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**International Journal of Pharmaceutical Research & Allied Sciences, 2019, 8(2):48-57**


**Research Article**

**ISSN : 2277-3657**
**CODEN(USA) : IJPRPM**

# *Preventive and Curative Effects of Metformin, Nigella sativa, Punica granatum and Zingiber officinale on Male Reproductive Dysfunction in Diabetic Rats*

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## ABSTRACT

*Diabetes mellitus is a metabolic disorder, that affects almost all the cells of the body. Well documented complications of DM include, neuropathy, nephropathy, microangiopathy, and retinopathy. The negative impact of the disorder is also found in other cells, including male reproductive system. In this study, fifty adult Wistar albino male rats were used. The rats were divided into 10 groups, each group comprised of 5 rats. Diabetes was induced by intraperitoneal injection of STZ, effects of DM were observed on the sperm producing cells then preventive as well as curative effects of Metformin, Punica granatum, Nigella sativa and Zingiber officinale were observed. It was found that all the substances prevent as well as repair/cure the damage caused by DM to the seminiferous tubules and other structures. The results of this study show that Metformin though prevented and caused repair to the damaged cells as well, but was not as effective as Punica granatum, Nigella sativa and Zingiber officinale.*

**Key words:** *Diabetes Mellitus, Metformin, Punica Granatum, Nigella Sativa, Zingiber Officinale, Infertility.*

## INTRODUCTION

Diabetes mellitus (DM) is a chronic, complicated metabolic disorder characterized by hyperglycemia, which often results from defects in insulin secretion, insulin action, or both. Moreover, DM is associated with severe disturbances of carbohydrate, fat, and protein metabolism [1].

World Health Organization detailed that Saudi Arabia positions seventh in the world for the diabetes rates. Roughly seven million of the community in Saudi Arabia are diabetic and around 3 million are pre-diabetic. In fact, diabetes has roughly increased ten times among the Saudi population in the previous three decades [2].

DM-induced unfavorable effects on male reproductive system, this might be attributed to hormonal alterations in the hypothalamic-pituitary-gonadal axis or through the immediate interactions of insulin with the testes and sperm cells, as both the testes and sperms themselves produce insulin [3]. Insulin expression in the testes also seems to be influenced by diabetes [4].

Diabetes mellitus are correlated with different complications, with the inclusion of oxidative stress (anti-oxidant/oxidant imbalance) as the major factor that is responsible for these complications [5]. The oxidative stress in the reproductive tissues forms the basis of reproductive system failure observed in individuals with DM [6]. Diabetes Mellitus induces molecular alterations which negatively affect sperm quality and function as well as fertility [6].

Metformin (dimethylbiguanide) is a drug that acts by the reduction of blood glucose level and improvement of glucose tolerance without altering the plasma insulin profile [7, 8].

Direct effects of metformin include the inhibition of the activity of respiratory electron transport chain in mitochondria [9] as well as activation of cytoplasmic protein kinase referred to as AMP activated protein kinase (AMPK) [10]. AMPK is an important sensor of cellular energy homeostasis and is sensitive to the AMP:ATP ratio [11, 12].

A number of in vitro studies have revealed positive effects of metformin on sperm parameters improvement, which involved improvement of the quality (motility and morphology) of frozen sperm [13], and promoted motility and viability of chicken sperm production [14, 15].

*Nigella sativa* is an annual herb belonging to the Ranunculaceae family, which is found in the countries surrounding the Mediterranean Sea, Pakistan and India. This plant is famous in Arab countries and other parts of the Mediterranean region; it has been used as a natural remedy [16, 17].

*Nigella sativa* seeds are used broadly in traditional medicine in many countries for the treatment of numerous ailments, that is due to the reported antiviral, anti-inflammatory, anti-schistosomiasis and immunomodulatory activities [18]. Many experiments confirmed that, most of the therapeutic properties of *N. sativa* are due to thymoquinone, which is the major active component of *Nigella sativa* oil [19].

Experimental studies using *Nigella sativa* seeds found an increase in reproductive organ weight, sperm motility, and count in cauda epidydimides and testicular ducts. Spermatogenesis increases at the primary and secondary spermatocyte [20, 21].

Protection of testis against oxidative stress may be attributed to the antioxidant effects of the bioactive compounds found in *Nigella sativa* oil [22]. Thymoquinone acts as inhibitor of non-enzymatic lipid peroxidation. This helps in decreasing oxidative stress and protection of the antioxidant enzymes of testis [23].

*Punica granatum* (Punicaceae) is a nutrient dense fruit rich in phytochemical compounds [24]. Many investigators have documented that pomegranate and its extracts have a marked antioxidant activity and free radical scavenger effect [25].

