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Beneficial effects of gymnemic acid on three-dimensional vascular architecture and expression of vascular endothelial growth factor of intrarenal segmental and interlobar arteries in diabetic rat kidney

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ABSTRACT

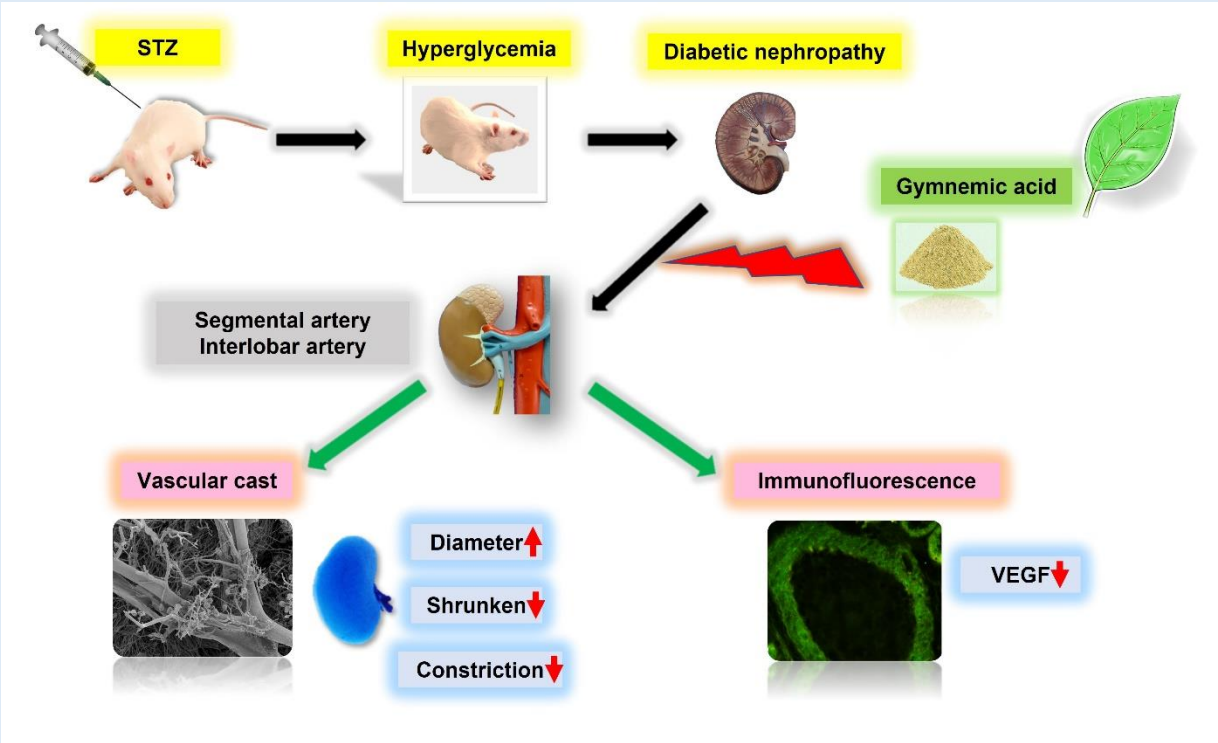
Background: A high prevalence of atherosclerotic vascular lesions has been associated with renal disease and diabetes and is a major cause for increasing deaths from cardiovascular disease. The present study aimed to determine the beneficial effects of gymnemic acids on the kidney microvasculature and to establish their anti-angiogenic properties that are related to the expression of vascular endothelial growth factor (VEGF) protein of segmental and interlobar arteries in induced diabetic rats.

Methods: Rats were divided into five groups including the control group (C), control treated with gymnemic acid (CGM), diabetic animals (DM group) that were rendered diabetic by a single dose [60 mg/kg body weight (BW)] of a streptozotocin (STZ) injection, diabetic rats treated with gymnemic acid (400 mg/kg BW) (GM), and diabetic rats treated with glibenclamide (4 mg/kg BW) (GR). After 8 weeks, kidney tissues were collected for histological analysis. In rats with DM, the segmental arteries exhibited increased wall thickness. The kidney microvasculature was examined using the vascular corrosion casting method.

Results: Rats with DM presented a decreasing diameter of segmental and interlobar arteries. They were evidently redeveloped and restored in the GM and GR groups. As determined by immunofluorescence, the expression of VEGF was significantly reduced in both the GM and GR groups.

Conclusions: The present study demonstrated that gymnemic acid from *Gymnema sylvestre* may be a promising medical herb for use in the treatment of diabetes and kidney disease.

Keywords: diabetes mellitus, segmental artery, interlobar artery, gymnemic acid, vascular architecture



INTRODUCTION

Metabolic alterations associated with diabetes lead to damaging of blood vessels and the resulting imbalanced blood circulation for cells and tissues. It can cause pathological injuries, such as glomerular hypertrophy, glomerulosclerosis, tubulointerstitial inflammation, and fibrosis in diabetic nephropathy (DN) (1). DN is observed in patients who are diabetic worldwide and is the leading cause of chronic kidney disease (CKD) (2). Diabetes and an underlying CKD intensify the risk of mortality from cardiovascular disease (3).

The kidneys receive blood supply from paired arteries on the lateral side of the abdominal aorta (4). Each renal artery enters the hilum and continues to branch into segmental arteries that pass between the lobes of the kidneys to form the interlobar arteries, which extend between the renal pyramids. Segmental and interlobular arteries carry blood from the renal artery to the kidneys. Vascular lesions are specific to diabetic kidneys. The glomerular filtration rate (GFR) was lower than normal and increased afferent and efferent arteriolar resistance, leading to the progression of renal dysfunction. Segmental branch of renal artery and interlobar artery stenosis can be a cause of renovascular hypertension, and in one large angiographic series was found to occur in 11% of patients (5). The interlobar arteries then yield branches that run through the border of the cortex and medulla of the kidneys to form the arcuate arteries. Each arcuate artery branches into some interlobular arteries that provide for the afferent arterioles supplying the glomeruli.

