Effects of curcumin on testis microvascular complication in streptozotocin-induced diabetes rats

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ABSTRACT

Background: Diabetes has a significant impact on the impaired function of the reproductive system in the testis.

Objective: The aim of this study was to investigate the effects of curcumin from turmeric on structural changes of testicular tissues and microvasculature in STZ-induced diabetic rats.

Methods: Rats were injected with a single dose of streptozotocin (60 mg/kg BW) to induce a diabetic condition. Three days after the injection, rats with a blood sugar level > 250 mg/dl were considered diabetic. Rats were allocated to one of three groups, diabetic (DM), diabetic rats receiving curcumin (DMC) (200 mg/kg BW), and control (C). Testicular tissues were studied eight weeks after the treatment using the histological technique. Testicular microvasculature was examined using the vascular corrosion cast technique under a scanning electron microscope (SEM).

Results: The testicular tissue of DM group showed damaged seminiferous tubules, spermatocytes, and spermatids with necrosis and germ cells degeneration. Destruction of testicular arteries and veins in pampiniform plexus were demonstrated. The destroyed capillaries, arterioles, and venules demonstrated stenosis and shrinkage. The vessel diameter in all DM groups decreased. In the DMC group which received curcumin, the appearance of all seminiferous tubules, spermatogenic, and sertoli cell was normal. The reconstruction and restoration of testicular artery, pampiniform plexus, capillary network, arterioles, and venules

were clear. As a result of curcumin supplementation, the standard and healthy testis microvasculature was restored. The DMC vessels diameter increased back to a quasi normal condition, particularly at the artery, arteriole, and capillary levels.

Conclusions: The results provided evident for effects of curcumin could be associated with recovering and improvement of the testicular tissues and microvasculatures in diabetic condition.

Keywords: Diabetes mellitus, Streptozotocin, Curcumin, Testis, Microvasculature, Vascular corrosion cast, Scanning electron microscope

BACKGROUND

Diabetes mellitus is a prolonged disease that incorporates the damaging impact of hyperglycemia characterized by high blood glucose which results from abnormal production and secretion of insulin. A diabetic patient is unable to metabolize carbohydrates, proteins, or fats due to insufficient insulin production or inability of the receptors to respond to insulin. Insulin enables cells to internalize glucose, which is used as an energy source. However, the cells of a diabetic patient are unable to use glucose from the blood stream, resulting in hyperglycaemia, a condition characterized by abnormally high levels of blood glucose. When these high glucose levels are maintained a long time in the bloodstream, severe damage of the retina and kidney microvasculature occurs, even nerve damage [1, 2]. Epidemiological studies have demonstrated a high incidence of erectile disfunction (ED) in patients with diabetes mellitus (DM) and a correlation between DM and ED [3]. ED was previously thought to occurr concomitantly with a hormonal imbalance, with low testosterone levels and high gonadotropin and oestradiol. However, subsequent studies failed to verify this association. As the testes produce sperm cells and the hormone testosterone, a defective spermatogenesis may be the result of defective testicular from the diabetes [4, 5]. Short-term hyperglycemia impairs the fertilizing ability of sperms taken from rat epididymis [6]. Diabetes is known to reduce rat body weight, decrease reproductive organs, and diminish the count of sperms taken from the testis and epididymis, which is associated with a decrease in plasma testosterone levels. The causes of this plasma testosterone decrease in the diabetic syndrome are unknown. Testicular atrophy and hypogonadism occurs in diabetic men [7, 8]. Discrete ultrastructural lesions of apical Sertoli cell cytoplasm, spermatogenic disruption, and morphological interstitial testicular tissue abnormalities suggest alterations in the microvasculature [9].

Blood vessels comprise of three tunicae or layers, tunica intima, tunica media, and tunica adventitia. The inner most layer is the tunica intima, which covers the vessel luminal side with a single layer of endothelial cells. This layer is surrounded by a matrix of extracellular connective tissue made of proteoglycans and collagen, and then by a layer of elastic cells. Depending on the vessel size, this elastic layer can have various thickness. The next layer, the tunica media, consists of smooth muscle cells surrounded by a thin elastic lamina. The tunica media is the thickest layer of a vessel. The outer most layer, the tunica adventitia, consists of a collagen matrix with intercalated fibroblasts and vascular smooth muscle cells.

The atherosclerosis develops with similar histological changes in diabetic and non-diabetic patients. After an endothelial injury, smooth muscle cell proliferate, foam cells develop and infiltrate the area. Then, platelets are activated, accelerating inflammation. Changes in blood

flow results in altered haemodynamic forces and injury to the endothelial cells from external sources, which determine where in the vessel the lesion will occur [10].

The cytotoxic action of streptozotocin (STZ) involve DNA bases alkylation occurring at specific sites and damage by free-radicals produced during STZ metabolism. DNA damage caused by STZ can be severe, resulting in cell death by apoptosis or necrosis. Diabetic individuals and experimental animals develops high oxidative stress [11]. Turmeric is traditionally considered an anti-inflammatory remedy. Recent studies have shown that curcumin, the active compound of turmeric possesses potent antioxidant and anti-inflammatory activities, have been investigated in several systems [12-15]. Curcumin can modulate several transcriptional factors [16-22], cytokines [23-27], growth factors (28-30), kinases (31-33), and other enzymes [34-36].

As several diseases result from dysregulated inflammation, it is important to find anti-inflammatory agents that are safe and effective. Morphological changes in testis of diabetic rats are known (37). However, it is unknown which morphological changes in the testicular blood vessels may result from the treatment and supplementation with curcumin. The present stud thereby aims to determine the effects of curcumin on testicular blood vessels in experimentally-induced diabetic rats.

