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***Original Article***

# Physiological and Histological Effects of Ginseng Oil on Reproductive Efficiency in Adult Male Rats

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**Abstract**

Diabetes mellitus (DM) is a collection of metabolic illnesses known as chronic hyperglycaemia. It is one of the most common chronic diseases caused by insulin functions or secretions deficiency, which may cause carbohydrate and lipoprotein metabolism to be disrupted. The pituitary-gonadal axis malfunctions, testicular tissue dysfunctions and poor quality of sperms are all symptoms of DM, which is one of the most common causes of reproductive abnormalities. The current study has been designed to demonstrate the impacts of treatment with Ginseng oil oxidative stress-induced physiological and histological alterations in the male reproductive system of rats with subcutaneous (s/c) injection alloxan. The study was done on 30 mature male Wistar rats randomly divided into three equal groups (n=10). The first group, which served as the negative control, the second group (positive control) injection with (s/c) a single alloxan dosage (120 milligrams per kilogram of body weight), the third group was given alloxan and treated with ginseng oil (0.5cc at dosage (5 g /kg body weight daily) for 30 days. The percentage of live sperms increased significantly ($P \leq 0.05$) in the group that was given Ginseng oil orally compared to the alloxan group, the percentage of dead sperms and sperm abnormalities dropped, and the total sperm count was decreased. In the rat testis, (s/c) given alloxan (120 mg/kg), aberrant spermatids were present with a decrease in the sperm numbers in the lumens of seminiferous tubules, as well as a division in the irregular germ cells. The current study concluded that Ginseng oil exerted an antioxidant effect on the male reproductive system of rats injected with subcutaneous (s/c) alloxan.

**Keywords:** Ginseng oil, Reproductive system, Allox

## 1. Introduction

Diabetes mellitus (DM) is a collection of metabolic illnesses known as chronic hyperglycaemia. It is one of the most common chronic diseases caused by insulin functions or secretions deficiency, which may cause carbohydrate and lipoprotein metabolism to be disrupted (1). The pituitary-gonadal axis malfunctions, testicular tissue dysfunctions and poor quality of sperms are all symptoms of DM, which is one of the most common causes of reproductive abnormalities (2). In diabetic patients, the increased levels of reactive oxygen species (ROS) may reduce antioxidant enzyme function. Excessive production of reactive oxygen species (ROS) and a reduction of endogenous antioxidant defences leave tissues vulnerable to oxidative stresses, which can contribute to diabetes problems (3). Various natural antioxidants can help to reduce oxidative stress. As natural antioxidants, much attention has been given recently to herbs and spices, and several plant remedies have been shown to improve sperm parameters (4).

Ginseng is one of the most widely used herbal medicines; ginsenoside (saponin), polyacetylene,

polyphenolic chemicals, and acidic polysaccharides are among the medicinal ingredients ginsenosides are the most pharmaceutically active. Up to the present time, 38 ginsenosides were isolated from ginsengs, with 5 primary ginsenosides (Rb1, Rb2, Rc, Re & Rg1) accounting for > (80%) of total ginsenoside (5).

The major group among total phenolic components is flavonoids, which are in higher quantity in Ginseng (6). Ginseng reduces overall chromosomal aberrations, sperm abnormalities, and testosterone concentration while increasing sperm quantity and motility (5, 7). Hwang, Kim (8) found that Ginseng enhances the quality and lifespan of sperms in Guinea pigs exposed to tetrachloro-dibenzo P-dioxin (TCDD) and stimulates Spermatogenesis, according to research. Ginseng can also be a beneficial factor for protecting against the toxic impacts of the endocrine disrupter, particularly testicular toxicity (8).

The epididymal sperm counts in a ginseng-treated group were found in another study to be considerably higher for (56) days than its counts in the controls. Ginseng-enhanced glial cell-derived neurotrophic factor (GDNF). These findings point to a possible link between higher GDNF levels and Spermatogenesis. Ginseng also affects Spermatogenesis by inducing the expression of GDNF. Ginseng has been shown to lessen the adverse effects of cytostatic chemotherapeutics (9). Through that, Park, Shin (10) reported that Ginseng treatment caused the elevation of cAMP-responsive element modulator (CREM) mRNA with protein expressions in rats. Such findings suggest that Ginseng can lead to sperm total number and motility improvement via activating CREM, with no serious adverse effects (10).

The current study has been designed to demonstrate the impacts of treatment with Ginseng oil oxidative stress-induced physiological and histological alterations in the male reproductive system in rats.

