

Study on the mechanism of Jin Gui Shen Qi Pill in the treatment of erectile dysfunction based on bioinformatics analysis

Jingjing Xiang, MD^a, Chaoyang Wang, PhD^a, Xiaoming Yu, PhD^a, Jing He, MD^{b,c,*} 

Abstract

Erectile dysfunction (ED) is a male disease, which is easy to cause disharmony in sexual life. However, at present, there are few drugs with small side effects in clinic. Jin Gui Shen Qi Pill (JGSQP) is a traditional Chinese medicine compound with obvious clinical effect in treating ED. Therefore, it is imperative to explore clinical drugs based on inhibiting the pathological characteristics of ED. First, the active ingredients and action targets in JGSQP were screened by applying Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) and SWISS Target Prediction. Further, a systematic pharmacological analysis platform for traditional Chinese medicine, and the ED targets were screened by applying Gene Cards and Online Mendelian Inheritance in Man databases to construct drug active ingredient-target-disease mapping, followed by gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and protein-protein interaction (PPI) network analysis. Finally, Molecular docking and molecular dynamics simulations were used to screen the active ingredients of JGSQP acting on PDE-5, and analyze the ligand-receptor interaction relationship and binding free energy. The results showed that there were 212 potential targets of JGSQP for ED disease, and GO analysis revealed that the main pathways were positive regulation of DNA-binding transcription factor activity, regulation of vascular diameter, and negative regulation of vascular diameter, etc. KEGG analysis revealed that the main pathways were HIF-1 signaling pathway, prolactin signaling pathway, fluid shear stress, and atherosclerosis, etc. PPI network analysis revealed that the core targets TGFB1 and EGFR have important roles. Molecular docking and molecular dynamics simulations showed that the main components acting on PDE-5 were MOL000546, MOL011169, MOL000279, MOL000273 and Sildenafil. MOL000546 was able to bind stably to PDE-5. The multi-component, multi-target, and multi-pathway action characteristics of JGSQP were confirmed by network pharmacology, which predicted the possible mechanism of action of JGSQP in the treatment of ED and provided a theoretical reference for further experimental validation.

Abbreviations: CCSMC = corpus cavernosum smooth muscle cell, ED = erectile dysfunction, EGFR = epidermal growth factor receptor, ERK 1/2 = extracellular regulated protein kinase 1/2, GO = gene ontology, JGSQP = Jin Gui Shen Qi Pill, KEGG = Kyoto encyclopedia of genes and genomes, MCODE = molecular complex detection, PDE-5 = type 5 phosphodiesterase inhibitor, PKC = protein kinase C, PPI = protein-protein interaction, RMSD = root mean squared error, TCMSP = traditional Chinese medicine systems pharmacology database and analysis platform, TGFB1 = transforming growth factor beta-1.

Keywords: erectile dysfunction, Jin Gui Shen Qi Pill, molecular docking simulation, molecular dynamics simulation, network pharmacology

1. Introduction

Erectile dysfunction (ED) refers to the inability of men to meet their normal sexual life because of penile erection.^[1] According to statistics, the number of people with ED is expected to increase to 322 million worldwide by 2025.^[2] The concern is that the prevalence is expected to increase in the coming

decades.^[3] The prevalence of ED in men under the age of 40 is about 1.10%, while the majority in men over 60 is as high as 20.40%. ED is not only negatively impacting the quality of life but is also associated with many co-morbidities such as depression, diabetes, metabolic syndrome, and cardiovascular disease. An American scholar reported that patients with ED will suffer from different degrees of inferiority complex, which

This research was supported by the National Natural Science Foundation of China (81703954).

The authors have no conflicts of interest to disclose.

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

The data used in this study were obtained from free public databases. Animal experiments and clinical trials were not involved in the research, so ethical approval was not required.

^a Hubei University of Chinese Medicine, Wuhan, Hubei, China, ^b Hubei Provincial Hospital of Traditional Chinese Medicine, Wuhan, Hubei, China, ^c Hubei Provincial Academy of Traditional Chinese Medicine, Wuhan, Hubei, China.

* Correspondence: Jing He, Hubei Provincial Hospital of Traditional Chinese Medicine, No. 4 huayuanshan, Wuchang District, Wuhan City, Hubei Province, China (e-mail: hejing@hbhctcm.com).

Copyright © 2022 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Xiang J, Wang C, Yu X, He J. Study on the mechanism of Jin Gui Shen Qi Pill in the treatment of erectile dysfunction based on bioinformatics analysis. *Medicine* 2022;101:46(e31668).

Received: 20 September 2022 / Received in final form: 12 October 2022 / Accepted: 13 October 2022

<http://dx.doi.org/10.1097/MD.00000000000031668>

will have a serious negative impact on men's sexual health and happiness, as well as a huge economic burden on patients.^[4]

With the aging of the global population, fertility rates are declining in many countries, and functional ED is a major cause of fertility decline.^[5] China has opened up the national 3-child family planning policy to improve the fertility rate. Therefore, ED is not only a male disease but also a significant problem that hinders the development of our society and needs to be addressed urgently.