MATERIAL AND METHODS
Induction and assessment of diabetes
The study was carried on male Wistar rats (200-250 g). The experimental procedure of the animal research was approved by Srinakarinwirot University Medical Center Animal Care Committee., Thailand and the protocols were reviewed and approved. All animals were kept and maintained in a controlled animal laboratory environment on alternative 12-hour light/dark periods ($25+2°C$), fed with standard rat chow and with access to water ad libitum. Experimental diabetic rats were induced by streptozotocin (STZ) (Sigma, St, Louis, MO, USA) 60 mg/BW dissolved in 0.1 mol/l citrate buffer injected intravenously into the lateral tail vein. Control rats received injection with citrate buffer alone. The blood sugar level was measured and analyzed by one-touch glucometer after the last injected with STZ for three days. Rats with a blood sugar level > 250 mg/dl were considered diabetic. Control and diabetic rats were randomly assigned to one of three groups: control rats (C), diabetic rats (DM), and diabetic rats supplemented with curcumin (DMC) in corn oil daily (99.99% pure, 200 mg/kg BW, Sigma, St. Louis, MO, USA). All animals were clinically observed and weighed weekly. At the end of 8 weeks after STZ injection or curcumin supplementation, the rats in each group were killed, the testis removed, dissected, and immediately fixed in Bouin's solution (Sigma, St, Louis, MO, USA) in preparation for histological study under a light microscope. For vascular corrosion casting technique, the rats of each group were used for studying the microvasculature of the testis by methyl methacrylate injection (Batson's no. 17) (Polysciences, Warrington, PA) and corroded with 10% KOH solution combined with scanning electron microscopic (JEOL JSM-5400 SEM) observation.
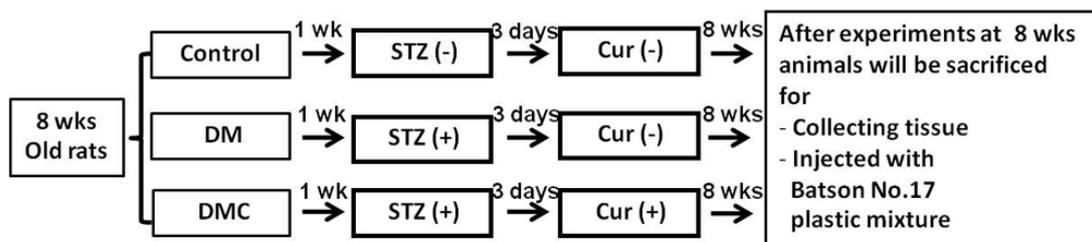
Figure 1. A flowchart of the experimental design.

Histological preparation

The testis was fixed in Bouin's solution overnight and then dehydrated in graded series of ethanol through 70, 80, 90, 95, and 100% with two changes for 1 hour each. Three changes of xylene with 30 minutes each were used as clearing reagent before filtration. The tissue was embedded in paraffin, sectioned at 5 µm thick, and then stained with hematoxylin and eosin (H&E). All sections were examined and photographed using an Olympus light microscope (BX-50, Olympus, Japan).

Vascular corrosion casting technique

Each animal of every group was deeply anaesthetized with thiopental (i.p.), laid down on the metal mesh placing in the stainless tray. The thoracic cavity was opened by subcostal incision to create a chest flap that was closed clamped and retracted to expose the heart. Then, heparin, 0.5 ml (5,000 IU/ml) was quickly injected into the left ventricle and allowed to circulate for 1 to 2 minutes. The left ventricle was then pierced with a blunt no. 18-gauge needle and its tip directed towards the lumen of the ascending aorta. A needle was held in place with an arterial clamp. The right atrium was cut and opened, being the outlet part for blood, and fluid was injected. Approximately 400-500 ml of 0.9% NaCl solution was infused through a cannula until the effluent was clear. Then, Batson's no. 17 plastic mixture was immediately injected into the cannula, following the perfusion with NaCl solution through the ascending aorta. Each animal was left at room temperature to allow the casting medium to settle. The testis was excised and immersed in warm water (80°C) to assure polymerization of the plastic. Testes were dissected and immersed in 10% KOH for 30-60 days to let the tissue disintegrate, leaving a casting of the vessels. The specimens were rinsed in slow running tap water and washed several times with distilled water. The preparation was then dissected, trimmed under a stereomicroscope, and left to air dry. The vascular cast of testis was placed on a metal stub with double side adhesive tape, sprayed with carbon paint, and coated with gold on a sputtering apparatus. Finally, the casts were examined under the scanning electron microscope (JEOL JSM-5400) at accelerating voltage of 10 KV.

Statistical analysis

The diameter of testis blood vessels, the average diameter and epithelial thickness of seminiferous tubules were measured by SemAfore computer software program. The results were expressed as mean ± standard error of the mean. Statistical analysis was performed by

using ANOVA followed by Bonferroni posttest. A *p*-value smaller than 0.05 was considered statistically significant.

RESULTS

Histological observations of testicular tissue

The histological investigations of testicular tissue and blood vessels among three different groups of rats (control, DM, and DMC) throughout the 8 week experiment demonstrated that the testis in control group was encapsulated by thick fibrous connective tissue called tunica albuginea (Figure 2A) and subcapsular arteries (Figure 3A). All seminiferous tubules were surrounded by interstitial connective tissue. The interstitial connective tissue revealed the cluster of interstitial Leydig's cells and intratesticular vessels (Figure 2B). Seminiferous tubules were lined with many layers of normal organization and had compacted with spermatogenic cells. Spermatogonia lie on the basement membrane; spermatocytes are displayed above them; spermatids are arranged above the spermatocytes in one to two layers. In an ordinary seminiferous tubule, four age groups of cells develop at the same time and in correct organization with each other. As each age group of the cells grows, Sertoli cells support their movement through the epithelium until the fully formed sperm are released into the tubular lumen.
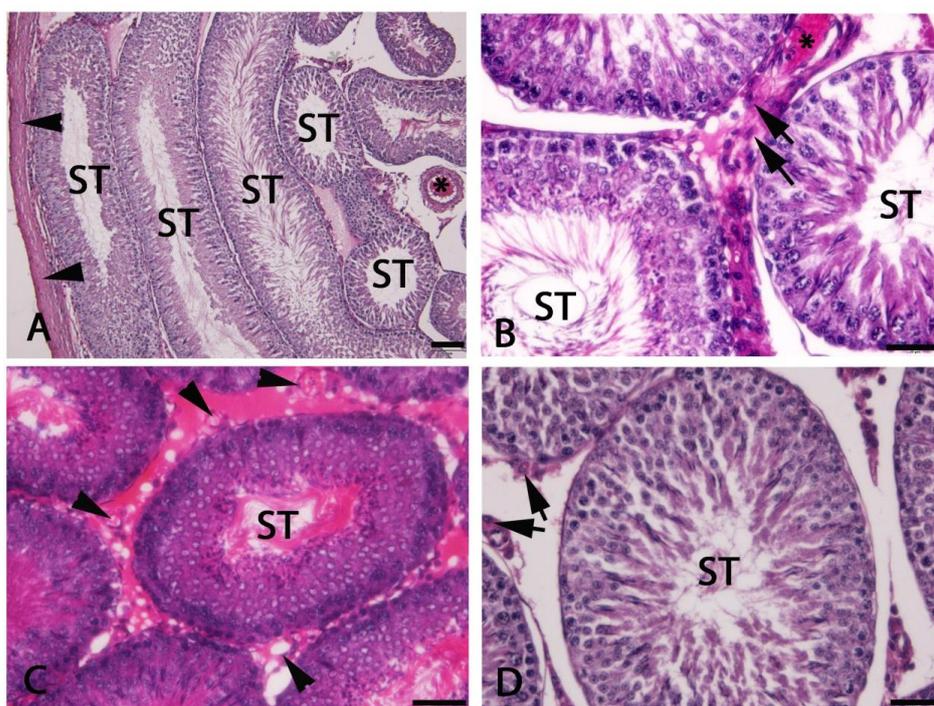

