Nutrition & Metabolism

RESEARCH

Open Access

# Quinoa ameliorates polycystic ovary syndrome via regulating gut microbiota through PI3K/AKT/mTOR pathway and autophagy

Jinfang Dou[1†], Yanxiang Wu[1†], Rentong Hu[2†], Jiaxian Liu[3,4], Yuelin Zhang[1], Xianjie Zhen[1], Tao Wu[1], Chuyue Zhang[1], Yutong Liu[3,4], Ruifang Zheng[5*] and Guangjian Jiang[1*]

## Abstract

**Background** Polycystic ovary syndrome (PCOS) is a unity of endocrine and metabolic disorders, associated with PI3K/AKT/mTOR, autophagy, and gut microbiota. Quinoa is a valuable food source, which contains rich minerals, unsaturated fatty acids, and has a positive modulating effect on metabolic diseases. However, its effects and potential mechanisms on PCOS have not been reported yet. Therefore, the purpose of this study is to investigate the effect of quinoa on PCOS rats by regulating PI3K/AKT/mTOR, autophagy, and gut microbiota.

**Methods** Ten–week-old female Sprague-Dawley (SD) rats have received letrozole for 24 days for induction of PCOS and subsequently were treated with a quinoa diet for 8 weeks. Vaginal smears were used to analyze the estrous cycle of rats. Hormone and biochemical indexes were analyzed by kit assays and glucometer. The pathological changes of ovary, pancreas, duodenum and colon were observed by HE staining. PI3K, AKT, mTOR and autophagy-related proteins in the ovary and colon were measured by western blot and immunohistochemistry staining. Tight junction proteins in colon were measured by immunohistochemistry staining. 16 s rDNA sequencing was used to detect the changes of intestinal microbiota in rats. Network pharmacology and molecular docking were used to study the possible targets and mechanisms of quinoa on PCOS. Spearman correlation analysis was used to study the relationship between intestinal microbial abundance and hormone levels of PCOS rats at the phylum and genus level.

**Results** Quinoa significantly improved estrous cycle and biochemical parameters of PCOS-like rats, and the pathological state of ovary, pancreas, duodenum and colon tissues. Especially, quinoa significantly regulated the expression of PI3K, AKT, mTOR and autophagy-related proteins in the ovary. Quinoa may repair the intestinal barrier by upregulating the expression of tight junction proteins in the colon, and regulate autophagy-related factors in

†Jinfang Dou, Yanxiang Wu and Rentong Hu contributed equally to this work.

*Correspondence:
Ruifang Zheng
872780352@qq.com
Guangjian Jiang
bucmjiang@163.com

Full list of author information is available at the end of the article

colon. Additionally, quinoa increased the abundance of Lactobacillu, Bacteroides and Oscillospira, and decreased the Firmicutes/Bacteroidetes ratio and the Blautia, and Prevotella, reversing the dysregulation of the gut microbiota. Correlation analysis showed that there is a strong correlation between gut microbiota with significant changes in abundance and hormone related to PCOS.

**Conclusion** Our result indicated that effect of quinoa on PCOS maybe associated with activation of the PI3K/AKT/mTOR signaling pathway, inhibition of autophagy, and regulation of intestinal flora.

**Keywords** Quinoa, Polycystic ovary syndrome, PI3K/AKT/mTOR, Autophagy, Sex hormone, Gut microbiota

## Introduction

Polycystic ovary syndrome (PCOS) is a complex, heritable reproductive-metabolic disorder in the women of child-bearing age, characterized by hyperandrogenism, ovulatory dysfunction and polycystic ovaries [1]. The disease, with a prevalence ranging from 4 to 21% among reproductive-age women [2], affects the entire long cycle of fertility and is considered to be a specific female reproductive risk factor [3]. In addition, PCOS also disturbs the metabolic and psychological health of women at childbearing age [4], which is related to diabetes, cardiovascular disease, depression, anxiety and endometrial cancer, and deteriorates their quality of life [5–8]. However, the mechanisms underlying these impairments remain not fully clarified.