Currently, the representative drug of Western medicine for ED is sildenafil.^[6] The drug is a 5-phosphodiesterase (PDE-5) inhibitor. After oral administration, it can be quickly absorbed by the human body and combined with PDE-5 to inhibit its activity, to improve the level of cyclic guanosine phosphate (cGMP) in the corpus cavernosum of the penis, cause the relaxation of the smooth muscle of the corpus cavernosum, and cause the blood to flow into the corpus cavernosum. However, taking the drug will dilate the systemic circulation vessels and produce many side effects, such as dizziness, headache, and indigestion. In addition, sildenafil can combine with PDE-6 in the retina to produce abnormal visual adverse reactions. 30% to 50% of ED patients failed to follow this treatment regimen due to adverse events, lack of efficacy, or drug cost.^[7]

In clinical practice, the use of Chinese medicine to examine the cause of the disease and identify the evidence can often achieve better potency. For example, Wang Jun^[8] used JGSQP combined with Valsartan to improve ED and sexual quality in diabetic ED patients with kidney yang deficiency. Zhang Yi^[9] found that JGSQP combined with tamsulosin significantly improved the International Index of Erectile Function (IIEF-5) score, lower urinary tract symptoms, and erectile function in patients with prostate enlargement with ED without significant adverse effects. JGSQP is composed of Di Huang, Shan Yao, Cornu Cervi Pantotrichum, Fu Ling, Mudan Pi, Alisma orientalis, Gui Zhi, Monkshood, Achyranthes bidentatai, and Plantago.^[10] Experimental studies have shown that JGSQP can antagonize the reproductive toxicity caused by Imatinib and increase sperm count.^[11] In addition, JGSQP can increase Fas/FasL expression and reduce testicular germ cell apoptosis in mice with kidney yang deficiency.^[12]

In 2007, the concept of network pharmacology was first put forward. Network pharmacology technology uses nodes and connections to establish a network model of individual connections, abstractly expresses the interaction of complex biological systems as a network, and realizes the system identification of organisms by analyzing the composition and characteristics of complex networks.^[13] Network pharmacology is a biological system network analysis strategy based on high-throughput omics data analysis, computer virtual computing, and network database retrieval.^[14,15] Network pharmacology is developed from the theories of systems biology, multidirectional pharmacology, genomics, proteomics, and other disciplines, based on the technologies of omics, high-throughput screening, network visualization and network analysis. With the rapid development of bioinformatics, systems biology and multidirectional pharmacology, web-based drug discovery is considered to be a cost-effective drug development method.

Based on the characteristics of traditional Chinese medicine, network pharmacology analyzes the relationship between different components of traditional Chinese medicine, studies the biological pathway signals of diseases, and establishes the overall action relationship between active components and targets and diseases, which is consistent with the overall view of traditional Chinese medicine treatment. At present, network pharmacology is mostly used in the screening of active drug components, the prediction of specific drug action mechanisms, the analysis of main active component targets, and the development of combined drugs. Interestingly, as a research idea, network pharmacology can also be used to explain the compatibility law of traditional

Chinese medicine and find new indications for traditional Chinese medicine. Network pharmacology is becoming a powerful and attractive tool to reflect traditional Chinese medicine's multi-component and multi-target characteristics. Based on the network pharmacology method, it can help explain many problems in the material basis of the efficacy of traditional Chinese medicine.

JGSQP has therapeutic effect on ED, but its specific therapeutic mechanism is still unclear, and its key target is not clear. The active ingredients acting on PDE-5 need to be excavated. In order to further reveal the mechanism of JGSQP in the treatment of ED, this study intends to use network pharmacology, molecular docking and molecular dynamics simulation to further explore the mechanism of action.

2. Methods

2.1. Related databases and software

Chinese medicine and bioinformatics databases: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, <http://lsp.nwu.edu.cn/tcmsp.php>),^[16] a systemic pharmacology database and analysis platform for Chinese medicine, SWISS Target Prediction (<http://www.swisstargetprediction.ch/>), a target prediction tool, protein interaction database STRING (<https://string-db.org/>), protein database UniProt (<https://www.uniprot.org/>), Online Mendelian Inheritance in Man (<https://omim.org/>), Gene cards database (<https://www.genecards.org/>),^[17] the functional enrichment analysis tool Metascape (<http://metascape.org/>), the protein structure database Research Collaboratory for Structural Bioinformatics protein data bank (RCSB PDB, <http://www.rcsb.org/>), the protein modeling tool Swiss model (<https://www.swissmodel.expasy.org/>). Related software: ChemWin 5.0 for chemical structural formula mapping, Open Babel 2.4.1 for chemical data conversion, Pyrx 0.8 for molecular docking, Visual Molecular Dynamics (VMD) 1.9.3 for visual molecular dynamics, Nanoscale Molecular Dynamics (NAMD) 2.13b for molecular dynamics simulation, Integrated molecular simulation software Discovery Studio 2020.

2.2. Active components and target collection of JGSQP

The names of each herbal medicine in JGSQP were entered into the TCMSP database separately, and the oral bioavailability (OB) and drug-likeness (DL) were used as screening parameters. The screening parameters were set as OB $\geq 30\%$ and DL ≥ 0.18 ,^[18] and the target proteins corresponding to the active ingredients of each drug were screened and imported into the database. The other components of JGSQP were searched for in the literature, and the targets were predicted using SWISS Target Prediction and summarized with the targets in TCMSP. The corresponding target proteins were entered into the UniprotKB database in Uniprot (<http://www.uniprot.org/>)^[19] to check the corresponding genes. The search condition was set as Organization: Homo Allens (Human), and the conversion of protein and gene was performed to construct the ingredient-target-gene database.

