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Exploring the Mechanism of Action of Glyasperin A in Intervening Menopause Based on Network Pharmacology and Molecular Docking Technology

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Abstract [**Objectives**] To investigate the mechanism of action of glyasperin A (GAA) in intervening menopause using network pharmacology and molecular docking technology. [**Methods**] All target names of the active ingredients were screened using TCMSP, 3D model molecules converted into SMILES online tool, Swiss target prediction and literature search. The relevant target genes corresponding to menopause were identified using the Genecards database. Venn 2.1.0 was then used to generate the corresponding Venn diagram. Finally, the protein-protein interaction (PPI) network was constructed using Cytoscape 3.9.1 software. The core targets that were screened underwent enrichment and analysis using the Gene Ontology (GO) biological process and KEGG pathways with the assistance of the DAVID database and bioinformatics. The molecular docking was then verified using AutoDock and Pymol software on the core targets. [**Results**] This study screened 100 target genes of active ingredients. In the PPI network, ESR1 and AKT1 were found to have a higher degree. The GO and KEGG enrichment analyses revealed that the biological processes primarily involved platelet activation, regulation of circadian rhythms, and regulation of mRNA stability. The signalling pathways included hepatitis B, cytotoxicity, and gastric cancer. The molecular docking results indicated that the key active ingredients and proteins bound well, as evidenced by their small binding energies. [**Conclusions**] Using a systematic network pharmacology approach, this study predicts the basic pharmacological effects and potential mechanisms of GAA in intervening menopause, which provides a foundation for further research on its pharmacological mechanisms.

Key words Network pharmacology, Molecular docking, Menopause, Glyasperin A

1 Introduction

Menopausal syndrome is a collection of symptoms resulting from hormonal fluctuations and decreases before and after menopause in women. The primary symptoms include hot flashes, sweating, irritability, and chest tightness, which can significantly affect the patient's daily life. According to traditional Chinese medicine, premenopausal and postmenopausal conditions are believed to be caused by an imbalance of yin and yang in the kidneys[1]. According to *Plain Questions . The Universal Truth*, menopausal syndrome is caused by the pathogenetic mechanism of weak qi and blood in the conception vessel and Taichong pulse in women at the age of 49 years. The treatment of menopausal syndrome is based on nourishing yin and tonifying the kidneys, detoxifying the liver, and strengthening the spleen[2]. Menopause is a significant physiological period for women. The gradual decline of ovarian function can cause physical and psychological changes. Menopausal insomnia is a typical manifestation, mainly characterized by persistent difficulty in falling asleep, waking up easily or early, and poor sleep quality during menopause. Currently, China is experiencing a rapid increase in population aging, leading to a rise in the number of female patients suffering from insomnia during menopause. This condition significantly impacts their quality of life and overall physical and mental health. The cause of menopausal insomnia is not yet fully understood, but it is commonly believed to be closely linked to changes in female sex hormone levels[3]. During menopause, women experience a gradual decline in ovarian function and a decrease in sex hormone secretion. This can lead to neurological dysfunction and trigger various menopausal syndromes[4]. Menopause is a significant stage in a woman's life cycle, marking the transition from childbearing to old age. It is a prolonged period that carries a high risk of disease. The prevalence of menopausal symptoms is increasing annually due to the fast-paced lifestyle and heightened work demands[5]. Currently, a relatively low proportion of Chinese women take the initiative to seek medical treatment for menopause-related symptoms. According to a community survey conducted in Shanghai in 2020, only 25.97% of patients with menopausal symptoms chose to seek medical treatment. Many women did not receive adequate guidance and treatment. Menopausal syndromes, formerly known as perimenopausal syndromes, include symptoms such as loss of emotional control, hot flashes, sweats, menstrual disorders, sleep disorders, and osteoporosis[6].

Menopause is a significant physiological period for women. While modern medical research acknowledges that some men also experience menopause, it primarily refers to the unique state of women during the perimenopausal stage. During this period, a woman's ovarian function will gradually decline until it ceases completely. This results in the cessation of the menstrual cycle, also known as menopause. According to Western medicine, menopause is caused by a decrease in estrogen secretion, which in turn affects the functioning of the hypothalamic-pituitary-ovarian nervous system, adrenal nervous system, and other systems. This im-

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balance leads to a loss of synthesis and secretion balance of various neurotransmitters and hormones, resulting in systemic symptoms. Estrogen replacement therapy is recommended in Western medicine for the relief and treatment of menopause. Its purpose is to replenish the lack of estrogen in a woman's body and maintain the balance of the nervous system to a certain extent. Estriol is a naturally synthesized estrogen in the human body. Nelestrol is a derivative of estriol with similar functions and effects. It can effectively alleviate various menopausal symptoms after being converted by relevant enzymes. However, it is important to note that long-term use of this drug may lead to endometrial hyperplasia, uterine bleeding in mild cases, and a significantly increased incidence of endometrial polyps and neoplasia in severe cases, and even carcinogenic risk. Therefore, there are still some differences in clinical estrogen replacement therapy in Western medicine[7].