Quinoa (*Chenopodium quinoa Willd.*), is an annual plant original from the Andes. Quinoa seeds are recognized to produce a diverse array of secondary metabolites, including phytosterols, flavonoids, saponin, terpenoids, phytoecdysteroids, phenols acids, bioactive peptides [9, 10]. A previous vivo study has implicated that quinoa intake could reduce plasma and liver cholesterol, and lessen obesity associated inflammation [11]. Several studies, including ours, suggest that quinoa may have a positive modulating effect on metabolic diseases [12–15]. We found that the PI3K/AKT signaling pathway contribute to PCOS by performing network pharmacology. Many literatures have reported that PI3K/AKT signaling pathway is one of the ways to intervene in PCOS, involved in regulating glucose and lipid metabolism, autophagy, estrogen receptor [16–18, 58].The PI3K/AKT/mTOR pathway is a common regulator of autophagy. Autophagy is a self-digesting process, an evolutionarily conserved and highly regulated catabolic process that provides energy for metabolism at a basal level, promotes homeostasis, and enables adaptation to environmental changes [19]. The importance of autophagy in the metabolic disorders associated with PCOS is increasingly recognized. In women with PCOS, autophagy marker protein increased in a dose-dependent manner with testosterone levels [20]. Autophagy also plays an important role in the regulation of sex hormones including progesterone and estrogen in PCOS women [21].

The gut microbiome includes the largest and most diverse microbiome in the human body and benefits the host in a variety of ways, such as maintaining the immune system, providing nutrition and digesting dietary macronutrients [22]. Emerging data suggest that PCOS is associated with changes or abnormalities in intestinal microbial diversity and composition [23, 24, 57]. In addition, manipulation of the gut microbiota can affect the PCOS phenotype [25]. It was found that insulin resistance (IR) in PCOS patients was related to the change of intestinal flora and fecal microbiota transplantation in women with PCOS can lead to the development of PCOS phenotype and IR in mice [26].

Therefore, the primary objective of our study was to explore whether the mechanism of quinoa on PCOS is related to PI3K/Akt/mTOR pathway, autophagy and gut microbiota. We wished that the results of this work will contribute to improve the integrated and value-added utilization of quinoa in the functional food and nutritional products industry.

## Materials and methods

### Experimental food and animals

Quinoa was provided from Gansu Chunjie Plateau Agricultural Technology Co., Ltd. (SC10162062300206). The quinoa seeds were washed with distilled water, and seeds were ground into powder and mixed with water, then by heating at microwave for 8 min to remove the moisture. Finally, the quinoa food was made to form standard rat food [13, 15]. Ten–week-old female Sprague-Dawley (SD) rats were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and bred and lived at the animal facility of Beijing University of Chinese Medicine (BUCM). After two weeks of adaptive feeding, the rats selected for the experiment had a regular cycle. All animals were provided with food and water during our study. The ethical approval number for this project is BUCM-4-2018112901-4051.

### Experimental design

After a week without any interventions, the twenty rats were random selected to receive letrozole (GYZZH199991001, Jiangsu Hengrui pharmaceutical Co., Ltd., China) at a dose of 1 mg/kg dissolved in 0.5%

CMC once daily for 24 days for induction of PCOS [27, 28]. Estrous-cycle stage was analyzed using vaginal epithelial cell smears taken daily for 8 consecutive days [28, 29]. The PCOS-like rat model was considered to be successfully evaluated if the estrous cycle disorder and body weight obviously increased in letrozole-induced rats than control rats at the end of this period. Ten PCOS-like rats from letrozole induced group were numbered and divided into the following two groups with 5 rats in each group. (i) Control group: fed with ordinary diet; (ii) PCOS group: fed with ordinary diet; (iii) Quinoa group: fed with quinoa (20 g/kg/day) combined with ordinary diet for 8 weeks. The body weight of rats was recorded every week during the course of the study. At the end of experiment, rats were anesthetized with 0.4% pentobarbital (1 ml/100 g) intraperitoneally, and disinfected with 75% ethanol, and their abdominal cavities were opened. Blood samples were collected from the abdominal aorta of each group. The right ovaries, duodenum, colon, and pancreas were fixed with a 4% polyformaldehyde solution. The left ovaries and feces of the rats were collected and frozen at -80 ℃. Finally, rats were euthanized via cervical dislocation.