2.3. ED disease target collection application

Gene cards and Online Mendelian Inheritance in Man databases were searched with "Erectile dysfunction" as the keyword, and the disease targets were obtained by removing duplicate targets from the intersection. The Uniprot database was used to check the disease targets and drug targets.

2.4. GO, KEGG, PPI network analysis

The intersecting genes were entered into the Cluego plug-in of Cytoscape 3.7.2 software for Gene Ontology (GO) enrichment

analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis to elucidate further the mechanism of action of JGSQP for ED. Protein-protein interaction (PPI) network analysis and core target analysis were performed using Metascape and MCODE.

2.5. Docking of active ingredients with PDE-5 molecules

All the active ingredients of Pill were used as ligands, and the PDE-5 structure with PDB ID 3HC8 was selected as the receptor for molecular docking. Sildenafil was downloaded from Pubchem, and the original structure of PDE-5 was processed by the Swiss model and Pyrx for residue complementation and hydrogenation. All ligands and receptors were imported into Pyrx software for molecular docking.

2.6. Molecular dynamics simulation and root mean squared error (RMSD) analysis

A 30ns molecular dynamics simulation was performed using Nanoscale Molecular Dynamics (NAMD) on the structure of the complex of the small molecule with the lowest docking energy of the system and the receptor to investigate the stability of the complex. The 2 small molecules were analyzed using Swiss param, and the corresponding topology and force field parameter files were prepared. The complex was immersed in a cubic water box using Visual Molecular Dynamics (VMD), and Na⁺ and Cl⁻ were added to make the NaCl concentration 0.15 mol/L and make the overall charge neutral. The force field used in the simulation is the CHARMM force field, the step size is set to 2fs, and other parameters are set to default values.

2.7. Molecular mechanics generalized born surface area (MM-GBSA) free energy calculation

The relative binding free energy between ligand and receptor was calculated using the MM-GBSA method.^[20,21] The motion trajectories of the receptor-ligand complex, receptor and ligand were first extracted separately from the kinetic simulation trajectories. The generalized Born implicit solvent (GBIS) model was used to calculate the extracted trajectories, and the solvent dielectric constant was set to 78.5, and the temperature was set to 310 K. The binding free energy was calculated as follows

$$\Delta G_{\text{binding}} = G_{\text{tot}}(\text{complex}) - G_{\text{tot}}(\text{receptor}) - G_{\text{tot}}(\text{ligand})$$

$$G_{\text{tot}} = E_{\text{internal}} + E_{\text{elect}} + E_{\text{vdW}} + G_{\text{solv}} - T \Delta S_{\text{conf}}$$

E_{internal} , E_{elect} , E_{vdW} , G_{solv} , $-T \Delta S_{\text{conf}}$ denote internal energy (including bond, angle, and dihedral energy), electrostatic free energy, van der Waals interaction, solvation free energy, and conformational entropy, respectively. Since all 3 trajectories originate from the same trajectory, the internal energies will cancel each other, so they are not calculated. In addition, the conformational entropy calculation is more complicated and has minimal effect on the calculation of the MM-GBSA binding free energy of the protein-ligand complex, so it is also not calculated. The ELECT energy term in the named output file already contains G_{solv} , so only the ELECT and VDW energy terms are used to estimate the binding free energy.

3. Results

3.1. GO and KEGG analysis results

3.1.1. Main ingredients of JGSQP. The compounds were retrieved from the TCMSP database: 2 from Dihuang, 16 from Yam, 20 from Cornus, 16 from Poria, 11 from Mudanpi, 10 from Zedoary, and 7 from Cinnamomum, 21 from Radix et Rhizoma, 20 from Niubizi and 9 from Plantago. A total of 114 active ingredients were obtained by deleting duplicate compounds.

3.1.2. GO enrichment analysis of intersecting genes. Using the Cluego 2.5.6 plug-in of Cytoscape 3.7.2 software, species were selected from Homo Sapiens, and the enrichment threshold $pV < 0.01$ was set. The smaller the p , the more closely the pathway is related to the disease. The results obtained a total of 117 relevant entries, mainly including regulation of apoptotic process in muscle cells, positive regulation of small molecule metabolic process, response to mechanical stimulation, core promoter binding, regulation of priMIRNA transcription by RNA polymerase II, regulation of smooth muscle cell proliferation, negative regulation of transmembrane transport, etc.

The top 5 results of the biological process analysis were positive regulation of DNA-binding transcription factor activity, regulation of blood vessel diameter, negative regulation of blood vessel diameter, positive regulation of vasculature development, and smooth muscle cell proliferation.

The top 3 results of the cell function analysis were the integral component of the presynaptic membrane, platelet alpha granule lumen, and plasma membrane raft.

The top 5 results of the molecular functional analysis were G protein-coupled amine receptor activity, ligand-activated transcription factor activity, adrenergic receptor activity, nuclear receptor activity, and heme-binding.

3.1.3. KEGG enrichment analysis. In Figure 1a, 59 ED-related pathways were analyzed, including the HIF-1 signaling pathway 75.81%, prolactin signaling pathway 1.61%, fluid shear stress, atherosclerosis 1.61%, calcium signaling pathway 9.68%, and amphetamine addiction 11.29% (Fig. 1b).

