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Study on the effects of Mogroside V in inhibiting NLRP3-mediated granulosa cell pyroptosis and insulin resistance to improve PCOS

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Abstract

Objective Polycystic Ovary Syndrome (PCOS) is a prevalent endocrinopathy in reproductive-aged women, contributing to 75% of infertility cases due to ovulatory dysfunction. The condition poses significant health and psychological challenges, making the study of its pathogenesis and treatment a research priority. This study investigates the effects of Mogroside V (MV) on PCOS, focusing on its anti-inflammatory and anti-insulin resistance properties.

Methods Forty-five female Sprague–Dawley rats were divided into three groups: control, PCOS model, and MV treatment. The PCOS model was induced using a high-fat diet and letrozole. The MV treatment group was subsequently administered MV after the establishment of the PCOS model. The study monitored body mass, assessed estrous cycle changes, and measured serum hormone levels. Transcriptome sequencing and bioinformatics were used to identify differentially expressed genes related to inflammation and insulin resistance. Expression of pyroptosis and insulin resistance markers was analyzed using qRT-PCR, Western blot, and IHC. Additionally, an in vitro model assessed MV's impact on inflammation and insulin resistance.

Results The PCOS group exhibited elevated serum testosterone (T), luteinizing hormone (LH), insulin, and fasting glucose levels, along with increased insulin resistance (HOMA-IR) and decreased estradiol (E2), which were reversed by MV treatment. Transcriptome analysis identified significant gene expression changes between groups, particularly in pathways related to NLRP3 inflammation and insulin metabolism. MV treatment normalized the expression of ovarian pyroptosis factors (NLRP3, Caspase-1, GSDMD) and inflammatory cytokines (IL-1β, IL-18). In cellular models, MV increased E2 levels, reduced LDH release, and decreased the expression of insulin resistance and pyroptosis markers. Correlation analysis showed pyroptosis factors were positively correlated with HOMA-IR and IGF1, and negatively with IGF1R and E2 levels.

Conclusion MV improves PCOS by reducing pyroptosis and insulin resistance, enhancing insulin sensitivity, and promoting estrogen synthesis, thereby restoring granulosa cell function and follicular development.

Keywords Polycystic Ovary Syndrome, Mogroside V, Pyroptosis, NLRP3, Granulosa Cells

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Introduction

Polycystic Ovary Syndrome (PCOS) is a prevalent endocrinopathy within the field of gynecology, affecting 5–18% of women worldwide [1]. The primary clinical characteristics of PCOS include hyperandrogenism, irregular menstruation, ovulatory dysfunction, and the presence of polycystic ovaries with ultrasonic examination [2]. Patients with PCOS exhibit a high degree of heterogeneity and diverse clinical manifestations [3], often coupled with an increased risk of metabolic disorders, particularly hyperinsulinemia, which is characterized by insulin resistance [4, 5]. Insulin resistance is observed in both the ovarian and endometrial tissues of PCOS patients [6, 7] and may disrupt the function of the hypothalamic-pituitary-ovarian axis, resulting in ovulatory dysfunction, menstrual irregularities, amenorrhea, and infertility [8]. In PCOS patients, local insulin resistance in the ovaries leads to abnormal hormone synthesis which can interfere with the normal development of follicles and subsequently cause ovulatory dysfunction [9]. Elevated insulin and androgens levels can contribute to an imbalance in hormone levels within PCOS patients [10].

An increasing number of research suggests that follicular dysfunction may be a core factor in the infertility associated with PCOS [11–13]. The primary functions of the ovaries are to produce mature oocytes and secrete reproductive hormones that are crucial for the development and ovulation of follicles [14]. During the maturation and development of the follicle, Granulosa Cells (GCs) play crucial roles to the activation and growth of primordial follicles, providing essential nutritional support for the development and maturation of oocytes [15], particularly in the late stages of antral follicle development. Extensive research has confirmed that GCs dysfunction is a critical cause of aberrant follicular development [15]. In studies of PCOS patients and corresponding animal models, an increase in cystic ovarian follicles and a significant reduction in the number of follicles and corpora lutea have been observed, along with a loose arrangement and thinning of the GC layers [11, 16]. Researches have found an increase in GCs apoptosis was a significant contributing factor to the impairment of follicular development in PCOS rats with insulin resistance [17].

Chronic low-grade inflammation may be closely associated with complications such as elevated androgen levels and insulin resistance [18, 19]. Increasing evidence suggests that patients with PCOS generally exhibit a physiological state of chronic low-grade inflammation [20]. The levels of inflammatory markers, such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP), are markedly higher in the serum and follicular fluid of PCOS patients compared with healthy individuals [21, 22]. An increased infiltration of lymphocytes and macrophages has been observed in the ovarian tissue of PCOS patients [23], suggesting that systemic inflammation and local ovarian inflammation may coexist in these individuals. More and more research found that chronic low-grade inflammation in ovarian can lead to ovarian dysfunction [22, 24, 25]. Inflammatory responses can trigger a novel form of programmed cell death known as pyroptosis [26]. Pyroptosis is an inflammatory form of programmed cell death characterized by the classical pathway where the activated NLRP3 inflammasome associates with the ASC protein, recruits pro-caspase-1, and cleaves it into active caspase-1. Concurrently, active caspase-1 also cleaves GSDMD, and it's fragment translocates to the cell membrane, leading to membrane damage and ultimately cell death [27, 28]. The main features of pyroptosis include the release of pro-inflammatory cytokines, such as IL-1β and IL-18, and the formation of pyroptotic bodies [29, 30]. Studies have found elevated levels of the inflammatory cytokines IL-18 and IL-1β, as well as activated NLRP3 inflammasomes the follicular fluid and granulosa cells of PCOS patients characterized by hyperandrogenemia, leading to pyroptosis, which ultimately affects oocyte quality and pregnancy outcomes [31]. Ibrahim et al. found that the NLRP3/Caspase-1 pathway is activated in letrozole-induced PCOS rat models [32].

In China, traditional Chinese medicine is commonly regarded as a significant complement to modern Western medicine. Mogroside V(MV) is considered the core active ingredient of Siraitiae Fructus, which has been discovered to possess various biological activities, including hypoglycemic effects, lipid-lowering properties, antioxidant effects and fatigue relief. Additionally, MV exhibits anti-inflammatory properties, improves pulmonary function, protects the nervous system, displays anticancer activity, promotes reproduction [33] and reduces intracellular ROS levels [34]. In recent years, studies have shown that MV plays a positive role in promoting normal follicular development,also candelay aging of oocytes [35], promote maturation of porcine oocytes [36], protect the intestinal epithelial barrier function of ulcerative colitis mice and reducing the production of inflammatory factors through in vivo and in vitro experiment [37]. Our previous studies have also confirmed that MV improves ovarian metabolic disorders in PCOS rats by regulating the expression of key enzymes in the glycolysis pathway, thereby promoting ovarian morphology, functional recovery, and fertility enhancement [38]. These findings suggest the potential of MV in treating various inflammatory diseases, although its specific mechanisms of action remain to be elucidated.

Drawing from the existing literature and the foundational research of our group, the current study aims to

investigate the effects of MV on inflammation, insulin resistance, and granulosa cell pyroptosis in a PCOS rat model induced by letrozole and a high-fat diet. This will be achieved by integrating ovarian transcriptomics analysis and employing molecular and cellular biological experimental techniques. Concurrently, we will construct models of granulosa cell pyroptosis and insulin resistance at the cellular level and intervene with MV. The study will explore the role of MV in inhibiting granulosa cell pyroptosis and insulin resistance at the cellular level and analyze the interrelationships among various factors. The objective is to preliminarily elucidate, both in vivo and in vitro, how MV may protect the development of follicles in PCOS by alleviating symptoms of inflammation and insulin resistance.

Materials and methods

Animals

Forty-five female Sprague–Dawley (SD) rats, aged five weeks with an average weight of 171 ± 11 g, were procured from the Laboratory Animal Center of the University of South China (Hengyang, China; permit number: SYXK (Xiang) 2020–0002). Upon arrival, the animals were allowed unrestricted access to food and water and were maintained in a controlled temperature environment (23 ± 2°C) under a regulated dark–light cycle. Following a one-week acclimatization period, the rats were randomly assigned to one of three experimental groups, each consisting of 15 individuals: a control group (Ctrl), a PCOS model group (PCOS), and a MV treatment group (PCOS-MV). The Ctrl group was fed a standard diet and administered intragastrically with 0.1% carboxymethyl cellulose (CMC) as a vehicle, for 30 consecutive days. Both the PCOS group and the PCOS-MV group were fed a high-fat diet and intragastrically administered letrozole (1 mg/kg·d) to establish a PCOS rat model, for 30 days [38].

After successful modeling, the Ctrl group continued with the standard diet, the PCOS group maintained the high-fat diet, and the PCOS-MV group switched to a standard diet and received intragastric administration of MV (600 mg/kg/d), for an additional 30 days. At the end of the experiment, the rats were euthanized via intraperitoneal injection of 20% urethane, and serum and ovarian tissue samples were collected from each group. The animal experimental procedures adhered to established guidelines and received approval from the Laboratory Animal Welfare Ethics Committee of the University of South China (permit number: USC2020031602).