DN is one of the major microvascular-associated complications of diabetes. Its progression is associated with several changes in the basement membrane of the glomeruli, capillaries, and renal tubules, and there is evidence of interstitial fibrosis. Hyperglycemia from

diabetes stimulates the excessive production of reactive oxygen species (ROS) in mesangial cells and podocytes (6). ROS can increase the glomerular extracellular matrix deposition that regulates the expression of profibrotic connective tissue growth factor (7). Hyperglycemia also upregulates the advanced glycation end-products (AGEs) in the glomerular basement membrane and the extracellular matrix of mesangial cells (8). Finally, mechanical stress associated with intraglomerular hypertension can cause the damage of podocytes and glomeruli (9).

Angiogenesis is the process of forming new blood vessels form pre-existing vessels, which boosts the physiological function of tissues important for the progression of diseases, such as during inflammation and cancer (10). Vascular endothelial growth factor (VEGF) is often associated with angiogenesis and increases endothelial numbers, caused by an imbalance in cell proliferation and apoptosis (8) in glomerular endothelial cells. (11). Glomerular hypertension may be the condition in angiogenesis in diabetes (12). Previously, DN was induced in an animal model by streptozotocin (STZ) injection to observe the endothelial nitric oxide synthase (eNOS) knockout (eNOSKO), and abnormally developed blood vessels and angiogenesis were observed in the kidneys (13). The abnormal vessels found in diabetes were presented by a thin wall of the basement membrane, swollen endothelial cells, and an increase in vascular permeability (14).

Gymnemic acids are triterpenoid compounds isolated from *Gymnema sylves*tre and used in the treatment of asthma, eye complaints, inflammations, and snakebites. They also exhibit antimicrobial and sweet taste bud-repressing activities and are also known to have hepatoprotective and anti-hypercholesterolemic properties (15). As recently reported in patients with type 2 diabetes, an extraction of *Gymnema sylvestre* exerted anti-diabetic effects *in vivo*, as observed by increased plasma insulin and C-peptide levels, and additionally decreased blood glucose concentrations in a small cohort of patients with hyperglycemia (16). The absorption of glucose occurs in the simple columnar epithelium of the external layer of the small intestine. Gymnemic acid binds the Na+ glucose symporter in the intestine and stops the glucose molecule from binding to the receptor, thus preventing excess glucose absorption (17). The demonstrated neuroprotective effects of *Gymnema sylvestre* extract in sciatic tissue in diabetic rats may be associated with their inhibitory effects on the excessive activation of inflammatory molecules and oxidative stress mediators (18). The present study aimed to determine the kidney microvascular protective effects of gymnemic acids and to establish their anti-angiogenic properties related to the expression of VEGF protein in rats with STZ-induced diabetes.

METHODS

Animal experiments: The present study was carried out on 8-week-old male Wistar rats (n= 80) weighing ~200-250 g at the beginning of the experiment. The experimental procedures described herein followed the guidelines stipulated by the Animal Ethics Committee of the Prince of Songkla University, and the protocols were reviewed and approved. All animals were maintained in a controlled animal laboratory environment on alternative 12-h light/dark periods (23±2˚C) and humidity (50±10%) with alternating 12-h light/ dark cycles.

Induction and assessment of diabetes: Diabetes was induced in the rats by an intraperitoneal injection of a single dose of STZ [60 mg/kg body weight (BW); Sigma-Aldrich; Merck KGaA] dissolved in 0.1 M citrate buffer (pH 4.5). The control rats received an injection of a single dose of 0.1 M citrate buffer alone. Rats with blood sugar levels >250 mg/dl were considered diabetic (19). Blood sugar levels from the lateral tail vein were analyzed using a blood glucose meter (Accu-Chek Active meter and test strips, Roche Diagnostics).

Three days after the final STZ injection, the control and diabetic rats were randomly divided into five groups as follows: the normal control rats that received a balanced standard diet (C group; n=10); gymnemic control rats that received a balanced standard diet supplemented with gymnemic acid (purified >75% by HPLC analysis) (Xi'an Huilin Bio-Tech Co., Ltd.) in 0.5 ml of 0.5% Tween-80 solution (CGM group; n=10); diabetic rats that received a balanced standard diet (DM group; n=20); diabetic rats that received a balanced standard diet supplemented with gymnemic acid (GM group; n=20) 400 mg/kg BW (20) in 0.5 ml of 0.5% Tween 80 solution; and diabetic rats treated with 4 mg/kg BW of glibenclamide (GR group; n=20) in 0.5 ml of 0.5% Tween-80 solution (21). Before the experimental planning, the literature was reviewed to examine the doses of substances used in the experiment from previous researchers. The substance used in this study were not toxic to the animals. They have been proven effective for reducing blood glucose and free radicals. So, the single dose of glibenclamide and gymnemic acid were chosen in this study. All animals were weighed and clinically observed on a weekly basis. Blood glucose measurement was measured once a week at the end of the week for 8 weeks, meaning the first week in this scenario was 7 days after the experiment began (end of the week). This means that substances or drugs had been started for 7 days before measurement. Therefore, the blood sugar values in each group should be different at the end of the first week. The rats in each group were divided in half (n=10) and tissues were collected for use in H&E staining and immunofluorescence studies. The other half of the rats in each group were used for analyses using an injection with resin for vascular corrosion casting combined with SEM (n=10). Following gymnemic acid supplementation at the end of 8 weeks, the experimental rats were euthanized by an overdose of sodium pentobarbital (200 mg/kg; intraperitoneal injection). Blood urine nitrogen (BUN) and creatinine (Cr) levels were analyzed by the Southern Lab Center Saha Clinic (using a Siemens ADVIA 1800 System Analyzer; Siemens Healthineers).

Immunofluorescence analysis: In order to determine the levels of VEGF, the cut renal tissues were deparaffinized in xylene, hydrated, and permeabilized in PBS with 0.1 Triton X-100 (PBST) for 30 min. Blocking was performed using horse serum in PBS for 1 h at room temperature, followed by incubation with anti-VEGF antibody (1:100; mouse monoclonal antibody, Thermo Fisher Scientific, Inc.) at 4˚C overnight. The sections were exposed to the fluorescein horse anti-mouse IgG antibody (1:200; Vector Laboratories, Inc.) in blocking solution to detect VEGF for 2 h at room temperature.

Images were examined and photographed under a fluorescence microscope (Olympus D73 equipped with CellSens software). The VEGF percentage of cell expression was determined using National Institutes of Health (NIH) Image J software 1.52 to measure the fluorescence intensity. A total of 5 segmental and interlobar arteries were randomly selected from each specimen. The optical density (OD) was normalized to measure the level of immunostaining at the area of segmental and interlobar arteries. The *OD* was determined as follows: OD = ID - (A x MGV). The integrated density of the selected artery region is ID, A is the area of the selected artery region, and MGV is the mean gray value of the background readings. The mean OD of these glomeruli was used for statistical analysis (22).