3.2. PPI network analysis

To further explore the therapeutic mechanism of JGSQP for ED treatment, a PPI network analysis was performed on 47 potential targets with the help of the Metascape online tool (Fig. 2a). The PPI network analysis using Molecular Complex Detection (MCODE) identified 3 clusters (Fig. 2b), and thus these 3 clusters were the most relevant for ED treatment. Cluster 1 contained 8 nodes and 13 edges. The core node of this cluster is Transforming growth factor beta-1 (TGFB1), which belongs to a multifunctional protein that regulates the growth and differentiation of various cell types and is involved in the ERK 1 and ERK 2 cascades mediating positive regulation.^[22]

Cluster 2 contains 5 nodes and 7 edges. The core node of this cluster is the Epidermal growth factor receptor (EGFR), which belongs to the tyrosine kinase receptor family that transmits extracellular signals to the intracellular and is also involved in the positive regulation of extracellular regulated protein kinase 1/2 (ERK 1/2) cascade transduction.^[23]

Cluster 3 contains 4 nodes and 6 edges. The cluster comprises nodes Androgen receptor (AR), PRKCA, E2F1, PTEN. AR is a steroid hormone receptor that regulates the expression of eukaryotic genes and affects cell proliferation and differentiation in target tissues.^[24] Protein kinase C alpha type (PRKCA) can directly phosphorylate the targets RAF1, BCL2, CSPG4, TNNT2/cTnT, or activate MAPK 1/3 (ERK 1/2) and RAP1GAP-mediated signaling cascades.^[25] Transcription factor E2F1 is a transcription factor that mediates cell proliferation, male germline stem cell neogenesis, and sperm development. Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase (PTEN) is a bispecific protein phosphatase involved in the regulation of male mating behavior.

3.3. Molecular docking validation of JGSQP

In order to elucidate the mode of action of the protein with the compound at the molecular level, the active components of

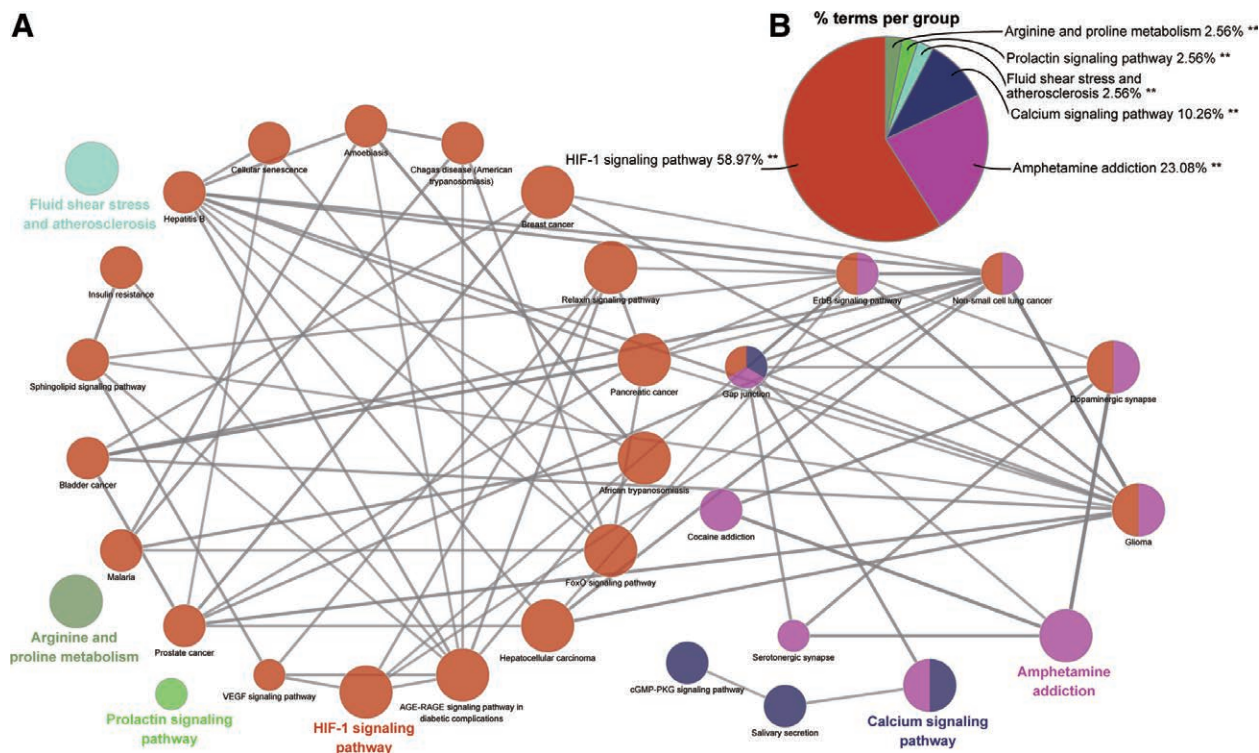


Figure 1. KEGG pathway enrichment analysis, KEGG = Kyoto Encyclopedia of Genes and Genomes.