Several studies showed that pomegranate had a positive effect on the reproductive system potency of male rats [26-28].

Ginger *(Zingiber officinale)* is a plant which is familiar with traditional alternative medicine and is used to treat a number of diseases [29]. Gingerols and zingerone have been noticed as two important active constituents of ginger that have various biological effects [30].

Some of ginger constituents have been shown to control anti-inflammatory in addition to antioxidant effects and antihyperglycemic effects [31, 32].

Ginger was reported to have positive effects on sperms sperm mobility and viability and could be beneficial to maintain healthy male reproductive system [33].

## MATERIALS AND METHODS:

### Plant Material:

Seeds of *Nigella sativa,* Ginger (*Zingiber officinale*), and Pomegranate were acquired from the regional market and the mandatory preparations were performed in the perusal of the set research methodology**.**

### Animals:

Fifty adult Wistar albino male rats, of 8 weeks old and weighing 250±10g, were obtained from the animal house in faculty of pharmacy, Northern Border University. Male rats were housed in temperature controlled rooms (25°C) with constant humidity (40–70%) and 12h/12h light/ dark cycle prior to be used in the experimental protocols. All animals were treated in accordance with the Principles of Laboratory Animal Care.

The experimental protocol approval was taken from the ethics committee, in accordance with the rules and regulations for the care and use of laboratory animals prepared by Deanship of Scientific Research at Northern Border University.

**Induction of Diabetes with STZ:**

Diabetes was induced by a single intra peritoneal (i.p.) injection of streptozotocin (STZ, Sigma-Aldrich, St Louis, MO, USA) in 0.1 M citrate buffer (pH 4.0) at a dose of 60 mg/kg body weight [34]. Blood glucose concentration and changes in body weight were monitored regularly.

The Wistar male rats will be divided into 10 groups comprising five animals in each group as follows:

- Group 1: Control rats will be given only 5cc Normal saline (0.9% NaCl).
- Group2: Control rats will be given ginger (100mg/kg/rat) daily.
- Group 3: Control rats will be given *Nigella Sativa* (80 mg/kg).
- Group 4: Control rats will be given Pomegranate.500mg/rat/day
- Group 5 will be given Metformin 150 mg/kg/day
- Group 6: Diabetic control (55 mg/kg, single intra peritoneal injection of STZ)
- Group 7: Diabetic group (55 mg/kg, single intra peritoneal injection of STZ) will receive 100mg/kg/day ginger.
- Group 8: Diabetic group (55 mg/kg, single intra peritoneal injection of STZ) *Nigella Sativa* 80 mg/kg/day
- Group9: Diabetic group (55 mg/kg, single intra peritoneal injection of STZ) received Pomegranate). 500mg/rat/day
- Group 10: Diabetic group (55 mg/kg, single intra peritoneal injection of STZ) will receive Metformin150mg/kg/day.

**Histological Examination:**

The histological examination was done according to the standard protocol of clinical pharmacy department, Northern border university.

Animals were given anesthesia with diethyl ether and were sacrificed by cervical dislocation then perfused with 10% neutral-buffered formalin. Epididymis, testis, epididymis and seminal vesicle were taken out, weighed, trimmed and fixed in Bouin overnight, after that were processed to obtain paraffin blocks. Serial paraffin sections were prepared, 5 μm thickness and stained with hematoxylin and eosin (H & E) and Masson trichrome (MT) as described by Drury and Wallington [35].

**RESULTS:**

**Histopathological findings:**

The testis of the negative control and all the preventive group animals show normal**,** seminiferous tubules, normal orderly maturing germ cells, arranged from base to lumen and interstitium also appear normal (Figures 1,2,3,4, and 5). Photomicrograph of the control testis (Hematoxylin and eosin stain) shows numerous seminiferous tubules. Within these seminiferous tubules spermatogonia, spermatocytes and spermatozoa are visible. Interstitial connective tissue and leydig cells are clear.


