The Protective Role of Royal Jelly against the Biochemical and Structural changes of Penile Corpora Cavernosa in Diabetic Rats

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Abstract

Diabetes mellitus (DM) is a leading cause of erectile dysfunction (ED). Understanding the structure of erectile tissue within the penile corpora cavernosa and their pathological changes in these tissues is essential for developing protective and therapeutic strategies. As the current diabetes management does not protect against ED, promising natural agents such as royal jelly (RJ), which has variable bioactive components that possess antioxidant, anti-inflammatory and antidiabetic properties are needed.

This study aimed to investigate the effect of induced DM on the biochemical and structural components of the corpora cavernosa and to evaluate the protective effect of RJ on these parameters. Forty adult albino male rats were randomly divided into 4 groups: the control group, the RJ group: received oral RJ (100 mg/kg/day), the diabetic group: subjected to induction of DM by using Streptozotocin (60 mg/kg) intraperitoneally; and the diabetic and RJ groups: subjected to DM induction and received RJ. All rats were sacrificed after 60 days; blood was drawn to estimate differences in diabetes parameters, testosterone levels, oxidative/antioxidant markers and nitrous oxide (NO) concentrations. Additionally, penile tissues were fixed in formalin for histological and immunohistochemical studies. STZ-induced DM results in marked hyperglycemia, decreased insulin, testosterone, and NO levels; and oxidative/antioxidative imbalance. Histologically, corpora cavernosa showed a decrease in collagen fibers, elastic and smooth muscle fibers with a disturbed normal architecture. Treatment of diabetic rats with RJ markedly decreased these biochemical and structural alterations.

In conclusion, RJ cotreatment is a promising practice for diabetes-induced corpora cavernosal damage possibly through its antihyperglycemic, antioxidant, and androgenic effects.

1. INTRODUCTION

Erectile dysfunction (ED) is a widespread problem affecting men across all age groups and is more than a serious quality of life problem for sexually active men(1). ED refers to the persistent inability to achieve or sustain a satisfactory penile erection that is pleasant for sexual performance(2). The etiology of ED is multifactorial and associated with various risk factors including aging, neurological diseases, lifestyle factors (smoking, alcoholism, lack of exercise, unhealthy diet and overweight) and chronic disorders, such as diabetes mellitus (DM) and hypertension(3, 4).

Currently, DM is a great public health concern that has a negative influence on patient quality of life due to its steadily increasing incidence and wide range of multiorgan complications such as neuropathy, retinopathy, nephropathy and cardiovascular disease(5, 6). Furthermore, many studies have reported a negative impact of DM on male reproductive organs and fertility(7, 8).

In this regard, DM appears to be a major determinant of ED, where diabetic men are three times more likely to develop ED more than nondiabetic men are(9). Research conducted on diabetic patients has suggested that the development of ED in relation to diabetes involves multiple factors, likely connected

to central and peripheral neuropathologies, impaired signaling for blood vessel dilation, dysfunction of the endothelium, problems with venous blood flow, low levels of gonadal hormones, harmful effects of oxidative stress, inflammation, and psychological factors(10–12).

Several molecular and cellular mechanisms have been proposed to explain how DM leads to ED. Oxidative stress represents a principal mechanism implicated in the pathogenesis of diabetic complications due to the overproduction of reactive oxygen species (ROS) and impaired antioxidant protective mechanisms; causing membrane destruction due to peroxidation of membrane lipids and protein glycation. Moreover, other mechanisms, such as impaired endothelial and neuronal nitric oxide (NO) synthesis and activity plus an imbalance between vasorelaxant and vasoconstrictive mediators favoring vasoconstriction, have been suggested to be involved(13, 14).

Currently, noninvasive and invasive management methods exist to improve ED; however, phosphodiesterase type 5 inhibitors are the most recommended first-line treatment. Invasive management involves intracavernosal injection of vasoactive materials, an intraurethral suppository of prostaglandin E1, penile prostheses and vacuum-assisted erectile devices(15, 16). However, those strategies only cope with the symptoms of ED and do not address any underlying pathogenesis; in addition, not all patients respond to this type of treatment. Furthermore, adverse effects such as headache, nasal congestion, flushing, vision loss, dyspepsia and myocardial infarction may further limit this pharmacological interference(17, 18).

Many previous studies have shown the role of several natural nutraceuticals with antioxidant activity in the management of DM and control of oxidative stress(19, 20). Among these nutraceuticals, royal jelly (RJ) is one of the most valued and high-quality products and has been used in traditional medicine to treat various diseases(21). RJ is secreted by the special glands of worker honeybees and contains many components such as proteins (9–18%), sugars (7–18%), lipids (3–8%) and unsaturated fatty acids. Other minor components include minerals (Fe, Na, Ca, K, Zn, Mg, Mn, Cu), amino acids, vitamins (A, B, C, and E), enzymes, hormones, polyphenols, and nucleotides(22, 23).

Earlier studies revealed that RJ has many health-promoting properties such as antitumor, immune-modulatory, anti-inflammatory, and antioxidative activities plus scavenging ability against ROS(24). Additionally, it was reported to have vasodilative, antihypertensive and antihyperglycemic effects(25, 26). Further studies have revealed its beneficial effects on the male reproductive system such as gonadotropic effects, increased fertility and reproductive capacity(27), and increased sperm concentration and motility(28).

Reviewing the literature, there is insufficient data on the effect of RJ in the treatment of DM-related male ER. Therefore, this research was conducted in rats to investigate the effect of experimentally induced DM on biochemical parameters, as well as the architecture and distribution of connective tissue components and smooth muscle cells in the corpora cavernosa, and to evaluate the protective effect of RJ on these parameters.

2. MATERIALS and METHODS

2.1. Chemicals

Streptozotocin (STZ) (Sigma–Aldrich Chemical Institute, St. Louis, MO, USA) and all the other chemicals, solutions, and kits used in this research were purchased from local scientific agents in Jeddah. Fresh RJ was obtained from hives in the Sarawat Mountains, Asir region, Saudi Arabia, and stored at − 20˚C until use.

2.2. Animals and experimental design

Forty adult male Sprague Dawley rats (weighing 200–240 g) were used in this study. They were kept in separate metallic cages with free access to a normal diet composed of purina rat chow and water ad libitum and were maintained under controlled conditions comprising a 12-h light–dark cycle, a room temperature of 22–25°C, and a relative humidity of 40–50%. After acclimatization for one week, the animals were randomly divided into four groups of 10 rats each:

- **Group I (control group)**: No manipulation was performed on the rats that were given distilled water only.
- **Group II (RJ group)**: Rats received 100 mg/kg/day of RJ orally. This dose was taken from a previous study of(29).
- **Group III (diabetic group)**: DM was induced in the rats.
- **Group IV (diabetic and RJ groups)**: DM was induced in rats orally administrated RJ as described above.