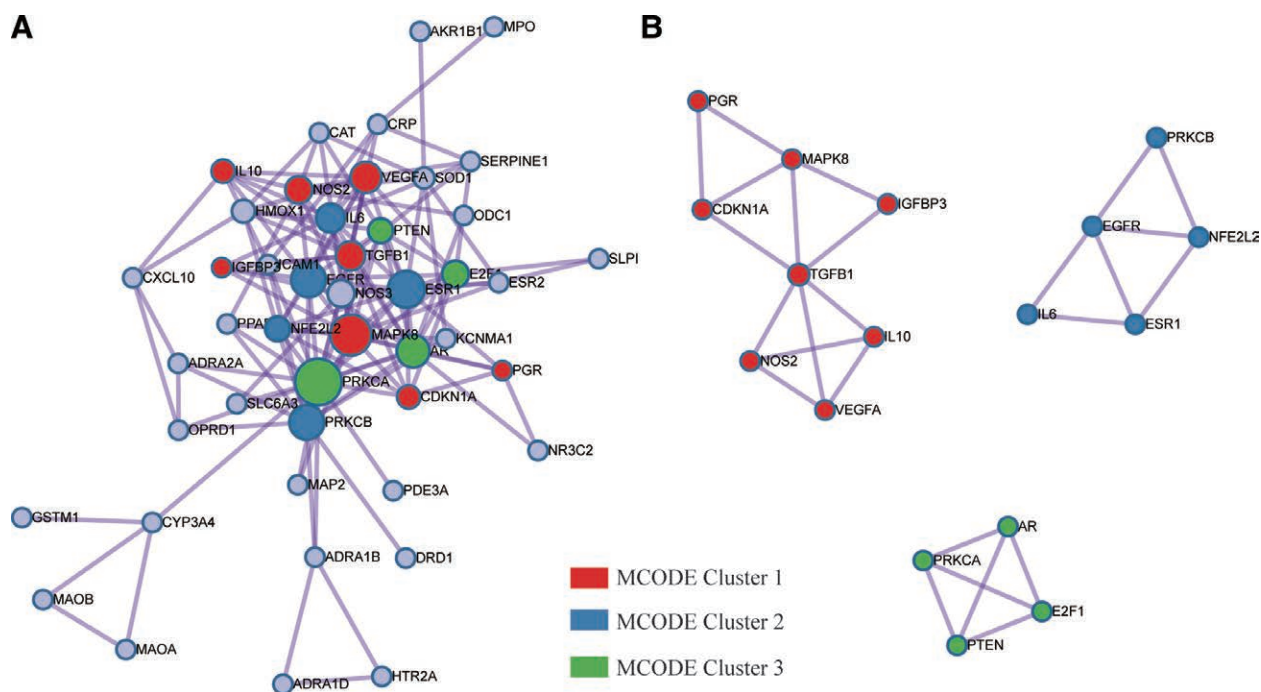


Figure 2. PPI network analysis and MCODE Cluster, PPI = protein-protein interaction, MCODE = molecular complex detection.

JGSQP were docked to PDE-5, and the theoretical binding patterns are shown in Table 1. As can be seen from the table, the residues of PDE-5 that produce conventional hydrogen bonds with small-molecule MOL000546 are GLN241, those that make Pi-sigma bonds are HIS79, and those that have Alkyl or Pi-alkyl forces are LEU191, PHE286, ILE234, ALA233, PHE252, VAL248; with small Molecular MOL011169 residues that generate van der Waals forces are ALA233,

ILE234, residues that generate conventional hydrogen bonds are LEU148, residues that generate Alkyl or Pi-alkyl forces are LEU231, TYR78, VAL248, PHE286, PHE252, HIS79, MET282; with small-molecule MOL000279 residues that generate Alkyl or Pi-alkyl forces are HIS79, MET282, TYR78, VAL248, PHE286, PHE252, LEU231; residues that generate conventional hydrogen bonds with the small molecule MOL000273 are TYR78, ASP230, LEU231, residues that

Table 1**Molecular docking.**

Ligand	Binding affinity	Hydrogen bond	van der Waals	Pi-sigma	Alkyl or Pi-alkyl	Two dimensional interaction	Three dimensional interaction
MOL000546	-11.4	GLN241		HIS79	LEU191, PHE286, ILE234, ALA233, PHE252, VAL248		
MOL011169	-11.2	GLU148	ALA233, ILE234		LEU231, TYR78, VAL248, PHE286, PHE252, HIS79, MET282		
MOL000279	-10.3				HIS79, MET282, TYR78, VAL248, PHE286, PHE252, LEU231		
MOL000273	-10.3	TYR78, ASP230, LEU231			PHE286, VAL248		
Sildenafil	-9.8			MET147	PHE252, PHE286, LEU191, ILE244		

generate Alkyl or Pi-alkyl forces are PHE286, VAL248; residues that generate Pi-sigma bonds with the small molecule Sildenafil are MET147, and residues that generate Alkyl or Pi-alkyl forces are PHE252, PHE286, LEU191, and ILE244.

3.4. Molecular dynamics simulation

3.4.1. RMSD and root mean square fluctuation. As shown in Figure 3, the protein-ligand complex is surrounded by generate. Then, molecular dynamics simulation was carried out for 30 ns. The RMSD of PDE-5 and the ligand (MOL000546) are shown in Figure 4. The RMSD shows that PDE-5 stabilizes around 13 ns, and after that, the overall fluctuation does not exceed 0.5 nm. From the root mean square fluctuation (Fig. 5), it can be seen that the hydrogen bonding residues HIS79 and Pi-sigma residues HIS79 with MOL000546 are both more stable at around 1.75 nm, indicating that the residues are more durable and the binding pocket does not change significantly.

3.4.2. Binding free energy results. The last 5 ns molecular dynamics simulation trajectory was taken for the binding energy calculation, and the binding free energy of PDE-5 complexed with MOL000546 was calculated using the MM-GBSA method (Fig. 6). It can be seen from the figure that the van der Waals free energy contributes more to the binding free energy, while the electrostatic free energy is negative. The binding free energy is basically around -20 kcal/mol.