Vascular corrosion cast technique/SEM: The thoracic cavity of each animal was opened by subcostal incision. 0.5 ml of heparin (5,000 IU/ml) was then rapidly injected into the left ventricle, with its tip directed towards the lumen of the ascending aorta with a blunt (no. 18) gauge needle. The right atrium was cut and opened, being the outlet for blood. In total, ~400 ml NSS solution was infused through a cannula until the effluent was clear. Following perfusion with NSS solution, PU4ii resin (VasQtec) (https://vasqtec.com)

was injected. The kidneys were removed and soaked in 10% KOH for 1 month to corrode the tissue. After the specimens were washed several times with distilled water, they were allowed to air dry. Finally, the casts were examined at 10-15 KV at an accelerating voltage of 10 kV under a scanning electron microscope (JEOL JSM-5400; JEOL Ltd.) The diameter of the kidney blood vessels was measured using SemAfore, 5.2 (JEOL Ltd.).

Statistical analysis: The results are expressed as the means ± standard error of the mean. Statistical analysis was performed using ANOVA followed by the Bonferroni post hoc test. A P-value <0.05 was considered to indicate a statistically significant difference.

RESULTS

Effect of gymnemic acid on blood glucose levels: The effect of gymnemic acid administration on blood glucose levels in diabetic rats is presented in Table I. The blood glucose levels of the rats in the C, CGM, DM, GM, and GR groups were examined during the 8-week experimental period. Blood glucose levels were significantly elevated in the DM group rats (P<0.001) as compared with the control group rats until termination of the study at 8 weeks. Following the administration of gymnemic acid and glibenclamide, the blood glucose levels of the rats in the GM and GR groups were reduced weekly when compared with those of the DM group rats.

Table 1. Comparison of blood glucose levels in different groups. (mg/dl)

Week	C	CGM	DM	GM	GR
1	109.71±5.99	109.67±2.78	461.00±45.70[a]	362.89±49.29[a]	182.62±38.92[c]
2	98.57±2.57	106.00±2.21	307.33±52.08[b]	269.22±35.60[e]	181.00±40.81
3	102.43±1.82	96.33±2.46	386.56±51.01[a]	188.00±41.51[d]	166.00±38.94[c]
4	102.71±1.96	105.17±1.81	379.67±49.46[a]	240.89±48.70[d]	115.75±10.49[c]
5	110.71±2.44	110.33±2.64	338.00±45.65[a]	289.78±50.06[b]	119.25±10.58[c]
6	96.14±2.02	92.67±2.40	358.89±48.92[a]	249.78±49.68[e]	107.00±4.77[c]
7	88.71±3.13	89.83±1.83	333.33±44.12[a]	259.00±45.53[b]	102.13±7.57[c]
8	101.43±2.22	96.67±3.01	334.67±53.02[a]	213.44±40.73	102.12±4.60[c]

Data are presented as mean ± SEM, during 8-week experiments for each group; n = 10 rats (C and CGM) 20 rats (DM, GM and GR) [a] $p<0.001$, [b] $p<0.01$, [e] $p<0.05$ compared with control group [c] $p<0.001$, [d] $p< 0.01$ compared with DM group. Statistical analysis was performed using two way ANOVA followed by the Bonferroni post hoc test.

Effect of gymnemic acid on BUN and Cr levels: The effect of gymnemic acid supplementation on BUN and Cr levels in diabetic rats is presented in Table 2. The BUN and Cr levels of the rats in the C, CGM, DM, GM, and GR groups were compared during the 8-week experimental period. They were significantly increased in all DM, GM (P<0.001), and GR group rats, as compared with the control rats until the termination of the study at 8 weeks. The BUN and Cr levels of the GM and GR group rats were significantly reduced weekly when compared with the DM group rats (P<0.01 and P<0.001, respectively

Table 2. Blood urea nitrogen (BUN) and creatinine. (mg%)

Parameters	C	CGM	DM	GM	GR
BUN	22.85± 1.28	24.3±1.11	49.4±6.86[a]	30.03±1.38[c]	20.85±1.99[b]
Cr	0.31±0.02	0.36±0.03	0.39±0.02	0.32±0.05	0.37±0.03

Data are presented as mean ± SEM, during 8-week experiments for each group; n = 10 rats (C and CGM) 20 rats (DM, GM and GR). [a] $p<0.001$ compared with control group, [b] $p<0.001$, [c] $p<0.01$ compared with DM group.

***Immunofluorescence analysis*:** To determine whether VEGF expression was increased in the diabetic kidney, they were quantified as described in the Materials and methods. The immunohistochemical segmental and interlobar artery sections in the control (Figs. 1A and 2A) and CGM (Figs. 1B and 2B) rat kidneys were found to exhibit a small amount of VEGF-specific antibody. The increased expression of VEGF (bright green staining) in the segmental (Fig. 1C) and interlobar (Fig. 2C) arteries was observed mainly in the smooth muscle layer of the arterial walls and endothelial cells in DM group rats. The quantification of VEGF expression (Figs. 1F and 2F) revealed a significant increase in expression in the diabetic rats compared with the control rats (P<0.001). Following the supplementation of gymnemic acid (Figs. 1D and 2D) and glibenclamide (Figs. 1E and 2E), VEGF expression was significantly decreased when compared with the DM group rats (P<0.001).

