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Role Of Ginseng And Royal Jelly In Diminishing The Reproductive Toxicity Of Diabetic Male Rats

Hani M. Abdelsalam[1*], Abdelaziz A Diab[1], Atef G. Hussien[2], Josef A. Aziz[3] and Sondos M. Helmi[1]

[1] Department of Zoology, Faculty of Science, Zagazig University, Egypt.
[2] Department of Biochemistry, Faculty of Medicine, Zagazig University, Egypt.
[3] Department of Anatomy, Faculty of Medicine, Zagazig University, Egypt.

Corresponding Author: E-mail addresses: hmmsama@hotmail.com
Postal address: Faculty of Science, Zagazig University, Zagazig, Asharquia, Egypt. Postal code: 44519

ABSTRACT : **Panax ginseng is known for its antioxidant capabilities, which help to boost the immune system. Royal jelly (RJ) has been demonstrated to reduce oxidative stress damage in reproductive organs when used as an antioxidant. The goal of this study is to use ginseng and Royal jelly to reduce the toxicity of diabetes on male rats' reproductive functioning. Control, Diabetic, Diabetic + Ginseng (G) group, Diabetic + Royal jelly (RJ), and Diabetic + Ginseng + Royal jelly group are the five groups of forty male albino rats. Serum sexual hormones (FSH, LH, and testosterone) were measured biochemically, as well as semen analysis and testis sections from all groups for histological investigation. Our findings showed that after streptosotzin (STZ) administration, treatment with ginseng and Royal jelly caused a significant improvement in all biochemical parameters, with hormonal levels in the Diabetic + Ginseng, Diabetic + Royal jelly, and Diabetic + Ginseng + Royal jelly groups being restored to normal levels. Ginseng and Royal jelly also caused a significant increase in FSH, LH, and testosterone concentrations, despite these levels being significantly decreased by STZ. All sperm parameters (count, motility deformation, and morphology) improved after treatment with ginseng and Royal jelly, according to sperm analysis. A histopathological examination revealed that ginseng and Royal jelly can help restore the testis architecture after it has been distorted by STZ. Finally, ginseng and Royal jelly treatment can help to mitigate the unfavourable effects of STZ administration.**

KEYWORDS Royal Jelly- Ginseng- Diabetes- Sexual Hormones- Semen analysis

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I. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterised by persistent high blood sugar levels. In 2015, there were roughly 415 million diabetic patients globally, with that figure expected to rise to 642 million by 2040 [1]. Type 1 diabetes (T1D), type 2 diabetes (T2D), and others (e.g., Maturity-Onset Diabetes of the Young (MODY)) are the most common types of diabetes [2]. Diabetes mellitus has a negative impact on several organs, including the testis [3]. Diabetes causes the thickening of basal membranes of seminiferous tubules and blood vessel walls, the creation of big cells, and significant degenerative injury in testicular tissue of rats, according to Atalay Uslu et al [4].

Royal jelly (RJ) is a honeybee substance secreted by the worker honeybees' hypopharyngeal and mandibular glands, and it is a necessary sustenance for the queen and larvae during their first three days of life [5]. RJ has

previously been shown to have beneficial benefits on fertility, reproduction potential [6] and the prevention of reproductive failure [7].

Ginseng leaves have long been utilised in Korea as a folk medicine for diabetic treatment. Because most studies on the use of ginseng as a diabetes therapy focused on the roots rather than the leaves, little has been written about the anti-diabetic effectiveness and compounds found in ginseng leaves. Researchers studied hypoglycemic activities in diabetic rat models and concluded that ginsenosides are the primary source of these activities [8]. Surprisingly, rats given 5% Panax ginseng in their chow for 60 days had considerably higher blood testosterone levels, whereas rats given 1% Panax ginseng had no impact [9]. The principal active component of Panax ginseng, ginsenoside Rg1 (10 mg/kg), is responsible for the increase in serum testosterone levels and improvement in copulatory behaviour observed. 15 Ginsenoside Rb1 (10 g/kg), a major ginsenoside present in American ginseng, has been shown to increase LH secretion by directly acting on the anterior pituitary gland [10]. The usage of Asian ginseng extract significantly increased plasma total and free testosterone, follicle stimulating hormone, and LH levels in a clinical research including 66 patients [11].

