Mechanism of the Xuefu Zhuyu decoction in treating diabetes mellitus erectile dysfunction based on the CaSR/Gq-PLC-PKC pathway

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Author contributions
Wu YY conceived and designed the study. Zhang CH and Li GJ collected the data. Wu YY, Zhang CH, and Li GJ verified and analyzed the data. Jiang LX, Fan HQ, and Fang HL performed the experiments. Zhang CH and Li GJ drafted the manuscript. All the authors participated in the final approval of the manuscript.

Competing Interests
The authors declare no conflicts of interest.

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Abbreviations
XFZG, Xuefu Zhuyu decoction; PKC, protein kinase; PKC-δ, protein kinase Cδ; P-P38, phospho-P38 mitogen-activated protein kinase; P38, P38 mitogen-activated protein kinase; p-JNK, phosphorylated c-Jun amino-terminal kinase; JNK, c-Jun amino-terminal kinase; DMED, diabetes mellitus erectile dysfunction; CaSR, calcium-sensitive receptor; ED, erectile dysfunction; DM, diabetes mellitus; NO, nitric oxide; TCM, traditional Chinese medicine; PLC, phospholipase C; DAG, diacylglycerol; STZ, streptozotocin; APO, apomorphine; ET-1, endothelin-1; ELISA, enzyme-linked immunosorbent assay; NADH, nitric oxide; DBS, phosphate buffered saline; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1.

Abstract
Background: To investigate the mechanism of Xuefu Zhuyu decoction in the treatment of diabetes mellitus erectile dysfunction. Methods: Rats with diabetes mellitus erectile dysfunction were developed using streptozotocin and randomly assigned into model, low-dose herbal, and high-dose herbal groups. All rats were administered normal saline or the corresponding drugs by oral gavage for 4 weeks. The related indices were detected using enzyme-linked immunosorbent assays, immunohistochemistry, western blotting, and transmission electron microscopy. Results: The levels of lectin-like oxidized low-density protein receptor-1, endothelin-1, NADH oxidase, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 in the model group were significantly higher than they were in the mock group but lower than they were in the herbal treatment group. The level of nitric oxide was lower in the model group than was in the mock group but higher than the level in the herbal treatment group. The calcium-sensitive receptor, phospho-protein kinase Cδ/protein kinase Cδ, phosphorylated c-Jun amino-terminal kinase/c-Jun amino-terminal kinase, and phospho-P38 mitogen-activated protein kinase/P38 mitogen-activated protein kinase expression levels in the model group were higher than were in the mock group but lower than were in the herbal treatment group. The structures of the Corpus cavernosum penis endothelial cells were significantly improved in the herbal treatment group than they did in the model group. Conclusion: Xuefu Zhuyu decoction can decrease injury to the endothelium, improve vascular endothelial diastolic and contractive function, and inhibit vascular fibrosis in rats with diabetes mellitus erectile dysfunction. This mechanism may be related to the CaSR/Gq-PLC-PKC pathway.

Keywords: Xuefu Zhuyu decoction; diabetes mellitus erectile dysfunction; CaSR/Gq-PLC-PKC; JNK; P38

Citation


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**Highlights**
1. DMED model was construct using rats.
2. Confirming the curative effect of XFZYD on DMED
3. Uncovering the mechanism of XFZYD in treating DMEM, which was related to the CaSR/Gq-PLC-PKC pathway

**Medical history of objective**
Xuefu Zhiyu decoction is the boiled water extract of 11 herbs. It was first recorded more than 200 years ago in the book "Correction of Errors in Medical Classics". Xuefu Zhiyu decoction is a representative prescription that promotes blood circulation to remove blood stasis. It can improve blood circulation and disperse stasis, eliminate stagnation, soothe the liver, and disperse blood stasis while generating body fluid. During the past years, Xuefu Zhiyu decoction has been widely used to treat several diseases that are caused by blood stasis, such as traumatic brain injury, erectile dysfunction, coronary heart disease, and so on.

**Background**
Erectile dysfunction (ED), characterized by the inability of the penis to achieve or maintain an adequate erection to provide a satisfactory sexual life, is the most common male sexual dysfunction [1]. The prevalence rate of ED in China is 26.1% and seriously affects the men’s quality of life and the stability of society and families [2]. Diabetes mellitus erectile dysfunction (DMED), a common complication of diabetes mellitus (DM), is a unique type of ED. Patients with diabetes have a higher incidence, an earlier onset of ED, and more severe symptoms than do those without diabetes [3]. Recent clinical and experimental studies have reported that traditional Chinese medicine (TCM) is effective and safe for treating DMED and is expected to be an effective means to delay the progression of diabetic complications while treating ED [4]. As a classic prescription in traditional Chinese medicine, Xuefu Zhiyu decoction (XFZYD), composed of eleven drugs, has been widely used in the clinical treatment of cardiovascular and cerebrovascular diseases, as a representative prescription for activating blood and resolving stasis [5]. However, there are few reports on XFZYD in erectile function.