Glycyrrhiza uralensis is known for its detoxifying properties and ability to ease pain. It is often referred to as "Guolao" and is used in traditional medicine to harmonize other remedies. Studies have shown that *G. uralensis* increases the body's tolerance to poisons by regulating cytochrome P450 proteins (CYP) in the liver to inhibit activation of poisons, inducing metabolism of poisons, and reacting with poisons chemically[8-9]. Additionally, it has immunosuppressive effects that may be potentiated in the treatment of immune disorders. Glyasperin A (GAA) is extracted from various parts of the plant, including the roots, stems, leaves, fruits, and seeds. GAA is a flavonoid compound isolated from the acetone extract of macadamia leaves[10]. It is also an isopentenyl flavonoid, which is a significant branch of flavonoids[11]. The addition of the isopentenyl substituent group, which possesses lipophilic properties, enhances the affinity of isopentenyl flavonoids for biofilms and improves their bioavailability[12]. Compared to flavonoids[13], this class of compounds exhibits significant pharmacological activity, particularly in anti-inflammatory, anti-tumor, antibacterial, neuroprotection, osteoporosis improvement, diabetes treatment, and testosterone production promotion. Licoagrochalcones were isolated from *G. glabra*. *In vivo* studies indicate that the extract may contain additional compounds that contribute to its estrogenic activity[14]. Phytoestrogens are the chemical components of plants that exhibit estrogenic or anti-estrogenic activity. Chinese herbal plants have long been used for women's health care and treating gynecological diseases. The pharmacological effects of phytoestrogens found in these herbs must not be disregarded. Several studies have demonstrated that administering estrogen replacement therapy to menopausal women significantly reduces their menopausal symptoms and prevents the development of osteoporosis and cardiovascular disease. However, long-term estrogen use can have adverse effects, including hypercoagulability, hypertension, and edema. Additionally, it increases the risk of breast and endometrial cancer. For this reason, while seeking estrogen substitutes, many scholars have found that phytoestrogens can alleviate menopausal symptoms without producing the adverse effects of estrogen mentioned above. Therefore, isoprene has become a hot topic of research in recent years, with significant development potential and broad application prospects.

However, the mechanism of action of GAA is complex, and there are limited studies on its pharmacological effects. Therefore, further in-depth research and improvement are necessary.

2 Data and methods

2.1 Acquisition of target genes of active ingredients The compounds in the MOL2 structure of GAA, downloaded from the TCMSP database, were uploaded to the 3D model molecules converted into SMILES online tool. The resulting SMILES numbers were then used to predict the corresponding targets using the Swiss Target Prediction database (http://www.swisstargetprediction.ch/).

2.2 Menopause gene search Target proteins related to menopause were searched using the Genecard database (http://www.genecards.org/) with "menopause" as the keyword.

2.3 Venn diagram creation and PPI construction The study utilized the Venn 2.1.0 analysis tool to compare and analyze the action targets of GAA with those related to menopause. The Venn diagram was plotted to obtain the common targets of the components and diseases. The 29 intersecting targets were imported into the STRING database to obtain the protein-protein interaction (PPI) information. The results were then imported into the Cytoscape software for visualization and topology analysis of the network. Finally, the PPI network was constructed, and the core action targets were screened out.

2.4 GO and KEGG enrichment analysis The targets deemed effective were uploaded to DAVID software, and gene ontology (GO) function enrichment analysis and visualization processing were performed using humans as the study subjects. GO comprises biological process (BP), cellular component (CC), and molecular function (MF). Meanwhile, we performed KEGG pathway enrichment analysis to determine the main pathways of action of GAA in menopause. The results were visualized using bioinformatics (https://www.bioinformatics.com.cn).

2.5 Molecular docking verification The PPI network diagram ranked the core proteins (ESR1, AKT1, PTGS2, PPARG, CYP19A1) as the top 5 in terms of degree value. Molecular docking was performed using Autodock software to dock each of these proteins with the active ingredient, GAA. The core protein PDF structures were downloaded from the RCSB PDB (https://www.rcsb.org/), and the ligand MOL2 structure files were downloaded from TCMSP. The proteins and small molecules were dehydrogenated, hydrogenated, and charged using Autodock 1.5.6 and the pymol software. The treated proteins and small molecules were then subjected to molecular docking. The visualization of the docking results between small molecule active ingredients and proteins was carried out using Pymol software.