II. MATERIAL AND METHOD

Animals and experiment design:

In this investigation, forty healthy male adult albino rats weighing 150-180 grammes were employed. The animals were procured from the Theoodor Bilharz Research Institute in Cairo, Egypt, and were kept in regular conditions with free access to food and water. All animals were kept in sanitary plastic cages in well-ventilated rooms with exhaust fans, fed a normal pellet diet, and had access to unlimited water. All animal procedures were carried out with the agreement of Egypt's National Research Center's Ethics Committee and in accordance with guidelines for the correct care and management of laboratory animals.

The rats were divided into five groups at random, each having eight rats:

1-Control group: To acquire the normal control references, the animals were left untreated.

2-Diabetic group: Animals were given 60 mg/kg body weight of streptozotocin (STZ) intraperitoneally for three days to induce diabetes.

3-Diabetic + Ginseng (G) group: Animals were given 60 mg/kg body weight streptozotocin intraperitoneally for three days to induce diabetes, then given powdered extracts of G dissolved in saline at a concentration of 300 mg/ml. The animals were given G solution orally for 30 days in a row.

4-Diabetic + Royal jelly (RJ) group: Animals were given streptozotocin (60 mg/kg body weight) in the intraperitoneal cavity for 3 days to develop diabetes, then gavaged with RJ (300 mg/kg/day) for 30 days.

5-Diabetic + Ginseng + Royal Jelly group: Animals were given streptozotocin (60 mg/kg body weight) for 3 days, then treated with G (300 mg/ml) and RJ (300 mg/kg/day) for 30 days.

Sample collection:

The rats were anaesthetized for 30 days before being murdered. The testis samples were taken out and kept away from the light. Samples of testis were washed three times in cold isotonic saline (0.9 percent v/w) and kept at (-20 °C) until analysis.

Blood was collected in test tubes from this dislocation, which was then centrifuged to remove serum, which was maintained in an Eppendorf tube in a deep freezer at (-20 °C).

Biochemical analysis:

Hormonal assay:

1-Determination of FSH and LH: [12]

The method for the quantitative determination of FSH is a sandwich chemiluminescence immunoassay.

2-Determination of Testosterone: [13]

The LIAISON® Testosterone assay's method for quantitative determination of testosterone is a direct, competitive, chemiluminescence immunoassay (CLIA).

Histopathological studies:

After decapitation Small portions of testis were removed from all groups of rats immediately and simultaneously and fixed in 10% neutral formalin solution, according to the procedure of [14].

Semen Analysis

After the dissection, the epididymis was collected as soon as possible and placed in a clean Petri dish. The cauda epididymis was then removed from the rest of the epididymis and sliced into numerous pieces, which were then immersed in 3 ml pre-warmed phosphate buffer saline (PBS) solution and incubated for 10 minutes at 37 °C to allow sperm to escape the epididymal lumen. The sperm suspension was pipetted numerous times and FT-IR spectrum analysis was performed on it [15].

Sperm count:

Epididymal sperm were obtained by slicing the cauda region of the epididymis into small pieces and soaking them in 1 ml human tubal fluid (HTF)+4 mg/ml bovine serum albumin (BSA) for 30 minutes at 37°C in 5%

CO2. A hemocytometer was used to count the sperm. The results were expressed in millions of sperm per millilitre. As a sample, a few drops of the diluted sperm suspension were placed on a Neubauer's enhanced counting chamber (depth: 0.1 mm) and left for 5 minutes [16].

Sperm morphology:

By examining sperm smears taken from the left cauda epididymides, we were able to determine sperm morphology. In order to examine spermatozoa malformations, an aliquot of the sample was used to prepare the smears [17]. The morphology of spermatozoa was determined using the eosin/nigrosin stain. After that, the slides were examined using a light microscope at a magnification of 400. On each slide, 300 spermatozoa were examined for abnormalities in the head and tail [16].

Sperm motility:

A light microscope (Olympus Co., Tokyo, Japan) at 400 magnifications was used to visually assess the percentage of sperm motility. One drop of sperm suspension was deposited on a glass slide, which was then covered with a lamella for this procedure. In several microscopic fields of vision, the number of sperm with rapid progressive forward movement (RPFM), slow progressive forward movement (SPFM), circumferential motion (CM), and those that remained motionless (ML) were counted, and the percentages of motile and non-motile sperm were calculated. In each sample, estimates of mobility were collected from ten separate fields [17].