DMED belongs to the category of "consumptive thirst" combined with "impotence" in Chinese medicine, and its pathogenesis is mainly due to the obstruction of penis collaterals by blood stasis [6]. Therefore, the treatment of DMED with XFZYD is in line with the etiological and pathological characteristics of the disease. Related clinical research reports have also demonstrated that XFZYD can improve both DMED and the development of diabetic microangiopathy complications [7, 8].

The hyperactivated protein kinase (PKC) pathway has been shown to be an important mechanism by which diabetes-induced vascular endothelial dysfunction leads to the formation of various complications of vascular fibrosis [9, 10]. Of the diverse molecular pathways that activate the PKC pathway, CaSR/Gq-PLC-PKC is a canonical pathway, in which the CaSR is activated by Gq protein-coupled phospholipase C (PLC) under the influence of a high-glucose environment, and it further promotes the breakdown of phosphatidylinositol-4,5-bisphosphate, which produces diacylglycerol (DAG) and inositol triphosphate. Inositol triphosphate, which binds to high-affinity receptors in the nucleus, opens calcium channels, allowing the release of internal calcium ions from the nucleus into the cytoplasm, and binding to DAG coactivates PKC on the surface of the cell membrane [11].

In this study, streptozotocin (STZ)-injected rats with induced DMED were selected as the study model, and the association of the CaSR/Gq-PLC-PKC pathway, which is closely related to diabetic angiopathy, with the effects of XFZYD and erectile function improvement was investigated. Notably, our findings clarify the mechanism by which XFZYD improves vascular endothelial function, reduces endothelial injury, and inhibits vascular fibrosis.

**Materials**

**Animals**
Fifty white male SPF Sprague-Dawley rats, aged 3 months and weighing 200 ± 10 g, were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. After purchase, pairing experiments confirmed that all experimental rats had normal sexual function [12]. The rats were then acclimatized at room temperature (17–25 °C) and 50–80% relative humidity for 1 week.

**Experimental drugs**
The XFZYD formulation granule is composed as follows: 12 g *Persicae Semen*, 9 g *Carthamus tinctorius* L., 9 g *Angelicae Sinensis* Radix, 9 g *Rehmannia glutinosa* (Gaertn.) *Lischos*. ex Fisch. et Mey., 9 g *Achyranthes bidentata* Blume, 5 g *Chuanxiong Rhizoma*, 5 g *Platycodon grandiflorus*, 6 g *Radix Panoniae Rubra*, 6 g *Pructus Auranti*, 6 g *Glycyrrhiza uralensis* Fisch., and 3 g *Bupleurum chinense*.

All experimental drugs were provided by the granule room of traditional Chinese medicine in the Second People's Hospital of Fujian Province, and the same batch number was sealed and kept in reserve to be converted by the animal experimental dose standard when used.

**Experimental reagents**
The experimental reagents are as follows: STZ (SIGMA, Louis, Inc. MO, USA), apomorphine (APO) injection (Supernus Pharmaceuticals, Rockville, MD, USA), calcium-sensitive receptor (CaSR) antibody (ab223360; Abcam, Shanghai, China), phospho-protein kinase Cβ antibody (2055S; Cell Signaling Technology, Danvers, MA, USA), protein kinase Cβ (PK-β) antibody (2058S; Cell Signaling Technology), phosphorylated e-Jun amino-terminal kinase (p-JNK) antibody (ab76572; Abcam), c-Jun amino-terminal kinase (JNK) antibody (ab208035; Abcam), phospho-P38 mitogen-activated protein kinase (P-P38) antibody (ab4822; Abcam), P38 mitogen-activated protein kinase (P38) antibody (ab170099; Abcam), rat endothelin-1 (ET-1) enzyme-linked immunosorbent assay (ELISA) kit (CSB-ENDH79; CUSABIO BIOTECH Co., Ltd., Wuhan, China), Nitric Oxide (NO) assay kit (A013-2-1; Nanjing Jiancheng Bioengineering Institute, Nanjing, China), rat Lectin-Like Oxidized Low-Density Lipoprotein Receptor 1 (LOX-1) ELISA kit (RTF001932; Assaygenie, Irish, Dublin, Ireland), NADH Oxidase (NOX) activity assay kit (B0630; Beijing Solarbio Science & Technology Co., Ltd. Beijing, China), electron microscope fixative (PN00015; Wuhan Pinoufei Biological Technology Co., Ltd. Wuhan, China), and Embed 812 (90529-77-4; SPI Supplies, West Chester, PA, USA).