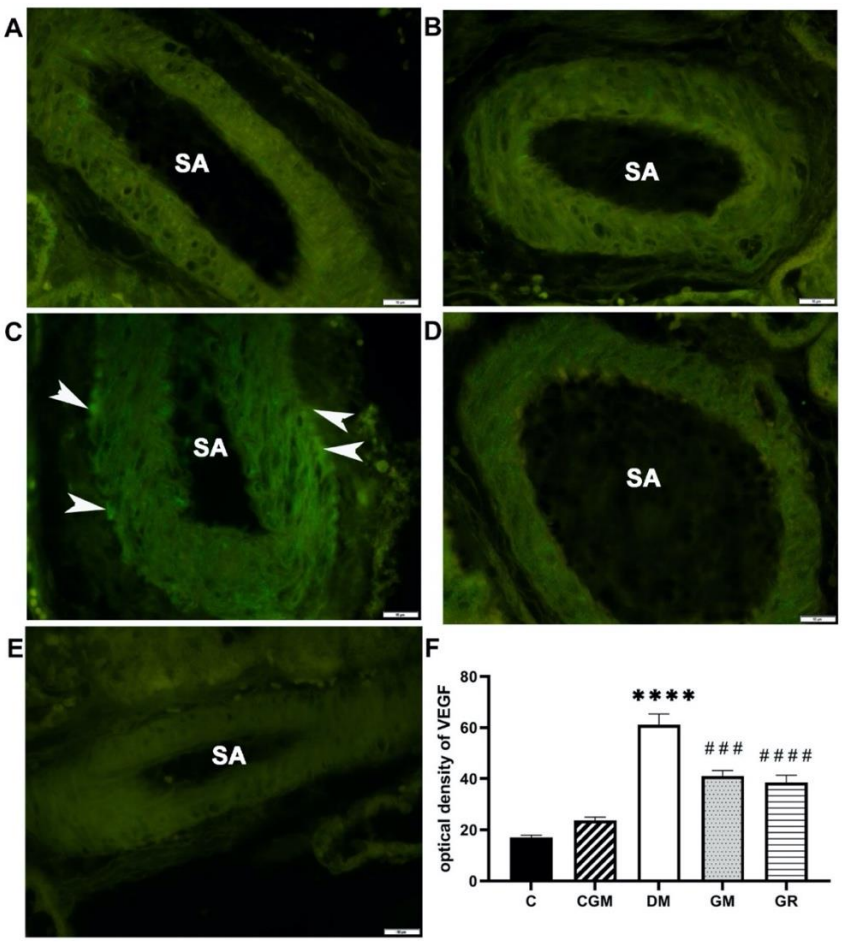


Figure 1. Photomicrographs immunohistochemical sections of SA with VEGF specific antibody in rat kidneys; control (A), CGM (B), DM (C), GM (D), and GR (E) rats. White arrowheads indicate the example of VEGF-immunoreactive smooth muscles in tunica media. Bar = 20 µm. The relative VEGF optical density was analyzed (F). Values are mean + SE, ***P<0.0001 when compared with control group, ###P<0.001, ####P<0.0001 when compared with DM group.

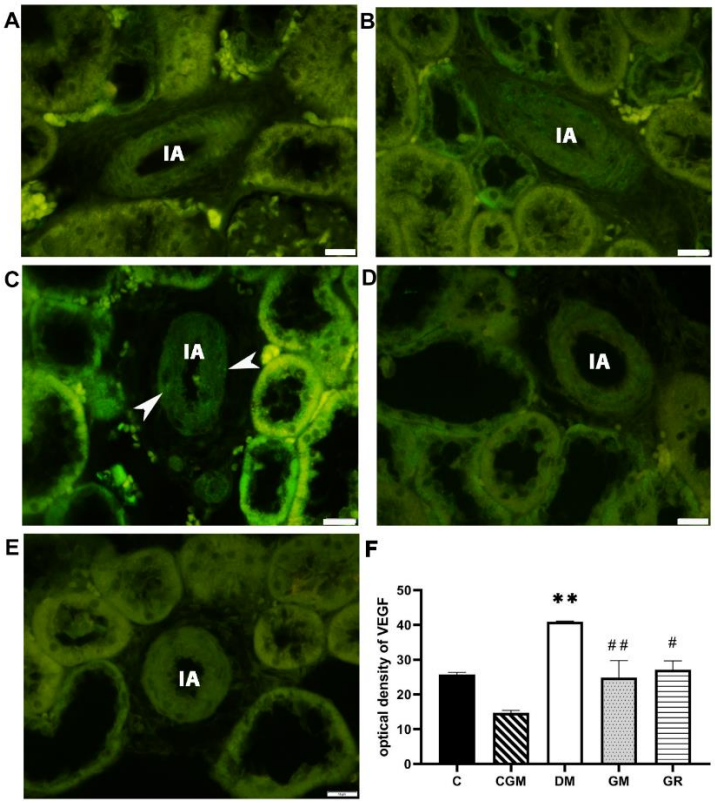


Figure 2. Photomicrographs immunohistochemical sections of IA with VEGF specific antibody in rat kidneys; control (A), CGM (B), DM (C), GM (D), and GR (E) rats. White arrowheads indicate the example of VEGF-immunoreactive smooth muscles in tunica media. Bar = 20 µm. The relative VEGF optical density was analyzed (F). Values are mean + SE, **P<0.01 when compared with control group, ##P<0.01, #P<0.05 when compared with DM group.

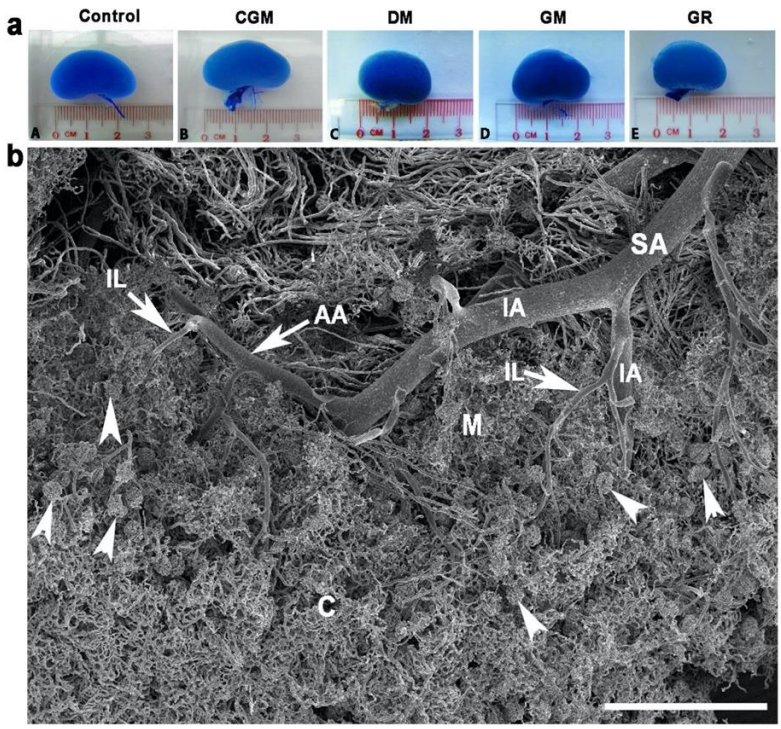


Figure 3. SEM micrographs of whole vascular cast of kidneys (a) in control, CGM, DM, GM, and GR rats. The low magnification of kidney vascular corrosion cast revealed a highly vascularized area of cortex (C) and medulla (M) in the control group (b). Each renal artery enters the hilum and then branches into segmental arteries. The segmental artery (SA) became interlobular arteries (IA) and arcuate arteries (AA), respectively. Arcuate arteries run between the cortex and medulla to form interlobular arteries before branching to the glomeruli (white arrowhead). Bar = 1 mm.