Transcriptome assay

RNA-seq library preparation and sequencing of ovarian samples ($n = 4$ rats per group) were conducted by Novogene Corporation (Beijing, China). The initial RNA for database construction comprised 3 mg of total RNA. The NEBNext® Ultra TM RNA Library Prep Kit (Illumina, USA) was utilized for library construction, and the Illumina platform executed the sequencing with a 150 bp paired-end strategy. Preprocessing steps involved removing reads with adapters, reads with undetermined base information (N), and low-quality reads (those with Qphred ≤ 20 bases constituting over 50% of the read length) from the original data. The index of the reference genome and the alignment of clean reads to the reference genome were performed using HISA T2 v2.0.5.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from cryopreserved ovarian tissue utilizing AG RNAex Pro RNA Regent (Accurate Biology, Cat#AG21101), followed by reverse transcription into cDNA utilizing the TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (Transgen-Biotech, Beijing, China). For primer synthesis, the target gene sequence was first retrieved from the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/) website. Then, the sequence was input into the official website of Sangon Biotech (Shanghai) for primer synthesis. RT-qPCR was executed in a 10 μl reaction volume employing 2 × ChamQ Universal SYBR qPCR Master Mix (Accurate Biology) and the Applied Biosystems QuantStudio 3 instrument (Thermo Fisher Scientific). *Gapdh* served as the internal reference for obtaining ΔΔCt values to calculate fold increases, and gene expression levels were determined utilizing the $2^{-\Delta\Delta Ct}$ method. Detailed primer sequences for amplification are provided in Table 1. Replication of all RT-qPCR experiments occurred three times for robustness.

Western blot

Ovarian tissue stored at −80°C, were homogenized in prechilled RIPA buffer (500 μL RIPA lysis buffer [CWBIO, Beijing, China]: PMSF [Solarbio] = 94:6). After centrifugation at 12,000 rpm for 30 min at 4°C to remove debris, protein concentration in the supernatant was determined using the BCA Protein Assay Kit (CWBIO). The proteins were denatured by boiling at 100°C for 10 min, separated on 10% SDS–polyacrylamide gels, and transferred to PVDF membranes. After blocking with 5% skim milk for 2 h at room temperature, primary antibodies were applied and incubated overnight at 4°C. Following this, a horseradish peroxidase-conjugated secondary antibody was applied for 2 h at room temperature. The

Table 1 Primary primers and associated information

Gene	Primer Sequence (5′–3′)	Accession NO
RAT NLRP3	F: GAGCTGGACCTCAGTGACAATGC R: AGAACCAATGCGAGATCCTGACAAC	XM_006246457.4
RAT Caspase-1	F: GACCGAGTGGTTCCCTCAAG R: GACGTGTACGAGTGGGTGTT	NM_012762.3
RAT GSDMD	F: ATTGGCTCTGAATGGGATA R: CGGCACCAGTTCTCCA	NM_001400994.1
RAT IL-1β	F: CCCTTGTCGAGAATGGGCAG R: GACCAGAATGTGCCACGGTT	NM_031512.2
RAT IL-18	F: CGACCGAACAGCCAACGAATCC R: GTCACAGCCAGTCCTCTTACTTCAC	NM_019165.2
RAT IGF1	F: TGGTGGACGCTCTTCAGTTC R: TCCGGAAGCAACACTCATCC	XM_039078402.1
RAT IGF1R	F: TGACACGTGGCGATCTCAAA R: AGCTCGGAGGAATCAGGACT	NM_001414181.1
RAT GAPDH	F: GAGTCCACTGGCGTCTTCAC R: GAGGCATTGCTGATGATCTTGAG	NM_017008.4
HOMO NLRP3	F: ATCTCACGCACCTTTACCT R: CAAGAGTCCCTCACAGAGTAG	XM_054334082.1
HOMO Caspase-1	F: TTTCCGCAAGGTTCGATTTTCA R: GGCATCTGCGCTCTACCATC	XM_017018396
HOMO GSDMD	F: GGAGCTTCCACTTCTACGATG R: GAGTCTGCCAGGTGTTAGGG	NM_024736.7
HOMO IL-1β	F: CTCGCCAGTGAAATGAT R: AAGCCCTTGCTGTAGTG	NM_000576.3
HOMO IL-18	F: ATGGCTGCTGAACCAGTAGAAGAC R: TCCGGGGTGCATTATCTCTACAGTC	NM_001243211.2
HOMO IGF1	F: AGGCTGGAGATGTATTGCG R: GGGCTGATACTTCTGGGTC	XM_054371952.1
HOMO IGF1R	F: CTACAACATCACCGACCCG R: CAAAGACGAAGTTGGAGGC	XM_054377836.1
HOMO GAPDH	F: GGATTTGGTCGTATTGGGCG R: TCCCGTTCTCAGCCATGTAG	NM_001357943.2

chemiluminescence of protein bands was detected using eECL (CW0049M, CWBIO) and visualized with the Tanon-5500 Chemiluminescence Imaging System. Densitometric analyses of protein expression levels were performed using Image J software. Details regarding the antibodies used in the experiment are provided in Table 2.

Immunohistochemistry

Following deparaffinization with xylene and rehydration in an alcohol series, 5-μm-thick ovarian tissue sections underwent blocking of endogenous peroxidase activity with 0.3% H_2O_2 in methanol, incubating for 30 min. Subsequently, the sections were subjected to an overnight incubation at 4°C with primary antibodies. The negative control group was treated with PBST. Later, the sections were exposed to a secondary antibody conjugated with horseradish peroxidase for 90 min, followed by the avidin-biotinylated horseradish peroxidase complex for an additional 30 min. Immunoreactive protein signals were visualized using the 20×Metal Enhanced DAB Substrate Kit. The sections were then counterstained with hematoxylin, dehydrated, and sealed. Quantitative analysis of immunohistochemical photomicrographs was conducted using Image J software. Details regarding the antibodies used in the experiment are provided in Table 2.

Cell culture and treatment

The human ovarian granulosa cell line (KGN) was procured from Zhejiang Meisen Cell Technology Co., Ltd. KGN cells were cultured with Dulbecco's Modified Eagle's Medium–high glucose (DMEM, Sigma, USA) supplemented with 10% fetal bovine serum (FBS, Invitrogen Gibco, USA) and maintained in an atmosphere of 5% CO_2 at 37℃. KGN cells were seeded at a density of 1×10^5 cells per well in 6 cm diameter culture dishes. After 24 h of adherence, the cells were added to the culture dish according to the selected drug concentration. Based on the literature and our research group's previous studies, we selected the concentrations of LPS at 1 μg/mL [39], Nig at 20 μmol/L [40], INS at 4 μg/mL [41], and MV at

Table 2 Primary antibodies and their corresponding dilution concentrations

Antibody	Accession no	Company	IHC	WB
NLRP3	T55651	Abmart Shanghai Co.,Ltd	1:400	1:1000
Caspase-1	A0964	ABclonal Technology Co.,Ltd	1:500	1:1000
GSDMD	TA4012	Abmart Shanghai Co.,Ltd	1:300	1:1000
IL-1β	A16288	ABclonal Technology Co.,Ltd	1:200	1:1000
IL-18	A1115	ABclonal Technology Co.,Ltd	1:200	1:1000
IGF1	A11985	ABclonal Technology Co.,Ltd	1:100	1:1000
IGF1R	A0243	ABclonal Technology Co.,Ltd	1:100	1:3000
β-tubulin	AC008	ABclonal Technology Co.,Ltd		1:5000
Rabbit IgG-HRP	SA00001-2	Protein Tech Group Inc		1:5000
Mouse IgG-HRP	SA00001-1	Protein Tech Group Inc		1:5000
HRP-conjugated Streptavidin	SA00001-0	Protein Tech Group Inc	1:200	
biotin-conjugated affinipure goat anti-rabbit IgG	SA00004-2	Protein Tech Group Inc	1:200	

50 nmol/mL [38] for the model construction. Subsequent experiments were performed 24 h after drug treatment.

Enzyme-Linked Immunosorbent Assay (ELISA)

Following anesthesia, blood samples were obtained by puncturing the abdominal aorta of the rats. The blood samples were left to sit for 2 h and then centrifuged at 3000 × g for 15 min at 4°C to isolate the serum. The serum collected was subsequently analyzed the concentrations of E2, INS, IL-1β, and IL-18 using commercial enzyme-linked immunosorbent assay kits (Abcam, Cambridge, UK), following the manufacturer's guidelines.

Statistical analysis

Statistical analyses were executed employing GraphPad Prism 8 (GraphPad Software, San Diego, California). The data are expressed as means ± standard error of the mean (SEM) and were subjected to scrutiny through one-way analysis of variance (ANOVA). A significance threshold of $p < 0.05$ was applied to discern statistical significance. Each experimental iteration was conducted independently on a minimum of three occasions.

Data analyses were performed using the online platforms accessible at https://bio-cloud.aptbiotech.com/ and https://www.omicstudio.cn/tool, where Principal Component Analysis (PCA), volcano analysis, clustering analysis, and Venn analysis were conducted. Subsequent KEGG pathway analyses were executed utilizing the resources available at https://david.ncifcrf.gov/ and https://magic.novogene.com/customer/main#/homeNew.

Results

Intervention with Mogroside V can restore the imbalance of serum hormone levels in PCOS rats

Our research group successfully established a PCOS rat model by co-administration of letrozole and a high-fat diet in the preliminary stage. Following intervention with MV, the PCOS rats exhibited restored regular estrous cycles and improved fertility. Genomic analysis revealed that MV can promote follicular development in PCOS rats by modulating the glycolysis pathway [38].

Serum testosterone (T), estradiol (E2), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels were determined using ELISA. The results showed that compared to the control group, serum testosterone levels were significantly elevated in the PCOS group ($p < 0.001$), while estradiol levels were decreased ($p < 0.001$), and LH levels were elevated ($p < 0.001$). FSH levels did not show significant changes among the groups, but the LH/FSH ratio was increased ($p < 0.001$). Following intervention with MV, hormone levels were restored to levels similar to those in the control group (Fig. 1). These findings suggest that MV intervention can restore the imbalance of serum hormone levels in PCOS rats.