The rats in the four groups were sacrificed 60 days after the onset of the experimental procedures by ether overdose during which blood was taken immediately through cardiac puncture. Serum samples were collected from clotted blood using a centrifuge operated at 3000 rpm for 10 min. The serum supernatants were collected and stored in a refrigerator at 4°C prior to biochemical analyses. The penile tissue was obtained via a circular incision with following the removal of the shaft skin and the foreskin. Then, the penis was laid on a horizontal surface and cut longitudinally and transversely into equal pieces. Some penile tissue sections were immediately frozen in liquid nitrogen and stored at -70^{o}C until homogenization, while the other sections were immediately fixed in 10% neutral buffered formalin (NBF) for 48 hours for further histological and immunohistochemical studies.

2.3. Induction of DM

Rats were fasted for 12 hours before receiving one-time intraperitoneal (IP) injection of newly prepared streptozotocin (STZ), a cytotoxic drug that is known to destroy the beta cells of the pancreas and thus induce diabetes (60 mg/kg body weight) via fusion in 0.1 M citrate buffer (pH 4.5). Therefore, STZ-injected animals were given a 5% glucose solution for 24 h to overcome the decrease in blood glucose levels induced by the drus. On the 3rd day after STZ injection, blood glucose levels were detected by

obtaining blood samples via the tail vein using a blood glucose testing kit. A blood glucose level of 250 mg/dl or more was considered diagnostic and confirmed the onset of the diabetes. The animals were kept on a high carbohydrate diet to maintain diabetes(30).

2.4. Assessment of the diabetic parameters

The fasting blood glucose (FBG) level was determined in rats in the different groups using an Accu-Chek glucometer (Roche, Germany). The glycated hemoglobin (HbA1c) level was determined using high-performance liquid chromatography and a commercial kit. The serum insulin (SI) concentration was determined by an enzyme-linked immunosorbent assay (ELISA).

2.5. Determination of the serum testosterone concentration

Serum testosterone concentration was measured by a commercial testosterone kit (Demeditec Diagnostics GmbH, Kiel, Germany). The amount of testosterone was expressed as ng/dL.

2.6. Preparation of penile homogenates and measurement of oxidative and antioxidative parameters

The frozen penile pieces were defrosted before being mixed with 2 ml of ice-cold Tris–HCl (pH 7.4) supplemented with 1% protease inhibitor and homogenized using a Teflon homogenizer (Heidolph Silent Crusher M) at 4,000 rpm. Buffer was subsequently added to adjust the final volume to be 10-fold the tissue weight. Using a spectrophotometer (Shimadzu UV 1700Japan), supernatants were utilized to determine the activities of lipid peroxidation and antioxidative enzymes at specific absorbances using specific kits and according to the manufacturer's instructions at specific absorbance. The MDA content was assayed using the thiobarbituric acid test. Superoxide Dismutase (SOD) activity was determined by assaying the autooxidation and illumination of pyrogallol at 440 nm for 3 min. Catalase (CAT) activity was measured by assaying the hydrolysis of H_2O_2 and the resulting decrease in absorbance at 240 nm over a 3 min period at 25°C. Glutathione Peroxidase (GPX) activity was measured using H_2O_2 as a substrate. The reaction was monitored indirectly as the oxidation rate of NADPH at 240 nm for 3 min.

2.7. Measurement of penile nitric oxide (NO) and endothelial nitric oxide synthetase (eNOS) levels

The levels of NO and eNOS were measured in the supernats of penile homogenates using colorimetric ELISA kits (Cloud-Clone Corp., Houston, USA) at 450 nm using a microplate spectrophotometer reader (BioTek, Winooski VT, USA) according to the manufacturer's instructions. The results are expressed as µmol/mg protein.

2.8. Light microscopic and immunohistochemical study

The transversely and longitudinally cut fixed penile sections were processed to prepare paraffin sections of 5 µm in thickness. The sections were stained with Haematoxylin and Eosin stain (H&E) to demonstrate the general histology, Masson's trichrome (MT) stain to demonstrate the collagen fibers

and Orcein stain to demonstrate the elastic fibers. For immunohistochemical staining, the avidin-biotin technique of Suvarna *et al*. (2018) was used to identify smooth muscle cells (SMCs), using monoclonal anti-actin alpha-smooth muscle antibody (A2547-Sigma Company) at a dilution of 1:100 in phosphate-buffered saline (PBS) as the primary antibody(31). Briefly, the sections were dewaxed in xylene, hydrated in a decreasing series of ethanol to water and washed in PBS for five minutes. After heat-induced epitope retrieval with citrate buffer, the sections were treated with 0.3% hydrogen peroxide in methanol to reduce endogenous peroxidase activity. Subsequently, the cells were incubated in a moist chamber for 30 minutes at 37^{o}C with 1% goat serum in PBS. Next, the cells were incubated in a moist chamber for 12–14 hours at 4^{o}C with the primary antibody. After 5 min of rinsing, the sections were incubated with the secondary antibody (biotinylated or anti-mouse IgG H + L, Vector, Burlingame, CA) for 2 hours at room temperature. The sections were then counterstained with hematoxylin. Finally, all histological sections were examined, and representative photos were taken with an Olympus BX41 research optical photomicroscope equipped with an Olympus DP25 digital camera (Olympus, Tokyo, Japan).

2.9. Histomorphometric analysis of the corpus cavernosum

Quantitative assessments of the collagen, elastic, and smooth muscle fibers of the corpus cavernosum were carried out using the ImageJ software (version 1.8.0, National Institutes of Health, Bethesda, USA). Briefly, from each slide of rat penis from different groups, six randomly selected fields of corpus cavernosum were evaluated at X200 magnification by two separate observers without knowledge of the studied groups. The areas of collagen fibers stained blue in the Masson's trichrome sections, areas of elastic fibers stained red in the Orcein sections and areas of positive brown staining in the SMC immunostained sections were evaluated as a percentage of the total tissue area.

2.10. Statistical analysis

The data acquired in this study are expressed as the means ± SDs and were analyzed using the Statistical Package for the Social Sciences, version 23 (SPSS Inc., Chicago, Illinois, USA). The significance of differences between groups was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P values < 0.05 were considered to be statistically significant.

3. RESULTS

3.1. Diabetic parameters

As shown in Fig. 1, compared with those in the control group, the FBG and HbA1c in the STZ-induced diabetic rats were significantly increased (P < 0.001). However, these levels were significantly decreased in the diabetic rats treated with RJ than in the diabetic rats (P < 0.001). Additionally, compared with that in the control group, the SI level in the STZ-induced diabetic rats was significantly lower (P < 0.001). However, a significant increase in this level was observed in the diabetic rats treated with RJ compared with the diabetic rats (P < 0.01).