## 2. Materials and Methods

### 2.1. Experimental Animals

The experiment was performed at the Veterinary Medicine College–University of Basrah's animal home. Wistar male rats (n=30) were sexually mature at 15-18 weeks, and weight (225±25gm) was used. They were kept under unified standard environmental conditions at a temperature of 24-28°C, and the humidity rate was about 50 %). They were fed standard pellets (protein 18.8%, barely 37%, corn 15 %) (ad libitum), and fresh, clean water was supplied at libitum. Animals were categorized into 3 groups at random, control of the first group, second and third groups injected with a single dose of alloxan subcutaneously (120 mg/kg body weight) (11), the third group treated with orally ginseng oil (5 g/kg/day) for 30 days.

### 2.2. Semen Fluid Analysis

#### 2.2.1. Sperm Analysis according to Adamkovicova, Toman (12)

After scarification, each animal's caudal epididymis was dissected and placed in a clean Petri dish with 5ml of rewarmed normal saline. Microsurgical scissors are used to cut the epididymis tissue into small pieces, which are then placed in a water bath for (5) minutes at (37°C) so that the sperms be able to swim out to the media and to allow efficient studying of the epididymal sperm characteristics (13).

#### 2.2.2. Sperm Concentration

The sperms were counted using a Neubauer hemocytometer chamber, previously used to count RBCs and WBCs. The epididymis was placed in a Petri plate with 5 mL 0.9 % normal saline. Using a sharp scalpel, cut the epididymis into 6-10 pieces. A clean piece of gauze was used to filter the suspension from the previous stage in the test tube. A drop from the filtrate was poured into Neubauers slide chamber, which was already covered with a cover slip. The sperms were counted on the five ruled squares for RBC counting with the objective lens (40×). In one $mm^3$, the sperms were computed according to the following equation:

$Sperm/mm^3 = N \times 10{,}000$ (N=Number of sperms in (5) squares)

#### 2.2.3. The sperm motility percentages

The motility of epididymal sperm has been determined using a principle of graduation proposed by

Chemineau, Cagnie (14) according to the following steps:

1. On a clean and warm slide, one drop of diluted epididymal sperm was placed and covered by a cover slip at 37°C.
2. The sperms were inspected under a microscope at a magnification of 40×.

The percentage is calculated based on the movement of progressive forward sperm, as well as the power and speed of their movement (Table 1).

**Table 1.** Strength and speed of sperm migration (14)

| Type of motion | Degree | Percentage |
|---|---|---|
| Sperms move rapidly and straightly. | 5 | (90–100) |
| Sperms move rapidly, and some of them with a circular movement | 4 | (75–85) |
| Sperms move on a straight slope, with no shivering movement | 3 | (45–65) |
| Some sperms move with the simple irregular shivering movement | 2 | (20–40) |
| Sperms move very slowly, with shivering and swinging tail | 1 | 10 |
| Sperms without movement | 0 | 0 |

### 2.2.4. Abnormality of Sperms

The percentage of abnormal sperms was counted on the slide, which was used for measuring the irritability in the epididymis via examining 200 sperms under the 100× lens of the light microscope (15). On a warm, clean slide, diluted sperm was dropped.

The warm Eosin–Nigrosine dye was applied to stain the semen and carefully mixed with a glass rod. The smear is performed by placing the slide in an angular position upon the semen's slide and dragging it in a horizontal position. For a while, the slide has been left for drying. A microscope has viewed the slide at a magnification of 40 times. Living sperms were shown in white, while dead ones were shown in red. The following substances were the Eosin- Nigrosine constituents: Eosin stain (1.67 g), Nigrosine stain (10 gm), Sodium citrate (2.9 g), and Distilled water (100 ml).

## 3. Results

The results of a recent study showed in table 2 that when rats were treated with alloxan (120 mg/kg), it caused a significant reduction ($P \leq 0.05$) as compared with the controls in terms of sperm count but, When compared to the alloxan group, the sperm count in the alloxan+Ginseng oil group increased significantly ($P \leq 0.05$). It was also discovered that compared to the control group, alloxan reduced enormous sperm motility substantially ($P \leq 0.05$). Massive sperm motility was dramatically increased in the alloxan+Ginseng oil group compared to the alloxan group.

In addition, as can be seen from the table, alloxan reduced individual sperm motility significantly ($P \leq 0.05$) compared to the controls and the Ginseng oil+alloxan group; individual sperm motility was much higher in the last group than in the alloxan group.