Figure 2. Light micrographs of testicular tissue of rats stained with H&E. (A) control group, demonstrated the rat testis was encapsulated by tunica albuginea (arrowhead) and have many seminiferous tubules (ST) inside (10X). Bar = 50 µm. (B) Each ST was surrounded by interstitial connective tissue (arrow) that revealed the cluster of Leydig's cell and blood vessels. (asterisk) ST had compacted of spermatogenic cells. (40X). Bar = 20 µm. (C) DM group, illustrating ST revealed irregular in shape. The number of spermatogenic cell layer was reduced and germ cells degeneration. Interstitial spaces (arrow) showed exudate and apparently thickened blood vessels (arrowheads) (40X). Bar = 20 µm. (D) DMC group, showing recovering ST and spermatogenic cells and Leydig cells (arrows). (40X). Bar = 20 µm.
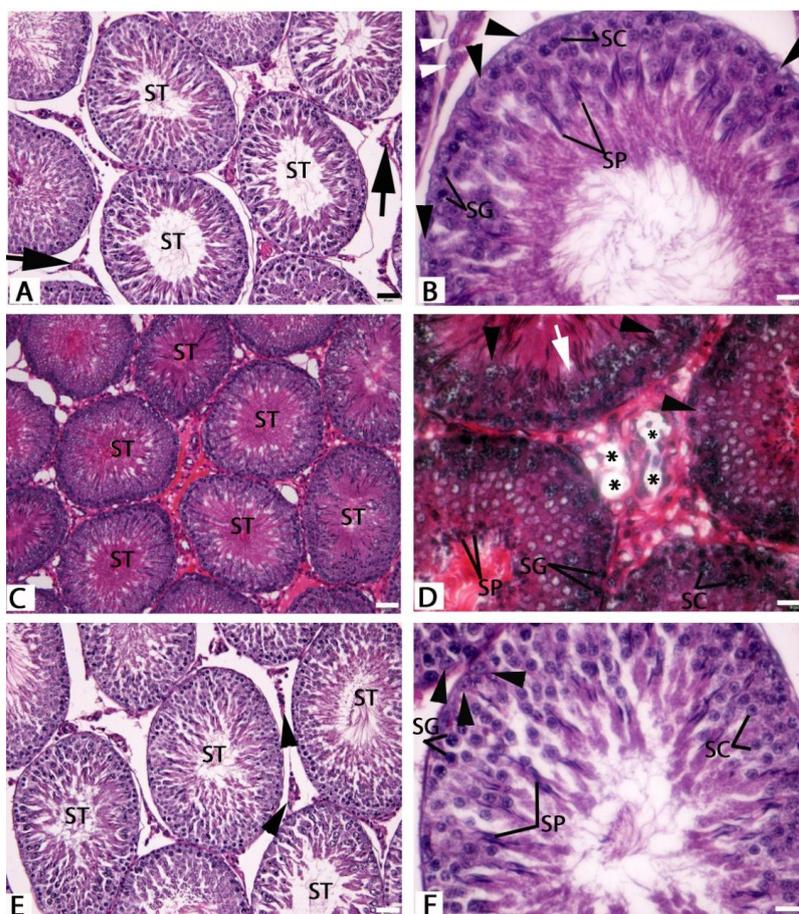
Figure 3. Light micrographs of testicular tissue of rats stained with H&E. (A) Control group, the seminiferous tubules (ST) were surrounded by interstitial connective tissue with Leydig's cells (arrows) (20X). Bar = 20 µm. (B) ST epithelium was structurally intact and showed normal association of germ cells. The tubules had the cluster of Leydig cells (white arrowhead). This presentation indicated normal interstitial tissue, Sertoli cells (Black arrowhead). (60X). Bar = 10 µm. (C) DM group, illustrating the small diameter of ST (20X). Bar = 20 µm. (D) DM group, demonstrating the germ cells degeneration. Many ST were presented vacuoles in Sertoli cells (black arrowhead). Moreover, the multinucleated cells (white arrow) with two or three nucleus were revealed in some of seminiferous tubules. The thickened blood vessels (asterisks) were also seen. (60X). Bar = 10 µm. (E) DMC group, illustrating redeveloping of ST and Leydig's cells (black arrowhead). (20X). Bar = 20 µm. (F) DMC group, ST were presented normal spermatogenic and sertoli cell and Leydig cells (60X). Bar = 10 µm. SG = spermatogonium, SC = spermatocytes, SP = spermatid

All types of spermatogenic cells demonstrated normal cellular attachment (Figures 1B, 2A and B). This presentation suggested that the interstitial tissue, Sertoli cells, and epithelium thickness were normal. The seminiferous tubules showed all the characteristic developmental stages of spermatogenesis. Spermatozoa were seen in the lumen of the tubules. The testicular tissue of DM group demonstrated abnormal histology. The damage in seminiferous tubules had irregular shape and small diameter. The illustrated seminiferous epithelium was altered from control rats in the distribution pattern. (Figures 1C, 2C). The number of spermatogenic cell layers was reduced, the spermatocytes and spermatids had necrosis, and the seminiferous tubules revealed a reduced number of sperms. Some tubules had few layers of spermatogenic cells with apparent no sperms and germ cells degeneration (Figures 1C, 2D). Many tubules presented vacuoles in Sertoli cells.

The multinucleated cells with two or three nuclei were also revealed in some of the seminiferous tubules. The basement membrane of seminiferous tubules thickened and interstitial connective tissue illustrated more abundant collagen fibers (Figures 2C, D).