3.2. Testosterone level

As shown in Fig. 2, significantly lower serum testosterone levels were detected in diabetic rats than in control rats ($P < 0.001$). However, cotreatment with RJ improved of testosterone levels nearly to the normal level ($P < 0.01$).

3.3. Nitrous oxide and endothelial nitrous oxide synthetase levels

As shown in Fig. 2, significantly lower levels of NO and eNOS was observed in diabetic rats than in control rats ($P < 0.001$). However, cotreatment with RJ resulted in a nearly normal recovery of these levels ($P < 0.05$ and $P < 0.05$).

3.4. Oxidative/antioxidative markers

As shown in Fig. 3, compared with those in the control group, the MDA level in the penile tissues, a marker of oxidative stress was significantly increased in STZ-induced diabetic rats when ($P < 0.001$). However, a significant decrease in this level was observed in the diabetic rats treated with RJ ($P < 0.001$). Additionally, the penile SOD, CAT, and GPx activities were lower in the diabetic group than in the control group ($P < 0.001$). Cotreatment with RJ in diabetic rats significantly increased the levels of these enzymes ($P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively).

3.5. Histological and immunohistochemical findings:

Low-power images of the penis of the rats in the control and RJ groups generally showed a normal architecture in the form of three erectile bodies, two dorsal corpora cavernosa and one ventral corpus spongiosum containing the urethra. The corpora cavernosa made up most of the penis's length and were surrounded by the thick capsule "tunica albuginea" (TA) and separated by an incomplete septum. Each corpus cavernosus was surrounded entirely by the TA, which sends perpendicular columns or trabeculae into its center, these trabeculae are separated by vascular spaces (Fig. 4- A, B, C). The TA was made up almost entirely of dense collagen fibers with a lamellar arrangement, which were differentiated into an outer layer of wavy fibers that run longitudinally along the whole length of the corpora cavernosa and an inner layer that is oriented circularly. From its inner aspect, regularly shaped finger-like pillars (trabeculae) penetrate into the depth of the corpora, branch and anastomose together forming a mesh of irregular vascular spaces or sinusoids, which are lined by intact flattened endothelial cells. These trabeculae consisted mainly of collagen fibers, which appeared as dense and wavy bundles of fibers distributed in different directions (Fig. 5A and Fig. 6A).

In diabetic rats, examination revealed distinct pathological changes that affected both the TA and the microstructure of the corpora cavernosa. The TA became thinner with disruption of the lamellar arrangement of collagen fibers between the outer and inner layers, and some collagen bundles, especially in the inner circular layer, were fragmented. Moreover, the trabeculae appeared shrunken and

irregular in size and shape, with dense collagen fibers in different directions. Additionally, the vascular spaces were markedly dilated, with destruction, discontinuity and shedding of the endothelial cell lining (Fig. 5B and Fig. 6B).

However, in diabetic rats cotreated with RJ, less marked damage was observed than in diabetic rats. In the TA, most of the collagen bundles retained their form and regular lamellar arrangement, while the trabeculae appeared regular in size and shape, where their collagen bundles were less packed. The vascular spaces were less dilated with continuity of their endothelial cell lining (Fig. 5C and Fig. 6C).

3.6. Elastic fibers

Examination of the elastic fibers in the control rats revealed many elastic fibers that appeared as a loose meshwork of thin branched fibers and had irregular profiles that were intermingled with the collagen fibers in the TA; more fibers were observed between the collagen bundles of the trabeculae and mostly surrounding vascular spaces (Fig. 7A). In the diabetic rats, the elastic fibers were markedly decreased in length and became very short and fragmented with the presence of some disorganized and condensed fibers in the trabeculae (Fig. 7B). However, in diabetic rats cotreated with RJ (Fig. 7C), the elastic fibers within the TA and trabeculae were more abundant and appeared more or less similar to those of control rats.

3.7. Smooth muscle cells (SMCs)

Moreover, the immunohistochemical investigation of smooth muscle cells (SMCs) from control rats revealed spindle-shaped cells located around the trabeculae in the wall of vascular spaces, forming a subendothelial layer in the lumina of these spaces (Fig. 8A). In diabetic rats, there were markedly fewer immunopositivity SMCs around the trabeculae and wall of vascular spaces than in control rats; these SMCs were shrunken and deeply stained (Fig. 8B). However, compared with those in the diabetic group, there was more immunopositivity with better orientation of SMCs around the trabeculae and vascular spaces in the diabetic rats cotreated with RJ (Fig. 8C).

3.8. Histomorphometric results

As shown in Fig. 9, the mean area of collagen fibers in the corpora cavernosa was 34.27% in the control group and significantly increased ($P < 0.001$) to 56.48% in the diabetic group. However, cotreatment of diabetic rats with RJ resulted in a marked decrease ($P < 0.001$) in the percentage of collagen fibers (33.57%). Furthermore, compared with that in the control group (16.84%), the mean percentage area of elastic fibers in the diabetic group was significantly lower ($P < 0.001$) (11.82%). However, cotreatment of diabetic rats with RJ resulted in a marked increase in the percentage of elastic fibers (14.67%) ($P < 0.01$). Moreover, the mean percentage of SMCs in the control sections was 24.53%, which was significantly ($P < 0.001$) lower in the diabetic rats (14.85%). However, cotreatment of diabetic rats with RJ resulted in a marked increase in the percentage of SMCs (20.87%) ($P < 0.05$).

4. DISCUSSION

This research was prompted by an increased occurrence of ED cases, particularly among diabetic men, which is also on the rise at the present time and reflects many adverse social and medical consequences. The main feature of diabetic ED is the structural alterations in penile erectile tissue, which cause poor responses to various types of invasive or noninvasive treatments (4, 32, 33).

Hence, the need for new medications is inevitable. Alternative and complementary medicines involving dietary supplements and herbal substances are increasingly being used in the management of ED (17). In this study, RJ was chosen as a protective agent against the harmful effects of DM on the corpora cavernosa on the basis of many previous studies demonstrating its antidiabetic (26, 34), antioxidant and anti-inflammatory effects (35, 36), as well as its gonadotropic effects and role in increasing fertility (37–39).