**Figure 1:** Seminiferous tubules of group 1 Negative control

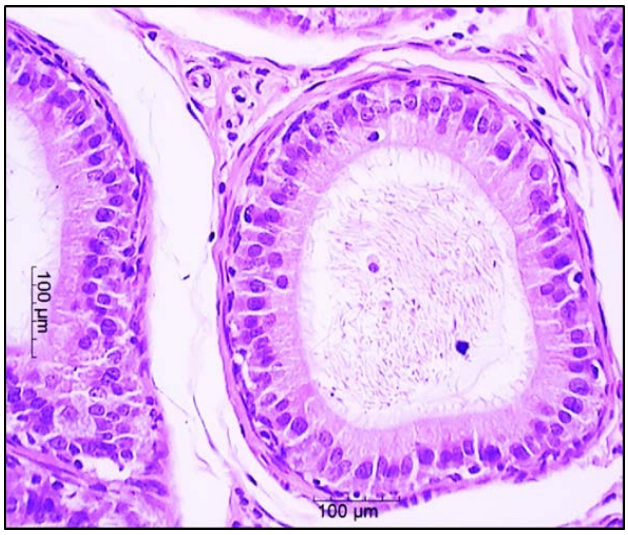


**Figure 2:** Coda Epididymis of group 2 (ginger)


**Figure 3**: Seminiferous tubules of group 3 (Nigella Sativa)

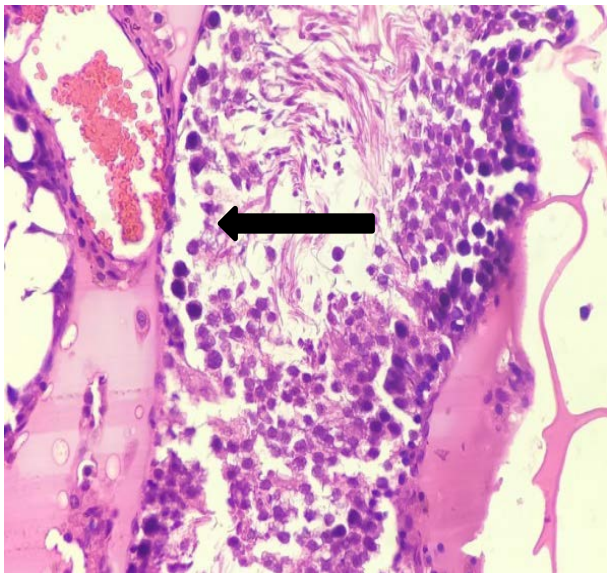

**Figure 4**: Seminiferous tubules of group 4 (Pomegranate)

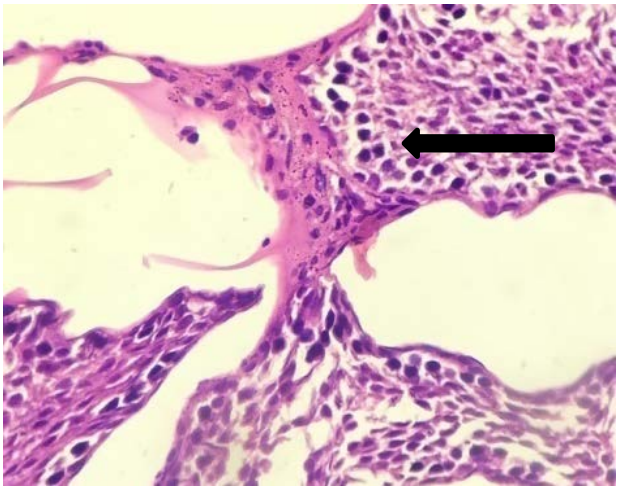

**Figure 5**: Seminiferous tubules of group 4 (Metformin)

In group 6 (DC) Photomicrograph of testis in diabetic rats (Hematoxylin and eosin) shows several seminiferous tubules. Spermatogonia and spermatocytes are decreased in number compared to the negative control. Spermatozoa are rare in the section. Leydig cells also appear to be decreased (Figure 6).

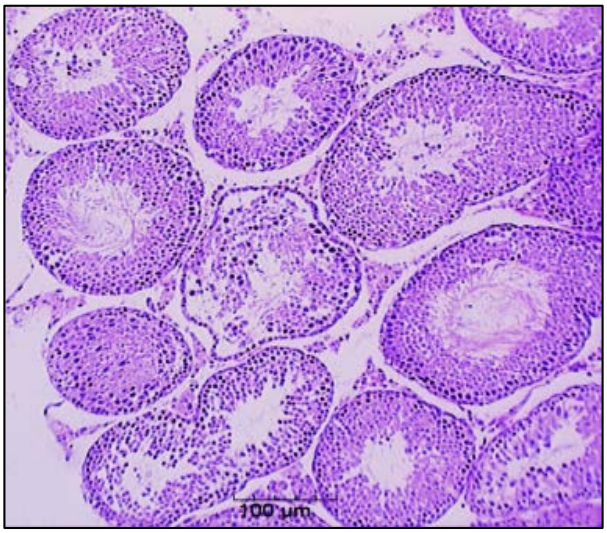


**Figure 6**: Testis in diabetic rats (group 6)

In the diabetic group (7) which was treated with Ginger, section studied shows number of small ducts, or ductules of the epididymis. Ductules are lined by ciliated tall columnar cells and each ductules is surrounded by smooth muscle. Interstitial connective tissue appears normal (Figure 7).