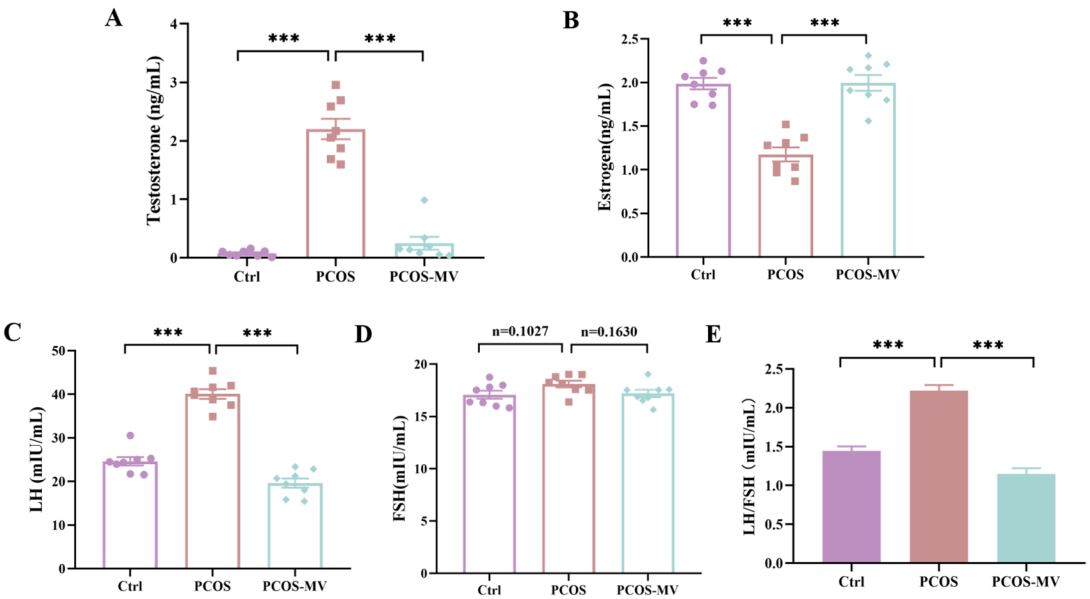

Fig. 1 Intervention with Mogroside V can restore the imbalance of serum hormone levels in PCOS rats across all groups. **A** The effect of Mogroside V intervention on serum T levels in PCOS rats. **B** The effect of Mogroside V intervention on serum E2 levels in PCOS rats. **C** The effect of Mogroside V intervention on serum LH levels in PCOS rats. **D** The effect of Mogroside V intervention on serum FSH levels in PCOS rats. **E** The effect of Mogroside V intervention on serum LH/FSH levels in PCOS rats. $n = 8$, ***$p < 0.001$

Intervention with Mogroside V regulates the ovarian transcriptome levels in PCOS rats

To further explore the molecular mechanisms underlying the restorative effects of MV on follicular development, we conducted transcriptome sequencing (RNA-seq) analysis on ovarian tissue. Initially, PCA was employed to assess the differences in gene expression among various treatment groups and the reproducibility among samples within each group. As depicted in Fig. 2A, the clustering of samples within each group indicates good reproducibility. Meanwhile, the dispersed distribution of samples between the PCOS and Ctrl groups, as well as between the PCOS and PCOS-MV groups, suggests significant differences in gene expression at the molecular level. Additionally, the closer distribution and partial overlap of samples between the PCOS-MV and Ctrl groups imply that Mogroside V intervention may facilitate the restoration of gene expression patterns towards a normal state. The heatmap of hierarchical clustering analysis for gene expression patterns (Fig. 2B) further illustrates the expression profiles of all genes, suggesting that the intervention with MV contributes to the recovery or improvement of biomarker alterations induced by PCOS. Volcano plot analysis (Fig. 2C) revealed that compared to the Ctrl group, there were 1376 upregulated and 2558 downregulated genes in the PCOS group; whereas in the comparison between the PCOS-MV and PCOS groups, there were 1943 upregulated and 1548 downregulated genes, respectively. Venn diagram analysis (Fig. 2D) showed that there were a total of 2404 differentially expressed genes (DEGs) among the three groups, with 2384 DEGs demonstrating significant improvement under the intervention of MV.

Considering that these DEGs may be involved in the molecular mechanisms underlying the ameliorative effects of MV on the development of PCOS follicles, we conducted a further KEGG analysis on these genes. The aim was to identify and understand the biological processes and molecular pathways associated with the changes in gene expression following MV intervention. The KEGG pathway analysis indicated that these DEGs are predominantly associated with signaling pathways such as PI3K-Akt, insulin secretion, PPAR, inflammation, cell death, and steroid biosynthesis (Fig. 2E). Subsequent cluster analysis of the DEGs enriched in these pathways further confirmed that treatment with MV effectively ameliorates the imbalances in pyroptosis, insulin resistance, and steroid levels in a PCOS rat model (Fig. 2F). The findings from these analyses provide a molecular basis for the potential application of MV in the treatment of PCOS.

Intervention with Mogroside V can ameliorate insulin resistance in PCOS rats

We measured fasting blood glucose and serum insulin concentrations in each group of rats, and calculated the insulin resistance index (HOMA-IR) accordingly. The obtained results are shown in Fig. 3A-C: compared to the Ctrl group, rats in the PCOS group exhibited significant increases in blood glucose, insulin levels, and insulin resistance index ($p < 0.01$); after treatment with MV, these indicators all showed decreases ($p < 0.05$).

To further explore the occurrence of insulin resistance in PCOS ovarian tissue, we utilized qRT-PCR, Western Blot, and IHC techniques to analyze the gene and protein expression levels of IGF1 and its receptor IGF1R in ovarian tissue from each group of rats. The results, as shown in Fig. 3D and F, revealed that compared to the Ctrl group, both the mRNA and protein expression levels of IGF1 in ovarian tissue of PCOS rats were significantly increased ($p < 0.001$), whereas after intervention with MV, these expression levels significantly decreased ($p < 0.001$). Conversely, the mRNA and protein expression of IGF1R were significantly decreased in the ovarian tissue of PCOS rats, but significantly increased after MV intervention. The IHC results revealed that both IGF1 and IGF1R were expressed in granulosa cells of the ovaries. The expression of IGF1 was significantly stronger in the PCOS group compared to the Ctrl group, while the expression of IGF1R was weaker in the PCOS group than in the Ctrl group. In the PCOS group, there was a significant decrease in the expression of IGF1, while the expression of IGF1R was notably increased when compared to the Ctrl group. Following MV intervention, IGF1 expression was markedly reduced, while IGF1R expression significantly increased in comparison to the PCOS group. These findings suggest that insulin resistance is present in the ovarian tissue of PCOS rats (Fig. 3G).

Intervention with Mogroside V can attenuate levels of inflammation and pyroptosis in the ovarian tissue of PCOS rats

Previous ovarian transcriptomic results observed increased expression levels of inflammatory and pyroptosis-related factors within the ovaries of PCOS rats. Specifically, pyroptosis-associated factors NLRP3, Caspase-1, GSDMD, IL-1β, and IL-18 were upregulated in the ovaries of PCOS rats and subsequently downregulated following intervention with MV. We assessed the expression of NLRP3, Caspase-1, GSDMD, IL-1β, and IL-18 in ovarian tissues from each group of rats at both the mRNA and protein levels using qRT-PCR, Western Blot, and IHC, respectively. As shown in Fig. 4A-E, qRT-PCR results indicated a significant increase in the mRNA expression levels of pyroptosis-related factors in the