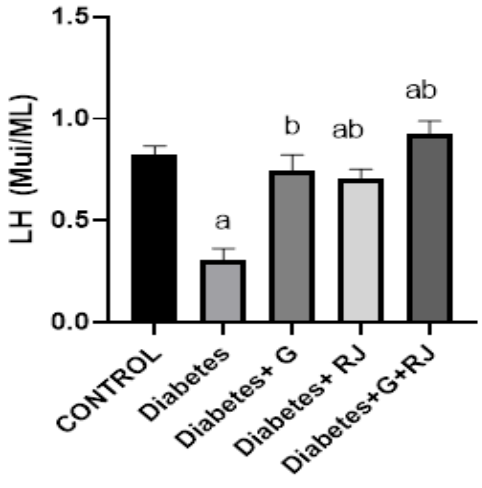
III. RESULTS

Table (1): Sperm count, motility and deformation in various studied groups

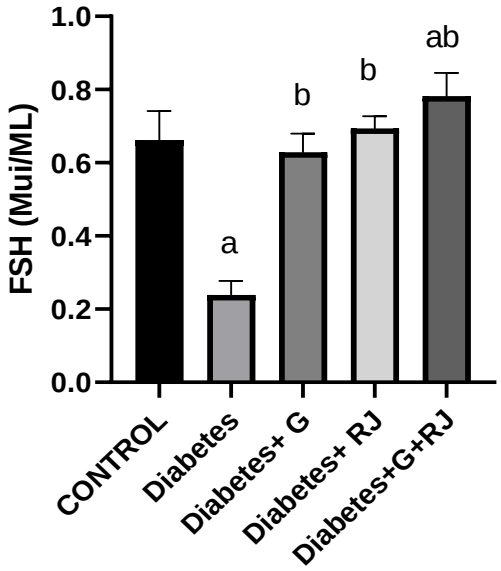
Parameter	CONTROL	Diabetes	Diabetes+ G	Diabetes+ RJ	Diabetes+G+RJ
Sperm Count (million/ml)	57.00± 3.52	16.63± 3.83 a	68.38± 1.77 b	76.0± 3.65 ab	83.5± 10.28 b
Sperm Motility (million/ml)	49.00± 0.88	15.75± 2.61 a	42.00± 1.53 b	39.63± 2.04 ab	51.38± 1.73 b
Sperm Deformation %	13.50± 1.19	39.38± 1.8 a	18.13± 1.51 b	16.63± 2.57 b	16.13± 1.2 b

All values are expressed as mean ±SD, *n*=8, significant vs control group, **b** significant vs Diabetic. A one-way ANOVA followed by post hoc Tukey test multiple comparisons between groups.

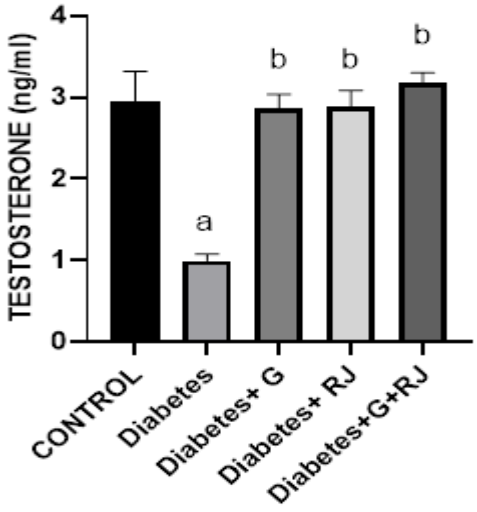
When compared to the control group, the Diabetic group had considerably reduced mean sperm count and sperm motility (P.001), whereas the Ginseng, royal jelly, and royal + ginseng groups had significantly greater mean sperm count and sperm motility. Furthermore, the Diabetic group showed a statistically significant increase in sperm deformation when compared to the control group (P.001), whereas the Ginseng, royal jelly, and royal + ginseng groups showed a statistically significant decrease in mean sperm count and sperm motility when compared to the Diabetic group. The statistical results of the various groups tested are shown in (Table 1).