In the present study, a significant increase in blood glucose and a decrease in serum insulin were observed in STZ-induced diabetic rats, which was also demonstrated in several studies (40, 41). However, RJ cotreatment resulted in a significant reduction in the elevated glucose concentration and a significant increase in the insulin concentration. It was reported that the administration of RJ to diabetic rats for eight weeks resulted in a significant improvement in glucose levels, insulin concentration and insulin resistance (34, 42). Another study also showed that feeding diabetic rats 100 mg/kg RJ for eight weeks lowered fasting blood glucose and elevated serum insulin concentrations (29). In this regard, it was reported that RJ decreases blood glucose levels via the insulin-like activity, which may improve insulin resistance (26, 37). Similarly, some human studies have shown that in patients treated with 1500 mg/day RJ for eight weeks, the mean of FBS and HbA1c levels decrease significantly (43).

In the present study, a marked reduction in the testosterone concentration was observed in diabetic rats compared to that in control rats. It has been indicated that men with DM are at increased risk of experiencing a decline in testosterone levels (hypogonadism) as well as other issues related to the penile arteries and nerves (44). Androgenic hormones may be critical for sustaining the penile structural integrity, as their deficiency is linked to degenerated corporal tissue and an increased incidence of erectile dysfunction. Several studies have revealed the significance of androgens in normal penile erection (45). Although the precise mechanism of this effect has not been fully elucidated, hypogonadism in some men may indirectly reduce the levels of pituitary hormones, which stimulate the production of testosterone in the testis (46). However, RJ cotreatment of diabetic rats significantly increased testosterone to nearly normal levels. Similarly, it was reported that RJ feeding increases testosterone levels in male individuals (47, 48).

Several cellular and molecular processes are hypothesized to explain the DM-related ED, where many reports have shown increased oxidative stress and reduced nitric oxide (NO) levels (49, 50). In this study, STZ induced oxidative stress was confirmed by increases in the MDA level and decrease in the SOD, CAT and GSH levels. It was reported that the oxidative stress is correlated with an increase in the production of ROS or a disturbance in the oxidant defense system in various tissues (51). In accordance with these

findings, various studies have shown that these parameters differ and change in diabetic rats compared to control rats, where hyperglycemia can augment oxidative stress (52). On the other hand, the results of this study showed that RJ treatment decreased the content of MDA and increased the levels and activities of antioxidant enzymes. The antioxidant effects of RJ were described in a previous animal study by Ghanbari *et al.* (2016), who showed that the administration of 100 mg/kg RJ to diabetic rats for six weeks decreased the level of MDA and increased antioxidant activity. Additionally, other respective human studies have demonstrated the effect of RJ on oxidative stress and inflammatory variables in patients with type 2 DM (29, 53), these studies reported that supplementation with 3000 mg/day RJ for eight weeks resulted in elevated total antioxidant capacity in diabetic patients.

It was also found that both NO and eNOS levels were significantly lower in the penile tissue of diabetic rats than in that of control rats. The endothelial cells produce NO by eNOS that has a crucial role in erectile function because it increases the blood flow by dilating the arterial vessels and increasing the size of corporal sinusoids through smooth muscle relaxation and subsequent penile erection (54, 55). In the penis, eNOS is normally confined to the sinusoidal and vascular endothelium, while nNOS is mostly scattered in the nonadrenergic and noncholinergic nitrergic nerve terminals (56, 57). Accordingly, NO synthase-dependent endothelial dysfunction induced by oxidative stress reduces local NO levels and smooth muscle relaxation and thus plays a chief role in the development and progression of DM-induced ED (58, 59). However, RJ cotreatment of diabetic rats significantly increased the levels of these two parameters to nearly the normal values. In agreement with these findings, it was reported that RJ's hypotensive and vasodilator mechanisms may be correlated with increased NO production. Additionally, RJ comprises muscarinic receptor agonists, which promote vasorelaxation through the NO/cGMP pathway (60).

Understanding the histological structure and various components of the corpora cavernosa led to an efficient approach for assessing functional alterations, which is a crucial step in addressing many questions concerning erectile pathophysiology and may ultimately help to treat some types of ED. It was documented that penile erection is comprises two successive steps: first, the passage of blood into the cavernosal sinusoids, leading to enlargement of the penis, and second, a decrease in venous outflow via veno-occlusion to uphold the enlargement and maintain rigidity of the penis. These two steps depend on the complicated balance and coordination of vascular and cavernous components of connective tissues and muscles (51, 61). As described in several studies, cavernous tissue is built up by many vascular sinusoids lined by an endothelial cell layer and surrounded by a rich trabecular network consisting of SMCs and connective tissue; additionally, cavernous tissue is formed of collagen and elastic fibers; and the correct organization and proportions of these elements are required for proper erectile function (62, 63).

In this study, the tunica albuginea (TA) of the corpus cavernosum was found to consist of dense bundles of collagen fibers and few elastic fibers. The collagen fibers were arranged in two layers: an outer longitudinal layer consisting of bundles running over the longitudinal axis of the corpus cavernosum, and an inner circular layer consisting of bundles moving circularly to cross those of the outer layer

perpendicularly. In agreement with these findings, several previous studies have reported that, in the outer layer, collagen fibers undulate and therefore elongate during erection, while in the inner layer, they increase in penile girth by stretching out (64). Moreover, it was reported that, from this inner layer, intracavernous pillars radiate to act as struts providing essential support to the erectile tissue (65). According to previous reports, the penile TA plays a crucial role in the mechanism of erection. This happens when lacunar spaces press the subalbugineal venous plexus against the tunica, increasing penile stiffness. Additionally, the TA protects the vascular and nerve components of the corpora from the increase in intracavernous pressure that occurs during the erection phase(66). It has been also described that the TA contains elastic fibers forming an irregular network on which the collagen fibers rest. These fibers allow the dispensability and free recoil of the cavernosal tissue during increases in blood flow and sinusoidal filling (63).

Our findings demonstrated that STZ-induced DM caused various alterations in the structural components of the corpora cavernosa, including collagen and elastic fibers, SMCs, trabeculae, and vascular sinusoids. Such histopathological changes have been documented in many previous studies in diabetic men and animal models of DM (49, 67, 68).

In this work, there were decreased amounts of collagen fibers and elastic fibers in both the tunica albuginea and trabeculae of the corpora cavernosa in diabetic rats compared to those in control rats. In agreement with these findings, recent evidence has shown that diabetes may cause alterations in collagen structure and impaired metabolism and, subsequently, mechanical function (69). Additionally, a high-glucose environment leads to alterations in the extracellular matrix, such as decreased collagen deposition and increased production of matrix metalloproteinases (70). However, some studies have reported controversial results by showing increased penile fibrosis in diabetic rats (71, 72). Although the exact mechanism linking diabetes and penile fibrosis remains unclear, it is believed that elevated blood sugar levels and associated vascular changes lead to the formation of fibrous tissue in the penile region.