4. Discussion

The pathogenic mechanism of ED is complex and regulated by a variety of factors, among which the diastole and contraction of the penile corpus cavernosum play a significant role.^[26]

The diastole of penile cavernous smooth muscle is mainly related to the nitric oxide/guanosine cyclic phosphate signaling pathway and cAMP signaling pathway. In the nitric oxide/cyclic guanosine phosphate signaling pathway, sexual stimulation of the penis promotes the release of NO from nitric oxide synthase (NOS), and NO as a signaling molecule activates guanylate cyclase (sGC), which promotes the conversion of GTP to cGMP and further activates the specific kinase PKG, causing a decrease in intracytoplasmic calcium ion concentration and an outflow of potassium ions, resulting in relaxation of penile smooth muscle, inward blood flow, and penile erection.^[27] In the cAMP signaling pathway, substances such as prostaglandin E, vasoactive intestinal peptide, and calcitonin gene-related polypeptide (CGRP) can enter the penile smooth muscle as signaling molecules and activate the specific kinase PKA. PKA acts similarly to PKG and will further alter calcium and potassium ions, leading to an erection.

The main signaling pathways of penile cavernous smooth muscle contraction are the RhoA/Rho kinase pathway, the protein kinase C/protein kinase C phosphatase inhibitor protein 17 (PKC/CPI-17) pathway, and the extracellular regulated protein kinase 1/2 (ERK1/2) pathway. Expression,^[28] in addition, ET-1, angiotensin II (Ang II) as peripheral transmitter can induce RhoA transfer from the cytoplasm to the cell membrane and activate downstream ROCK1/2. ROCK can phosphorylate

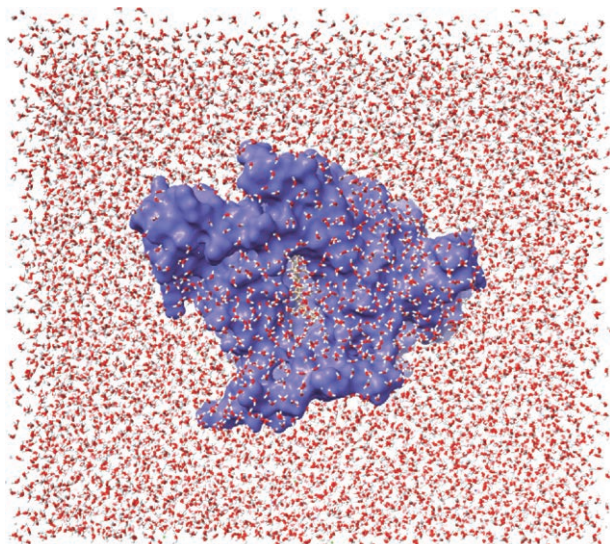


Figure 3. PDE-5 and MOL000546 surrounded by saline, PDE-5 = Type 5 phosphodiesterase inhibitor.

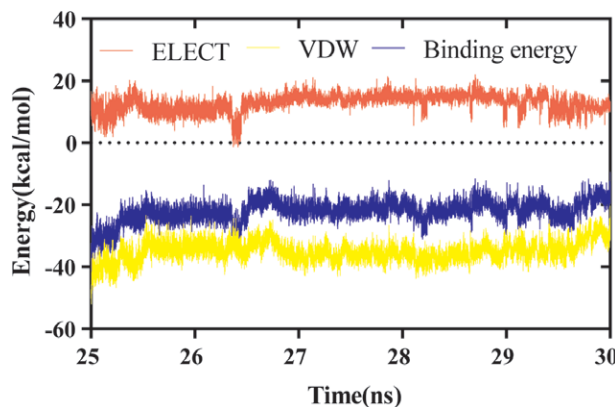


Figure 6. Binding free energy, VDW = van der Waals.

transmitter binding to cell membrane receptors activates PKC, which further acts on the downstream CPI-17 protein, a smooth muscle-specific type I protein phosphatase inhibitor protein that reduces MLCP activity, leading to smooth muscle contraction.^[29] In the ERK1/2 pathway, ERK1/2 is a member of the mitogen-activated protein kinase (MAPK) family. Phosphorylated ERK1/2 activates calcium channels, increases intracellular calcium ion concentration, and smooths muscle contraction. Secondly, phosphorylated ERK1/2 also activates the ET-1-induced contraction-related pathway of corpus cavernosum smooth muscle cell (CCSMC), inhibiting eNOS activity impeding CCSMC diastole, which then leads to the development of ED.^[30]

We found 47 intersecting targets of JGSQP and ED through network pharmacology experiments. The main results of biological process analysis by GO analysis were positive regulation of DNA-binding transcription factor activity, regulation of vascular diameter, negative regulation of vascular diameter, positive regulation of vascular development, and smooth muscle cell proliferation. The main results of the cellular function analysis are components of the presynaptic membrane, platelet alpha granule lumen, and plasma membrane rafts. The main results of the molecular functional analysis were G protein-coupled amine receptor activity, ligand-activated transcription factor activity, adrenergic receptor activity, nuclear receptor activity, and heme-binding. The main pathways identified by KEGG analysis were the HIF-1 signaling pathway 75.81%, prolactin signaling pathway 1.61%, fluid shear stress and atherosclerosis 1.61%, calcium signaling pathway 9.68%, and PPI network analysis revealed an essential role for the core targets TGFβ1 and EGFR. The present study suggests that JGSQP may act on TGFβ1 and EGFR to attenuate ERK 1/2 cascade conduction, inhibit CCSMC contraction, and regulate the activity of enzyme nitric oxide synthase to promote CCSMC relaxation, thus exerting a therapeutic effect on ED. Molecular docking and molecular dynamics simulation results suggest that MOL000546 and PDE-5 can bind more stably to the docking pocket, and the primary acting residues are GLN241, HIS79, LEU191, PHE286, ILE234, ALA233, PHE252, VAL248, and the binding free energy is about -20 kcal/mol.