**Main instruments and equipment**
A blood glucose meter and test strips (Roche Diagnostics, Basel, Switzerland), a microtome (Leica Biosystems, Wetzlar, Germany), Diamond Knives (DIATOME Ltd., Switzerland), and transmission electron microscopy (FEI, Hillsboro, OR USA) were used.

**Methods**

**Ethics committee approval**
The animal experimental protocols used in this study were approved by the Scientific Research Ethics Committee of the Second People's Hospital affiliated to Fujian University of Traditional Chinese Medicine (NO: FJSPH-IAC2022035. Date: 20220201).

**Reagent preparation**
- **Preparation of streptozotocin solution.** STZ powder (100 mg) was dissolved in 100 mL of sodium citrate-citric acid buffer in an ice bath to obtain a final concentration of 10 mg/mL. The solution was stored away from light for immediate use.
- **Preparation of apomorphine solution.** APO (4 mg) was dissolved in 0.5 mg/kg vitamin C and normal saline to a concentration of 40 µg/mL.
Modeling
Fifty healthy male Sprague-Dawley rats were numbered, weighed, and recorded after 1 week of adaptive feeding. They were randomly divided into a mock group (N = 10) and a pre-model group (N = 40) using the random number table method. All animal-related procedures of this study were in accordance with Regulations for the Administration of Affairs Concerning Ex-Perimental Animals (Revised in 2017, Order of the State Council of the People’s Republic of China).

All rats were fasted for 8 h. The rats in the pre-model group received a single intraperitoneal injection of STZ (60 mg/kg) in the left lower abdomen, while the rats in the control group received an equal amount of sodium citrate-citric acid buffer in the same area. After the injection, the rats were fed normally and observed for 2 weeks. The fasting micro blood glucose levels of the rats were measured weekly using a blood glucose meter after 8 h of fasting. Rats with blood glucose values of > 16.67 mmol/L were considered to have successfully established DM, and 26 rats were included in this study (Figures 1, Figure 2 attached) [13]. The body weight of the rats with DM reduced, and the urine volume, diet, and drinking water increased, while the rats of the mock group were in good condition.

Next, 26 rats with DM were placed in a glass box, the light was dimmed while maintaining enough visibility for observation, and we allowed the rats to adapt to the environment for 20 min. The rats were then subcutaneously injected with APO at a dose of 100 μg/kg in the neck and observed for 40 min after injection, and penile erection was recorded for each rat [14]. If the number of erections was ≥ 1, the erection test result was considered positive. Rats that were APO-positive were excluded, and rats that were APO-negative were...

Figure 1 The modeling process of our study and the effects of XFYD on alleviating vascular endothelial damage and improving vasomotor function. (A) The modeling workflow for establishing DMED rats. (B) XFYD alleviates vascular endothelial damage. (C) XFYD improving vasomotor function. Statistical comparisons were performed using one-way ANOVA followed by Tukey’s post-hoc test: \( P < 0.01, P < 0.001, P < 0.0001 \). XFYD, Xuefu Zhuyu decoction; DMED, diabetes mellitus erectile dysfunction; ANOVA, analysis of variance; APO, apomorphine.
included in the model group. In total, 21 rats had DMED, 18 of which were selected for further experiments (Supplementary Table 1).

**Grouping and administration**

The 18 model rats with DMED were randomly divided into three groups: model control, herbal high-dose, and herbal low-dose. Each group contained six rats, and six rats in the mock group were randomly selected for the experiment. Unselected rats were used in the preliminary experiment.

The mock group and the model group were given deionized water by gavage. The low- and high-dose groups were given the Xuefu Zhuyu decoction granule water suspension by gavage. Based on the amount of medicine used per unit body surface area, the amount of medicine used in rats is about 6.3 times that used in humans. The low- and high-dose groups were given 1.5 g/kg (1.5 times of that in humans) and 6 g/kg (6 times of that in humans) Xuefu Zhuyu decoction, respectively. During the experiment, the rats were weighed every 3 days, and the dosage was adjusted.