3 Results and analysis

3.1 Active ingredient target prediction After conducting the relevant searches and manipulations, we were able to remove duplicate targets and targets not documented in Uniport. This resulted in a final count of 100 targets for GAA.

3.2 Menopause gene search The Genecrads database was searched for menopause-related genes, and duplicates were removed, resulting in 1 164 genes. Screening was performed twice, with median Relevance scores of 0.579 and 0.969.

3.3 Venn diagram creation and PPI construction The genes related to drugs and menopause were imported into Venn 2.1.0 (http://bioinfogp.cnb.csic.es/tools/veeny/) to generate the corresponding Venn diagram (Fig.1). Subsequently, the results were visualized and analyzed for ntwork topology using cytoscape 3.9.1 software to construct a PPI network (Fig.2). The network comprised 29 nodes and 105 edges. The top 5 nodes were filtered based on degree value, including ethylene response transcription factor ESR1 (ESR1), serine/threonine kinase 1 (AKT1), prostaglandin G/H synthase 2 (PTGS2), peroxisome proliferator-activated receptor γ (PPARG), and Cytochrome P450 family 19 subfamily A member 1 (CYP19A1).

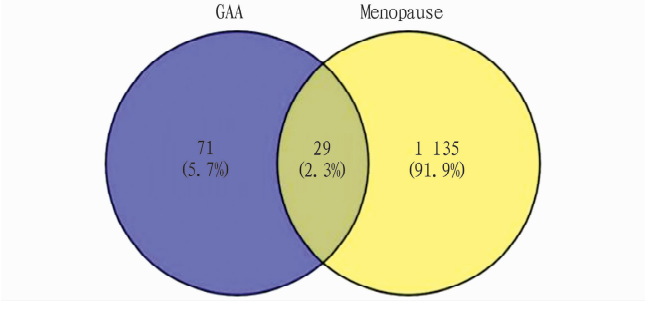


Fig.1 Venn diagram

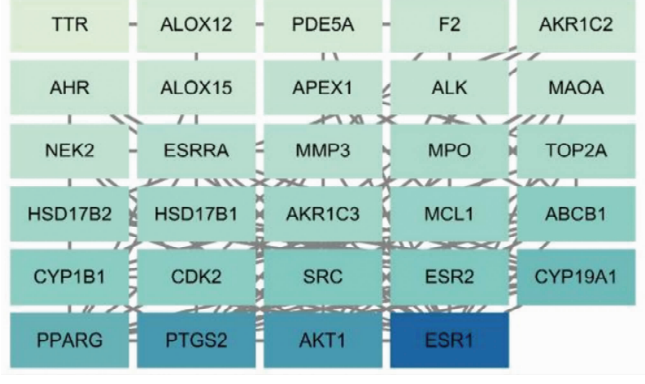


Fig.2 Protein-protein interaction diagram

3.4 GO enrichment analysis The top 10 entries for BP, CC, and MF were selected for GO analysis. The GO enrichment analysis results revealed that platelet activation, regulation of circadian rhythm, and regulation of mRNA stability were the most significantly enriched factors in BP analysis; caveola, male germ cell nucleus and chromatin the most significantly enriched factors in CC analysis; metal ion binding, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, and E-box binding the most significantly enriched factors in MF analysis (Fig.3).

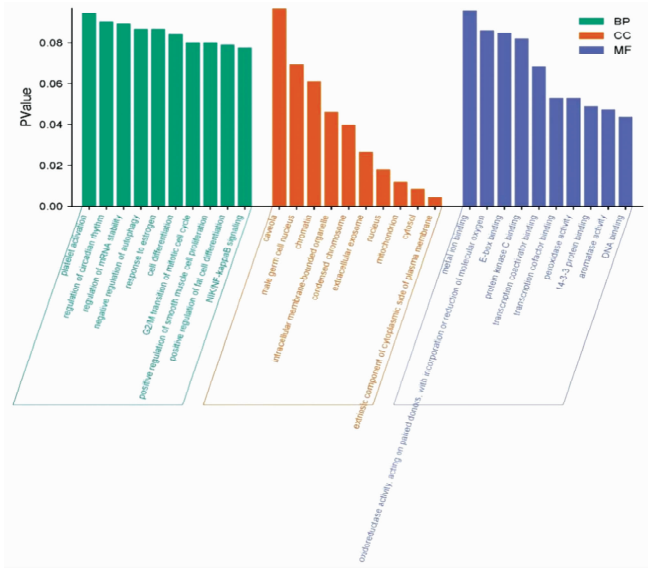


Fig.3 GO (BP, CC, MF) enrichment analysis

3.5 KEGG enrichment analysis The David database was used to perform KEGG functional enrichment analysis. The results, shown in Fig.4, included pathways such as hepatitis B, exocytosis, gastric cancer, breast cancer, MicroRNA in cancer, and platelet activation.