Moreover, interstitial spaces showed exudate (Figure 4C) and apparently thickened blood vessels (Figure 4D). The subcapsular arteries were encapsulated by tunica albuginea and the intratesticular arteries were located in the interstitial testicular tissue. Most of the small blood vessels had notably narrow lumens. The diameter of subcapsular artery and the intratesticular artery were seriously increased (Figure 4C and D). In the DMC group, all seminiferous tubules had normal spermatogenic cells (spermatocytes and spermatids) and Sertoli cells. Furthermore, the interstitial space between tubules had a normal appearance. Leydig cells also had a normal appearance but the thickness of the basement membrane and interstitial connective tissue decreased. The intratesticular artery appeared to have normal shape and size. The interstitial space between seminiferous tubules was also normal.
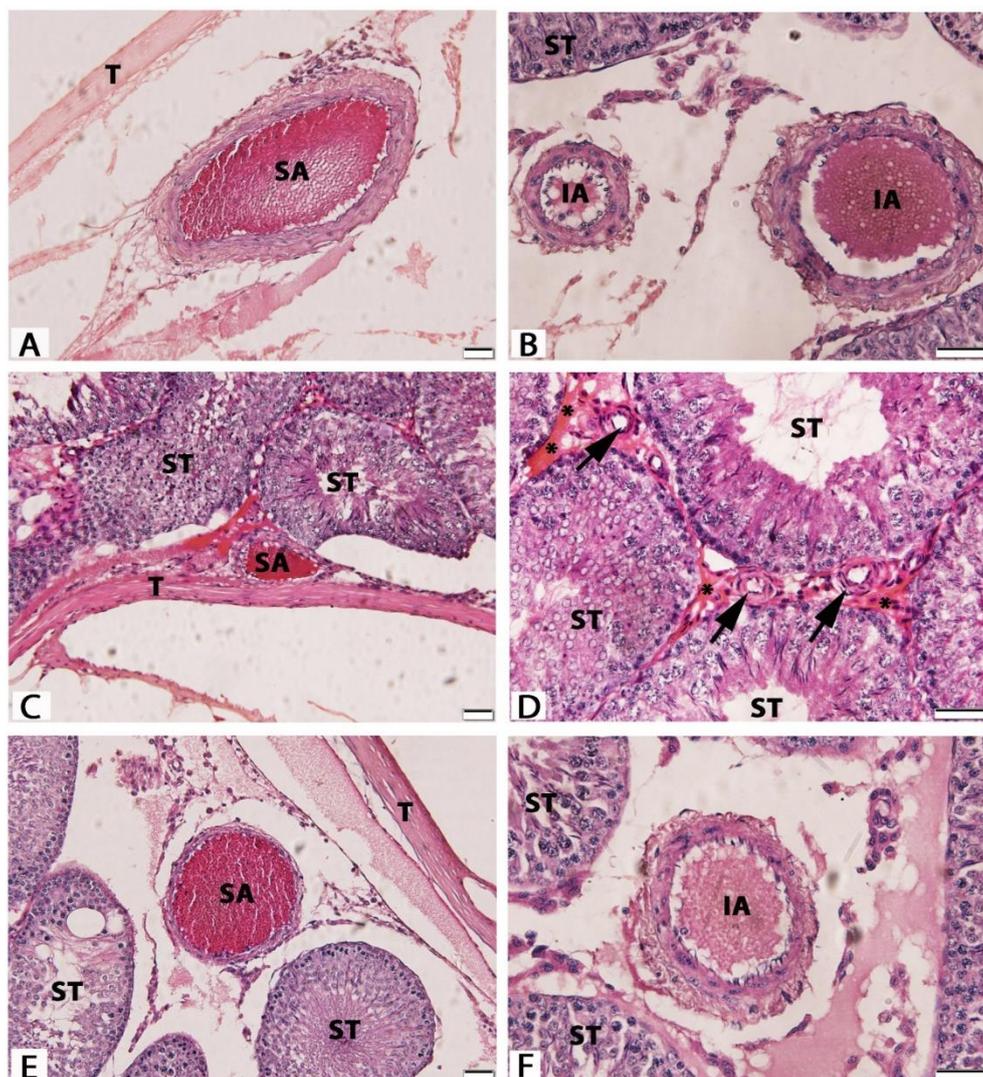

Figure 4. Light micrographs of blood vessels in testicular tissue of rats stained with H&E. (A, B) Control group, A: The subcapsular artery (SA) that lined near tuniga albuginea (T) (20X). Bar = 20 µm. B: The intratesticular arteries (IA) demonstrated normal pattern of three layers of the wall and surrounded by interstitial loose connective tissue between seminiferous tubules (ST) (60X). Bar = 10 µm. (C, D) DM group, C: The subcapsular artery (SA) demonstrated the small diameter (20X). Bar = 20 µm. D: The intratesticular arteries (arrows) had shrinkage revealing the pathology of blood vessels. The interstitial connective tissue around blood vessels had an abundance of collagen fibers (asterisk), ST: seminiferous tubule (60X). Bar = 10 µm. (E, F) DMC group, E: The seminiferous tubules (ST) and intratesticular artery (IA) were an improvement and were the control group. (20X). Bar = 20 µm. (F): the appearance of all intratesticular artery (IA) all had a normal shape and size. The interstitial space between seminiferous tubules was quite natural. ST: seminiferous tubule (60X). Bar = 10 µm.

The graph data of the average diameter (Figure 10) and epithelial thickness (Figure 11) of seminiferous tubules of DM group demonstrated significant decrease when compared to the control group ($p<0.001$, $p<0.001$ respectively). In contrast, they were significantly increased after treatment with curcumin in DMC rats ($p<0.01$, $p<0.01$ respectively).

Vascular corrosion casting technique to examine the testicular microvasculature
The low magnification of vascular corrosion cast technique with stereomicroscope revealed the testis in control group at 8 weeks to be highly vascularized. The proximal portion of testicular artery (TA) (Figure 5) was straight along the testicular vein (TV) that become pampiniform plexus (PP), a loose network of small veins found within the male spermatic cord. Testicular artery become convoluted and surrounded by the pampiniform plexus descending directly onto the dorsal surface of the testicular blood vascular bed (Figure 5). This artery turned at the caudal pole of the testis and ascended onto the ventral surface of the testicular blood vascular bed. On this ventral surface, the testicular artery demonstrated 5-7 marked convolutions. Testicular artery finally enters into the testicular parenchyma close to the rostral pole, giving rise to 4-5 parenchymal branches (Figure 5). Afterwards, the parenchymal branches in the interstitial space divided into arterioles of various diameters with mesh of capillaries surrounding the distal portions of the capillaries, converging to form small venules that drained the blood into the collecting veins, respectively. Two collecting veins at both sides of the testis joined together, draining the blood into the pampiniform plexus.
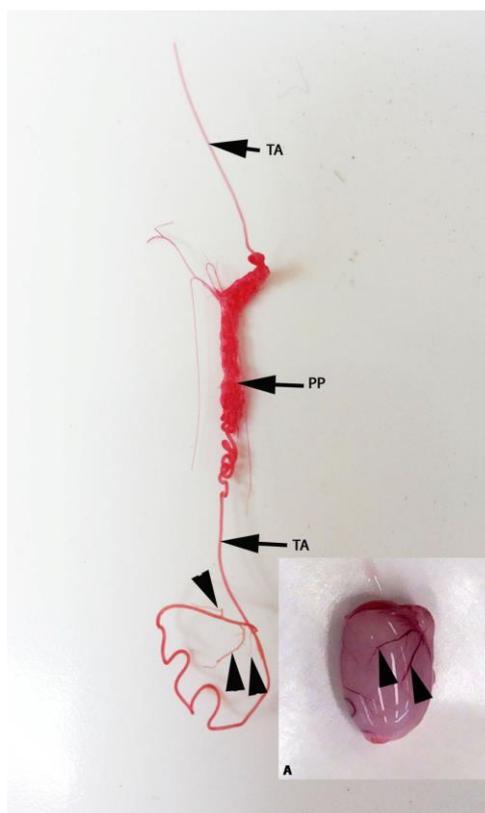

Figure 5. Photomicrograph of whole vascular cast in control rat's testis at 8 weeks. The proximal portion of testicular artery (TA) is somewhat straight. When the artery become the convoluting segments are surrounded by the pampiniform plexus (PP). Testicular artery finally penetrates into the testicular parenchyma near the rostral pole to give off 4-5 parenchymal branches (arrowhead) into testicular tissue.