**Evaluation of erectile function and detection of blood glucose level**

After 4 weeks of drug intervention, the rats in each group were weighed and evaluated using the APO erectile induction experiment, and penile erections and erection times were recorded. The method was the same as that used for the 2.2 model group selection. Simultaneously, blood glucose levels were detected in rat tail vein blood.

**Collection of animal samples**

Four weeks after the intervention, the rats in each group were fasted for 8 h, weighed, and intraperitoneally anesthetized with 10% chloral hydrate. Blood was then collected from the abdominal aorta, either with citrate theophylline adenosine dipyrindamole platelet activation-specific vacutainers or in ethylenediaminetetraacetic acid anticoagulant and serum tubes and centrifuged for 15 min (3,000 rpm), to separate the plasma from the serum. Subsequently, the rats were anesthetized and sacrificed, and the corpus cavernosum penis tissue was retained and stored at −80 °C for further examination and histological observation.

**Indicator detection**

**ELISA.** After blood collection, the serum was separated by centrifugation at 3,000 rpm for 15 min, and NO, ET-1, and LOX-1 levels were determined by ELISA.

**NOX activity assay.** The corpus cavernosum penis tissue was homogenized in an ice bath mortar and then centrifuged at 4 °C for 5 min (600 g). The supernatant was collected and centrifuged at 4 °C for 10 min (11,000 g). The precipitate was added to the corresponding reagents and mixed thoroughly; the protein was quantified using the bicinchonic acid method, and the NOX activity assay kit was used to determine and calculate viability units. **Immunohistochemical staining.** Freshly collected corpus cavernosum penis tissue was washed in phosphate buffered saline (PBS), and the central part was fixed in 4% paraformaldehyde, dehydrated, embedded, and sectioned at a thickness of 4 μm. After dewaxing, the cells were washed with 1% methanolic hydrogen peroxide, distilled water, and 0.1 M PBS at 26 °C, and the samples then underwent antigen retrieval and blocking. The samples were incubated with the primary antibody overnight at 4 °C, followed by secondary antibody incubation for 20 min at room temperature. Horseradish enzyme-labeled streptavidin working solution was added dropwise, and the samples were developed using diaminobenzidine, re-stained with hematoxylin, dehydrated, and sealed for preservation. The results were observed via microscopy, and positive sites were brownish-yellow in color.

**Western blotting.** An appropriate amount of penile corpus cavernosum tissue was homogenized with pre-cooled tissue lysis
solution and then centrifuged at 12,000 r/min for 15 min at 4 °C. The supernatant was extracted and used for protein quantification using the bicinchoninic acid method. The samples were added to 5 × protein loading buffer and denatured in a metal bath at 100 °C for 15 min. The proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred to polyvinylidene difluoride membranes. A 5% skimmed milk powder solution was used to seal the film at room temperature for 2 h, and the film was washed three times with a Tris Buffered Saline with Tween 20 using a shaker. The membrane was incubated overnight with antibodies against JNK, CaSR, PKC, P38, and glyceraldehyde-3-phosphate dehydrogenase at 4 °C. The membrane was then washed and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature, and the protein bands were observed and analyzed using an enhanced chemiluminescence detection system. Relative protein content was expressed as the ratio of the optical density of the target protein to that of the glyceraldehyde-3-phosphate dehydrogenase band.

Transmission electron microscopy sample preparation. Fresh corpus cavernosum penis tissue (≤ 1 mm) was placed in a fixative solution at 4 °C for 2–4 h, rinsed three times with 0.1 M PBS, fixed in 1% osmic acid fixative solution for 2 h, and rinsed again. The tissue was then gradient dehydrated and permeated overnight using a 1:1 mixture of acetone and 812 embedding agent. Then, pure 812 embedding agent was allowed to permeate into the samples overnight and polymerized at 60 °C for 48 h, before the samples were sectioned with a microtome (ultra-thin sections of 60–80 nm). Finally, the sections were double-stained with uranium and lead, dried overnight at room temperature, and observed under a transmission electron microscopy to collect images for the analysis.

Statistical analysis. SPSS software (version 21.0) was used for the statistical analysis, analysis of variance followed by Tukey’s post-hoc test was used for multiple comparisons. The data were expressed as means ± standard deviations with statistical significance of \( P < 0.05 \).