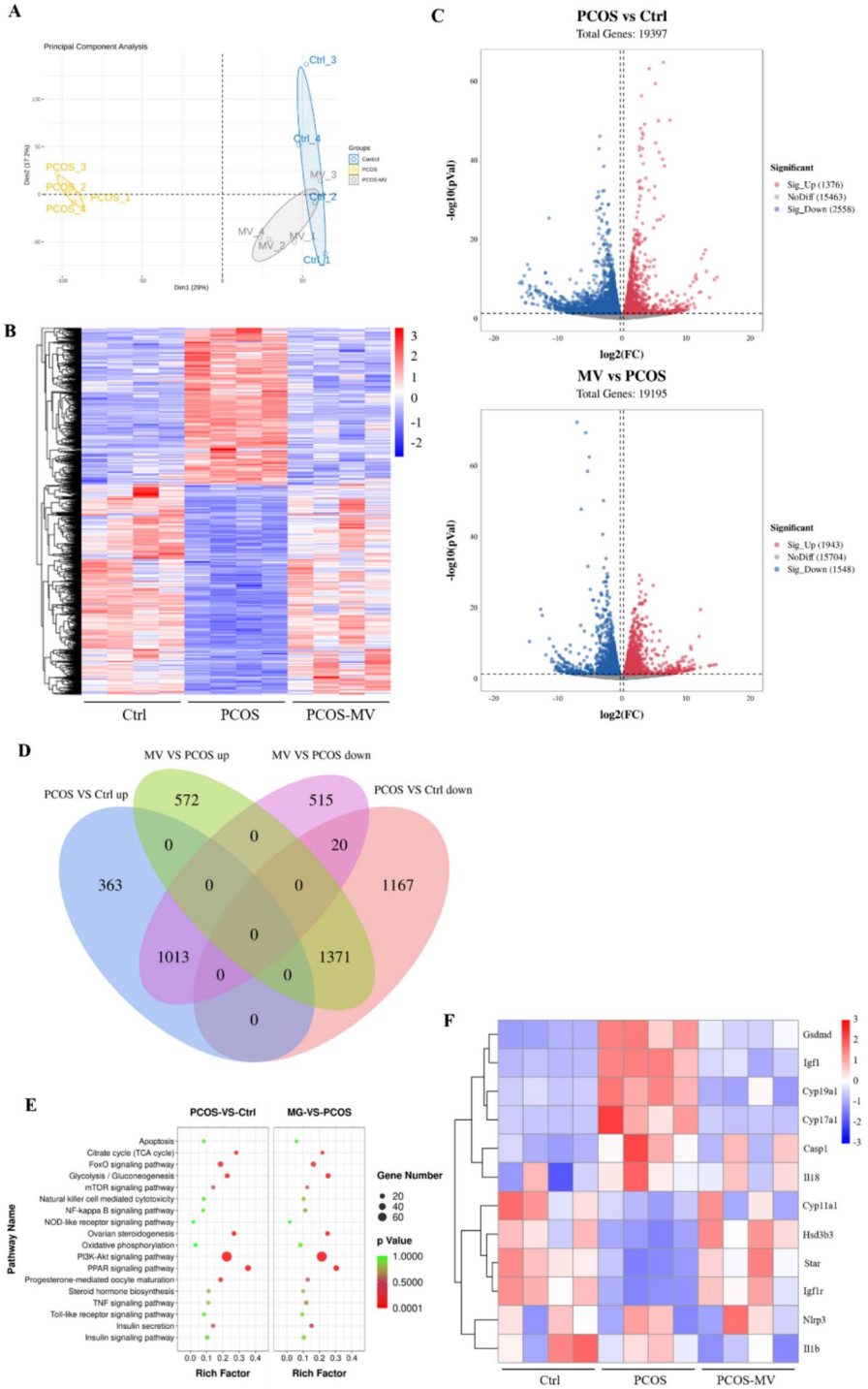

Fig. 2 Effects of Mogroside V on the Transcriptome of Ovary. **A** PCA analysis of all expressed genes in ovaries of Ctrl group, PCOS group, and PCOS-MV group. **B** Results of cluster analysis of the three sets of whole genes. **C** Volcano analysis plots of the three groups of DEGs. **D** Venn diagram of the three groups of DEGs. **E** The functional enrichment analysis of DEGs. **F** The heatmap of the changed DEGs identified in functional enrichment analysis. ($n = 4$)

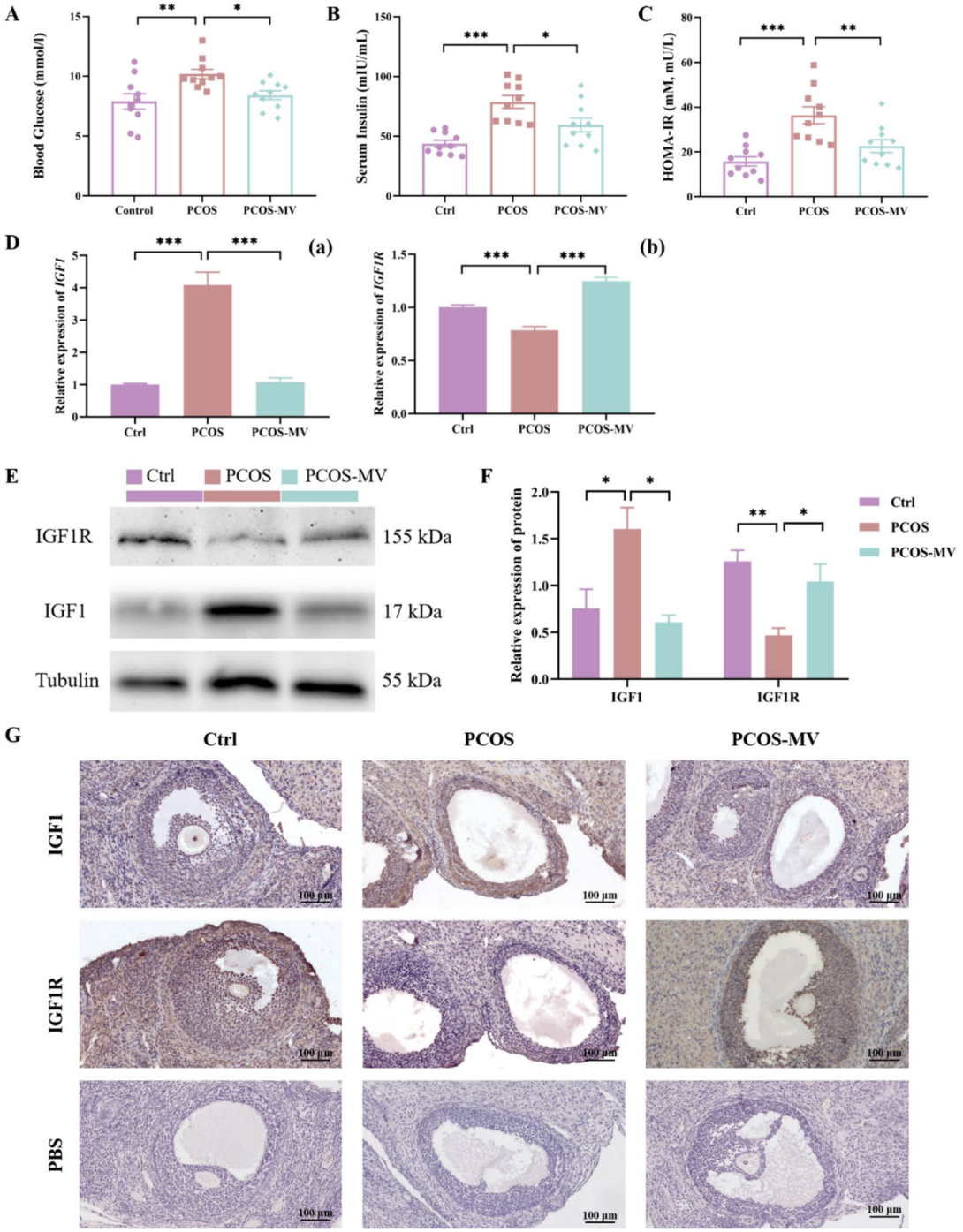

Fig. 3 Mogroside V enhanced the ovarian insulin sensitivity in PCOS rats. **A**-**C** The level of blood glucose (**A**), serum insulin (**B**), and HOMA-IR (**C**) of rats in each group. **D** qRT-PCR validation of IGF1 (a) and IGF1R (b) in ovaries ($n=4$). **E**–**F** Ovarian protein expression levels of IGF1 and IGF1R were determined by Western Blot and quantified by ImageJ software ($n=4$). **G** Immunohistochemistry analysis of the expression of IGF1 and IGF1R in ovaries. PBS was used as the negative control ($n=4$). $^{*}p<0.05$, $^{**}p<0.01$,. $^{***}p<0.001$

ovaries of PCOS rats, which were downregulated after MV intervention. The findings from Western Blot analysis were consistent with the trends observed in qRT-PCR (Fig. 4F-G). IHC results revealed the expression of pyroptosis-related factors within ovarian granulosa cells, with significantly higher expression in the PCOS

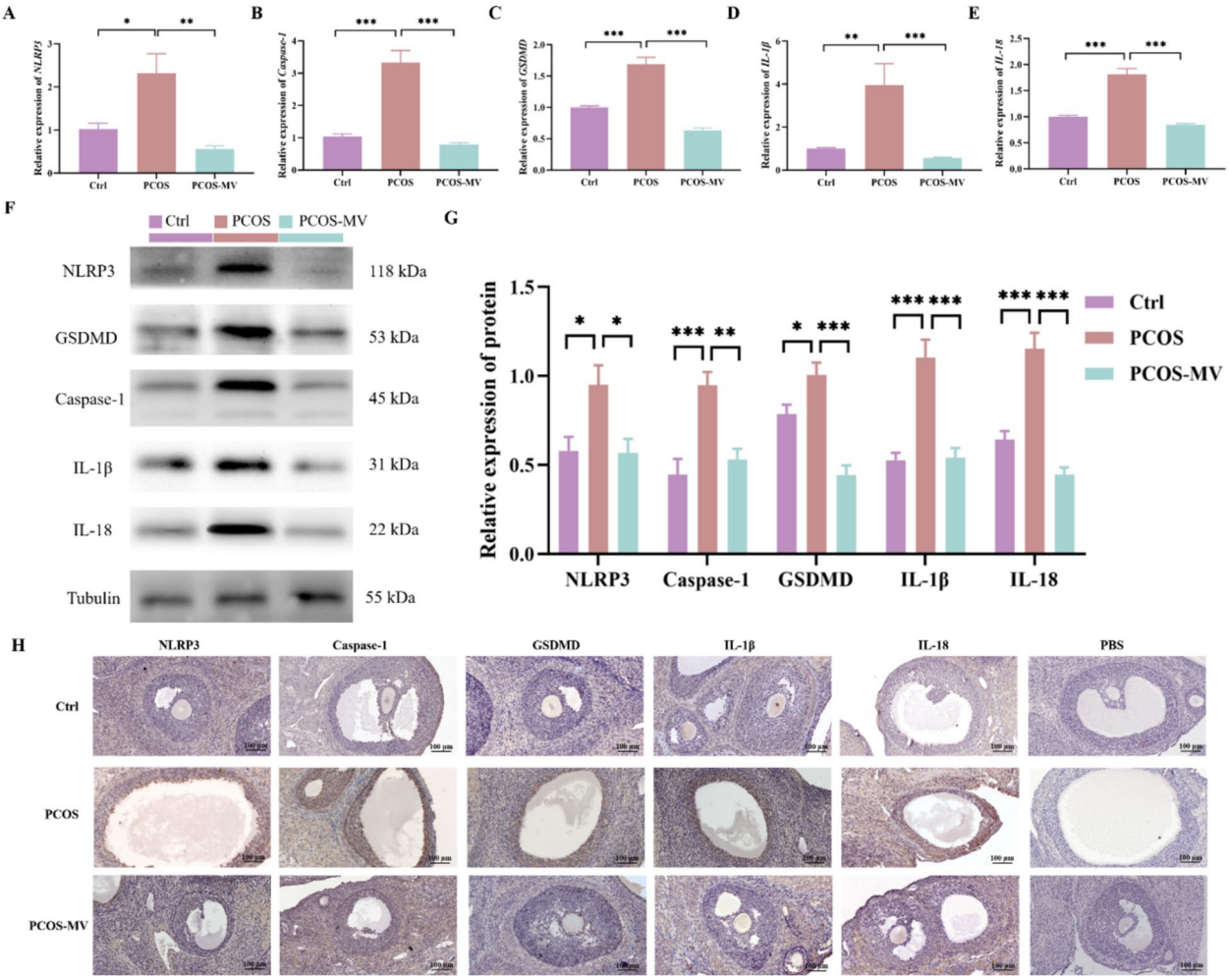

Fig. 4 Mogroside V enhanced the ovarian insulin sensitivity in PCOS rats. **A**-**E** Ovarian mRNA expression level of NLRP3 (**A**), Caspase-1 (**B**), GSDMD (**C**), IL-1β (**D**) and IL-18 (**E**) ($n=4$). **F**-**G** Ovarian protein expression levels of NLRP3, Caspase-1, GSDMD, IL-1β and IL-18 were determined by Western Blot and quantified by ImageJ ($n=4$). **H** Immunohistochemistry analysis of the expression of NLRP3, Caspase-1, GSDMD, IL-1β and IL-18 in ovaries. PBS was used as the negative control ($n=4$).* $p<0.05$,** $p<0.01$,.*** $p<0.001$

group compared to the control group, and a reduction in expression intensity following MV intervention (Fig. 4H).