#### Hormone profile and biochemical indexes

Trunk circulation blood samples were obtained and allowed to incubate for 4 h at room temperature. And all blood samples were centrifuged at 2,500 rpm for 15 min to separate serum samples, collected into 1.5 ml Eppendorf tubes, stored in -80 °C refrigerator for further analysis. The Estradiol kit (YZB13482019), total testosterone kit (YZB06152019), follicle stimulating hormone (FSH) kit (YZB07202019), luteinizing hormone (LH) kit (YZB06102019) and the fasting insulin index (FINS) kit (YZB06252019) from Tianjin Jiuding Medical Bioengineering Co., Ltd. were utilized for serum hormone levels. Fasting blood glucose (FBG) in rats were measured with a glucometer (One touch, California, USA). The HOMA-IR was calculated according to the following formula: [FINS (μIU/mL) × FBG (mmol/L) / 22.5].

#### Hematoxylin and eosin (HE) staining

The duodenum, colon, ovary and pancreas tissues were fixed in 4% tissue cell fixative (Beijing Baierdi Biotechnology Co., Ltd., Beijing, Chian) overnight, then gradient alcohol dehydration and embedded in paraffin, the 3 μm thick sections were sectioned, stained with HE. The images were acquired using an Olympus BX53 microscope (Japan).

#### Immunohistochemistry (IHC) staining

IHC staining was conducted according to the manufacturer's protocol (KIT-9701/9706; MXB Biotechnologies). As we've done before [13], paraffin-embedded colon and ovary sections were placed in the oven at 37 °C for 12 h, then degreased with xylene and hydrated with gradient ethanol. Sequentially, slides were incubated with the antigen retrieval solution, 3% $H_2O_2$ for 10 min, and incubated with a primary antibody overnight at 4 °C, including Claudin-5 (1:200; bs-10296R; Bioss), Occludin (1:200; ab216327; Abcam), AKT (1:100; A18675; ABclonal), mTOR (1:100; bs-1992R; Bioss), Bcl-2 (1:100; sc-7382; Santa Cruz), Beclin 1 (1:100; WL02237; Wanleibio), ULK1 (1:100; WL03067; Wanleibio) and p62 (1:500; GB11531; Servicebio) antibodies. After that, the slides were incubated with secondary antibodies for 10 min followed by DAB and hematoxylin staining. Finally, images were captured by laboratory microscopy, and IOD of the IHC positive areas was analyzed by using Image-Pro Plus 6.0 software.

#### Western blot

Proteins were extracted with RIPA cracking liquid (C1053; Applygen, Beijing, China) and protein concentration was determined by bicinchoninic acid (BCA) protein assay kit (P1511; Applygen, Beijing, China). Next, 10% SDS-PAGE was used for protein isolation, then the proteins were transferred onto nitrocellulose membranes and incubated with PI3K (1:1000; A18355; ABclonal), AKT (1:500; A18675; ABclonal), mTOR (1:500; bs-1992R; Bioss), Bcl-2 (1:200; sc-7382; Santa Cruz), Beclin 1 (1:500; WL02237; Wanleibio), ULK1 (1:1000; WL03067; Wanleibio), p62 (1:500; GB11531; Servicebio) and LC3B (1:1000; A5202; Selleck) antibodies against at 4 °C for the night. After that, we took secondary antibodies (1:5000; AS014; ABclonal) onto the membranes for reaction for 1.5 h at 25 ℃. The bands were visualized with a chemiluminescence reagent (PK10003; Proteintech, Rosemont, USA) and subsequently scanned and analyzed by Image J software. The intensity of the protein bands was normalized to β-actin.