Results

Xuefu Zhuyu decoction treatment alleviated vascular endothelial damage and improved vasomotor function in rats with DMED

In total, 40 rats in the pre-model group were injected intraperitoneally with 60 mg/kg STZ and fasted for 8 h, and their blood glucose levels were measured after 2 weeks. Rats with diabetes (blood glucose > 16.67 mmol/L) were selected, and 100 μg/kg of APO was injected subcutaneously into the neck after 20 min of acclimatization in a dark environment. After 40 min of observation, APO-negative rat screening was considered successful, and a total of 18 rats with DMED were obtained for the experiment. After sampling, the levels of endothelial damage markers (LOX-1 and NOX) and endothelial diastolic and contractile function markers (NO, ET-1) were measured. As shown in Figure 1B, rats with DMED had higher levels of LOX-1 and NOX than did the normal rats, while the LOX-1 and NOX levels were reduced after XFZYD treatment, indicating that the XFZYD alleviated endothelial damage. Moreover, when compared with normal rats, rats with DMED had a lower level of NO and a higher level of ET-1, and the XFZYD decoction recovered the DMED-induced changes (Figure 1C). Overall, these findings suggest that XFZYD treatment improves vasomotor function.

XFZYD treatment inhibited vascular fibrosis in rats with DMED

The expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) in the corpus cavernosum penis tissue was detected using Immunohistochemistry Staining. As presented in Figure 2, the endothelial cells in the mock group were slightly stained, while those in the model group were strongly positive, with brownish-yellow deposits. When compared with the model group, the low- and high-dose groups presented a reduction in both the range and degree of expression. These results indicated that XFZYD inhibits vascular fibrosis.

XFZYD treatment suppressed the CaSR/Gq-PLC-PKC pathway in rats with DMED

Compared with rats of the mock group, rats of the model group had the levels of CaSR, p-PKC-δ/pkδ-8, p-JNK/JNK, and p-P38/P38 proteins in the corpus cavernosum penis tissues significantly elevated, with statistical significance. The levels of CaSR, p-PKC-δ/PKCδ-8, p-JNK/JNK, and p-P38/P38 proteins in the low- and high-dose groups were significantly lower than they were in the model group, and with statistical significance (Figure 3).

Figure 3 The effects of XFZYD on inhibiting the CaSR/Gq-PLC-PKC pathway in rats with DMED. Statistical comparisons were performed using ANOVA followed by Tukey’s post-hoc test: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. XFZYD, Xuefu Zhuyu decoction; DMED, diabetes mellitus erectile dysfunction; ANOVA, analysis of variance.
XFZYD treatment ameliorated endothelial cell structural damage in the penile corpus cavernosum tissues of DMED

As displayed in Figure 4, the mitochondria (A, red arrow) and nuclei (A, blue arrow) were normal in endothelial cells of the rats in mock group. In endothelial cells of rats in the model group, nuclear chromatin was highly agglutinated (B, blue arrow) and organelles were damaged, decreased, or even absent (C, red arrow). The endoplasmic reticulum (D, red arrow) and mitochondria (E, red arrow) were swollen, the cristae of the mitochondria were missing and structurally incomplete, and the cell membranes were broken (E, blue arrow). The low-dose (F–G) and high-dose (H–I) groups showed obvious improvements in morphology, with no nuclear shrinkage and more intact mitochondrial structures (red arrow) than was observed in the model group.

Discussion

As societal norms and standards continue to change, the importance of a good sexual quality of life for male patients with DM is increasing, and DMED has a serious impact on physical and mental health and family relationships around the world. Phosphodiesterase-5 inhibitors are the first choice of treatment for DMED in modern medicine, and the efficacy of this treatment deserves recognition [15]. However, this treatment is more effective for non-diabetic patients than for those with DMED, and its ability to repair vascular endothelial dysfunction in patients with DM remains unclear [16]. Previous studies have reported that TCM is effective and safe for the treatment of DMED. In TCM theory, “blood stasis” is a notable pathological cause of the chronic vascular complications of DM and one of the basic pathological causes of impotence [17]. Therefore, a method for promoting blood circulation and removing blood stasis should be used for patients with DMED. The XFZYD is a representative prescription that promotes blood circulation to remove blood stasis, including *Persicae Semen* (Taoren), *Angelicae Sinensis Radix* (Danggui), *Chuanxiong Rhizoma* (Chuanxiong), *Carthami Flos* (Honghua), *Radix Padoniae Rubra* (Chishao), *Rehmanniae Radix* (Dihuang),...
**Aurantii Fructus (Zhiqiao), Bupleuri Radix (Chaihu), Platycodonis Radix (Jiegeng), Achyranthis Bidentatae Radix (Nixiu), and Glycyrrhiza uralensis Fisch.** (Gancao). It can improve blood circulation and disperse stasis, eliminate stagnation, and facilitate the generation of body fluid. The XFZYD has been used clinically to treat ED and improve the efficacy in diabetic vasculopathy [18].