Moreover, a marked reduction in the number of elastic fibers was found in the corpora cavernosa of the diabetic rats compared to that in the control rats. Accordingly, Abidu-Figueiredo *et al.* (2011) reported that the elastic fibers of the corpus cavernosum of diabetic rabbits decreased despite the increase in smooth muscle fibers, which revealed that alterations in the performance of elastic fibers could be directly related to the occurance of pathological processes leading to ED (68). Also, despite the multifactorial nature of ED, a decrease in the quantity of elastic fibers plays a chief role in decreasing the elastic capacity of the penis and its firmness during erection. Any reduction in the elastic fibers can lead to a decreased ability to resist distension during an erection, resulting in a decrease in pressure and eventually causing ED (62, 65).

It was documented that trabeculae are composed of endothelial cells and SMCs, in addition to an extracellular matrix composed of collagen and elastic fibers. The elastic fibers are formed of fibril collections and fibrillar glycoproteins, which lie in the extracellular space and are embedded in elastin.

This structure permits elongation and an increase in penile stiffness during erection, followed by a quick return to a flaccid state following detumescence (73, 74).

The vascular spaces (sinusoids) within the corpora cavernosa in this study were separated by dense bundles of collagen fibers, which were lined by endothelial cells, with SMCs distinctly localizing to the trabeculae, forming a narrow subendothelial layer that surrounds the lumina of the corpora cavernosa. However, examination of the penises of diabetic rats revealed that there was a distinct narrowing of the cavernous spaces, which seemed to be occupied by bands of thick collagen fibers with penile fibrosis. Moreover, quantitative analysis was performed to confirm these findings, which revealed a significant increase in the collagen/ smooth muscle ratio in diabetic rats. The same results were observed in several previous studies (75, 76).

In this study, the immunohistological findings showed that the SMCs in the STZ-induced diabetic rats were considerably lower than those in the control rats. Accordanly, in 7-month- old obese Zucker fa/fa rats, a type 2 DM model, **Kovanecz et al. (2009)** reported a marked decrease in penile SMC content. Additionally, other studies of the corpora cavernosa in STZ-diabetic rats have revealed significant reductions in smooth muscle cell and endothelial cell densities and decreased levels of eNOS (77). Moreover, it was reported that the SMC to collagen ratio was significantly lower in the penile tissue of diabetic rats than in that of healthy normal rats(78). SMCs plays an important role in maintaining penile vascular tone by cooperating with endothelial cells to help regulate blood flow within the cavernous space. Relaxation of SMCs leads to an increase in the inflow of blood into the lacunar spaces of the corpora cavernosa; thus, pressure stretches the relaxed trabecular walls, causing an expansion of the TA, which subsequently elongates and compresses the draining venules; hence, corporal SMCs play a key role in the process of erection(79, 80). Additionally, ED has been shown to be related to qualitative and quantitative changes in those structures, including reduced trabecular SMCs and elastic fibers, increased collagen, disruption of the arrangement of collagen fibers in the tunica albuginea lamella and loss of endothelial integrity(68).

In the present study, compared with that if the diabetic group, the histological sections of the RJ group showed obvious conservation of SMC content. The results revealed obvious restoration of the size and structure of the CS. However, few thick collagen fibers were still observed obliterating them. The quantitative analysis of Masson trichrome-stained sections revealed a significant decrease in the collagen/smooth muscle ratio in the diabetic group compared to that in the control group. Similar results were observed using human urine-derived stem cells either alone or genetically modified with fibroblast growth factor 2 (75). It was thought that this therapeutic approach improves erectile functions in type 2 diabetic rats by engaging resident cells and increasing smooth muscle endothelial expression and contents. Therefore, restoring the smooth muscle/total collagen ratio is important for relaxing the SM and facilitating the growth of endothelial cells in the CC. A decreased smooth muscle/total collagen ratio lowers the ability of sinusoids to expand, leading to veno-occlusive dysfunction(81).

5. CONCLUSION

The findings of this study demonstrated that DM had a negative impact on the corpora cavernosa through altered oxidative balance, increased inflammatory mediator levels and structural damage to various components of the corpora cavernosa, which is the chief cause of erectile dysfunction in diabetic patients. Moreover, RJ cotreatment is a good option for protection against cavernosal damage, possibly because of its antihyperglycemic, antioxidant, and androgenic effects.

Declarations

Ethics approval

This research proceeded after receiving the consent of the Medical Research Ethics Committee, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia (REF NO. 216-21) [HA-02-J-008].

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The authors declare that there are no conflicts of interest.

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Figures

FIGURES AND LEGENDS

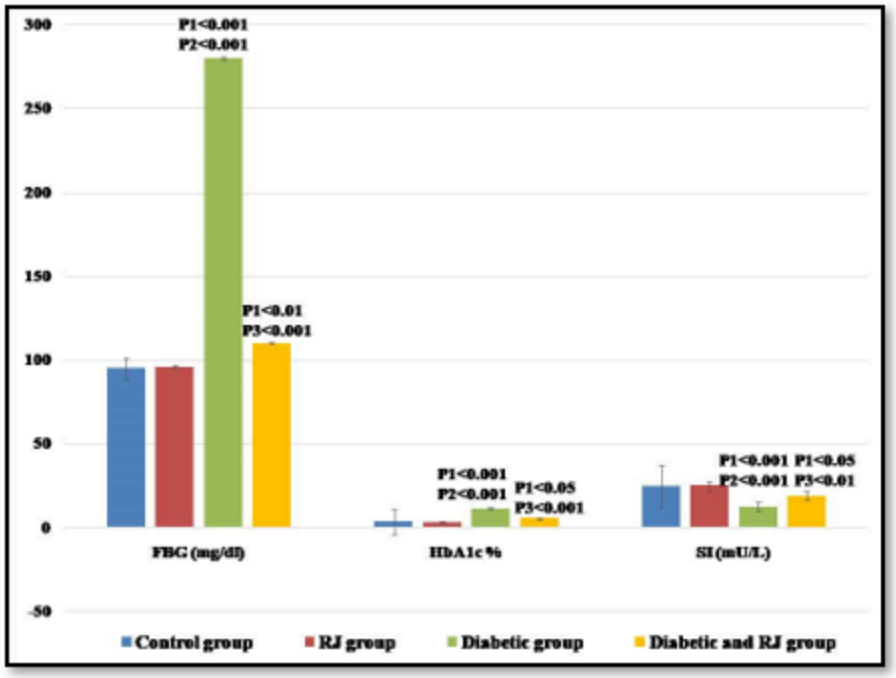

Figure 1

Graphical representations of the statistical analysis of the mean values of fasting blood glucose (FBG), HbA1c and serum insulin (SI) concentrations between the different groups.

