III, IV and V (Table 3). These findings could be clarified by the significant ( $p < 0.05$ ) increase in testosterone hormone that could increase the number and function of somatic and germinal cells of testis and resulted in increase the testis and epididymis weight as well as stimulates spermatogenesis by Sertoli cells (Parandin *et al.*, 2012). Moreover, reduced sperm counts and motility and higher incidence of abnormal sperm occurred in diabetes may be interpreted to a combined effect of reduced testosterone hormone (Tanaka *et al.*, 2001) and oxidative stress associated with diabetes (Attia *et al.*, 2009). Also, high plasma level of cholesterol or triglycerides was associated with poor semen quality and direct adverse effects on testicular function (Bashandy, 2007). These improvements in treated groups could be due to ameliorating of metformin, *Nigella sativa* and their combination to the previously mentioned effects (Chakraborty *et al.*, 2011; McLachlan *et al.*, 2002).

In addition, there might be direct effects of insulin on sperm quality. *In vitro* administration of insulin to retrieved epididymal spermatozoa leads to restoration of their motility to the of normal levels, suggesting a direct effect on spermatozoa due to defective carbohydrate metabolism. Studies have reported that insulin as well as Insulin-like Growth Factor I (IGF-I) and IGF-II promote the differentiation of spermatozoa into primary spermatocytes by binding to the IGF-I receptor (Nakayama *et al.*, 1999). Evidence also suggests that

both the sperm membrane and the acrosome represent cytological targets for insulin (Silvestroni *et al.*, 1992).

Data obtained in the present work revealed that using a combination of metformin (350 mg/kg body weight) and *Nigella sativa* (1 mL/kg body weight) (group IV) was more effective in improving the studied parameters than using either metformin alone (III) or *Nigella sativa* alone (V). This could be attributed to the synergism between metformin and *Nigella sativa* oil in regeneration of pancreatic  $\beta$ -cells and the subsequent improvement in glycemic control in addition to the antioxidant and hypolipidemic effects of *Nigella sativa* which leads to adjustment of the redox state of the diabetic rats and decrease the oxidative damage of the testes leading to marked improvement in semen quality.

## Conclusion

From the obtained results it could be concluded that *Nigella sativa* oil exerts therapeutic effect on diabetes and diminish the side effects of diabetes on the reproductive system and semen quality by preserving pancreatic beta-cell integrity, decreasing oxidative stress and improvement of lipid profile. Consequently, administration of metformin and *Nigella sativa* oil resulted in better regulation of diabetes and its related subfertility compared with metformin alone. So *Nigella sativa* oil can be used as an add therapy to conventional diabetic drugs for better glycemic control and enhancement of the fertility state of the diabetic patients.

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## Author's Contributions

All authors equally contributed in this work.

## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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