Vascular corrosion casting technique to examine the kidney microvasculature: From the study of the characteristics of arterial structures, it was found that the kidney size of the rats in the DM group was smaller than other groups (Figure 3A). The low magnification of kidney vascular corrosion cast revealed a highly vascularized area of cortex and medulla in the control group (Figure 3B). The segmental artery (SA) was branched from the renal artery that became interlobular arteries and arcuate arteries, respectively. Arcuate arteries run between the cortex and medulla to form interlobular arteries before branching to the glomerulus.

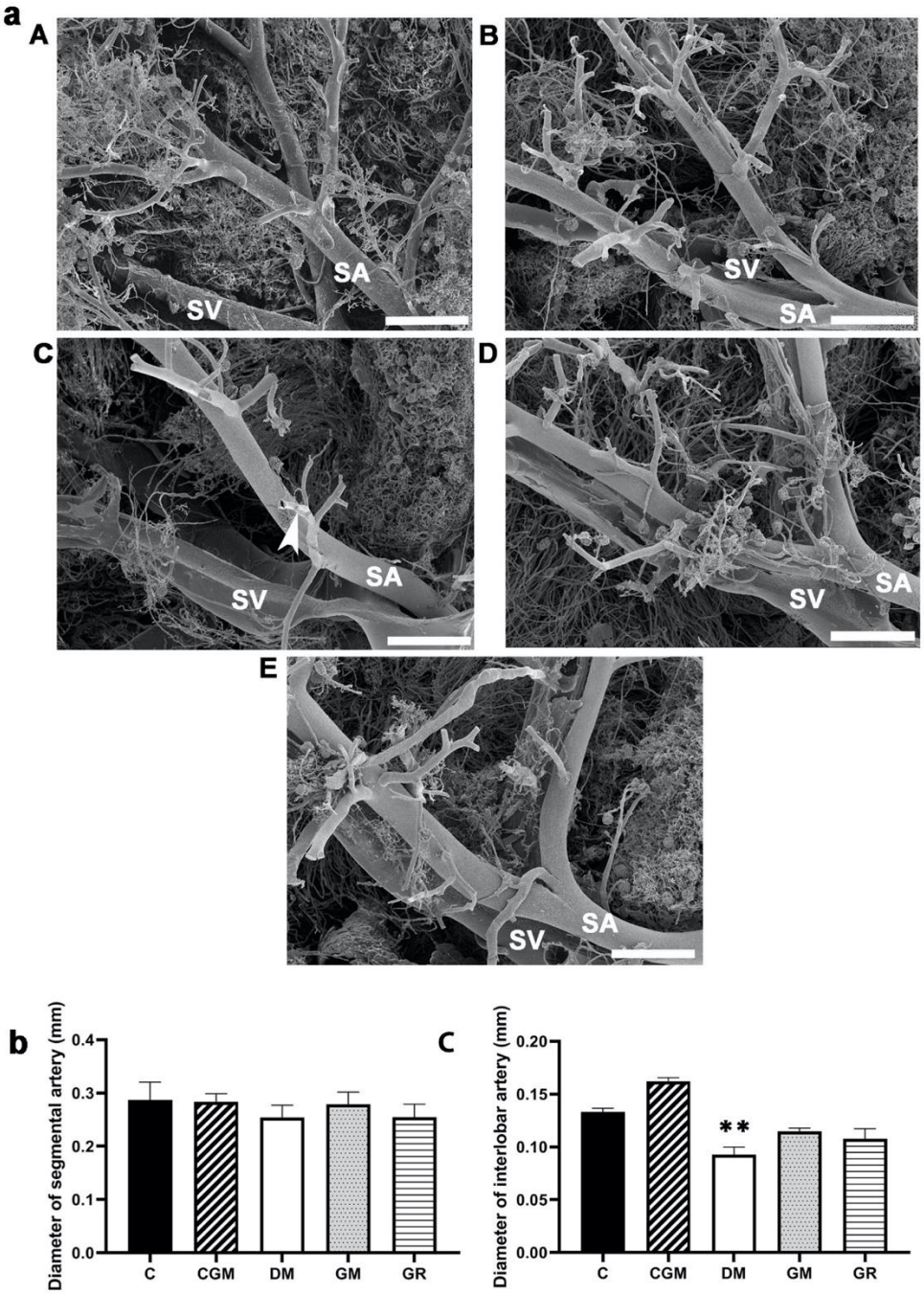


Figure 4. SEM micrographs of kidney vascular corrosion cast (A) in control (a), CGM (b), DM (c), GM (d), and GR (e) rats. The segmental artery (SA) and veins (SV), in DM group (c) demonstrated a smaller diameter of SA and IA, and shrunken interlobar artery (white arrowhead). In contrast, GM and GR groups presented the recovery and redevelopment of large blood vessels. Bar = 1 mm. The average diameter of SA (B) and IA (C) in five groups of rats at 8 weeks were seen. Values are mean + SE, **P<0.001 when compared with control group.

At high magnification, the segmental arteries and interlobar arteries in the kidneys of the control and CGM group rats (Fig. 4A and B) exhibited healthy and typical patterns of the segmental arteries and their branches. The DM group exhibited a smaller diameter of interlobar arteries. Shrunken and constricted interlobar arteries were observed (Fig. 4C), and also demonstrated two sprouting of blood vessels from the segmental artery (Figure 5). Moreover, in the GM and GR groups (Fig. 4D and E, respectively), the recovery and redevelopment of large blood vessels was observed.