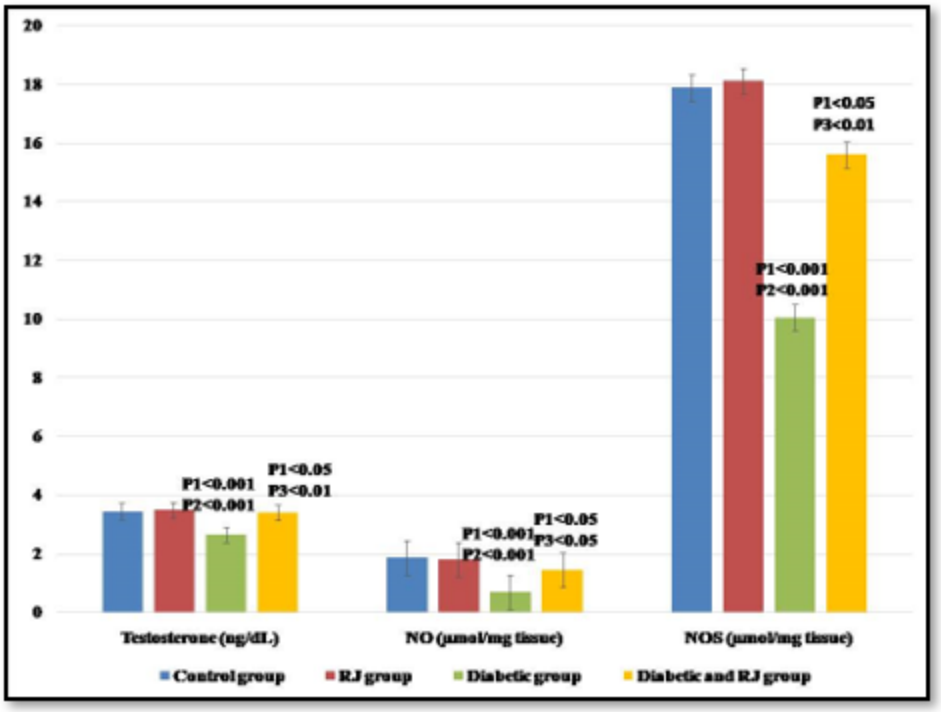

Figure 2

Graphical representations of the statistical analysis of the mean blood testosterone, nitrous oxide (NO) and endothelial nitrous oxide synthetase (eNOS) levels in the different groups.

The control group (G1), RJ group (G2), diabetic group (G3), and diabetic and RJ group (G4) were included.

P^1: significant versus G1; P^2: significant versus G2; P^3: significant difference from G4.

The values are expressed as the mean and SDs. Significance was determined using one-way ANOVA test followed by the Turkey test.

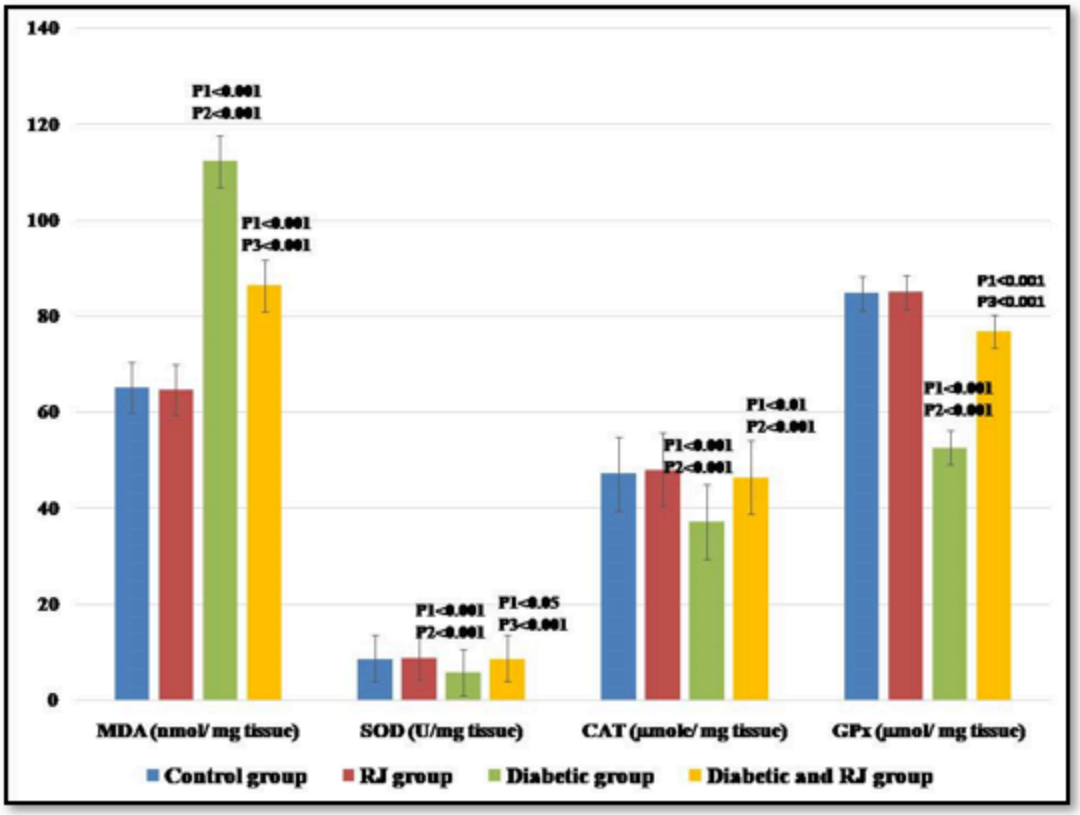

Figure 3

Graphical representations of the statistical analysis of the mean values of oxidative stress and antioxidant enzyme levels in the different groups.

The control group (G1), RJ group (G2), diabetic group (G3), and diabetic and RJ group (G4) were included.

P^1: significant versus G1; P^2: significant versus G2; P^3: significant difference from G4.

The values are expressed as the mean and SDs. Significance was determined using one-way ANOVA test followed by the Turkey test.