A



B



C

Figure (1): FSH, LH and Testosterone hormones in various studied groups.

FSH (A), LH (B), and Testosterone (C) levels in various study groups are shown in Figure 1. All values are reported as mean standard deviation (n=8). a significant vs control group, b significant compared Diabetic group. Multiple comparisons across groups were analysed using a one-way ANOVA followed by a post hoc Tukey test.

When compared to the control group, the Diabetic group had statistically considerably reduced mean FSH, LH, and testosterone levels (P.001), whereas the Ginseng, Royal Jelly, and Royal + Ginseng groups had significantly higher mean FSH, LH, and testosterone levels. The statistical results of the various groups tested are shown in (Fig 1).

Histopathological results: (Figs. 2,3)

The seminiferous tubules (ST) were rounded, continuous, and regularly organised, contiguous in histological structure. Sertoli and spermatogenic cells at various stages of maturation lined the tubules, which were resting on a thin basement membrane. In the lumen, there were clumps of spermatozoa. Clusters of Leydig cells were found in the small interstitial area.

Tubules in the Diabetic group were uneven and widely apart. The blood vessels were clogged, and the walls of the vessels had thickened significantly. The tubular lining epithelium had been severely damaged and was vacuolated. The spermatogenic cells were disordered, and apoptosis was high. There were only a few sperm in the lumen. In the interstitial space, there was vacuolization and deposition of homogeneous acidophilic material. Regeneration of the germinal epithelium with minimal vacuoles in both the ginseng and royal jelly groups. The thickness of the foundation membrane and the intertubular gap both decreased. Congestion and blood vessel wall thickness were also less noticeable. The rats who were given both ginseng and royal jelly showed a greater improvement.