The alloxan group's dead sperm ratio increased significantly compared to the control and alloxan+ginseng oil groups ($P \leq 0.05$).In the alloxan+ginseng oil group, the dead sperm ratio was decreased significantly in comparison with alloxan groups at ($P \leq 0.05$).

It is also evident from the table 2 that alloxan caused the abnormal sperm ratio to increase significantly ($P \leq 0.05$) in comparison with the controls. In the alloxan+ginseng oil group, the abnormal sperm ratio decreased significantly compared to the alloxan group.

### 3.1. Histological Results

The testes in the control group had normal seminiferous tubule anatomy and Spermatogenesis (Figure 1). Figure 2 showed suppression of Spermatogenesis, some tubules without spermatozoa, another with few spermatozoa, poor Spermatogenesis, interstitial space enlargement and congestion.

While the histological section of rats that administrated ginseng oil and alloxan show seminiferous tubules with an increased number of primary and secondary spermatocytes (increase spermatocytes), as shown in figure 3.

**Table 2.** The effect of ginseng oil and alloxan on sperm viability of male rats

| Parameters / Groups | Sperm count ($n\times10^6/mm^3$) | Massive motility (%) | Individual motility (%) | Dead Sperm (%) | Abnormal Sperm (%) |
|---|---|---|---|---|---|
| Control | $195.2\pm0.3^A$ | $90\pm0.4^A$ | $90\pm0.6^A$ | $11\pm1.4^C$ | $12.5\pm2.1^C$ |
| Alloxan | $66.5\pm1.0^C$ | $10\pm0.7^C$ | $10\pm0.6^C$ | $96\pm1.4^A$ | $31\pm1.4^A$ |
| Alloxan+ginseng oil | $155.7\pm1.5^B$ | $77.5\pm3.5^B$ | $57.5\pm3.5^B$ | $32.5\pm3.5^B$ | $20\pm1.4^B$ |

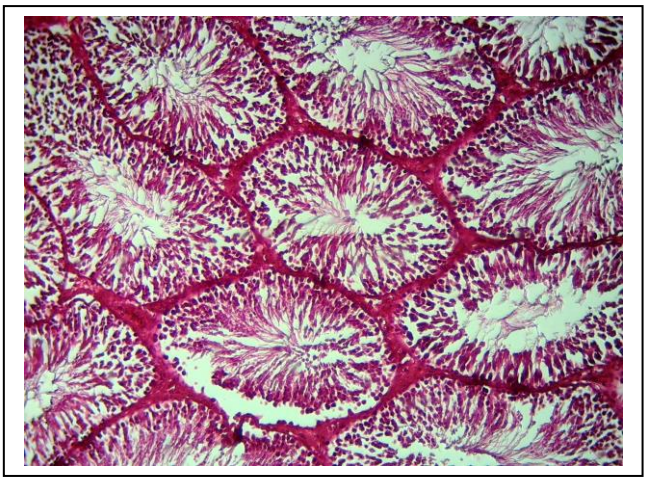

**Figure 1.** The histological tests structure of control, notice normal Spermatogenesis and presence of Spermatogenesis, 400×