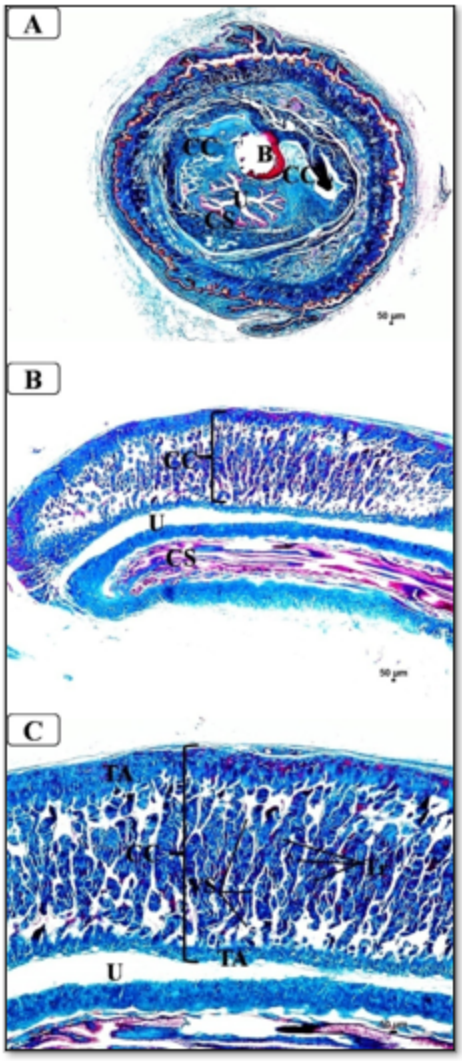

Figure 4

Photomicrographs of transverse (A) and longitudinal (B & C) MT-stained sections of penis from the control and RJ-treated groups showing the general architecture of three erectile bodies, two large corpora cavernosa (CC) dorsally, and the corpus spongiosum (CS) containing the urethra (U) ventrally. Notice that each CC is surrounded entirely by the TA, which sends perpendicular columns or trabeculae (Tr) into its center; these trabeculae are separated by vascular spaces (VS). B = baculum (os penis). A and B are MT X20, C is MT X40.

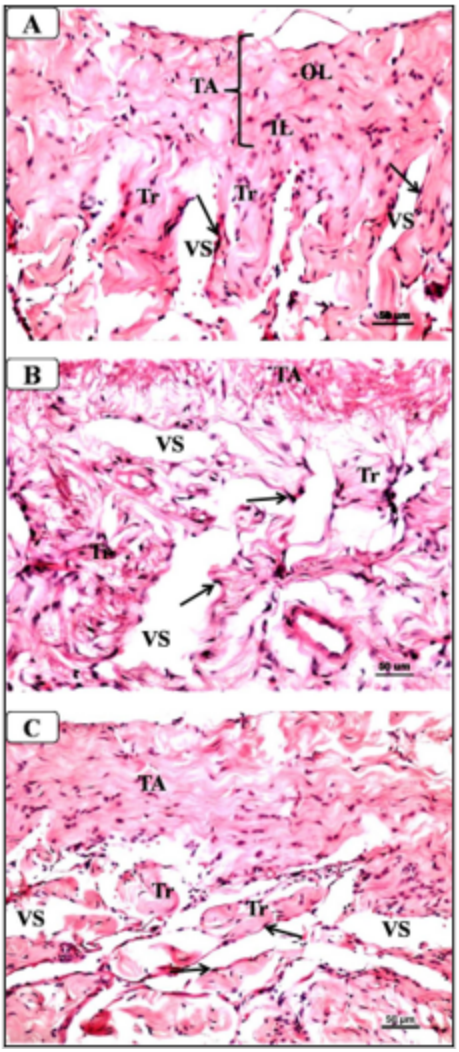

Figure 5

Representative photomicrographs of longitudinal sections of rat penis from different groups stained with H&E:

A)Control and RJ-treated groups showing a thick tunica albuginea (TA), in which the collagen fibers are arranged in two layers: an outer layer (OL) and an inner layer (IL). The trabeculae (Tr) were oriented perpendicular to the long axis of the penis and separated by vascular spaces (VS). These spaces are lined by endothelial cells with flat nuclei (arrow). H&E X200

B)Diabetic group showing a disturbed arrangement of collagen fibers in the tunica albuginea (TA) and trabeculae (Tr), which were fragmented and taken different directions. The presence of wide vascular spaces (VS) with discontinuous or detached endothelium linings is noticeable (arrows). H&E X200

C) Diabetic group cotreated with RJ showing regular arrangement of collagen fibers in the tunica albuginea (TA) and trabeculae (Tr). The vascular spaces (VS) with continuity of their endothelial lining are noticeable (arrows). H&E X200

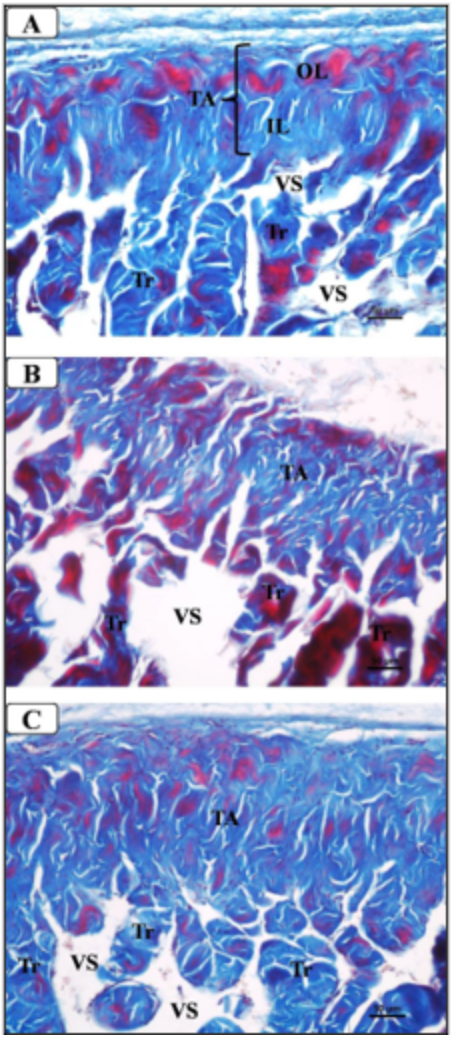

Figure 6

Representative photomicrographs of longitudinal sections of penis tissue from rats in different groups stained with Masson's trichrome (MT):

A)Control and RJ-treated groups showing regular arrangements of collagen fibers in the tunica albuginea (TA), which are differentiated into outer layer (OL), which consists of wavy fibers that run longitudinally, and the inner layer (IL), which is oriented circularly. The collagen fibers of the trabeculae (Tr) appeared dense and wavy. VS = vascular space. MT X200

B)Diabetic group showing a thin disrupted tunica albuginea (TA) lamellar arrangement of collagen fibers between the outer and inner layers. The trabeculae (Tr) appeared shrinking and irregular in size and shape dense collagen fibers. VS = vascular space. MT X200

C) Compared with diabetic rats, the diabetic rats cotreated with RJ exhibited less marked damage. In the TA, most of the collagen fibers retained their lamellar arrangement while the trabeculae appeared more regular in size and shape. VS = vascular space. MT X200