At high magnification, the testis vascular cast of large blood vessels in control group (Figure 6A, B) demonstrated that the testicular artery was in the typical pattern of coil artery in the spermatic cord and surrounded by pampiniform plexus. In contrast, in the DM group (Figure 6C), the testicular artery and veins in pampiniform plexus were destroyed and shrunk. Moreover, in the DMC group (Figure 6D), presented the testicular artery and pampiniform plexus recovered and redeveloped. At the level of small blood vessels in the testicular tissue, small arterioles came from the intratesticular artery in the interstitial spaces that gave branches into capillaries surrounding the seminiferous tubules. The distal portions of the capillaries converged to form small venules draining blood into the collecting vein. In the DM group, the destroyed capillaries were presented by the small diameter, stenosis and shrinkage of capillaries, arterioles, and venules respectively. After curcumin supplementation in DMC group, the redevelopment and restoration of capillary network, arterioles, and venules were evident.
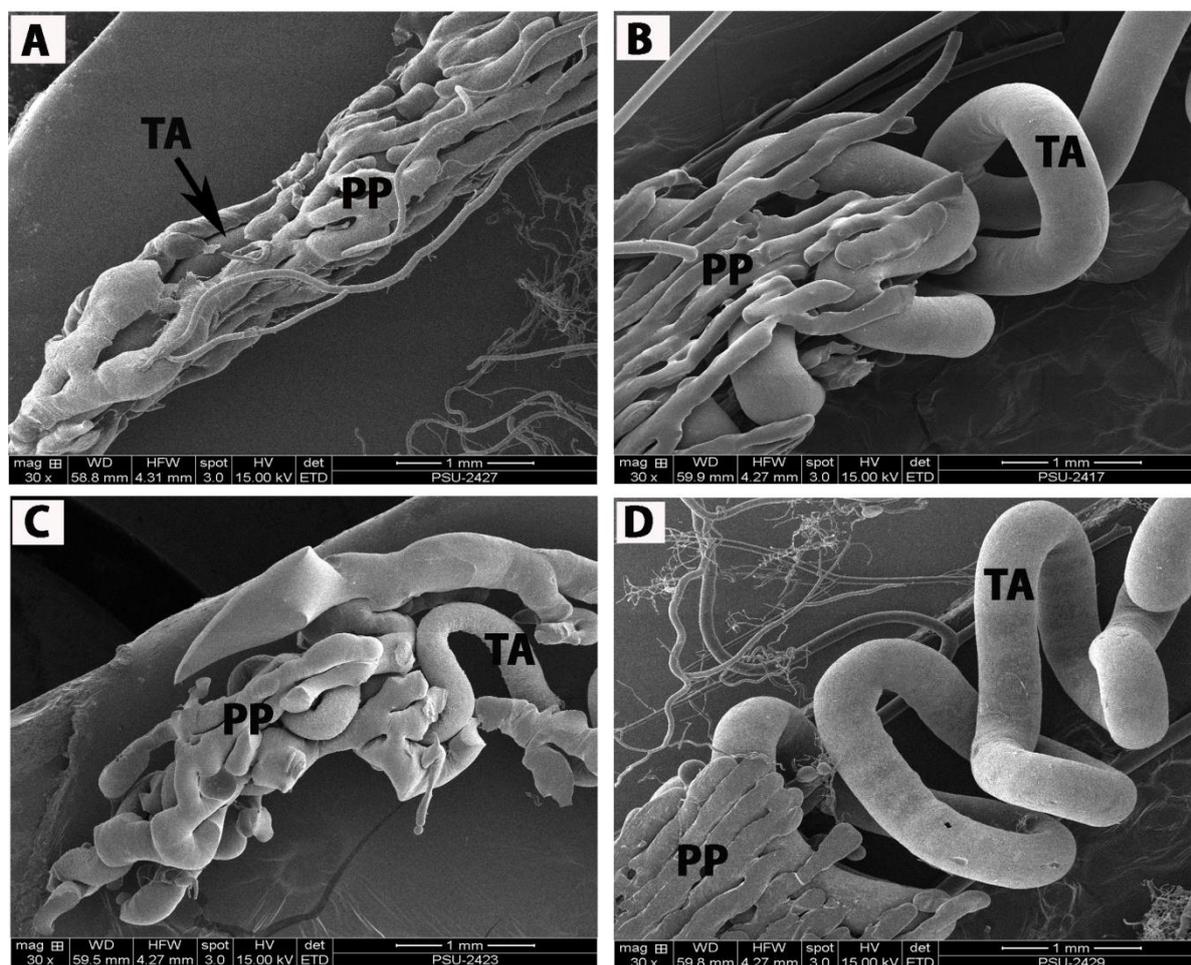