As this article mainly relies on database information, it still has some limitations. First, this study only collected the active ingredients in JGSQP from TCMSP database, and did not collect the active ingredients from other literatures or databases, so there may be omissions. Second, this study mainly relies on database information, and only considers the number of interactions in the network analysis, without considering the intensity of the interaction, the dosage of traditional Chinese medicine, the decoction method and other issues, so there is a deviation from the actual situation. Third, key targets and possible signal pathways of active ingredients of drugs were obtained through network pharmacology, but the accuracy of prediction was not

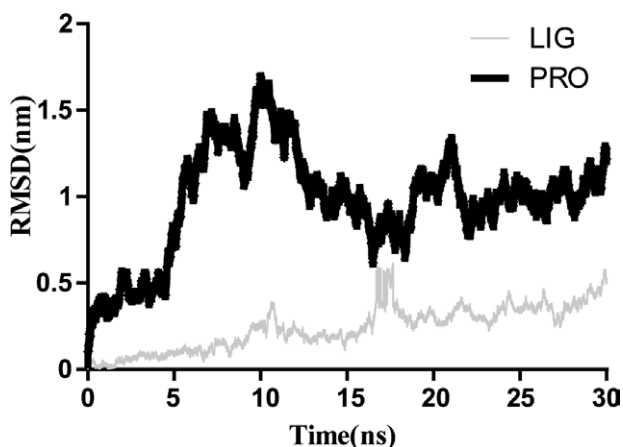


Figure 4. RMSD. RMSD = Root mean squared error.

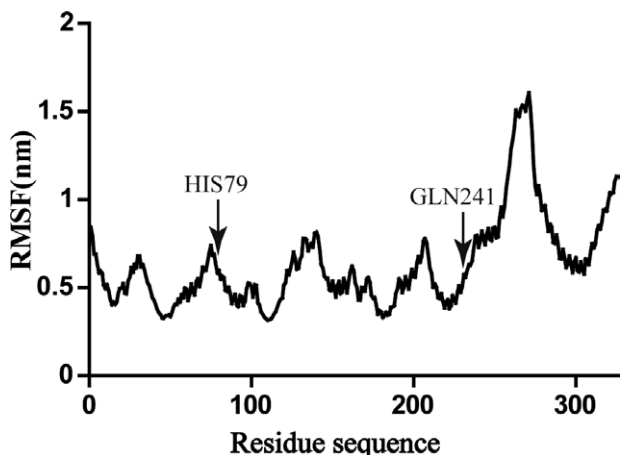


Figure 5. RMSF. RMSF = Root mean square fluctuation.

myosin light chain (MLC) with the participation of ATP and decrease myosin light chain phosphatase (MLCP) dephosphorylation, leading to contraction of penile cavernous smooth muscle. In the PKC/CPI-17 pathway, smooth muscle contractile

further confirmed by in vivo and in vitro experimental verification, lacking data support. These limitations need to be further supplemented and improved in future research.

5. Conclusions

This experimental study suggests that JGSQP can treat ED through multiple pathways, both by promoting the diastole of penile cavernous smooth muscle and inhibiting the contraction of penile cavernous smooth muscle and also by acting on the PDE-5 target to treat impotence. Therefore, this study reveals to a certain extent, the mechanism of action of JGSQP in the treatment of ED and provides theoretical references for further research.

Author contributions

Jingjing Xiang and Xiaoming Yu designed this study and performed the online database search. Jingjing Xiang and Jing He all contributed to the data collection and data analysis. Jingjing Xiang and Xiaoming Yu prepared the original draft. Jing He finished the revision of the manuscript. All authors have read and approved the final manuscript.

Funding acquisition: Xiaoming Yu.

Project administration: Chaoyang Wang.

Supervision: Chaoyang Wang.

Writing – original draft: Jingjing Xiang.

Writing – review & editing: Jingjing Xiang, Jing He.