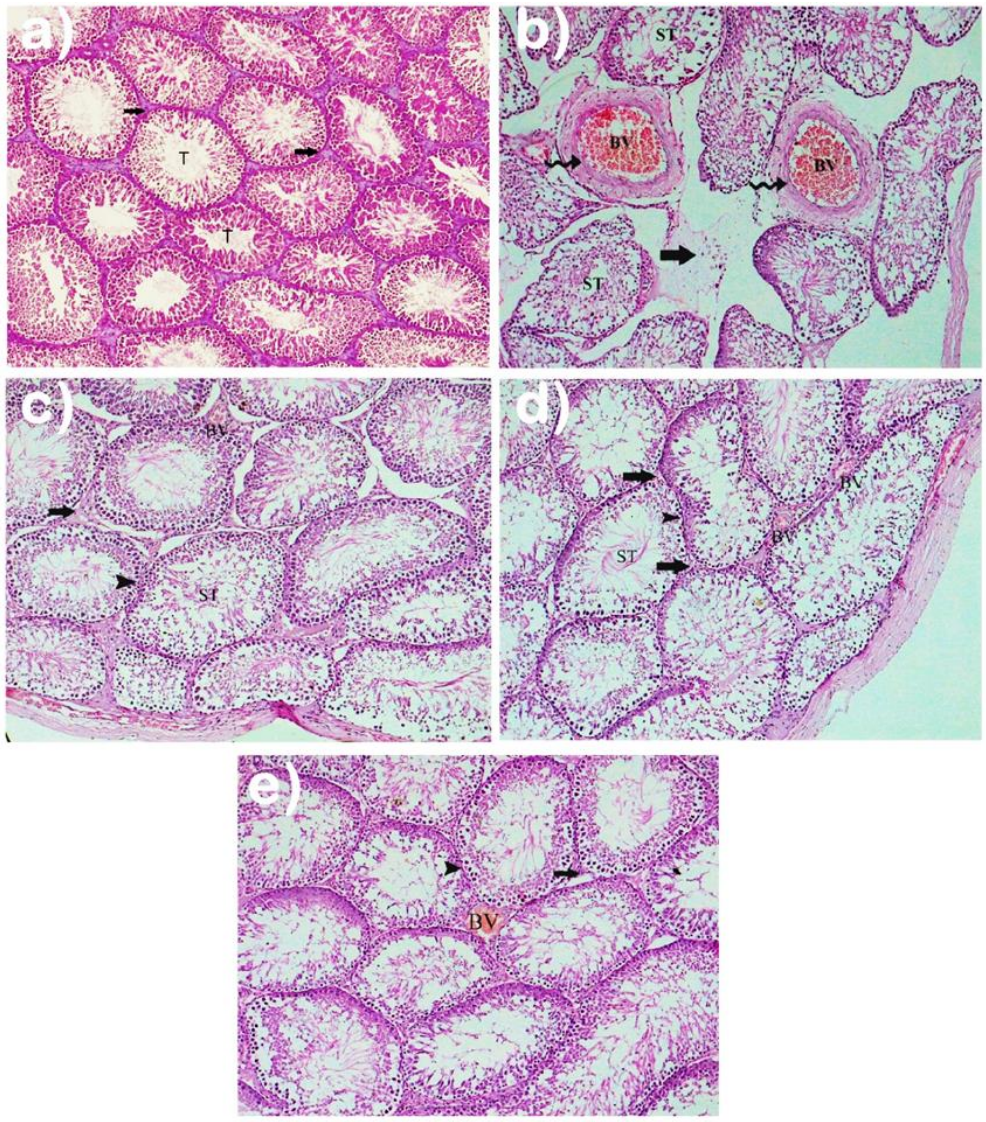


Figure (2): photomicrographs of slices from all groups' testes: a) Seminiferous tubules (ST) are rounded, with a thin basement membrane (arrowhead) and a narrow interstitial space in the control group (thick arrow). b) In the diabetic group, the seminiferous tubules (ST) are dilated and separated by a large intertubular gap (thick arrow). The walls of blood vessels (BV) have thickened and are congested (wavy arrow). c) The ginseng group has spherical seminiferous tubules (ST) with a thin basement membrane (arrowhead) and a limited interstitial space with minor congestion of blood vessels (BV). D) The royal jelly group has spherical seminiferous tubules (ST) with a thin basement membrane (arrowhead), as well as a limited interstitial space with modest congestion of blood vessels (BV). e) Ginseng + Royal Jelly exhibits seminiferous tubules with thin basement membranes that are practically normal (arrowheads). In the limited intertubular area, a mildly congested blood vessel (BV) can be detected.

(X 200, H&E)