The correlation between the expression intensity of pyroptosis-related factors in PCOS ovarian tissue and the expression intensity of HOMA-IR and IGF1

Based on the aforementioned results, we conducted a Spearman correlation analysis, and the results are depicted in Fig. 5. The upregulated pyroptosis-related factors (NLRP3, Caspase-1, GSDMD, IL-1β, and IL-18) exhibited a positive correlation with HOMA-IR and IGF1, and a negative correlation with IGF1R. They also showed a positive correlation with the reproductive hormone T and a negative correlation with the level of E2. HOMA-IR is a commonly used method for assessing an individual's state of insulin resistance. The positive correlation between the aforementioned pyroptosis-related factors and HOMA-IR suggests that in PCOS rats, as the expression of pyroptosis-related factors increases, the degree of insulin resistance also escalates, accompanied by an increase in androgen levels and a decrease in estrogen levels. This indicates that pyroptosis-related factors may contribute to increased insulin resistance in PCOS rats by promoting tissue inflammatory responses, thereby affecting hormone levels.

Intervention with Mogroside V can suppress the levels of inflammation and pyroptosis in KGN cells

In conjunction with our previous experimental studies and the findings presented herein, to further investigate the mechanisms by which MV improves follicular development in PCOS, we constructed a KGN cell pyroptosis model using LPS and Nig, followed by treatment with MV. The results revealed that compared to the Ctrl group,

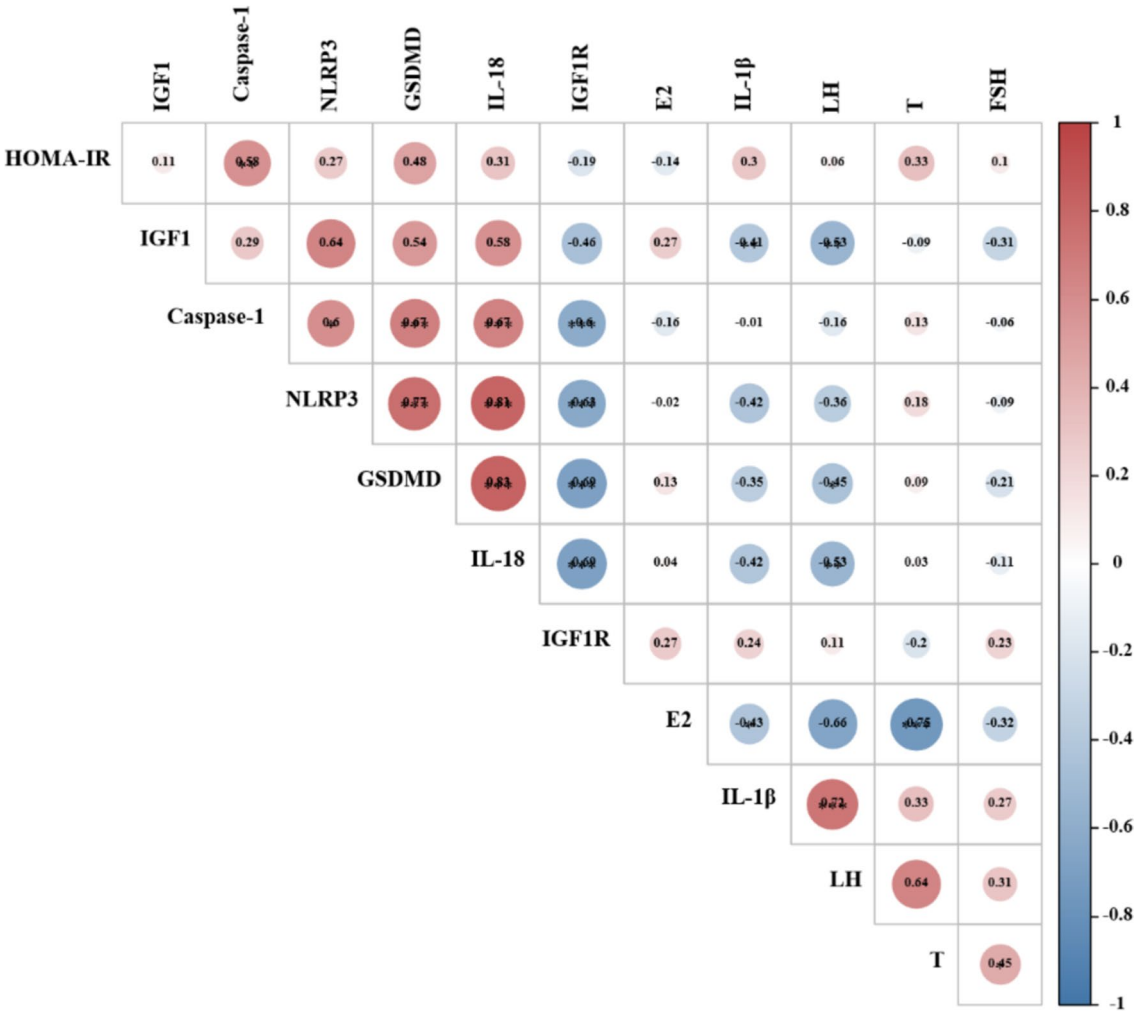

Fig. 5 Correlation coefficient matrix. Correlation analysis was performed using Spearman's correlation due to a skewed distribution of the data. The value of *r* represents the degree of correlation ($0 > r > 1$, positive correlation; $-1 < r < 0$, negative correlation)

the levels of IL-1β and IL-18 in the supernatant of the LPS + Nig group were significantly elevated. After treatment with MV, the levels of IL-1β and IL-18 in the supernatant of KGN cells (LPS + Nig + MV) were significantly reduced (Fig. 6A-B). To further verify the impact of MV on cell pyroptosis, lactate dehydrogenase (LDH) release in the cell culture supernatant of KGN cells subjected to different treatments was measured using a cytotoxicity detection kit. The findings indicated that the release of LDH in the supernatant of the LPS + Nig group was significantly higher than that of the Ctrl group, and this release was decreased after MV intervention (Fig. 6C). Additionally, the content of E2 in the supernatant of the LPS + Nig group was reduced, while it increased after MV intervention (Fig. 6D). These results suggest that MV treatment can significantly reduce the high levels of inflammation induced by LPS and Nig in KGN cell supernatants, decrease LDH release, and restore E2 levels.

The expression of pyroptosis-related factors, including NLRP3, GSDMD, Caspase-1, IL-1β, and IL-18, at both the mRNA and protein levels in cells from each group was examined using qRT-PCR and Western Blot. The results showed that the mRNA expression levels of these factors were significantly higher in the LPS + Nig group compared to the Ctrl group, and this overexpression was effectively mitigated after MV treatment (Fig. 6E). The results obtained from Western Blot were consistent with those from qRT-PCR (Fig. 6F-G). Moreover, these experimental outcomes corroborate the in vivo results, further demonstrating that MV can inhibit the expression of the NLRP3/Caspase-1/GSDMD pathway and inflammatory cytokines, thereby exerting its anti-pyroptotic effects.