#### 16s rDNA sequencing

Fresh fecal samples were taken from all rats, collected into 1.5 ml sterile EP tubes, rapidly snap frozen in liquid nitrogen, and stored at -80 °C until further analysis. Then the genomic DNA from rat feces samples was extracted using a specific DNA extraction kit, and then the DNA was detected by 1.2% agarose gel electrophoresis. With the V3 and V4 hypervariable region of the 16 S rDNA hypervariable regions were amplified by Phusion® High-Fidelity PCR Master Mix with GC Buffer (New England Biolab, USA) using the following primers (5′ to 3′): 338 F (5'ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). All samples were sequenced using the paired-end strategy on Illumina platform. After library establishment (SRP381975), the microbiome bioinformatics were performed with QIIME

2 2019.4 [15] with slight modification according to the official tutorials (https://docs.qiime2.org/2019.4/tutorials/). The raw reads were filtered, merged, demultiplexed for quality control and clustered into operational taxonomic units (OTUS) using a 97% similarity cut-off from UCLUST. Finally, the library was sequenced on an Illumina platform, and 200 bp/450 bp single-end reads were generated.

#### Network pharmacology analysis and molecular docking

Components of quinoa were screened by searching PubMed and China National Knowledge Infrastructure (CNKI) database. The targets genes of active components of quinoa was obtained by the SwissTargetPrediction (http://www.swisstargetprediction.ch) and the candidate targets of polycystic ovary syndrome were obtained from Genecards Databases (http://www.genecards.org/). The Cytoscape 3.9.1 platform was used to obtain compound-disease common targets. And the intersection targets were used the online software DAVID Bioinformatics Resources 6.8 convert gene symbol to GENE_ID (https://david.ncifcrf.gov/conversion.jsp) for KEGG pathway enrichment analysis. The following criteria were applied to filter genes, $p \leq 0.01$and Benjamin $\leq 0.01$. The visualization was subsequently analyzed for enrichment by using the bioinformatics platform (https://hiplot.com.cn/). KEGG enriches the interaction information of biological pathways, intersects the targets, combines the screened drug components, and uploads them to Cytoscape 3.9.1 to construct the drug component target pathway network diagram. The core targets and the main active ingredients for the treatment of diabetes were determined according to the network topology parameters. The 3D structure information of quinoa components is obtained through TCMSP database, and at the same time, through PDB database (https://www.rcsb.org/) download the 3D structure of the target protein, use Autodock tools 1.5.7 software for pretreatment, find the docking active site with the help of POCASA 1.1, and then dock the treated components with the target protein in Autodock Vina 1.2.0 software. Finally, visualize the docking results through PyMOL 2.5.2 software. 2D structure display using Discovery Studio 4.5.

#### Statistical analysis

Data presented were analyzed with GraphPad Prism v8 software. Data are presented as mean ± SD unless otherwise indicated. For experiments that presented normal distributions and equality of variances, one-way ANOVA was employed; otherwise, non-parametric analyses were utilized for one-factor analyses. Microbial diversity parameters and composition analyses were evaluated using the Kruskal–Wallis test. Statistical significance was determined when the *P* value was less than 0.05.

## Results

#### Supplementation of quinoa reduced the body weight and ovary weight

After 4 weeks of quinoa intervention, there was no significant change in body weight of PCOS rats. (Fig. 1A). Following 8 weeks of quinoa supplementation, the body weight of PCOS rats was markedly decreased compared with the PCOS group ($P<0.05$) (Fig. 1B). Additionally, the intervention with quinoa reduced the ovary weight in PCOS-like rats ($P<0.05$) (Fig. 1C).

#### Effect of quinoa on the hormone levels in the PCOS-like rats

Serum T, LH concentrations and LH/FSH ratio were obviously higher in the PCOS-like rats than in the control rats ($P<0.001$, $P<0.01$, $P<0.05$). However, quinoa supplementation markedly decreased the serum T, LH concentration and LH/FSH ratio compared with the PCOS group ($P<0.001$, $P<0.01$, $P<0.05$) (Fig. 1D, E and G). Additionally, the serum E2 levels decreased in the PCOS group compared with the control group, while the quinoa supplementation markedly increased the serum levels of E2 in PCOS rats ($P<0.05$, Fig. 1H). FINS and HOMA-IR were significantly increased in the PCOS group compared to the control group during the experiment ($P<0.0001$, $P<0.01$), while the FINS and HOMA-IR levels decreased in quinoa group than PCOS group ($P<0.001$, $P<0.01$) (Fig. 1I-J). The estrous cycle of rats in the normal group is regular, and the diestrus of PCOS rats was prolonged; however, quinoa reversed the estrus cycle of rats to normal (Fig. 1M-O).