Figure 6. SEM micrographs of testis vascular cast of large blood vessels in rats at 8 weeks. (A, B) Control group, showing the testicular artery (TA) in the pattern of coil artery in the spermatic cord. It was surrounded by pampiniform plexus (PP). (C) DM group, demonstrated the destroyed and shrunken testicular artery (TA) and veins in pampiniform plexus (PP) (D) DMC group, presented the recovery and redevelopment of testicular artery (TA) and pampiniform plexus (PP) Bar = 1mm.
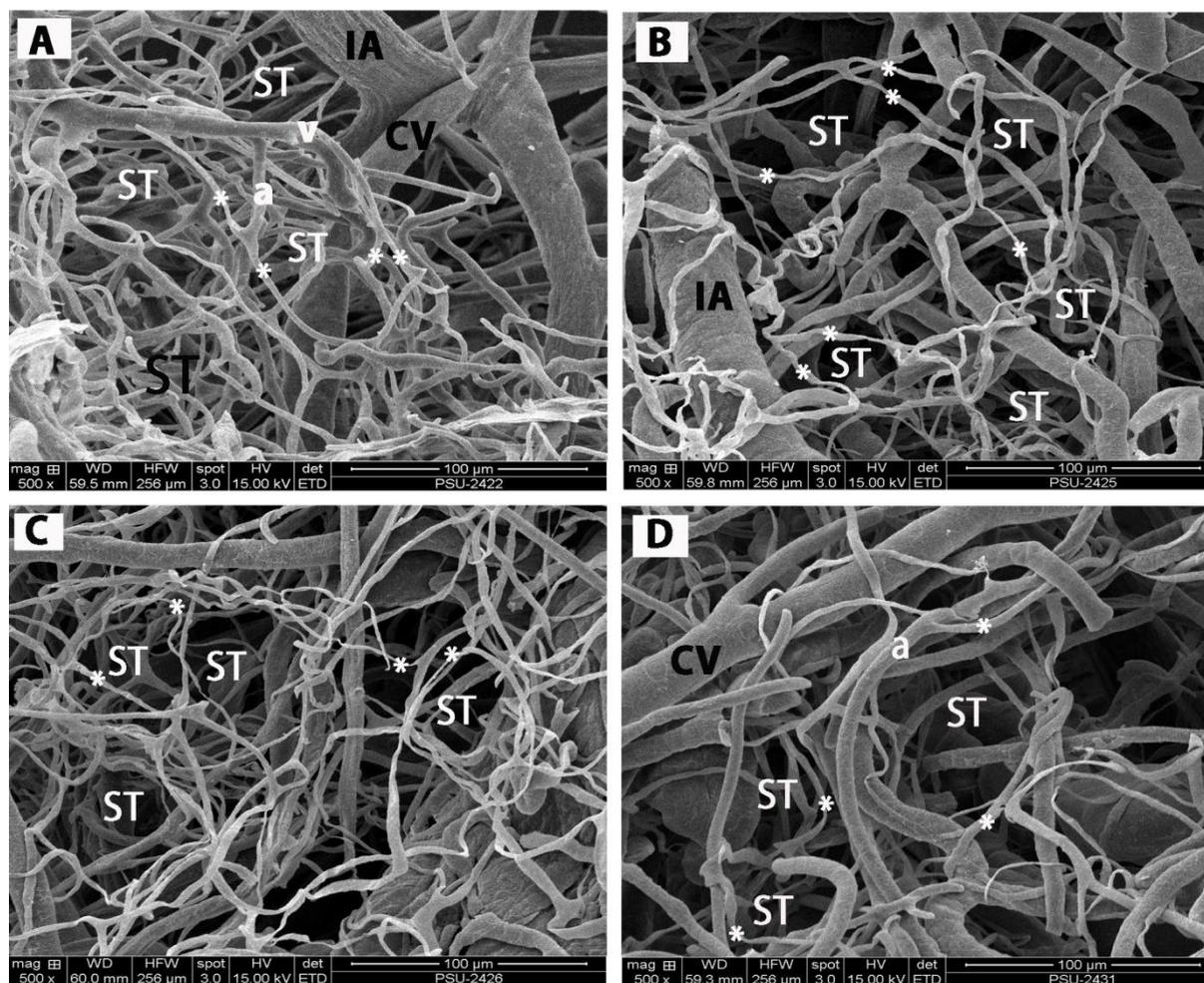
Figure 7. SEM micrographs of testis vascular cast of small blood vessels in rats at 8 weeks. (A) Control group, illustrating small arterioles (a) came from the intratesticular artery (IA) in the interstitial spaces gave branches into capillaries (white asterisk) rounded the seminiferous tubules (ST). Distal ends of the capillaries converged into small venules (V) and drained the blood into the collecting veins (CV), respectively. (B, C) DM group, destroyed capillaries were seen by presentation of stenosis and shrinkage of capillaries (white asterisk), arterioles and venules. (D) DMC group, the redevelopment and restoration of capillaries, arterioles and venules were evidently. Bar = 100 µm.

The comparative diameter sizes of all vessels among C, DM, and DMC groups of the 8 week experiments were demonstrated in large and small types of vessel: TA, TV, PP (Figure 7), Intratesticular artery (IA), Intratesticular vein (IV), arterioles, venules, and capillaries (Figure 8). Diameters of DM vessels were critically decreased and much more severe compared to the control group. TA, TV, IA, IV, and arterioles demonstrated remarkable decrease in sizes more than once within the venules and capillary, through stenosis and shrinkage. Although diameters of pampiniform plexus had no difference among the three groups of rats, low density and aggregration of blood vessels in pampiniform plexus of DM group was seen. Significantly, the signs of vessel restoration and improvement were also presented by the increase of diameters of all types of vessels in the DMC experiment. The diameters of all the vessels were measured and compared in Figures 7 and 8.
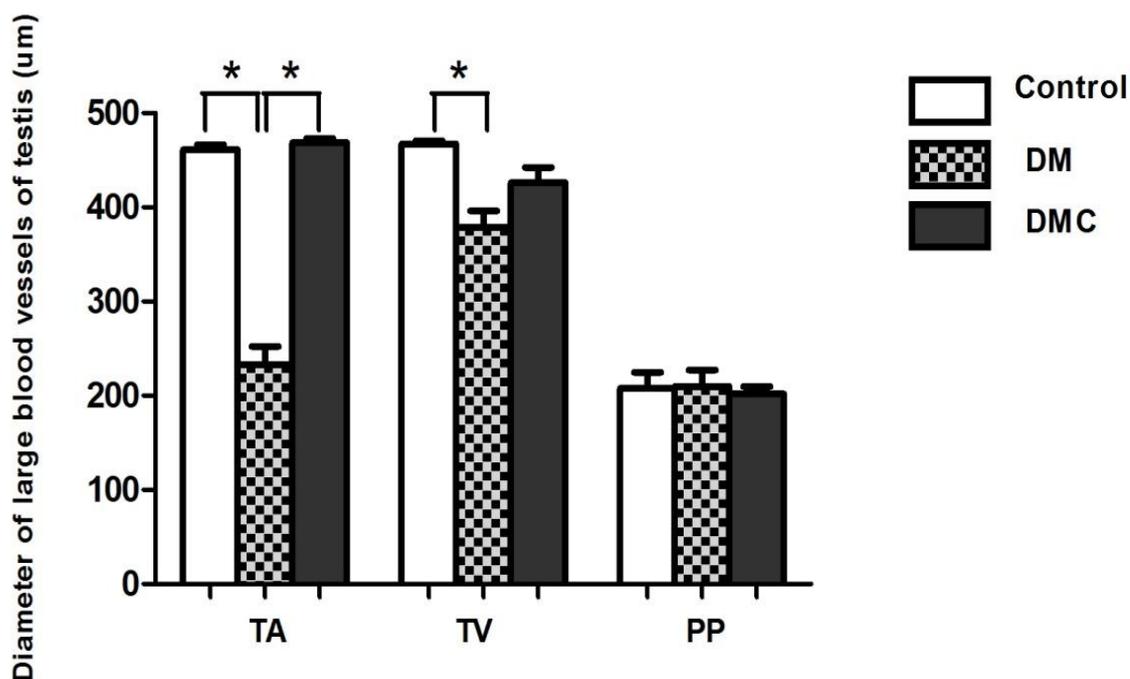
Figure 8. The average diameters of large blood vessels of testis: testicular arteries (TA), Testicular vein and Pampiniform plexus (PP) in control (C), diabetes (DM), and diabetes treated with curcumin (DMC) rats at 8 weeks. Values are mean + SE, *P<0.001
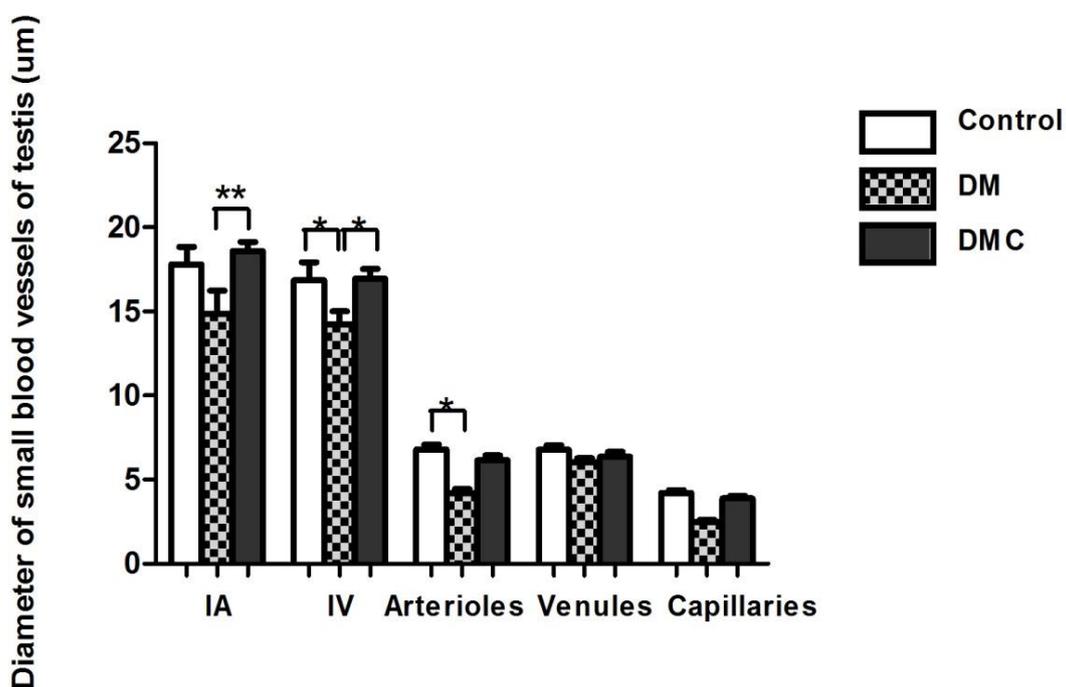