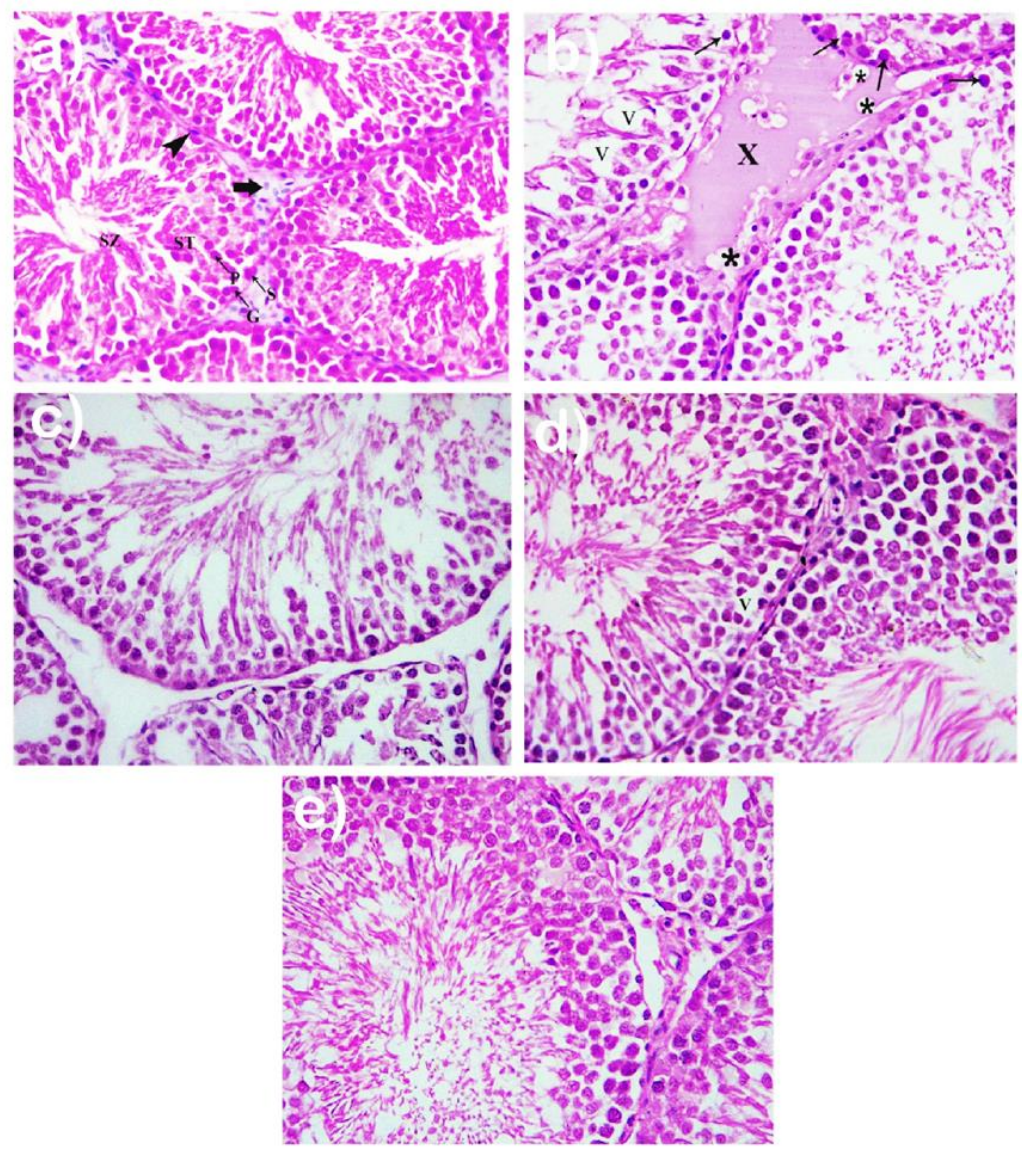


Figure (3): photomicrographs of slices from all groups' testes: a) Parts of seminiferous tubules ensheathed by myoid cells (arrowhead) and aggregates of sperms in the lumen are seen in the control group (SZ). Sertoli cells (S), spermatogonia (G), spermatocytes (P), and spermatids (SD) line the tubules. In the limited interstitial area, clusters of Leydig cells (arrowhead). b) In the diabetic group, the epithelium is destructed, with numerous vacuoles (v) and apoptotic cells (thin arrows). Vacuoles (asterisks) are visible in the interstitial space, which are filled with homogeneous acidophilic material (X). The ginseng and royal jelly groups both improved moderately. e) Ginseng + Royal Jelly improves the tubules significantly, resulting in an almost normal spermatogenic profile.

(H&E, X 400)