#### Effects of quinoa on ovary and pancreas morphological structure

As shown in Fig. 1K and L, the morphology of ovary and pancreas in PCOS-like rats were altered. HE staining show ovaries of the PCOS model group were disordered, with no oocytes and obvious cystic dilatation in the follicles. Furthermore, there were only two to three granular cell layers, ovarian interstitial hyperplasia, and thickening in the ovarian cortex. After treatment with quinoa, ovarian structure was partially recovered, and we found increased layers of granulosa cells and restored oocytes and corona radiata in the dominant follicles (Fig. 1K). The HE-stained sections of the pancreas showed that islet morphology appeared normal in control group. This difference is reflected in the looser and more variable organization of cells within islets, and the numbers of islets were reduced, cells exhibiting abnormal with enlarged, irregular nuclei in PCOS group. Interestingly, quinoa treated animals had an obviously greater number and morphology of total islets than the PCOS-like rats, which showed uniform size, regular shape, and clear boundaries of the pancreas (Fig. 1L).

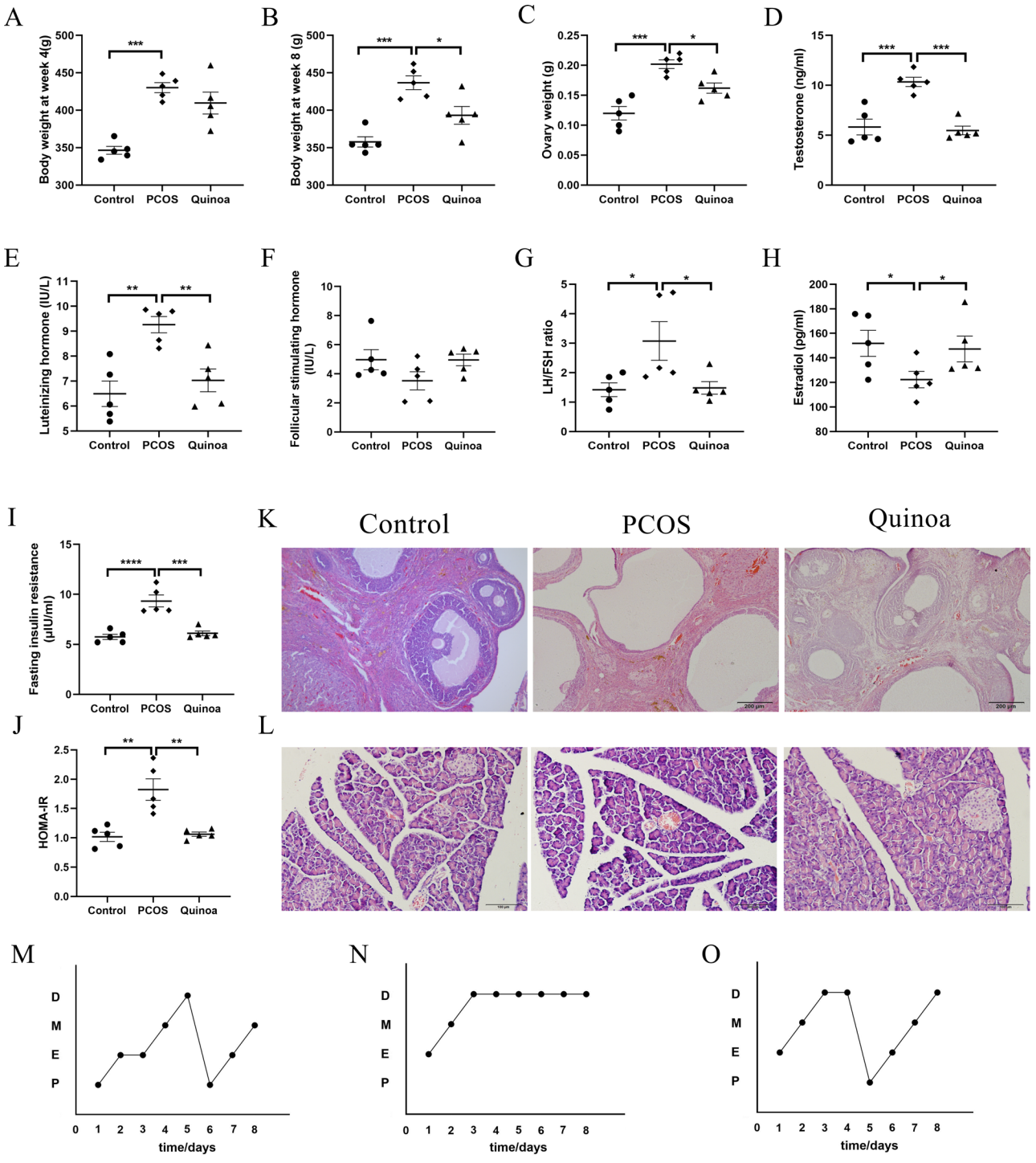


**Fig. 1** Quinoa improved the symptoms of PCOS rats. (**A**) Bodyweight at 4 weeks. (**B**) Bodyweight at 8 weeks. (**C**) Ovary weight. (**D**) Testosterone (T). (**E**) Luteinizing hormone (LH). (**F**) Serum follicle-stimulating hormone (FSH). (**G**) LH/FSH. (**H**) Estradiol(E2). (**I**) Fasting serum insulin (FINS). (**J**) HOMA-IR. (**K**) HE staining of ovary tissue sections. (**L**) HE staining of pancreas tissue. (**M**-**O**) Different estrous cycles in each group. The estrous cycle consisted of diestrus, proestrus, estrus, and metestrus. The estrous cycle consisted of diestrus (**D**), proestrus (**M**), estrus (**E**), and metestrus (**P**). Data are expressed as mean ± SD ($n = 5$). *$P < 0.05$.