Figure 9. The average diameters of intratesticular arteries (IA), intratesticular vein (IV), arterioles, venules, and capillaries in control (C), diabetes (DM), and diabetes treated with curcumin (DMC) rats at 8 weeks. Values are mean + SE, *P<0.01, **P<0.05
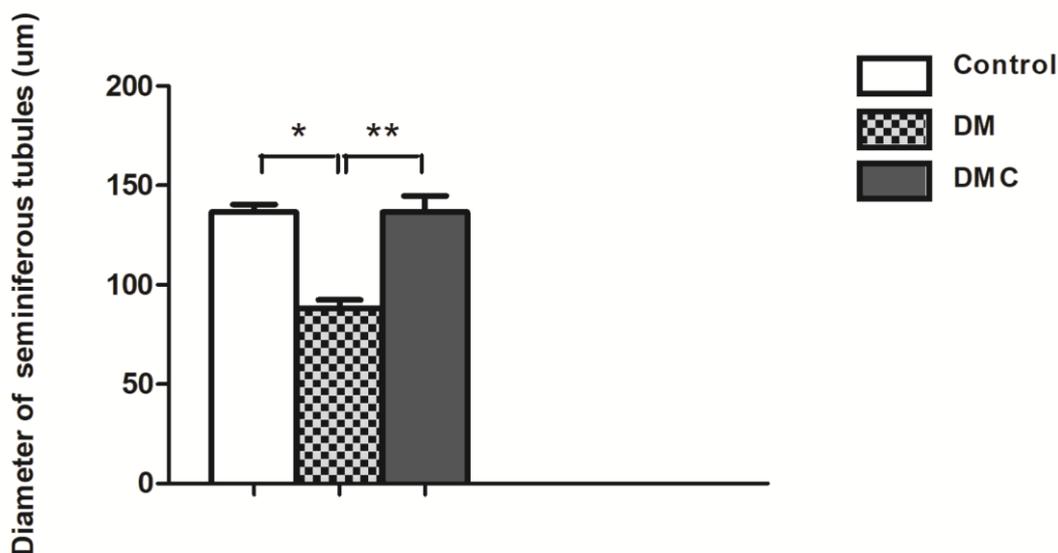
Figure 10. The average diameters of seminiferous tubules in the control (C), diabetes (DM), and diabetes treated with curcumin (DMC) rats at 8 weeks. Values are mean + SE, *P<0.001, **P<0.01
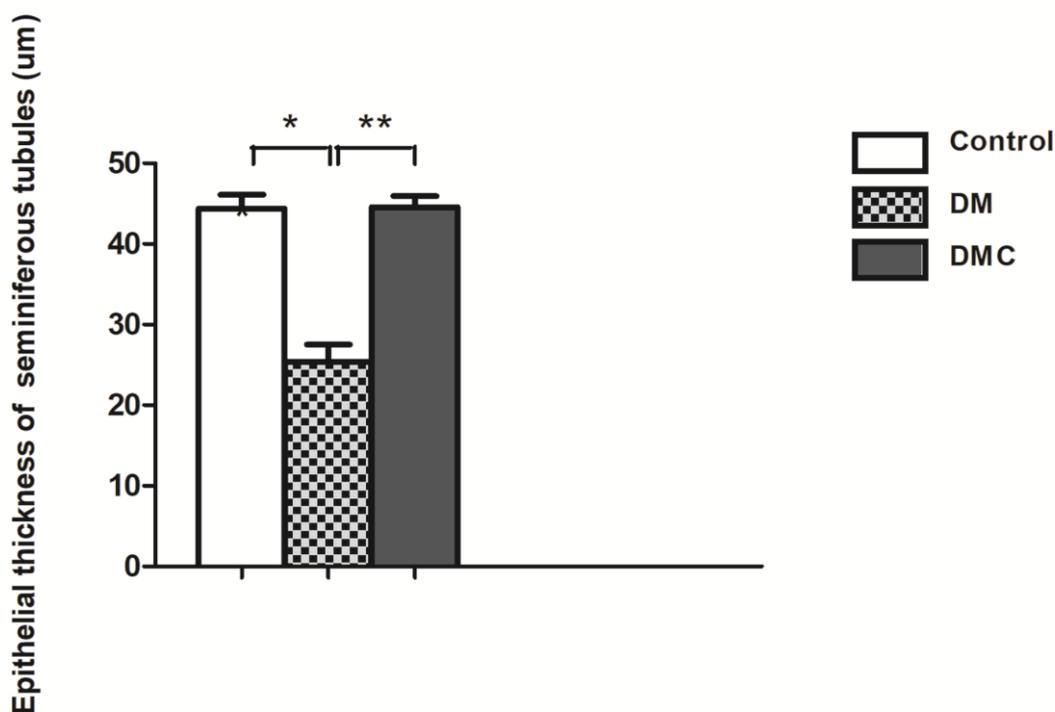

Figure 11. The average epithelial thickness of seminiferous tubules in control (C), diabetes (DM), and diabetes treated with curcumin (DMC) rats at 8 weeks. Values are mean + SE, *P<0.001, **P<0.01

The graph data of the DM large testicular blood vessels (Figure 8) demonstrated significant decrease in the diameter of TA and TV indices when compared to the control group (p<0.001). In contrast, the diameters of TA were significantly increased after treatment with curcumin in DMC rats (p<0.001). Additionally, the diabetic-diameters of small blood vessels (Figure 9), IV and arterioles decreased and were much more severe compared to the ones of control group

(p<0.01). Furthermore, the diameter of IA and IV significantly increased after treatment with curcumin in DMC rats (p<0.05, p<0.01 respectively).