Sperm Morphology

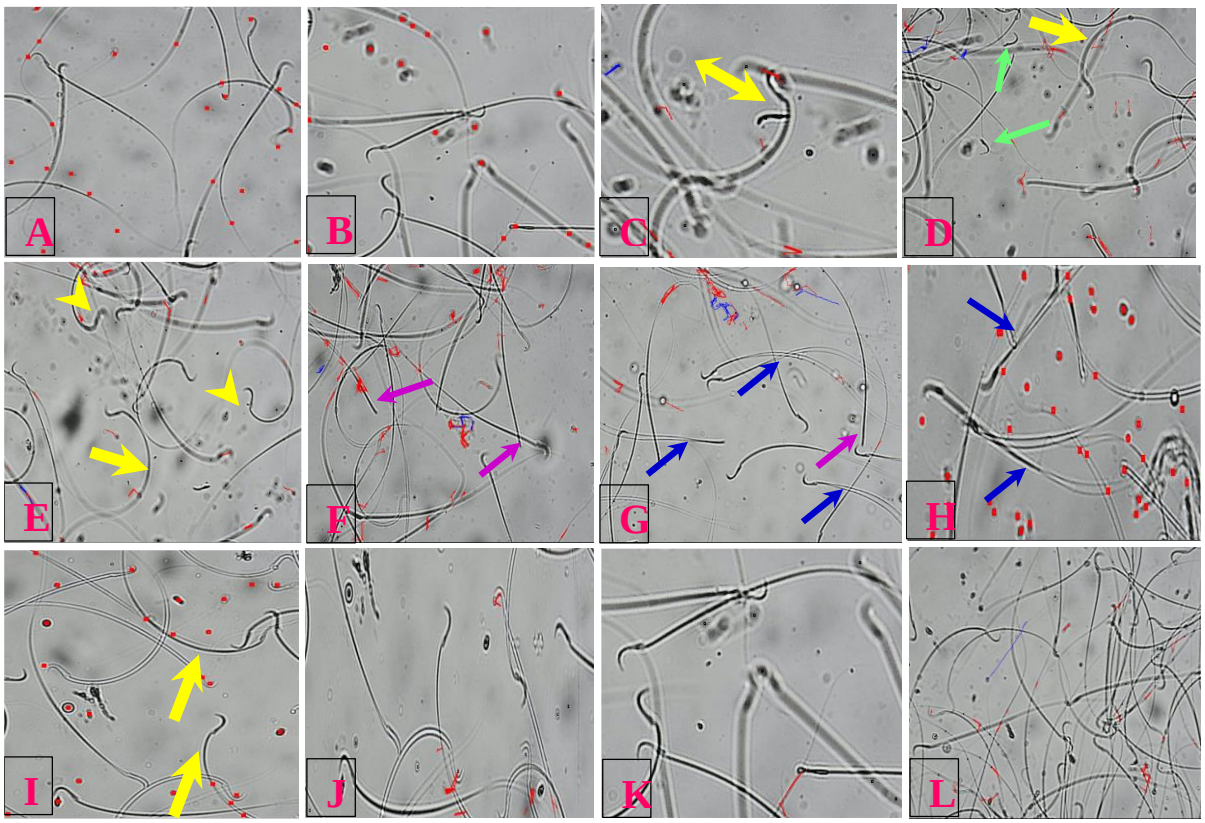


Fig. 4 Photomicrographs of sperm from different experimental groups. Normal spermatozoa with normal head and tail were seen in the control group (A,B); aberrant sperms with bent head (arrow head), bent tail (arrow), detached head (green arrows), bi-forked head (), pin head (violet arrow), bi-forked tail were seen in the diabetic group (D,E,F,G,H,I) (blue arrow). Normal spermatozoa with normal head and tail were found in the ginseng group (J), the royal group (K), and the royal + ginseng group (L), with a substantial number of them in the last group, which relates to the recovery that occurs after STZ treatment.

IV. Discussion

Diabetes is well-known for being linked to decreased male fertility and sexual dysfunction [18]. Diabetes has been linked to oxidative stress, which has been linked to sperm membrane lipid peroxidation, which may interfere with membrane fluidity and transport functions [19]. Short-term hyperglycemia lowered sperm counts in diabetic rats, according to Scarano et al.[20], whereas Amaral et al. (2006) found that sustained hyperglycemia negatively affects sperm concentration and motility in rats due to oxidative stress.

Our findings demonstrated that, as compared to the control group, the Diabetic group had considerably lower sperm count and sperm motility, whereas the Ginseng, royal jelly, and royal + ginseng groups had significantly greater mean sperm count and sperm motility. Furthermore, the Diabetic group had a statistically significant increase in sperm deformation compared to the control group, but the Ginseng, royal jelly, and royal + ginseng groups had a statistically significant drop in mean sperm count and sperm motility compared to the Diabetic group. These findings are consistent with those of Abu Bakar et al.[21], who found that Streptocotozin-induced diabetic rats were infertile because the number of sperms, percentage of sperm viability, and percentage of normal sperm morphology were all low, but the percentage of immotile sperm was high.

The following is how the findings were explained: STZ causes -cell necrosis in the pancreatic islet of Langerhans, resulting in insulin production failure [22]. Insulin deprivation causes GLUT glucose transport protein transcription and activation failure, according to a study by Kim and Moley [23]. GLUT is required for transferring glucose into sertoli cells in order to carry out the glycolysis process, which results in the formation of lactate [24]. Lactate is an energy source that is required for germ cell proliferation and sperm maturation [25]. When lactate isn't available for germ cell feeding, the spermatogenesis process is disrupted, and sperm quality

suffers as a result. High concentrations of free radicals caused sperm death, sperm DNA breakage, and altered the expression of fertility proteins, according to Ding et al. [26], indicating that they operate as antioxidants to protect cells against structural modification by advanced glycation end (AGE) products.