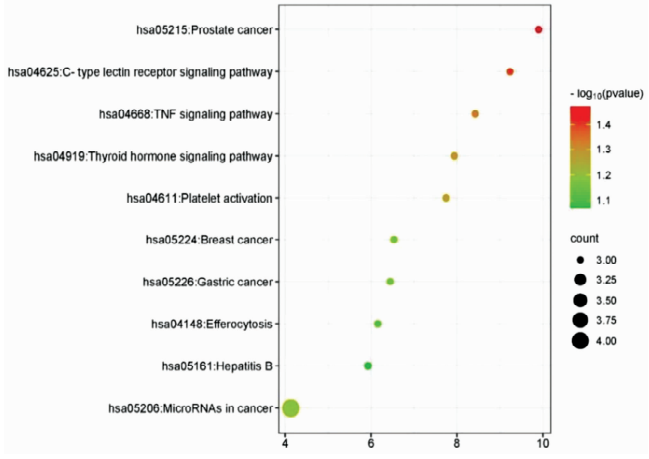
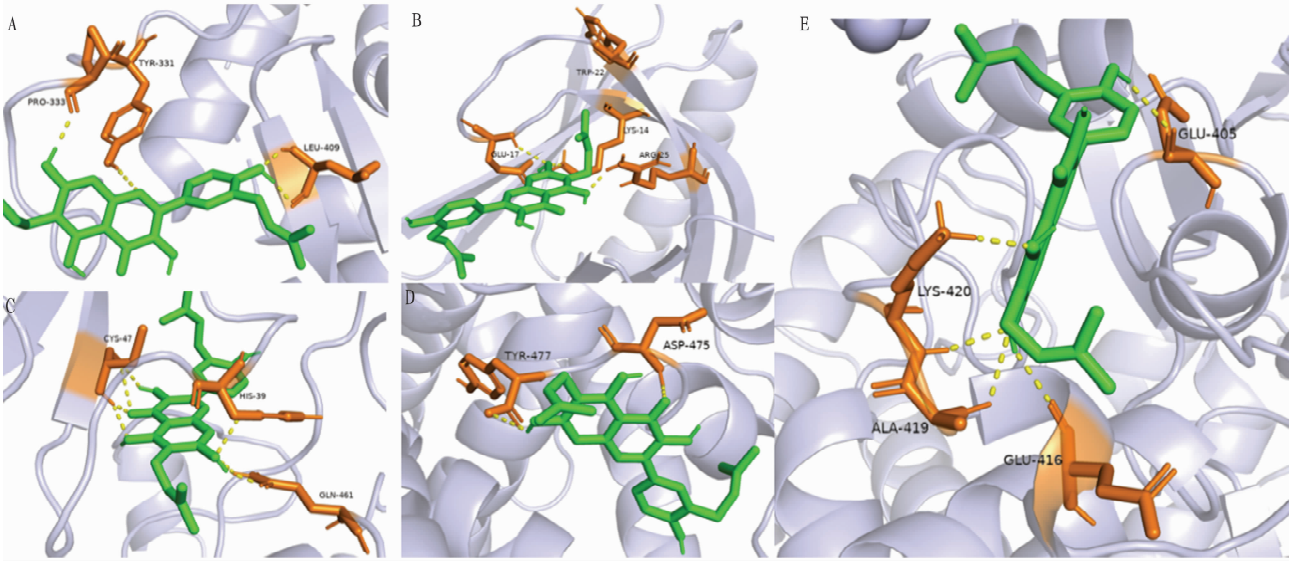


Fig.4 Bubble diagram of KEGG enrichment analysis

3.6 Molecular docking Table 1 displays the Autodock molecular docking results. The results were analyzed based on the binding energy (ΔGbind). A binding energy less than −5.0 kcal/mol indicates good binding, while less than −7.0 kcal/mol indicates strong binding activity[15]. Fig.5 shows that the active ingredient had a strong binding capacity to the relevant proteins. These proteins were hypothesized to be a crucial component of GAA for intervening in menopause.