References

- [1] Sand MS, Fisher W, Rosen R, et al. Erectile dysfunction and constructs of masculinity and quality of life in the multinational Men's Attitudes to Life Events and Sexuality (MALES) study. *J Sex Med.* 2008;5:583–94.
- [2] Goldstein I, Giraldo A, Maculaitis MC, et al. Real-world assessment of the impact of erectile dysfunction on sexual planning behavior and health- and treatment-related outcomes among men in 8 countries. *Sex Med.* 2020;8:338–49.
- [3] Foy CG, Newman JC, Berlowitz DR, et al. Blood pressure, sexual activity, and erectile function in hypertensive men: baseline findings from the systolic blood pressure intervention trial (SPRINT). *J Sex Med.* 2019;16:235–47.
- [4] Bauer SR, Breyer BN, Stampfer MJ, et al. Association of diet with erectile dysfunction among men in the health professionals follow-up study. *JAMA Netw Open.* 2020;3:e2021701.
- [5] Kun X, Yang L. The family planning policy, cost socialization and fertility ratio. *J Guizhou Univ Fin Econ.* 2022;40:69–78.
- [6] Nik-Ahd F, Shindel AW. Pharmacotherapy for erectile dysfunction in 2021 and beyond. *Urol Clin North Am.* 2022;49:209–17.
- [7] Beecken WD, Kersting M, Kunert W, et al. Thinking about pathomechanisms and current treatment of erectile dysfunction—"The Stanley Beamish Problem." Review, recommendations, and proposals. *Sex Med Rev.* 2021;9:445–63.
- [8] Wang J. Observation of valsartan combined with Jinguishenqi Bolus on diabetic erectile dysfunction patients. *Cardiovasc Die Electr J Int Trad Chin Western Med.* 2019;7:17–8.
- [9] Zhang Y, Liu B. The curative effect of tamsulosin combined with Jinkuishenqi pill in treating patients with benign prostatic hyperplasia coexisting with erectile dysfunction. *Chin J Hum Sex.* 2015;24:3–5.
- [10] Huang Q. Discussion about the evolution of Jinguishenqi pill. *Jiang Xi Trad Chin Med.* 2021;52:12–3.
- [11] Sihan Z, Lingling W, Xiaoxia C, et al. Effect of Jinkui Shenqi Pill on reproductive toxicity of imatinib-induced mice and its content in blood and bletilla striata. *Chin J Ethnomed Ethnophar.* 2020;29:10–15 + 38.
- [12] Zhu QJ, Li X, Yang J, et al. Effects of Jinguishenqi bolus on apoptosis of kidney-Yang deficiency model of testicular cells from the Fas/FasL pathway. *Lishizhen Med Materia Med Res.* 2017;2:272–76.
- [13] Li-wen H, Shan-jun C, Rong D, et al. Progress of network pharmacology applications in studying the complex mechanisms of action of traditional Chinese medicine. *Shandong Sci.* 2021;34:22–31.
- [14] Li S, Zhang ZQ, Wu LJ, et al. Understanding ZHENG in traditional Chinese medicine in the context of neuro-endocrine-immune network. *IET Syst Biol.* 2007;1:51–60.
- [15] Hopkins AL. Network pharmacology. *Nat Biotechnol.* 2007;25:1110–1.
- [16] Li DQ, Jiang F, Zhang HS, et al. Network pharmacology-based approach to investigate the mechanisms of Zingiber officinale Roscoe in the treatment of neurodegenerative diseases. *J Food Biochem.* 2022;46:e14068.
- [17] Stelzer G, Rosen N, Plaschkes I, et al. The GeneCards suite: from gene data mining to disease genome sequence analyses. *Curr Protoc Bioinform.* 2016;54:1.30.31–31.30.33.
- [18] Huang J, Cheung F, Tan HY, et al. Identification of the active compounds and significant pathways of yinchenhao decoction based on network pharmacology. *Mol Med Rep.* 2017;16:4583–92.
- [19] UniProt Consortium T. UniProt: the universal protein knowledgebase. *Nucleic Acids Res.* 2018;46:2699.
- [20] Kollman PA, Massova I, Reyes C, et al. Calculating structures and free energies of complex molecules: combining molecular mechanics and continuum models. *Acc Chem Res.* 2000;33:889–97.
- [21] Homeyer N, Gohlke H. Free energy calculations by the molecular mechanics Poisson-Boltzmann surface area method. *Mol Inform.* 2012;31:114–22.
- [22] Chen Q, Lee CE, Denard B, et al. Sustained induction of collagen synthesis by TGF- β requires regulated intramembrane proteolysis of CREB3L1. *PLoS One.* 2014;9:e108528.
- [23] Madeo A, Maggiolini M. Nuclear alternate estrogen receptor GPR30 mediates 17 β -estradiol-induced gene expression and migration in breast cancer-associated fibroblasts. *Cancer Res.* 2010;70:6036–46.
- [24] Cui J, Yang Y, Zhang C, et al. FBI-1 functions as a novel AR co-repressor in prostate cancer cells. *Cell Mol Life Sci.* 2011;68:1091–103.
- [25] Xu X, Han L, Zhao G, et al. LRCH1 interferes with DOCK8-Cdc42-induced T cell migration and ameliorates experimental autoimmune encephalomyelitis. *J Exp Med.* 2017;214:209–26.
- [26] Wang QX, Lu WJ, Meng H, et al. Mechanism of male erectile dysfunction and progress in prevention and treatment of traditional Chinese Medicine. *Chin J Basic Med Trad Chin Med.* 2020;26:1578–81.
- [27] Andersson KE. Mechanisms of penile erection and basis for pharmacological treatment of erectile dysfunction. *Pharmacol Rev.* 2011;63:811–59.
- [28] Li M, Zhuan L, Wang T, et al. Apocynin improves erectile function in diabetic rats through regulation of NADPH oxidase expression. *J Sex Med.* 2012;9:3041–50.
- [29] Baek I, Jeon SB, Kim J, et al. A role for Rho-kinase in Ca-independent contractions induced by phorbol-12,13-dibutyrate. *Clin Exp Pharmacol Physiol.* 2009;36:256–61.
- [30] Xu CX, Jiang R. [Raf/MEK/ERK1/2 signaling pathway and penile erection]. *Zhonghua Nan Ke Xue.* 2010;16:354–8.