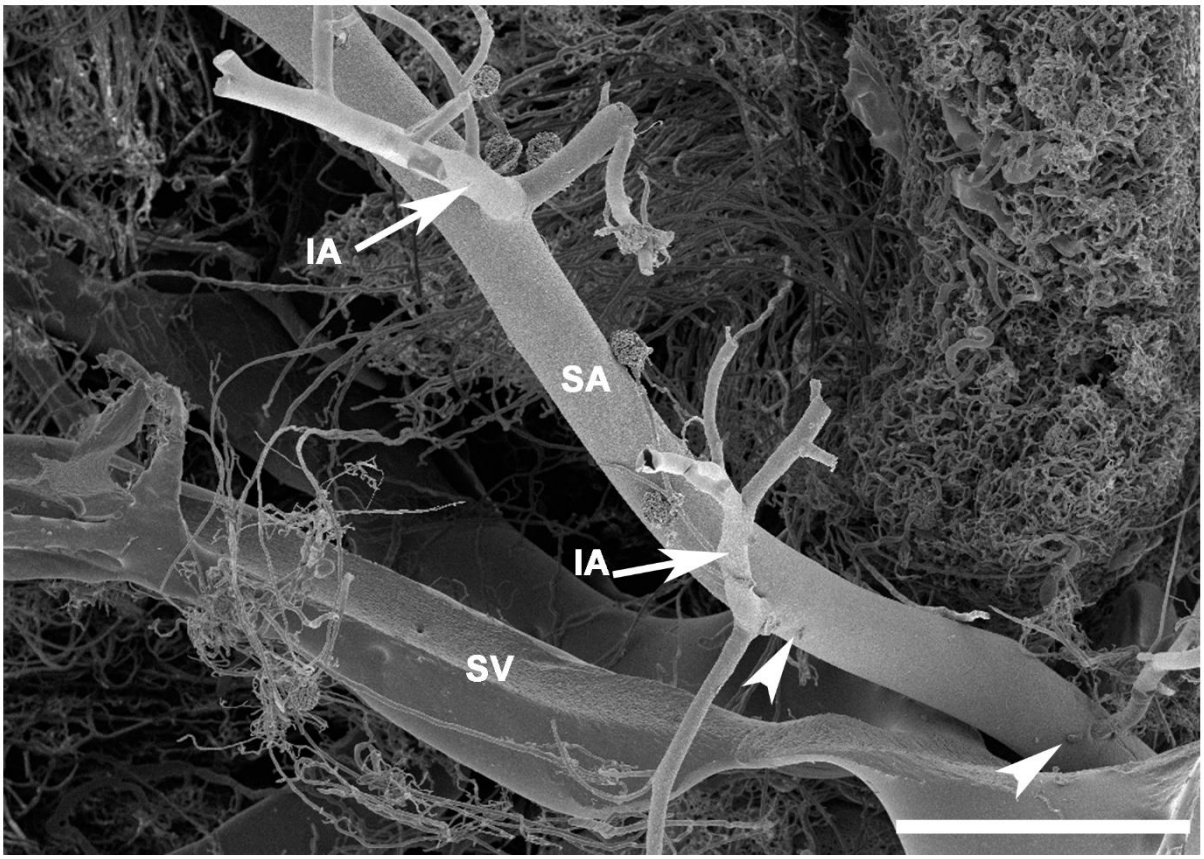


Figure 5. SEM micrographs of kidney vascular corrosion cast (a) in DM rats. The segmental artery (SA) and veins (SV). DM group demonstrated shrunken interlobar artery (white arrowhead). The two sprouting of blood vessels from the segmental artery were presented (white arrows). Moreover, in GM and GR groups, the recovery and redevelopment of large blood vessels was presented. Bar = 1 mm.

The compared diameter sizes of blood vessels of all groups at 8 weeks of the experimental period were demonstrated in all types of vessels, including segmental and interlobar arteries (Fig. 6b). The graph data of the DM groups rats revealed a decrease in the diameter of the segmental artery and a significant decrease in the interlobar artery indices when compared to the control group ($P<0.001$). Notably, signs of vessel restoration and improvement were also presented by the increase in the diameters of all types of vessels in the GM and GR groups.

DISCUSSION

DN is commonly observed in diabetic patients with significant microvascular complications. Hyperglycemia leads to the activation of protein kinase C (PKC) and results in the formation of advanced glycation end-products (AGEs) of the extracellular matrix (23). The excessive production of extracellular matrix components in the mesangium and glomerular basement membrane results in changes in the permeability of the filtration barrier (24). Blood tests for BUN and Cr are the simplest method for monitoring kidney function. The main activity of the kidneys is to

filter excess water and normal waste products out of the blood to generate urine. Urea is the end product of biochemical protein breakdown. In kidney disease, urea is not excreted normally, and thus accumulates in the body, causing an increase in blood urea levels. Cr is a breakdown output of creatinine phosphate, originating from the normal deterioration of muscles in the body, that is secreted from blood to urine and are associated with the glomerular filtration rate (23).

In a previous study, vascular corrosion casting and SEM microscopy demonstrated that treatment with gymnemic acid in a diabetic rat group helped regain the healthy architecture of brain vessels (25). In this study, the vascular cast kidney size of the DM group was smaller than other groups, and interlobar and segmental arteries exhibited a smaller diameter. They also presented shrunken and constricted interlobar and segmental arteries. Moreover, in the GM groups, the reorganization of large blood vessels was observed. The pathogenic mechanisms for microvascular complications in this study may be associated with oxidative stress, which damages various target organ systems (26). The importance of reactive oxygen species (ROS) in vascular injury lies in the fact that their production is positively regulated by a number of cytokines whose expression is increased following injury, by either oscillatory shear stress or mechanical disruption (27). The high glucose concentrations induce endothelial dysfunction in diabetes, which is associated with oxidative stress-free radicals (28). An overproduction of reactive oxygen species (ROS) is a reaction of mitochondrial malfunction and a consequence of hyperglycemia (29). Gymnemic acid improved antioxidant count, such as malondialdehyde glutathione, glutathione peroxidase, and catalase. It possesses glucose lowering effects, which are associated with reduced ROS in rats. Following injury, arteries are regenerated by cells that migrate from adjacent normal tissue by chemotactic factors for vascular smooth muscle cells (VSMCs), including transforming growth factor-β (TGF-β), basic fibroblast growth factor, platelet-derived growth factor (PDGF), and Ang II (29).