Table 1 Autodock docking results

MOLID	Active ingredient	Binding energy//kcal/mol				
		ESR1	AKT1	PTGS2	PPARG	CYP19A1
MOL005005	Glyasperin A	-4.44	-3.79	-8.44	-4.55	-5.04



NOTE A, B, C, D and E are the docking results of ESR1, AKT1, PTGS2, PPARG and CYP19A1 with GAA, respectively.

Fig. 5 PyMol visualization diagram

4 Discussion and conclusions

4.1 Discussion Menopausal syndrome, also known as perimenopausal syndrome, refers to a set of negative symptoms that occur in women before and after menopause. As women age, their ovarian function declines, and menstrual flow gradually stops[16]. Menopause is a natural physiological process. However, hormonal imbalances before and after menopause can cause physical and mental changes that may distress women[17]. Women experiencing menopausal syndrome may encounter symptoms such as fatigue, distress, and sleep disruption due to fluctuating estrogen levels[18]. Long-term sleep disorders can lead to cognitive and neurological decline, as well as an increased risk of chronic diseases, significantly impacting both work and personal life. Menopausal women may experience various syndromes due to a decrease in sex hormone secretion resulting from declining ovarian function. This can cause changes in both physiological function and psychological state. A survey indicates that approximately 80% of women experience at least one symptom related to menopause. The most common symptoms include weakness, irritability, sleep disorders, muscle, bone, and joint pain, as well as hot flashes and sweats. Menopause symptoms can impact women's physical and mental health both before and after menopause, and may increase the risk of chronic diseases such as genitourinary syndrome, osteoporosis, Alzheimer's disease, metabolic disorders, and cardiovascular pathologies. Studies have shown that menopause may be the onset of chronic diseases in old age. To alleviate menopausal symptoms and delay the onset of diseases, comprehensive lifestyle guidance and health management for menopausal women are recommended. Menopausal hormone therapy is appropriate for some women, while non-hormone therapy is recommended for others. In summary, menopause has a direct impact on health throughout old age. The active treatment of menopausal syndromes can improve the quality of life in the near future and promote health in old age.

KEGG enrichment analysis screened 10 major relevant pathways with significant -lgP values, including hepatitis B, cytotoxicity, gastric cancer, and breast cancer. The core targets identified were ESR1, AKT1, PTGS2, PPARG, and CYP19A1. The *ESR1* gene encodes the estrogen receptor 1 (ER) protein, which is expressed in around 70% of breast cancers. The level of ER expression is a crucial factor in guiding the classification and treatment of breast cancer subtypes. ER and its hormonal ligands play a fundamental role in normal mammary gland development and the etiology and progression of breast cancer, as established by numerous experimental and clinical studies[19]. PTGS2, also referred to as cyclooxygenase-2, is an inducible enzyme that synthesizes prostaglandins from arachidonic acid *in vivo*. In hypoxic environments, HIF1A can induce the production of PTGS2. HIF1A is constitutively expressed in the vas deferens and epididymis and participates in the inflammatory response[20]. The binding of estrogen to the ER triggers multiple events that activate the ER pathway and induce conformational changes in the LBD. This allows the estrogen-ER complex to interact with specific DNA sequences, known as estrogen response elements (EREs), as well as with co-activating and co-repressor proteins. These interactions regulate estrogen-responsive gene transcription, which plays important roles in various physiological and pathological processes, including carcinogenesis and tumor progression[21-22]. *AKT1* is an oncogene that can be activated through PI3K. Studies have shown that the *AKT1*

gene regulates cell proliferation, growth, and apoptosis primarily through the AKT/protein kinase B signaling pathway[23]. Liu Guangwei *et al.*[24] discovered that bacterial infection resulted in the down-regulation of protein kinase (AKT1). They also found that AKT1 deficiency led to severe disease progression, accompanied by neutrophil recruitment and enhanced bactericidal activity in mouse models of acute inflammatory lung injury and *Staphylococcus aureus* infection.

4.2 Conclusions This study employed network pharmacology and molecular docking methods to identify the core targets and pathways of GAA intervention in menopause. The mechanism of GAA intervention in menopause was predicted using a chemoinformatics database and a bioinformatics database. GAA may have an impact on menopause by affecting key targets such as ESR1, AKT1, PTGS2, PPARG, and CYP19A1, which regulate the pathways for gastric and breast cancer. In the future, we will conduct animal and cellular experimental studies focusing on key targets and pathways. This will provide a reference for the development of new drugs to intervene in menopause.

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