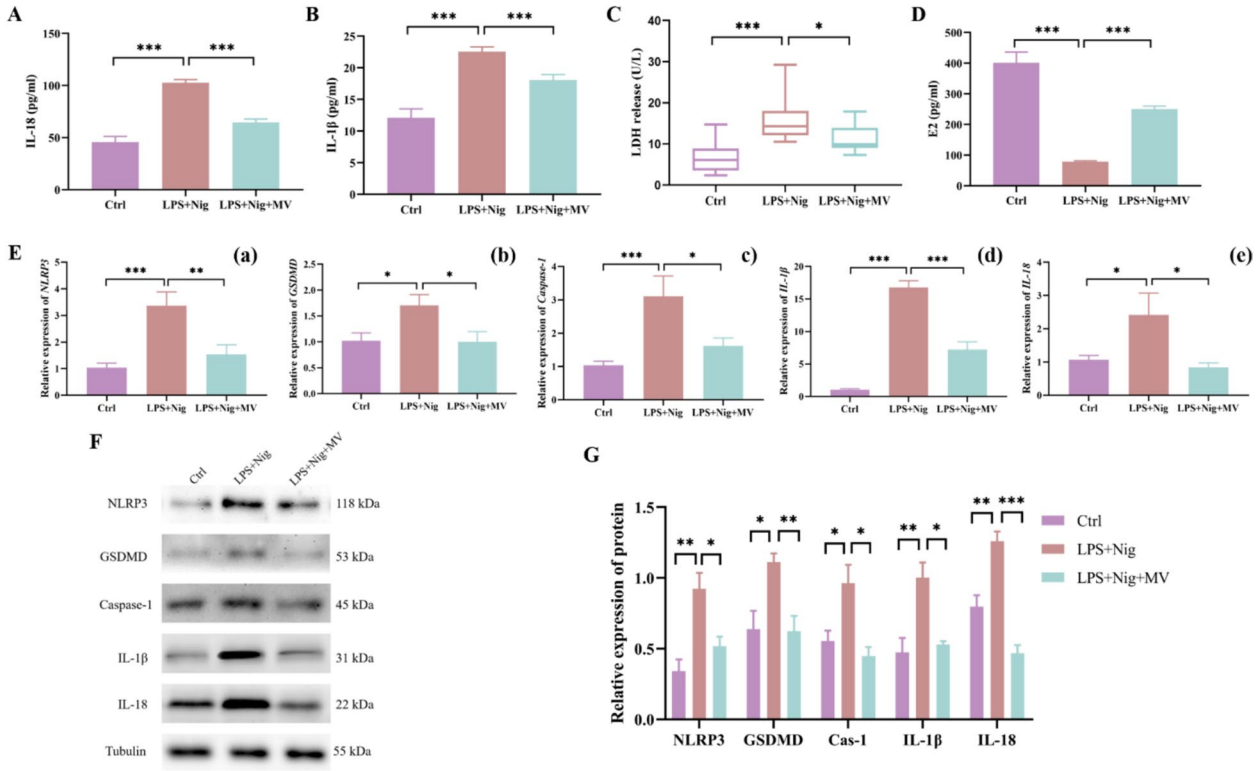

Fig. 6 Mogroside V treatment reduces inflammation and pyroptosis in KGN cells. **A** Expression levels of IL-18 in the supernatant of the KGN pyroptosis cell model ($n=5$). **B** Expression levels of IL-1β in the supernatant of the KGN pyroptosis cell model ($n=5$). **C** Release of LDH in the supernatant of the KGN pyroptosis cell model ($n=5$). **D** Expression levels of E2 in the supernatant of the KGN pyroptosis cell model ($n=5$). **E** qRT-PCR validation of NLRP3, GSDMD, Caspase-1, IL-1β and IL-18 ($n=3$). **F**-**G**. Protein expression levels of NLRP3, GSDMD, Caspase-1, IL-1β and IL-18 were determined by Western Blot and quantified by ImageJ software ($n=3$). $^{*}p<0.05$, $^{**}p<0.01$,. $^{***}p<0.001$

Intervention with Mogroside V can enhance insulin sensitivity in the KGN cell pyroptosis model

The extent of cellular pyroptosis determines the severity of inflammation, and the activation of NLRP3 can lead to insulin resistance through the induction of inflammatory responses.

We detected the content of INS in the supernatant of KGN cell lysates using the ELISA method and found that the content of INS was elevated in the LPS + Nig group, whereas it was reduced following treatment with MV (Fig. 7A). qRT-PCR (Fig. 7B-C) and Western Blot results (Fig. 7D-F) revealed that, compared to the Ctrl group, the expression levels of IGF1 were increased, while the expression levels of IGF1R were decreased in the LPS + Nig group, and intervention with Mogroside V effectively reduced the expression of IGF1 and increased the expression of IGF1R in KGN cells.

Based on these results, we conducted a Spearman correlation analysis, the results of which are depicted in the figures (Fig. 7G). There was a positive correlation between the pyroptosis factor NLRP3 and insulin concentration, IGF1, suggesting that upregulation of NLRP3 in the KGN cell pyroptosis model reflects an exacerbation of the inflammatory state, which is closely associated with insulin resistance, as well as alterations in hormone production and metabolism. A negative correlation was observed between NLRP3 and IGF1R, E2, indicating that as NLRP3 is upregulated and inflammation increases, the cellular response to IGF1 diminishes, which may be related to interference with the insulin signaling pathway. The decrease in E2 levels may reflect a reduction in estrogen synthesis or an impact on hormone metabolism, which is consistent with the characteristic hormonal imbalances observed in PCOS. Mogroside V may exert an interventional effect on insulin resistance, pyroptosis, and granulosa cell function by inhibiting the activation of NLRP3 and alleviating cellular inflammatory responses.

Intervention with Mogroside V can enhance the insulin sensitivity induced by insulin in KGN cells

Insulin resistance is a common occurrence in patients with PCOS and is associated with hyperinsulinemia. We constructed a KGN cell model of insulin resistance using INS and treated it with MV. The results showed that the

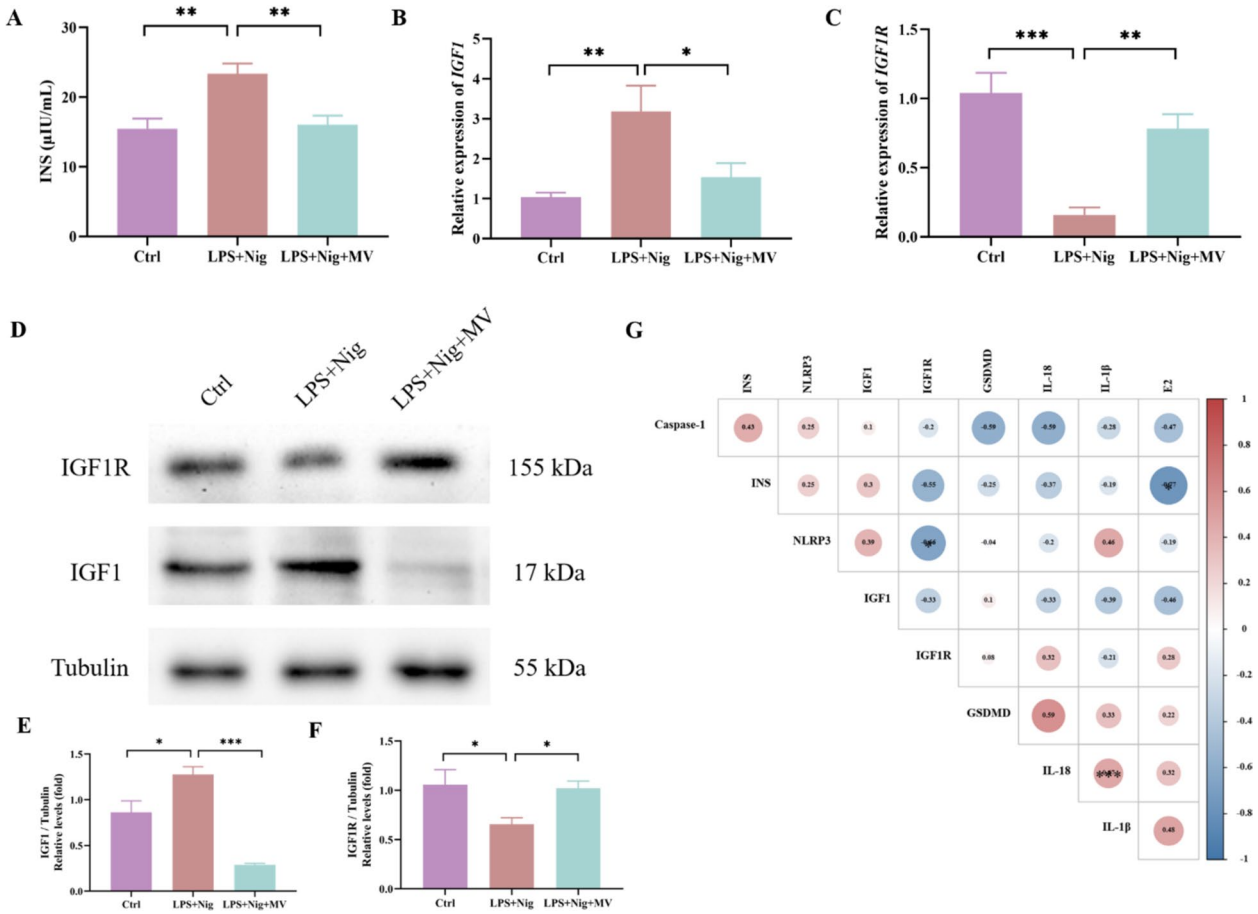

Fig. 7 Mogroside V enhances insulin sensitivity in KGN pyroptosis model. **A** Expression levels of INS in the supernatant of the KGN pyroptosis cell model ($n=5$). **B**-**C** qRT-PCR validation of IGF1 and IGF1R ($n=3$). **D**-**F** Protein expression levels of IGF1 and IGF1R were determined by Western Blot and quantified by ImageJ software ($n=3$). **G** Correlation coefficient matrix. Correlation analysis was performed using Spearman's correlation due to a skewed distribution of the data. The value of r represents the degree of correlation ($0 > r > 1$, positive correlation; $-1 < r < 0$, negative correlation). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

concentration of INS in the supernatant of the INS group cells was elevated, and this concentration was reduced following MV treatment (Fig. 8A), indicating the successful establishment of the model. qRT-PCR and Western Blot analysis revealed that, compared to the Ctrl group, the expression level of IGF1 was increased while the expression level of its receptor IGF1R was decreased in the INS group cells. Intervention with MV significantly reduced the expression of IGF1 and elevated the expression of IGF1R in KGN cells that were in a state of insulin resistance (Fig. 8B-F).

Intervention with Mogroside V can reduce the levels of inflammation and pyroptosis in the KGN cell model of insulin resistance

In PCOS, there is a close correlation between insulin resistance and the inflammatory state. Experimental results indicate that, compared to the Ctrl group, the levels of inflammatory cytokines IL-1β, IL-18, and LDH were significantly elevated, and the level of E2 was decreased in the supernatant of INS group cell cultures; however, in the MV treated group (INS + MV), the levels of IL-1β, IL-18, and LDH in the cell culture supernatant were significantly reduced, and the level of E2 was increased. These findings suggest that treatment with MV can markedly decrease the levels of inflammation induced by insulin in KGN cells, restore estrogen levels, and provide a certain degree of protection against insulin-induced damage to KGN cells (Fig. 9A-D).