DISCUSSION

Diabetes is a regular health problem which impairs the sexual function of females and males. The hyperglycaemic condition of diabetes interrupts cellular metabolisms and affects the oxidative environments of the tissues and cells. Hyperglycaemia can disturb the testicular function by reducing the fertility of diabetic rat (38). In this study, the seminiferous tubules atrophied, becoming irregular in shape and revealing a small diameter accompanied by the reduction of sperm production in diabetic rats. Spermatogenic cells were reduced and degenerated. In diabetic male rats, Kanter et al. (37) demonstrated a reduction in testis weight, diameters of seminiferous tubules, spermatogenic cells, and blood serum testosterone levels. Furthermore, a reduction in Sertoli cells leads to a reduction in the number of sperms. Sertoli cells play an important role in spermatogenesis or production of sperm, providing physical support, nutrition, and generation of hormone signals essential for spermatogenesis [39]. A condition which decreases Sertoli cells also leads to a decreased number of germinal cells [40]. In diabetes-induced hyperglycemia, reactive oxygen and nitrogen species chemically modify intracellular macromolecules, including carbohydrates, lipids, nucleic acids, and proteins [41]. A consequence of hyperglycemia is an overproduction of reactive oxygen species (ROS) as a reaction of mitochondrial malfunction. Hyperglycaemia may damage blood vessels by upregulation of protein kinase C (PKC). As the hexosamine and polylol pathways are activated, there is an increase in the production of advanced glycation end products (AGE) and an upregulation of RAGE receptors. Since glucose is taken up in several tissues, a mechanism independent of insulin, an increase in serum glucose raises the intracellular glucose concentration [42]. An increase in the generation of ROS alongside the weakening of the antioxidant defense system leads to oxidative stress. In diabetic patients, oxidative stress results from hyperglycaemia which stimulates the production of several reducing sugars via the polyol pathway and glycolysis pathways, decreasing the capacity of endogenous antioxidants. The effects of diabetes on the vasculature is considerable. Diabetes impairs the endothelium and smooth muscle cells. However, diabetes also perturbs platelets, lipoproteins, local vasoactive substances, clotting factors, and triglycerides. Diabetes enhances the local response to hypoxia and the formation of new collateral vessel [43]. In diabetic rats, the diameter of subcapsular artery and the intratesticular artery were seriously decreased. Diameters of DM vessels from vascular casting were critically decreased and much more severe compared to the control group. TA, TV, IA, IV, and arterioles revealed remarkable decreases in sizes more than ones of venules and capillary that were seen by presentation of stenosis and shrinkage. Interestingly, the signs of vessel restoration and improvement were also presented alongside the increase of diameters of all types of vessels in DMC experiment. Thus, a common long-term complication of diabetes is vascular disease which damages blood vessels all over the body.

Insulin resistance and oxidative stress are directly related in mediating the cardiovascular complications of diabetes. Insulin helps to maintain the important physiological function of endothelium. Insulin is able to induce NO release through the activation of the PI3K-Akt signaling cascade leading to serine phosphorylation of eNOS. In an insulin resistant condition, the PI3K signalling decreases, activating the mitogen-activated protein kinase (MAPK) and increasing the secretion of ET-1, resulting in endothelial dysfunction [44]. This disfunction is

characterized by an inability of arteries and arterioles to dilate properly in response to a suitable stimulus by vasodilators. This endothelial dysfunction is related to a decrease in the NO bioavailability, which may be the result of reduced NO synthesis by the endothelial cells or by deactivation of NO by caused by ROS [45].

Curcumin plays an important role in ameliorating diabetic vascular disease by modulating PKC-α, PKC-β2, and recovering STZ-induced testicular damage, oxidative stress, and apoptosis possibly via regulation of NADPH oxidase activity and the MAPK pathway [46-48]. In an experimental model of diabetic cardiomyopathy, curcumin prevents accumulation of AGE collagen and cross-linking of collagen in the tendon and skin [49]. Curcumin decreases oxidative DNA and protein damage via a decrease in endothelial nitric oxide synthase (eNOS) and an induction of nitric oxide synthase (iNOS). In STZ rats, curcumin debilitated diabetes-induced vascular dysfunction by enhancing the ratio of prostanoidmetabolites PGI (2)/TXA (2) via suppression of cyclooxygenase-2 (COX-2) activity, NF-κB, and PKC [50]. In hypertension associated diabetic rats, curcumin induces heme oxygenase-1 (HO-1) normalizing the vascular contractility via a reduction in TNF-α and ROS [51]. In STZ-rats [52] and mice [53], curcumin is able to improve the endothelial dysfunction by its own antioxidant activity and by inhibiting PKC.

According to vascular complication, our previous works have continuously established that curcumin restores and reforms microvascular architectures in the STZ-induced diabetes, (including in the liver) and choroid vessels in eyes and heart [54-56]. The research findings show the potential effects of curcumin as therapeutic agent on diabetic complications. Considering the intake of curcumin in the daily diet could be recommended the anti-inflammatory and antioxidant properties of curcumin should be carefully considered [57].

CONCLUSION

The results of our study demonstrated that the effects of curcumin could improve the destruction of the testicular microvasculature and tissues in diabetic rats. Interestingly, the signs of vessel restoration and improvement were presented by increasing the diameters of all types of vessels in DMC experiment that study by vascular corrosion casting. The vessels diameter increased back to a quasi-normal condition, particularly at the artery, arteriole, and capillary levels. The reconstruction and restoration of testicular artery, pampiniform plexus, capillary network, arterioles, and venules were restored. This study could be useful in examining the role of curcumin as a natural remedy in the damage of testicular and vascular tissue caused by diabetes.

List of Abbreviations: C, control; DM, diabetic; SEM, scanning electron microscope; STZ, streptozotocin; TA, testicular artery; TV, testicular vein; PP, pampiniform plexus; IA, intratesticular artery; IV intratesticular vein.

Competing Interests: The authors declare no conflict of interest.

Authors' Contributions: VA and WK designed and conducted the research and performed the vascular corrosion casting. MK and PB assisted for tissue preparation and staining methods. WK and VA wrote the manuscript and performed statistical analysis. All authors read and approved the final version of the manuscript.

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