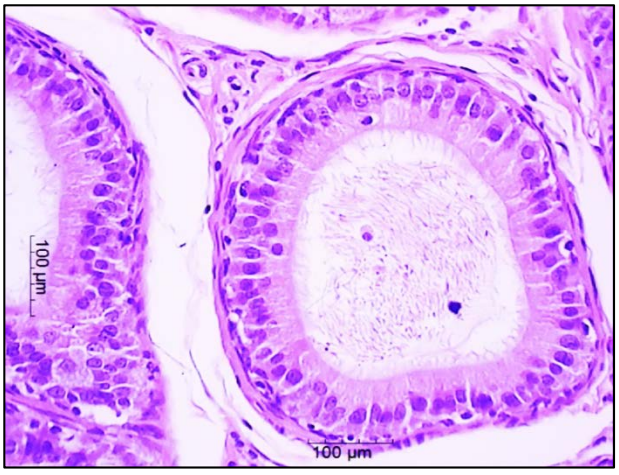


**Figure 7**: Testis in diabetic rats treated with Ginger (group 7)

In the diabetic group (8) which was treated with *Nigella sativa*, section studied shows number of small ducts, or ductules of the epididymis. Each ductule is lined by ciliated tall columnar cells and each ductules is surrounded by smooth muscle. No abnormality is seen (Figure 8).

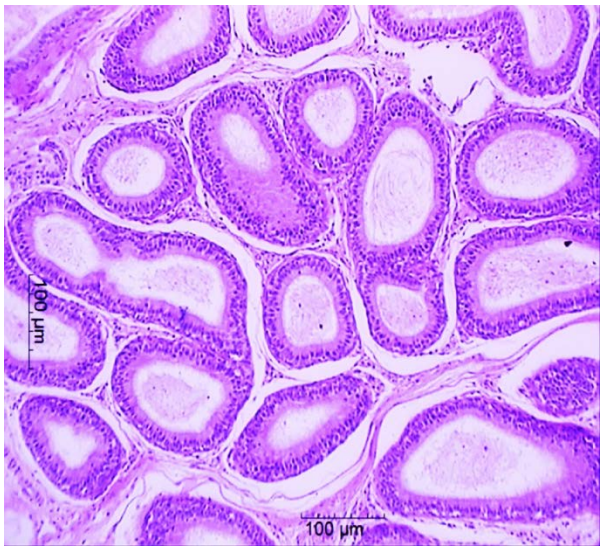


**Figure 8**: Testis in diabetic rats treated with Nigella sativa (group 8)

In the diabetic group (9) which was treated with pomegranate, photomicrograph of the control testis (Hematoxylin and eosin stain) shows numerous seminiferous tubules. Within these seminiferous tubules spermatogonia, spermatocytes and spermatozoa are visible. Interstitial connective tissue and leydig can also be seen appearing normal (Figure 9).

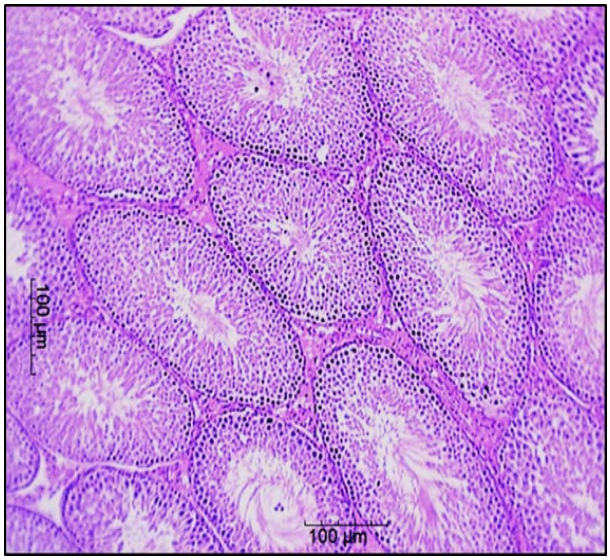


**Figure 9**: testis in diabetic rats treated with Pomegranate (group 9)

In the diabetic group (10) which was treated with Metformin, section studies show seminiferous tubules with interstitial leydig cells. Tubules showing normal spermatogonia, spermatocytes and spermatids. Mild reduction in the Interstitial cells of leydig is seen. Interstitium shows the less number of Sertoli cells and mild edema is clearly visible (Figure 10).