Analysis of the expression levels of pyroptosis-associated factors, including NLRP3, GSDMD, Caspase-1, IL-1β, and IL-18, at both the mRNA and protein levels in cells from different treatment groups revealed that, compared to the Ctrl group, the expression levels of these factors were significantly increased in the INS group. Following intervention with MV, the expression of these

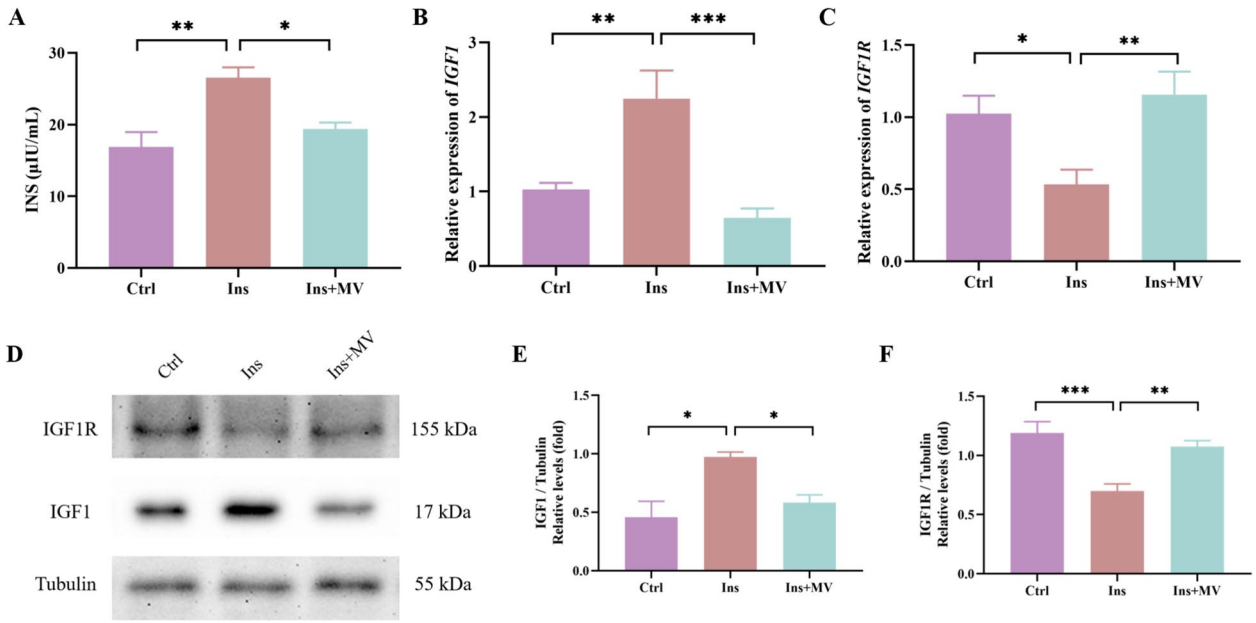

Fig. 8 Mogroside V boosts insulin sensitivity in KGN cells. **A** Expression levels of INS in the supernatant of the KGN pyroptosis cell model ($n=5$). **B**-**C** qRT-PCR validation of IGF1 and IGF1R ($n=3$). **D**-**F** Protein expression levels of NLRP3, GSDMD, Caspase-1, IL-1β and IL-18 were determined by Western Blot and quantified by ImageJ software ($n=3$). $^{*}p<0.05$,$^{**}p<0.01$,.$^{***}p<0.001$

factors was markedly reduced (Fig. 9E-G). These results suggest that MV has the potential to mitigate the inflammatory response and the extent of pyroptosis induced by insulin in KGN cells.

We conducted a Spearman correlation analysis on the factors examined in the KGN insulin-resistant cell model(Fig. 9H). As illustrated in the figures, a positive correlation was observed between NLRP3 and the concentrations of INS and IGF1, suggesting that the upregulation of NLRP3 may exacerbate insulin resistance. MV may improve granulosa cell pyroptosis and function by alleviating NLRP3-mediated insulin resistance.

Discussion

PCOS is a complex disease affecting female reproductive health, characterized by endocrine abnormalities and metabolic disorders. In a large epidemiological study, the prevalence of PCOS in the Chinese Han female population was found to be approximately 5.6% according to the Rotterdam PCOS diagnostic criteria [42]. The clinical manifestations of the disease exhibit high heterogeneity, primarily including ovulatory dysfunction, hyperandrogenemia, and polycystic ovarian morphology. Additionally, patients may face related issues such as insulin resistance, chronic inflammation, and weight gain. In the long term, PCOS patients may encounter a variety of complications, including dyslipidemia, type II diabetes, cardiovascular diseases, and psychological impacts [2, 43]. To date, there is no universally satisfactory treatment for PCOS. Clinically, the treatment often involves symptomatic therapies such as anti-androgen drugs, insulin sensitizers, and ovulation-inducing medications [44]. In recent years, there has been a growing interest in the research of MV for improving female fertility, and it has been shown to have certain therapeutic effects. However, the mechanisms by which it ameliorates PCOS remain to be elucidated.

Due to the numerous limitations associated with obtaining ovarian tissue samples from patients with PCOS, the construction of animal models has been widely utilized to simulate in vivo experiments of PCOS in order to delve into the underlying mechanisms of the disease's pathology. In this study, a PCOS animal model was successfully established by administering letrozole to SD rats coupled with a high-fat diet. The PCOS rats exhibited significant weight gain, hyperandrogenemia, menstrual cycle disorders, and a decrease in litter size; the ovaries showed polycystic changes, a reduction in the number of granulosa cell layers, a decrease in the number of corpora lutea, and abnormal follicular development [38]. Our study shows that the PCOS rat model exhibited significantly elevated serum testosterone and LH levels, an increased LH/FSH ratio, and decreased estrogen levels, consistent with hormonal imbalances reported in previous literature48, 49. This alignment further supports the diversity of

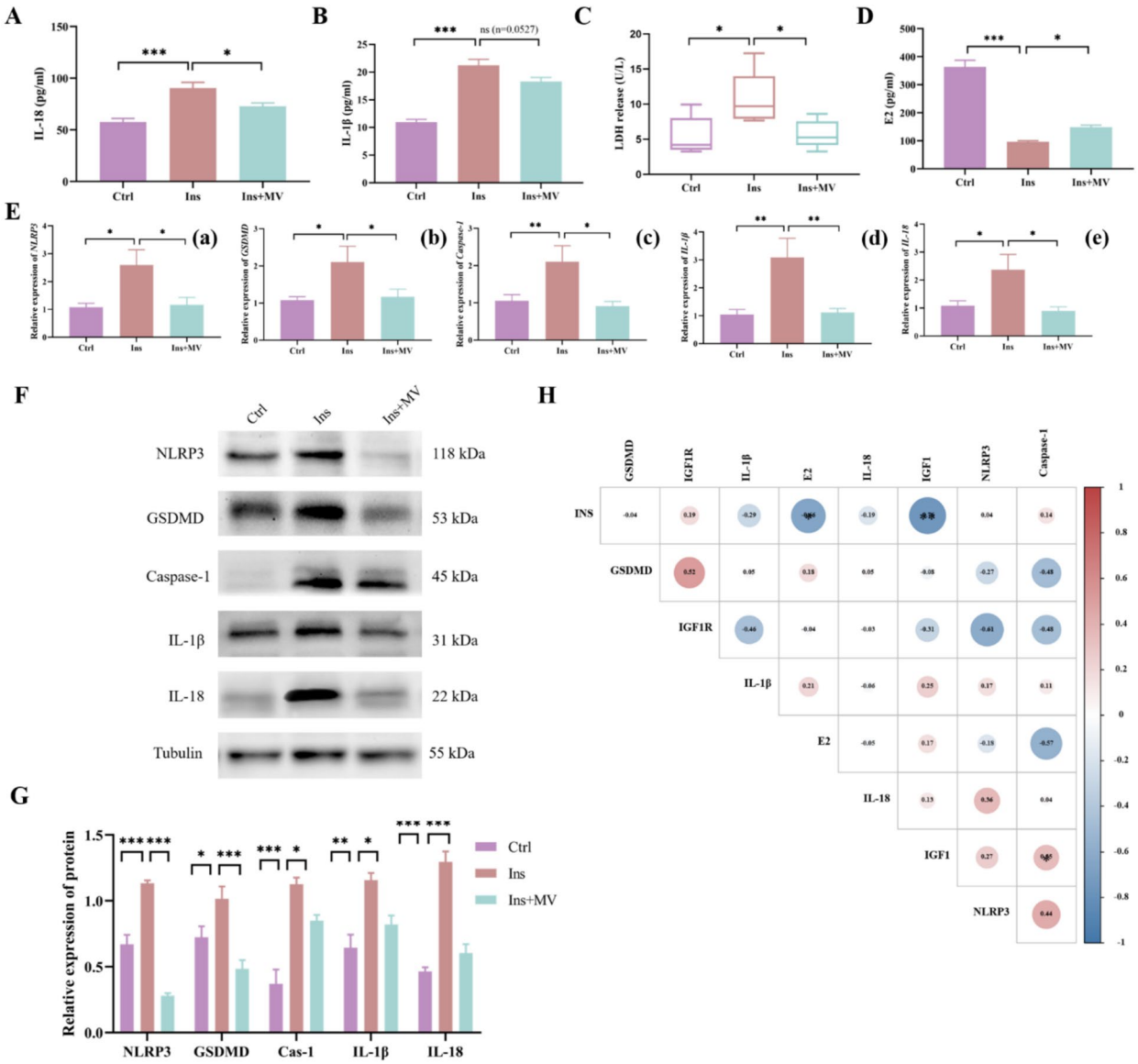

Fig. 9 Mogroside V reduces inflammation and pyroptosis in insulin-resistant KGN cells. **A** Expression levels of IL-18 in the supernatant of the KGN pyroptosis cell model ($n=5$). **B** Expression levels of IL-1β in the supernatant of the KGN pyroptosis cell model ($n=5$). **C** Release of LDH in the supernatant of the KGN pyroptosis cell model ($n=5$). **D** Expression levels of E2 in the supernatant of the KGN pyroptosis cell model ($n=5$). **E** qRT-PCR validation of NLRP3, GSDMD, Caspase-1, IL-1β and IL-18 ($n=3$). **F**-**G** Protein expression levels of NLRP3, GSDMD, Caspase-1, IL-1β and IL-18 were determined by Western Blot and quantified by ImageJ software ($n=3$). **H** Correlation coefficient matrix. Correlation analysis was performed using Spearman's correlation due to a skewed distribution of the data. The value of *r* represents the degree of correlation ($0>r>1$, positive correlation; $-1<r<0$, negative correlation). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

hormonal abnormalities in PCOS and indicates that our model successfully simulates PCOS endocrine features [45, 46]. Following intervention with MV, there was a decrease in the levels of T and LH in the serum of PCOS rats, along with a reduction in the LH/FSH ratio and a significant increase in the level of E2. This indicates that MV intervention can ameliorate the hormonal imbalance observed in the serum of PCOS rats.