The use of royal jelly increased sperm count, maturation, motility, and testosterone levels in the blood [27]. Furthermore, antioxidative activity and scavenging ability of RJ protein fractions against free radicals such as superoxide anion, DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, and hydroxyl radical have been observed [28]. Ginseng increased the number, maturation, and motility of sperm. With these facts in mind, it's clear that Ginseng minimises oxidative stress while simultaneously providing protection from it, allowing proper spermatogenesis in the testis [29].

The main conclusion of this study is that the male STZ-induced diabetic rat has aberrant sex hormone levels, with a decrease in FSH, LH, and testosterone in particular. It follows in the footsteps of Bentez and Pérez Dáz. FSH, LH, prolactin, and growth hormone levels were found to be lower in diabetic rats [30]. Diabetes lowers the levels of LH in the blood, which is required for optimal Leydig cell function (testosterone secretion) [30]. The pituitary of diabetic rats shows a blunted response, with reduced FSH and LH production in response to stimuli [31]. Furthermore, a link has been hypothesised between serum insulin/glucose levels and LH/FSH levels, albeit the mechanisms by which insulin, glucose, or both govern these two hormones are unknown [32]. A drop in testosterone levels could be linked to a drop in LH, a drop in Leydig cell population, or both. This has been observed in diabetic male rats [32].

Diabetes had a detrimental effect on the histological architecture of the testis in the current investigation, which was consistent with earlier research. Diabetes, according to Khaneshi and Nasrolahi [33], resulted in a decrease in the diameter and epithelial height of seminiferous tubules, as well as an increase in interstitial gaps. Apoptosis of spermatogenic cells and deposition of hyaline material in the interstitial spaces were observed in this study. This is in agreement with Zha, Bai [34], who stated that apoptosis is one of the main characteristics of diabetic testicular injury. Diabetes can impact reproductive functioning by increasing oxidative stress through the production of reactive oxygen species (ROS), which damage spermatogenic and steroidogenic cells' proteins, lipids, and DNA, resulting in testicular apoptosis[35]. The thickening and congestion of testicular blood vessels, which was also highlighted in the investigations of [35, 36], was a notable feature in the diabetic group. Furthermore, Piryaei and Najar [37] discovered that diabetic testes had more new vascularization than control testes. According to Sawiress and Ziada [38], ginseng extract increased antioxidant state and reduced diabetes-induced degenerative alterations in the seminiferous tubules. In the same way, royal jelly helped to prevent diabetic testicular alterations. [Ghanbari, Nejati [39] noted this as well, explaining that RJ alleviated diabetes-induced testicular dysfunction, most likely due to its antioxidant properties.

In terms of the impact of diabetes on sperm, our findings revealed an increase in STZ spermatozoa with aberrant sperm morphology. This result is consistent with Mangoli et al's findings [40]. The activation of the synthesis of advanced glycation end products (AGE), polyol pathway, hexosamine pathway, and protein kinase C (PKC) pathways in the hyperglycemic situation following the administration of STZ has been shown to cause sperm abnormalities [41].

Our data also revealed that Ginseng, Royal jelly, and royal + ginseng all had normal spermatozoa with normal heads and tails, with a substantial number of them in the last group, indicating the recovery that occurs after STZ treatment. P. ginseng treatment improved the proportion of sperm cells with normal shape and motility, as well as lowered the apoptotic indexes of spermatogenic cells in epididymoorchitis rats, according to Eskandari et al. [42]. P. ginseng treatment (200 mg/kg/day for 4 months) in aged rats improved sperm maturation and testicular functioning by lowering MDA and raising enzymatic and nonenzymatic antioxidant levels, according to Ramesh et al. [43].

In the male diabetic rat reproductive system, RJ has favourable effects on histological changes amelioration and antioxidant defence system strengthening [39]. In streptozotocin-induced diabetic animal models, RJ improves sperm kinematic characteristics, DNA fragmentation, and lipid peroxidation [44], and protects against stanozolol-induced sperm abnormalities and embryo toxicity [45].

Abbreviations: RJ: Royal jelly, G: Ginseng, FSH: Follicle stimulating hormone and **LH:** luteinizing hormone

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