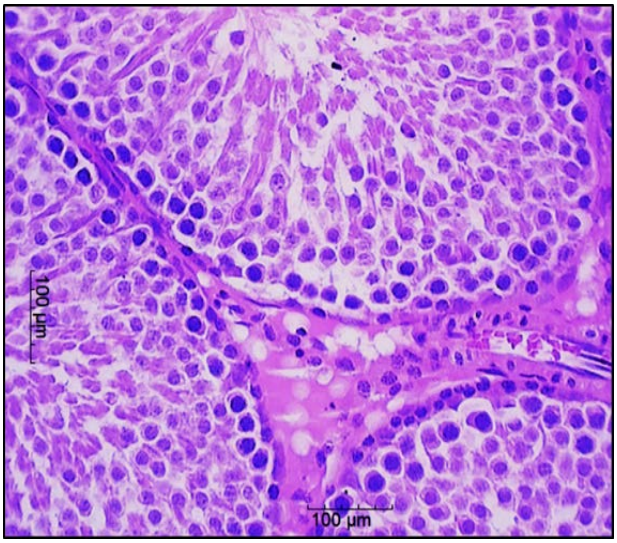


**Figure 10:** Testis in diabetic rats treated with metformin (group 10)

**DISCUSSION:**

Diabetes Mellitus is a condition that is related to oxidative stress and it causes damage to the majority of the vital organs and vessels, including brain and kidney. Reduction in fertility has also been observed in the Diabetics [36, 37]. The substances used in the study are known to contain anti-inflammatory and antioxidant effects at the same time they reduce insulin resistance [38].

The main effect of DM found on male reproductive system is the reduction in the number and motility of the sperms, which leads to infertility [39, 40] that is secondary to the damage caused by DM to sperm producing cells.

The current study results reveal that Metformin, *Nigella sativa*, Ginger and *Punica granatum* cause decrease in hyperglycemia at the same time prevent and repair the damaged sperm producing cells.

The results of our study regarding the effect of *Nigella sativa* on male reproductive system are in conformity with the studies conducted previously [41]. Increase in number as well as in motility of sperms was found in the diabetic rats and repair to the damage in the testis structure caused by diabetes was also found, that it correlates to the anti-inflammatory effect of *Nigella sativa* and its anti-oxidant constituents [42, 43].

In our study, it was discovered that Ginger (*Zingiber officinale*) alleviates the diabetes mellitus inflammation and damage to testicular morphology and improves the quality, number, and motility of the sperms. These effects may be due to decrease in oxidative stress and anti-inflammatory effects of ginger. The results of this study tally with the previous studies [44-46].

As *Punica granatum* is rich in flavonoids and antioxidants, so it is known to reduce oxidative stress [47]. In previous studies it has been found effective in male infertility, the results of our study showed that it improves the production of sperms and their quality as well. These results are similar to the studies conducted before [48, 49].

Metformin is very well known insulin sensitizer, its effects on insulin resistance are very much familiar. This drug is recommended by the American diabetic association for the prevention of type II DM and as first line therapy for treatment of Type II DM in obese patients [50, 51].

Some previous studies mention that Metformin prevents as well as cures diabetes induced injury to sperm producing cells and increases the number and quality of sperms [52-54].

However, the results of our study contradict the results of those studies. It was found that Metformin doesn't improve the inflammation in the sperm producing cells caused by STZ induced Diabetes in rats.

It would be premature to predict at this stage about the exact mechanism of actions for these preventive and curative effects, however, decrease in insulin resistance secondary to decrease in oxidative stress can be the possible mechanism, which has also been substantiated by the earlier research results.

**CONCLUSION:**

The results of our study show that the substances used in the study like *Punica granatum*, Ginger and *Nigella sativa* reduce the oxidative stress and inflammation, thereby, improve and repair the damage caused by Diabetes

Mellitus to the sperm producing cells. Improvement in the function of seminiferous tubules where sperms are produced, leads to increase in the production of increased number of sperms with good motility. In comparison to *Punica granatum*, Ginger, and *Nigella sativa*, Metformin was found less effective. It is highly suggested that, further studies to be conducted to verify the results of this study in combination with other biochemical and hormonal parameters. However, these substances can be used as adjuvant therapeutic agents for the management of male infertility and human clinical trials needed to be conducted before their use as anti-infertility agents.

**ACKNOWLEDGEMENT:**

The research project was approved and funded by Deanship of Scientific Research, Northern Border University, Saudi Arabia; grant NO (7273-PHM-2017-1-8-F).

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