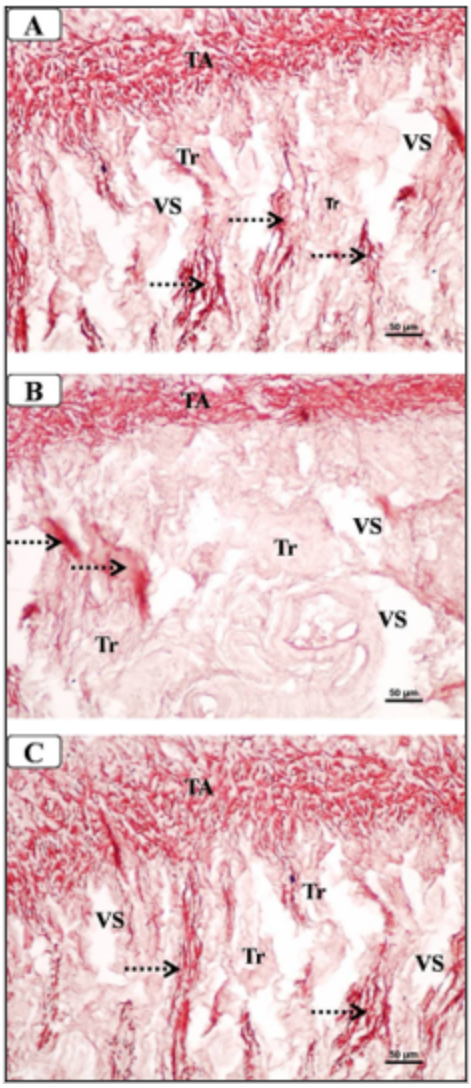

Figure 7

Representative photomicrographs of longitudinal sections of penis tissue from rats in different groups stained with Orcein:

A) Control and RJ-treated groups showing the presence of many elastic fibers (dotted arrow) in both the tunica albuginea (TA) and trabeculae (Tr). These elastic fibers (red color) appeared thin and branching. VS = vascular spaces. Orcein X200

B)Diabetic group showing marked decrease in elastic fibers with the presence of some disorganized and condensed fibers in the trabeculae (Tr) (dotted arrow). VS = vascular spaces. Orcein X200

C) Rats in the diabetic group cotreated with RJ showed more elastic fibers within the TA and trabeculae (TA) (dotted arrow) that appeared more or less similar to those of control rats. VS = vascular spaces. Orcein X200

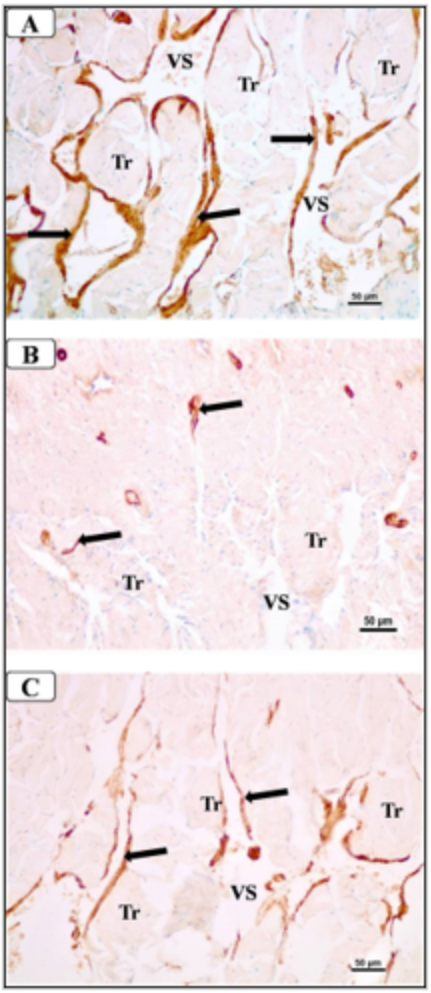

Figure 8

Representative photomicrographs of transverse sections of penis tissue from rats in different groups stained immunohistochemically with an anti-α-SMA antibody:

A) Control and RJ-treated groups showing the SMCs (thick arrow), which appeared as spindle-shaped cells located around the trabeculae (Tr) in the wall of vascular spaces (VS), forming a subendothelial layer. Anti-α-SMA X200

B)Diabetic group showing a marked decrease in SMCs (thick arrow) around the trabeculae and wall of vascular spaces (VS) between the trabeculae (Tr) compared to those in the control group. Anti-α-SMA X200

C) Compared with those in the diabetic group, diabetic rats cotreated with RJ showed more immunopositivity with better orientation of the SMCs (thick arrow) around the trabeculae and vascular spaces. Anti-α-SMA X200

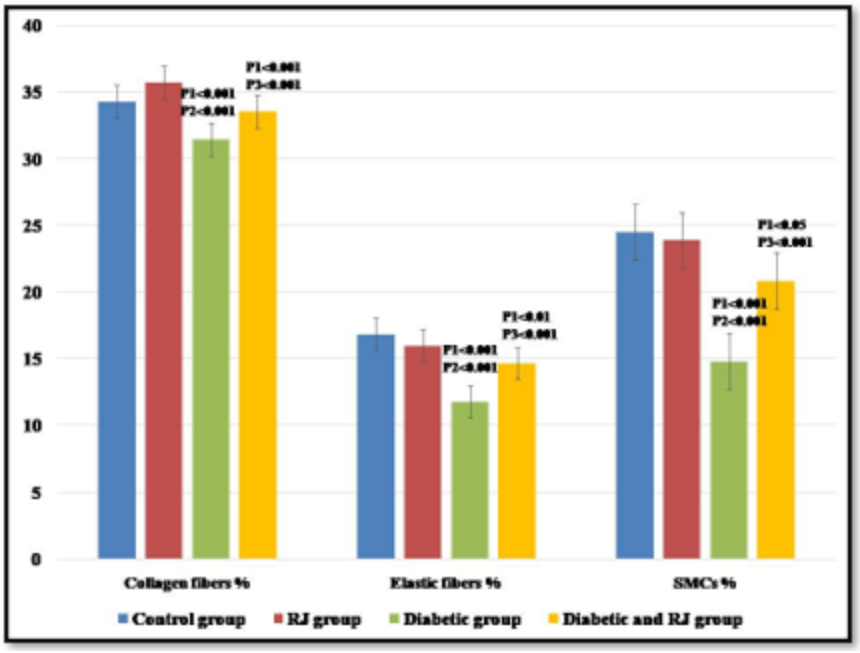

Figure 9

Graphical representations of the statistical analysis of the mean area percentages of of collagen fibers, elastic fibers and SMCs in the corpora cavernosa in the different groups.

The control group (G1), RJ group (G2), diabetic group (G3), and diabetic and RJ group (G4) were included.

P^1: significant versus G1; P^2: significant versus G2; P^3: significant differences from G4.

The values are expressed as the mean and SDs. Significance was determined using one-way ANOVA test followed by the Turkey test.