According to the immunofluorescence and vascular corrosion casting/SEM analyses, they can confirm that angiogenesis involves the construction of new blood vessels sprouting from pre-existing vessels that are presented from the segmental artery of DM rats. Angiogenesis is important for the physiological functions of tissues, and for the progression of diseases, such as inflammation and cancer (30). This process affects the growth, migration, and differentiation of endothelial cells inside the wall of blood vessels. VEGF plays a crucial role in vascular diseases and blood vessel homeostasis (23). It is considered to participate in the glomerular capillary hyperpermeability of macromolecules that potentially underlies diabetic albuminuria. Evidence for the role of VEGF is relatively straight forward in animal models of diabetes, establishing that VEGF is upregulated in the diabetic kidney (31). The effects of VEGF are redirected towards endothelial cell proliferation, which has deleterious consequences for diabetic vasculopathy (32).

Gymnemic acid is an active compound that is a mixture of saponins in *Gymnema sylvestre*. Gymnemic acids can prevent the absorption of sugar molecules by the intestine, which leads to a reduction in blood sugar levels (17). Gymnemic acids have also been found to increase the secretion of insulin, and play a possible role in regenerating insulin and β-cells (33). It has been previously reported that gymnemic acid can enhance the activities of hexokinase, glycogen synthetase, glyceraldehyde 3-phosphate dehydrogenase, and glucose 6-phosphate dehydrogenase enzymes. It also has the effect of reducing the activity of insulin-independent enzymes, glycogen phosphorylase, gluconeogenic enzymes, glucose 6-phosphatase, fructose 1,6-diphosphatase, and sorbitol dehydrogenase, which affects the increase of phosphorylase activity. This saponin of *Gymnema*

sylvestre exhibits antioxidant activity and radical scavenging activity (34).

CONCLUSION

In conclusion, the present study demonstrated that gymnemic acid from *Gymnema sylvestre* at the used dosage decreased the serum BUN and Cr concentrations. The effect of gymnemic acid on kidney angiogenesis in STZ-induced diabetes was investigated in the present study, and the levels of VEGF, the major angiogenic cytokine, and TGF-β, the master regulator of fibrosis, were reduced following treatment with gymnemic acid over the 8-week experimental period. Histologically, the kidneys exhibited a decreased wall thickness, an increased blood vessel diameter, increased glomerular tuft, and decreased Bowman's spaces. Gymnemic acid functioned by stimulating enzymes and natural antioxidant activity, which directly affected kidney tissue. Gymnemic acid administration also improved diabetes-induced endothelial dysfunction via a decrease in vascular superoxide production and PKC inhibition with hyperglycemia. Further studies are required to unravel the mechanisms responsible for the suppression of the VEGF and TGF-β1-induced accumulation of extracellular matrix by gymnemic acid. The elucidation of these mechanisms may be useful for the development of new therapeutic options for the prevention and treatment of DN.

Abbreviations: VEGF: expression of vascular endothelial growth factor, STZ: streptozotocin, DM: diabetes, GR: glibenclamide, GM: gymnemic acid, BUN: blood urea nitrogen, Cr: Creatinine, DN: diabetic nephropathy, CKD: chronic kidney disease, GFR: glomerular filtration rate.

Authors' contributions: WK and MK designed and conducted the research. RJ, NS and UV prepared the tissue and performed immunofluorescence. WK and MK performed vascular corrosion cast. WK wrote the manuscript and performed the statistical analysis. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no conflicts of interest.

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REFERENCES

1. Lim AK. Diabetic nephropathy - complications and treatment. Int J Nephrol Renovasc Dis 2014, 15(7): 361–81. https://doi.org/ 10.2147/IJNRD.S40172
2. Alicic RZ, Rooney MT, Tuttle KR. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. Clin J Am Soc Nephrol 2017, 12(12): 2032–45. https://doi.org/ 10.2215/CJN.11491116
3. Matovinović MS. Pathophysiology and Classification of Kidney Diseases. EJIFCC. 2009, 20(1): 2–11. PMID: 27683321; PMCID: PMC4975264.
4. Aristotle S, Sundarapandian FC. Anatomical study of variations in the blood supply of kidneys. J Clin Diagn Res 2013, 7(8): 1555–7. https://doi.org/ 10.7860/JCDR/2013/6230.3203
5. Stavros AT, Parker SH, Yakes WF, Chantelois AE, Burke BJ, Meyers PR, Schenck JJ. Segmental stenosis of the renal artery: pattern recognition of tardus and parvus abnormalities with duplex sonography. Radiology 1992, 184(2): 487-92. https://doi.org/ 10.1148/radiology.184.2.1620853
6. Susztak K, Raff AC, Schiffer M, Böttinger EP. Glucose-Induced Reactive Oxygen Species Cause Apoptosis of Podocytes and Podocyte Depletion at the Onset of Diabetic Nephropathy. Diabetes. 2006, 55(1): 225 LP – 233. PMID: 16380497.
7. Iglesias-de la Cruz MC, Ruiz-Torres P, Alcamí J, Díez-Marqués L, Ortega-Velázquez, R, Chen S, et al. Hydrogen peroxide increases extracellular matrix mRNA through TGF-β in human mesangial cells. Kidney Int 2001; 59(1):87–95. https://doi.org/ 10.1046/j.1523-1755.2001.00469
8. Nakagawa T, Kosugi T, Haneda M, Rivard CJ, Long DA. Abnormal angiogenesis in diabetic nephropathy. Diabetes 2009, 58(7): 1471–8. https://doi.org/10.2337/db09-0119
9. Zent R, Pozzi A. Angiogenesis in Diabetic Nephropathy. Semin Nephrol 2007, 27(2):161–71. https://doi.org/10.1016/j.semnephrol.2007.01.007
10. Shibuya M. Vascular Endothelial Growth Factor (VEGF) and Its

Receptor (VEGFR) Signaling in Angiogenesis: A Crucial Target for Anti- and Pro-Angiogenic Therapies. Genes Cancer 2011, 2(12): 1097–105. https://doi.org/10.1177/1947601911423031