Our study findings indicate that, in comparison with the control group, PCOS rats demonstrated elevated levels of HOMA-IR and increased expression of IGF1 in the ovaries, with a concomitant decrease in the expression of its receptor, IGF1R. Intervention with MV was found to effectively reduce the levels of HOMA-IR in rats, enhance insulin sensitivity, and alleviate insulin resistance. In patients with PCOS, insulin resistance is

a common phenomenon that is closely associated with reproductive and metabolic issues related to PCOS. As a compensatory response to insulin resistance, hyperinsulinemia, a physiological reaction in this pathological state, affects ovarian function, leading to increased androgen levels and impaired follicular maturation. Clinically, obese patients with PCOS often present with insulin resistance [47]. The IGF1R is the primary functional receptor for IGF1, and together they regulate cellular growth, differentiation, and metabolic processes. Within the female reproductive system, they are involved in the regulation of key physiological functions such as follicular development, the production and secretion of steroid hormones, the process of follicular atresia, and the maturation of oocytes. Furthermore, the expression levels of IGF are closely related to an individual's degree of insulin resistance [48]. In patients with PCOS, the levels of IGF1 and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) are significantly elevated, and a positive correlation exists between the two [49]. Beyond PCOS models, Liu et al. reported that MV significantly reduced serum insulin levels and HOMA-IR in diabetic mice, improving insulin resistance [50]. This aligns with our findings and further supports MV's potential in ameliorating metabolic disorders.

We also observed that the ovaries and granulosa cells of PCOS rats were in a state of inflammation, with elevated expression of pyroptosis-related factors, and that the expression levels of these factors in the ovaries were reduced following intervention with MV. Kelly et al. reported that PCOS patients experience a state of low-grade inflammation, which aligns with our observations of inflammatory responses in the ovaries and granulosa cells of the PCOS rats, including elevated expression of NLRP3 inflammasome and inflammatory factors. This common finding underscores chronic low-grade inflammation as an important pathological feature of PCOS. The primary cause of this inflammatory state is the abnormality in glucose and lipid metabolism. Chronic low-grade inflammation and metabolic disorders in PCOS mutually exacerbate each other, creating a vicious cycle characterized by abnormal follicular development, increased androgen synthesis, and reduced insulin sensitivity, thereby intensifying issues such as ovulatory dysfunction in PCOS patients [51]. The chronic inflammatory state is associated with elevated levels of various pro-inflammatory cytokines and chemokines. Studies have shown that both PCOS patients and animal models exhibit characteristics of chronic low-grade inflammation [52]. These findings are consistent with the results we have observed.

Chronic low-grade inflammation can trigger or exacerbate insulin resistance, and concurrently, insulin resistance may intensify inflammatory responses. This bidirectional interaction complicates and diversifies the clinical manifestations of PCOS. Studies have indicated that the activation of the NLRP3 inflammasome is a cause of exacerbated insulin resistance, and the deletion of NLRP3 in mice eliminates insulin resistance and inflammation [53]. The activation of the NLRP3 inflammasome is a significant factor leading to inflammation and insulin resistance induced by obesity [54]. Through correlation analysis, we found that the expression levels of NLRP3 were positively correlated with those of IGF1. This suggests that in PCOS, the upregulation of NLRP3 may be associated with the activation of these metabolic pathways.The results of the correlation analysis reveal a complex interplay between the upregulation of inflammatory and pyroptosis-related factors and insulin resistance in PCOS. This provides important clues for further research into the molecular mechanisms and potential therapeutic targets of PCOS.

Numerous studies have indicated that abnormal follicular development may be the underlying cause of persistent ovulatory dysfunction and the clinical endocrinological abnormalities observed in patients with PCOS. The process of follicular maturation is complex and delicate, and its structure primarily consists of the oocyte, granulosa cells (GCs), and theca cells [55]. The oocyte and its surrounding granulosa cells are within the same follicular microenvironment, engaging in mutual communication and forming a unified entity. Granulosa cells are also regulated by oocyte-secreted factors, and thus, the gene expression and functional state of granulosa cells can, to a certain extent, reflect the developmental capacity of the oocyte. GCs are often selected for in vitro experimental studies of PCOS to delve deeper into the pathogenesis of the disease [56, 57]. Hyperinsulinemia due to insulin resistance and fluctuations in androgen levels can affect the function of GCs [58, 59]. Since KGN cells retain several physiological characteristics of normal granulosa cells, such as expressing functionally competent FSH receptors and exhibiting steroid hormone production patterns and apoptosis through the Fas pathway similar to those observed in normal granulosa cells [60]. In this study, KGN cells were selected as the model for in vitro experimental research. By introducing LPS and Nig into KGN cells to establish a pyroptosis model, and adding INS to create an insulin resistance model, we observed inflammatory responses in both the cell supernatant and intracellular environments across all model groups. There was a significant increase in the expression of the NLRP3 protein and a decrease in insulin sensitivity. Following intervention with MV, improvements were noted in the aforementioned indicators. These experimental results suggest that MV may improve

the function of granulosa cells and, consequently, follicular development in PCOS patients by inhibiting NLRP3-mediated pyroptosis and insulin resistance.

The innovation of this study lies in its systematic evaluation of the potential ameliorative effects of the natural compound MV on insulin resistance in a PCOS rat model, as well as its regulatory role in the pathway of pyroptosis. Utilizing transcriptomics analysis, ELISA, qRT-PCR, and Western blot techniques, we not only confirmed the regulatory effects of MV on hormone levels but also revealed that it may improve the state of insulin resistance by modulating the expression of genes associated with inflammation and pyroptosis. These findings provide new insights into the molecular mechanisms underlying PCOS and offer a scientific basis for the potential therapeutic strategy of MV in treating PCOS. Future research should further explore the mechanisms of action of MV and assess its therapeutic potential in a clinical context.

Limitations and Prospects of This Study: This study employed animal and cellular models for research, which, although capable of simulating certain characteristics of PCOS and yielding positive results, still differ from the complexity of human PCOS. Future studies may consider clinical trials to evaluate the safety and efficacy of Mogroside V in patients with PCOS. While this study has shed light on the possible pathways through which Mogroside V improves PCOS, further in-depth research is still needed to elucidate its specific mechanisms of action and molecular targets. Given the complexity of PCOS, future research may explore the effects of MV in combination with anti-inflammatory agents or insulin sensitizers to achieve better therapeutic outcomes in PCOS treatment. Additionally, as patients with PCOS-IR often present with dysregulation of carbohydrate and lipid metabolism, further studies could include the monitoring of lipid metabolism-related indicators, such as total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein, adiponectin, and leptin, to obtain a more comprehensive assessment of metabolic characteristics.

Conclusion

In summary, through the establishment of a PCOS rat animal model in this study, it was found that Mogroside V could ameliorate both systemic and ovarian tissue inflammation, as well as insulin resistance. Additionally, it improved the dysregulated serum hormone levels associated with PCOS. Subsequently, by constructing KGN cell models of pyroptosis and insulin resistance, it was observed at the cellular level that Mogroside V improved the function of granulosa cells in PCOS by inhibiting NLRP3-mediated pyroptosis and insulin resistance, promoting the synthesis and secretion of estrogen, and thereby ameliorating the symptoms of PCOS. The potential mechanisms underlying these effects provide a scientific basis and a new perspective for the clinical use of Mogroside V in the treatment of PCOS. However, further research is still required to elucidate the specific molecular mechanisms involved.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13048-024-01563-5.

Supplementary Material 1.

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None.

Authors' contributions
Conceptualization, W.Y. and X.L.; Methodology, Y.M.; Software, W.Y. and Y.W.; Validation, Y.M., X.L. and M.L.; Formal Analysis, W.Y.; Investigation, Y.W.; Resources, X.L.; Data Curation, W.Y. and Y.W.; Writing – Original Draft Preparation, W.Y.; Writing – Review & Editing, W.Y., Y.M. and Y.W.; Visualization, W.Y.; Supervision, X.L.; Project Administration, Y.M., J.Z. and M.L.; Funding Acquisition, Y.M., J.Z. and M.L. All authors have read and agreed to the published version of the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All members of this team in this research work will follow the principle of humanitarian, ensure laboratory animal welfare ethics, and strictly abide by the laboratory animal center at the university of south China related rules and regulations, accept the experimental animal welfare ethical review committee of the guidance and supervision and inspection.

Competing interests
The authors declare no competing interests.

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