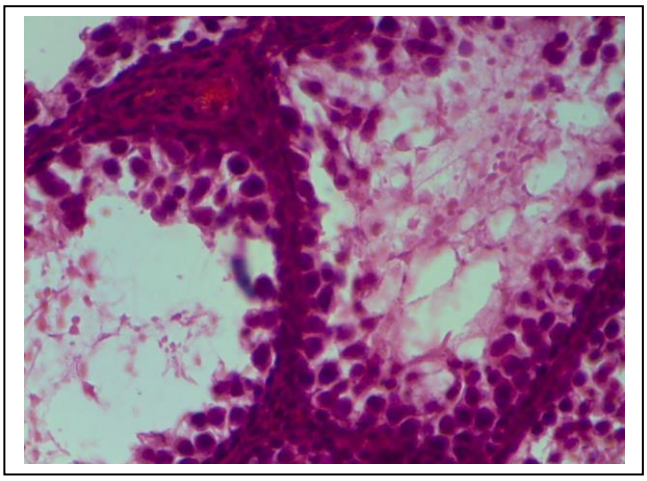

**Figure 2.** The histological tests structure of alloxan, Notice suppression of Spermatogenesis, 400×

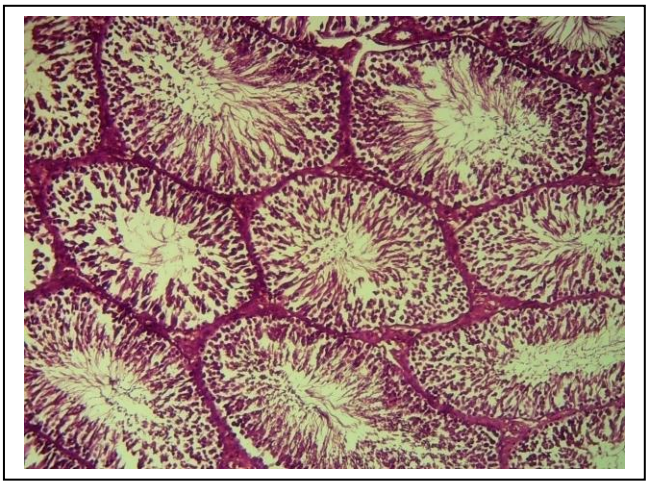

**Figure 3.** The histological tests structure of Ginseng oil with alloxan, Notice primary and secondary spermatocyte of Spermatogenesis, 400×

## 4. Discussion

Results of the current study in table 2 showed that the rats treated with alloxan had a significant ($P \leq 0.05$) decrease in total sperm counts, alive sperms, and individual sperm motility, whereas showing a significant ($P \leq 0.05$) increase in dead and abnormal sperm, when it compared with the control group these results agree with Green, Brand (16) which has the same results because of alloxan activity by producing free radicals which damage pancreas tissue resulting increase blood glucose as well as ROS in mitochondria converted to hydrogen peroxide the last ion react with hydroxyl group lead to damage the living cell.

In another study, diabetes has been linked to a decrease in pituitary-gonadal axis activity. It was also discovered that oxidative stress influenced germ cell death in adult diabetic rats' testicular tissue diabetes caused by insulin shortage (17).

Previous studies have shown that diabetes has a detrimental effect on the hypophysis-gonadal hormone axis, resulting in a decrease in gonadotropin and testosterone secretion, which causes spermatogenesis impairment and sperm motility defects (18).

On the other hand, our results of the current study showed the diabetic animals which administrated Ginseng oil had significantly increased ($P \leq 0.05$) in total sperm counts, alive sperms and individual sperm motility and a significant ($P \leq 0.05$) decrease in dead and abnormal sperm, as comparative with the alloxan group and these results agreed with Kumar, Sharma (7) and Hwang, Kim (8). They found that Ginseng protects the testis by lowering testicular acid phosphatase activity, lowering lipid peroxidation, and significantly enhancing alkaline phosphatase activity. Their ability to eliminate free radicals and protect the cell membrane from lipid peroxidations' and their influence on the endocrine system could explain their protective function (7, 8).

Ginseng has preventive and curative benefits on the damage of testis caused by ZEN, indicating that it could be a beneficial tool to avoid and treat any damage to the test caused by environmental pollutants (5). Hwang, Kim (8) stated that Ginseng enhances the lifespan as well as the quality of sperms of Guinea pigs exposed to tetrachloro-dibenzo P-dioxin (TCDD) and stimulates Spermatogenesis, according to research.

Histological results indicated testicular degeneration and suppression of Spermatogenesis due to alloxan activity; in diabetic groups, male sex cell numbers, Sertoli cells and Leydig cells are reduced. Furthermore, in people with diabetes, seminiferous tubule diameters, seminiferous epithelium heights and fertility rate was reduced. Diabetes can also raise the risk of degeneration of the seminiferous tubules. In diabetic rats, the sperm quality parameters and Sertoli cell number were significantly lower (2).

Furthermore, diabetic rat testes showed decreased seminiferous tubule width and tubular epithelium thickness (2). The spermatogenic cell decrease and seminiferous tube diameter reduction indicate failure of histopathological Spermatogenesis among rats with diabetes (7). Whereas the Ginseng oil administration repairs the damaged testicular tissue, Ginseng may provide significant protection against testicular damage caused by radiation. Ginseng supplementation before gamma radiation therapy effectively protected the germ cell population in mice from gamma radiation (7).