11. Hohenstein B, Hausknecht B, Boehmer K, Riess R, Brekken RA, Hugo CPM. Local VEGF activity but not VEGF expression is tightly regulated during diabetic nephropathy in man. Kidney Int 2006, 69(9): 1654–61. https://doi.org/ https://doi.org/10.1038/sj.ki.5000294
12. Osterby R, Bangstad HJ, Nyberg G, Rudberg S. On glomerular structural alterations in type-1 diabetes. Virchows Arch 2001, 438(2): 129–35. https://doi.org/ 10.1007/s004280000311
13. Nakagawa T, Sato W, Glushakova O, Heinig M, Clarke T, Campbell-Thompson M, et al. Diabetic endothelial nitric oxide synthase knockout mice develop advanced diabetic nephropathy. J Am Soc Nephrol 2007, 18(2): 539–50. https://doi.org/ 10.1681/ASN.2006050459
14. Wehner H, Nelischerm G. Morphometric investigations on intrarenal vessels of streptozotocin-diabetic rats. Virchows Arch A 1991, 419(3): 231–5. https://doi.org/10.1007/BF01626353
15. Kanetkar P, Singhal R, Kamat M. Gymnema sylvestre: A Memoir. J Clin Biochem Nutr 2007, 41(2): 77–81. https://doi.org/10.3164/jcbn.2007010
16. Al-Romaiyan, A, King AJ, Persaud SJ, Jones PM. A novel extract of Gymnema sylvestre improves glucose tolerance in vivo and stimulates insulin secretion and synthesis in vitro. Phytother Res 2013, 27(7): 1006–11. https://doi.org/10.1002/ptr.4815
17. Sahu NP, Mahato SB, Sarkar SK, Poddar G. Triterpenoid saponins from Gymnema sylvestre. Phytochemistry 1996, 41(4): 1181–5. https://doi.org/10.1016/0031-9422(95)00782-2
18. Fatani AJ, Al-Rejaie SS, Abuohashish HM, Al-Assaf A, Parmar MY, Ola MS, et al. Neuroprotective effects of Gymnema sylvestre on streptozotocin-induced diabetic neuropathy in rats. Exp Ther Med 2015, 9(5): 1670–8. https://doi.org/10.3892/etm.2015.2305
19. Komolkriengkrai, M, Nopparat J, Vongvatcharanon U, Anupunpisit V, Khimmaktong W. Effect of glabridin on collagen deposition in liver and amelioration of hepatocyte destruction in diabetes rats. Exp Ther Med 2019, 18(2): 1164–74. https://doi.org/ 10.3892/etm.2019.7664
20. Tiwari P, Mishra BN, Sangwan NS. Phytochemical and pharmacological properties of *Gymnema sylvestre*: an important medicinal plant. Biomed Res Int 2014, 2014: 830285. https://doi.org/ /10.1155/2014/830285
21. Feihua, W, Zhigui J, Jian J, Hypoglycemic effects of glabridin, a polyphenolic flavonoid from licorice, in an animal model of diabetes mellitus. Molecular Medicine reports 2013, 7: 1278-82. https://doi.org/10.3892/mmr.2013.1330 PMID: 23426874
22 Fu JM, Wei C, Zhang W, Schlondorff D, Wu J, Cai M, et al. Gene expression profiles of glomerular endothelial cells support their role in the glomerulopathy of diabetic mice. Kidney Int 2018, 94(2): 326–45. https://doi.org/10.1016/j.kint.2018.02.028
23. Kolset SO, Reinholt FP, Jenssen T. Diabetic nephropathy and extracellular matrix. J Histochem Cytochem 2012, 60(12): 976–86. https://doi.org/10.1369/0022155412465073
24. Forbes JM, Cooper ME, Oldfield MD, Thomas MC. Role of Advanced Glycation End Products in Diabetic Nephropathy. J Am Soc Nephrol 2003, 14(suppl 3): S254 LP-S258. https://doi.org/10.1097/01.asn.0000077413.41276.17
25. Sandech, N., Jangchart, R., Komolkriengkrai, M., Boonyoung, P., Khimmaktong, W."Efficiency of Gymnema sylvestre-derived gymnemic acid on the restoration and improvement of brain vascular characteristics in diabetic rats". Experimental and Therapeutic Medicine 2021, 22(6): 1420. https://doi.org/10.3892/etm.2021.10855
26. Zhang S, Xu H, Yu X, Wu YI, Sui D. Metformin ameliorates diabetic nephropathy in a rat model of low-dose streptozotocin-induced diabetes. Exp Ther Med 2017, 14(1): 383–390. https://doi.org/10.3892/etm.2017.4475
27. Papaharalambus CA, Griendling KK. Basic mechanisms of oxidative stress and reactive oxygen species in cardiovascular injury. Trends Cardiovasc Med 2007, 17(2): 48–54. https://doi.org/10.1016/j.tcm.2006.11.005
28. Khimmaktong W, Komolkriengkrai M, Boonyoung P, Anupunpisit V. Ameliorative effects of curcumin on microvascular complication in streptozotocin-induced diabetic rat penis. Functional Foods in Health and Disease 2019, 9(2): 92-107. https://doi.org/10.31989/ffhd.v9i2.553
29. Tanabe K, Maeshima Y, Sato Y, Wada J. Antiangiogenic Therapy for Diabetic Nephropathy. Biomed Res Int 2017, 2017: 5724069. https://doi.org/10.1155/2017/5724069
30. Taylor WR. Hypertensive vascular disease and inflammation: mechanical and humoral mechanisms. Curr Hypertens Rep 1999 1(1): 96–101. https://doi.org/10.1007/s11906-999-0079-5. PMID: 10981048.
31. Chen S, Ziyadeh FN. Vascular endothelial growth factor and diabetic nephropathy. Curr Diab Rep 2008, 8(6): 470. https://doi.org/10.1007/s11892-008-0081-3
32. Nakagawa T. Uncoupling of the VEGF-endothelial nitric oxide axis in diabetic nephropathy: an explanation for the paradoxical effects of VEGF in renal disease. Am J Physiol Physiol 2007, 292(6): F1665–72. https://doi.org/10.1152/ajprenal.00495.2006
33. Shanmugasundaram ERB, Gopinath KL, Shanmugasundaram KR, Rajendran VM. Possible regeneration of the islets of langerhans in streptozotocin-diabetic rats given gymnema sylvestre leaf extracts. J Ethnopharmacol 1990, 30(3): 265–79. https://doi.org/ 10.1016/0378-8741(90)90106-4
34. Periandavan K, Campus T, Nadu T. Protective role of gymnemic acid in curbing high fat diet and high fructose induced pancreatic oxidative stress mediated type 2 diabetes in Wistar rats. Int J Pharm Sci 2018, 9(5): 2130–9. https://doi.org/10.13040/IJPSR.0975-8232.9(5).2130-39