Prior studies have reported that DMED is associated with a variety of complex factors, including psychological factors, including factors affecting blood vessels, corpus cavernosum penis tissue, and nerves. However, the microcirculatory disturbance caused by penile vascular endothelial injury underlies the pathophysiological basis of DMED and provides starting point for patient treatment [19]. NO released by endothelial cells, is associated with endothelial dysfunction, and is the primary neurotransmitter of penile erection. Notably, NO functions in the transmission of nerve impulses between nerve cells, the absence of which affects penile erection [20]. The NOX enzyme is one of the most important reactive oxygen species-generating systems in the human body, and the increased production of oxygen free radicals can indirectly lead to ED. ET-1 is mainly secreted by endothelial cells and can strongly constrict blood vessels, causing structural remodeling and wall thickening [21]. Furthermore, LOX-1 plays a key role in oxidized low-density lipoprotein-induced endothelial disorders.

In this study, we used STZ combined with APO to build a DMED rat model, and two different XFZYD doses were used to treat the rats with DMEDs. We found that XFZYD reduced LOX-1 and NOX levels, decreased vascular endothelial damage, reduced ET-1 levels, increased NO levels, and improved the vasodilation and contraction of the vascular endothelium in rats with DMED. Altogether, our findings reveal that the XFZYD restored erectile function in rats with DMED by improving vascular endothelial damage, vasodilatation, and contraction.

Furthermore, high blood glucose concentrations in patients with DM can stimulate the vascular endothelium for a short period of time, which can elevate VCAM-1 and ICAM-1 expression, and the subsequent recruitment of immune cells to the endothelial surface further contributes to arteriosclerosis [22]. These pathological features are some of the direct causes of the early onset of penile endothelial damage and vascular fibrosis in patients with DM [23]. Notably, we found that the XFZYD could inhibit vascular fibrosys by inhibiting the expression of VCAM-1 and ICAM-1, thereby improving ED symptoms in DMED rats. Hyperactivation of the PKC pathway has been shown to be an important mechanism by which diabetes-induced vascular endothelial dysfunction leads to vascular fibrosis [9, 10]. Of the diverse molecular pathways that activate the PKC pathway, CaSR/Gq-PLC-PKC is a canonical pathway, in which the CaSR is activated by Gq protein-coupled PLC under the influence of a high-glucose environment. This pathway further promotes the breakdown of phosphatidylinositol-4,5-bisphosphate into DAG and inositol triphosphate, which binds to high-affinity receptors in the nucleus and induces the opening of calcium channels, allowing the release of internal calcium ions from the nucleus into the cytoplasm. Furthermore, the co-binding of calcium and DAG to PKC coactivates PKC on the surface of the cell membrane [11].

Activation of the PKC pathway can further increase the production of free oxygen radicals mediated by NOX, decrease the bioavailability of NO, and increase the release of endothelin, causing endothelium-dependent vasodilation and contraction dysfunction and can lead to ED. Simultaneously, PKC can initiate a mitogen-activated protein kinase signaling cascade that results in the activation of amino-terminal kinase JNK and P38 to subsequently increase the expression of the pro-apoptotic protein Bax and the assembly of apoptotic complexes. This results in the initiation of programmed vascular endothelial cell death through the release of cytochrome C from the mitochondrial inner membrane into the cytoplasm [24]. Our western blotting results indicate that the efficacy of the XFZYD may be achieved by inhibiting the CaSR/Gq-PLC-PKC pathway and reducing the expression levels of CaSR, PKC-δ, JNK, and P38 proteins.

In conclusion, this study revealed that the XFZYD can improve erectile function in rats with DMED by improving vascular endothelial diabetic and contractile functions, reducing endothelial damage, and inhibiting vascular fibrosis. The underlying mechanism may involve the inactivation of the CaSR/Gq-PLC-PKC signaling pathway. Due to the complex pathogenesis of DMED, further research that involves tissues transcriptome sequencing and pathway enrichment will be carried out to allow a further in-depth investigation of the mechanism of action of XFZYD in treating DMED and its influence on the CaSR/Gq-PLC-PKC signaling pathway.

**References**


10. Das Evcimen N, King G. The role of protein kinase C activation and the vascular complications of diabetes. *Pharmacol Res*