Depending on the present study, we conclude that all oxen group causes a decrease in the sperm count, massive sperm motility and individual motility while it causes an increase in the dead sperm ratio and abnormal sperm ratio. It is also evident that the alloxan+Ginseng oil group causes an increase the sperm count, massive sperm motility and individual motility while it causes a decrease in the dead sperm ratio and abnormal sperm ratio. It was observed in the histological section in the alloxan group change in tubules and interstitial space, and in the histological section of rats that administrated ginseng oil and alloxan group showed abnormal seminiferous tubules and spermatocytes (increased spermatocytes).

## Authors' Contribution

Study concept and design: A. A. M.
Acquisition of data: A. A. M.
Analysis and interpretation of data: B. F. H.
Drafting of the manuscript: A. S. M.
Critical revision of the manuscript for important intellectual content: A. S. M.
Statistical analysis: A. A. M.
Administrative, technical, and material support: A. A. M.

## Ethics

The Ethics Review Board at the Middle Technical University, Baghdad, Iraq.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

1. Zendjabil M. Biological diagnosis of diabetes mellitus. Curr Res Transl Med. 2016;64(1):49-52.
2. Gaunay G, Nagler HM, Stember DS. Reproductive sequelae of diabetes in male patients. Endocrinol Metab Clin. 2013;42(4):899-914.
3. Lushchak V. Free radicals, reactive oxygen species, oxidative stresses and their classifications. Ukr Biochem J. 2015:87(6):11-8.
4. Sinclair S. Male infertility: nutritional and environmental considerations. Altern Med Rev. 2000;5(1):28-38.
5. Hassan AM, Abdel-Wahhab MA. Antioxidant effect of parsley and panax ginseng extract standardized with ginsenosides Rg3 against alteration induced in reproductive functions in male mice. Egypt J Hosp Med. 2006;22(1):60-72.
6. Justesen U, Knuthsen P. Composition of flavonoids in fresh herbs and calculation of flavonoid intake by use of herbs in traditional Danish dishes. Food Chem. 2001;73(2):245-50.
7. Kumar M, Sharma MK, Saxena PS, Kumar A. Radioprotective effect of Panax ginseng on the phosphatases and lipid peroxidation level in testes of Swiss albino mice. Biol Pharm Bull. 2003;26(3):308-12.
8. Hwang SY, Kim WJ, Wee JJ, Choi JS, Kim SK. Panax ginseng improves survival and sperm quality in guinea pigs exposed to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. BJU Int. 2004;94(4):663-8.
9. Yang WM, Park SY, Kim HM, Park EH, Park SK, Chang MS. Effects of Panax ginseng on glial cell-derived neurotrophic factor (GDNF) expression and spermatogenesis in rats. Phytother Res. 2011;25(2):308-11.
10. Park WS, Shin DY, Yang WM, Chang MS, Park SK. Korean ginseng induces spermatogenesis in rats through the activation of cAMP-responsive element modulator (CREM). Fertil Steril. 2007;88(4):1000-2.
11. Mostafavinia A, Amini A, Ghorishi SK, Pouriran R, Bayat M. The effects of dosage and the routes of administrations of streptozotocin and alloxan on induction rate of typel diabetes mellitus and mortality rate in rats. Lab Anim Res. 2016;32(3):160-5.
12. Adamkovicova M, Toman R, Martiniakova M, Omelka R, Babosova R, Krajcovicova V, et al. Sperm motility and morphology changes in rats exposed to cadmium and diazinon. Reprod Biol Endocrinol. 2016;14(1):1-7.
13. Klinefelter GR, EarlGray Jr L, Suarez JD. The method of sperm collection significantly influences sperm motion parameters following ethane dimethanesulphonate administration in the rat. Reprod Toxicol. 1991;5(1):39-44.
14. Chemineau P, Cagnie Y, Guerin Y, Orgeur P, Vallet J. Manuel de formation pour l'insémination artificielle chez les ovins et les caprinsTraining manual on artificial insemination in sheep and goats: FAO, Roma, Italia. 1991.
15. Evan G, Maxwell W. Salamon's Artificial Insemination of sheep and goat. Butter woth, Sydney, Australia. 1987.
16. Green K, Brand MD, Murphy MP. Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes. Diabetes. 2004;(53):110-8.
17. Shoorei H, Khaki A, Khaki AA, Hemmati AA, Moghimian M, Shokoohi M. The ameliorative effect of carvacrol on oxidative stress and germ cell apoptosis in testicular tissue of adult diabetic rats. Biomed Pharmacother. 2019.
18. Bhattacharya SM, Ghosh M, Nandi N. Diabetes mellitus and abnormalities in semen analysis. J Obstet Gynaecol Res. 2014;40(1):167-71.