**$P < 0.01$. ***$P < 0.001$. ****$P < 0.0001$

### Network pharmacology prediction

A total of 117 chemical constituents of quinoa were retrieved through the initial literature search, 72 potentially active ingredients were obtained after intestinal absorption and drug-like screening, and we have marked with different colors (supplemental Table 1).

In total, 524 targets related with quinoa were screened through SwissTargetPrediction database, but the total of 14 components of quinoa-saponin-10, quinoa-saponin-6, quinoa-saponin-7, quinoasaponin-8, Tannins, saponin, omega-6, omega-3, (epi)-gallocatechin, Catechuic acid, Zeatin, Vitamin B6, serine, Epicatechin did not predict the relating targets. At the same time, the 4688 relation targets of PCOS were screened from the Genecards database (www.genecards.org). As shown in Fig. 2A, the 261 intersecting targets were obtained by introducing the target network between quinoa and PCOS into the CytSpace software.

To further find the possible mechanism of quinoa on PCOS, 170 pathways were acquired by KEGG enrichment analyses. As shown in Fig. 2B, the top 20 pathways were shown by bubble diagram, such as Pathways in cancer, PI3K-Akt signaling pathway, Chemical carcinogenesis - receptor activation, Lipid and atherosclerosis, Chemical carcinogenesis - reactive oxygen species, Neuroactive ligand-receptor interaction, Ras signaling pathway, MAPK signaling pathway, Proteoglycans in cancer, Rap1 signaling pathway, cAMP signaling pathway, EGFR tyrosine kinase inhibitor resistance, Endocrine resistance, and et al. It is noteworthy that PI3K/AKT signal pathway is the most obviously enriched except in tumor signal pathway.

Analogously, we used the DAVID 6.8 database did the GO enrichment analysis for 261 common targets. As Fig. 2C shown the top 30 key targets between quinoa and PCOS, which mainly involved in the plasma membrane, cytosol, cytoplasm, nucleus, signal transduction, protein

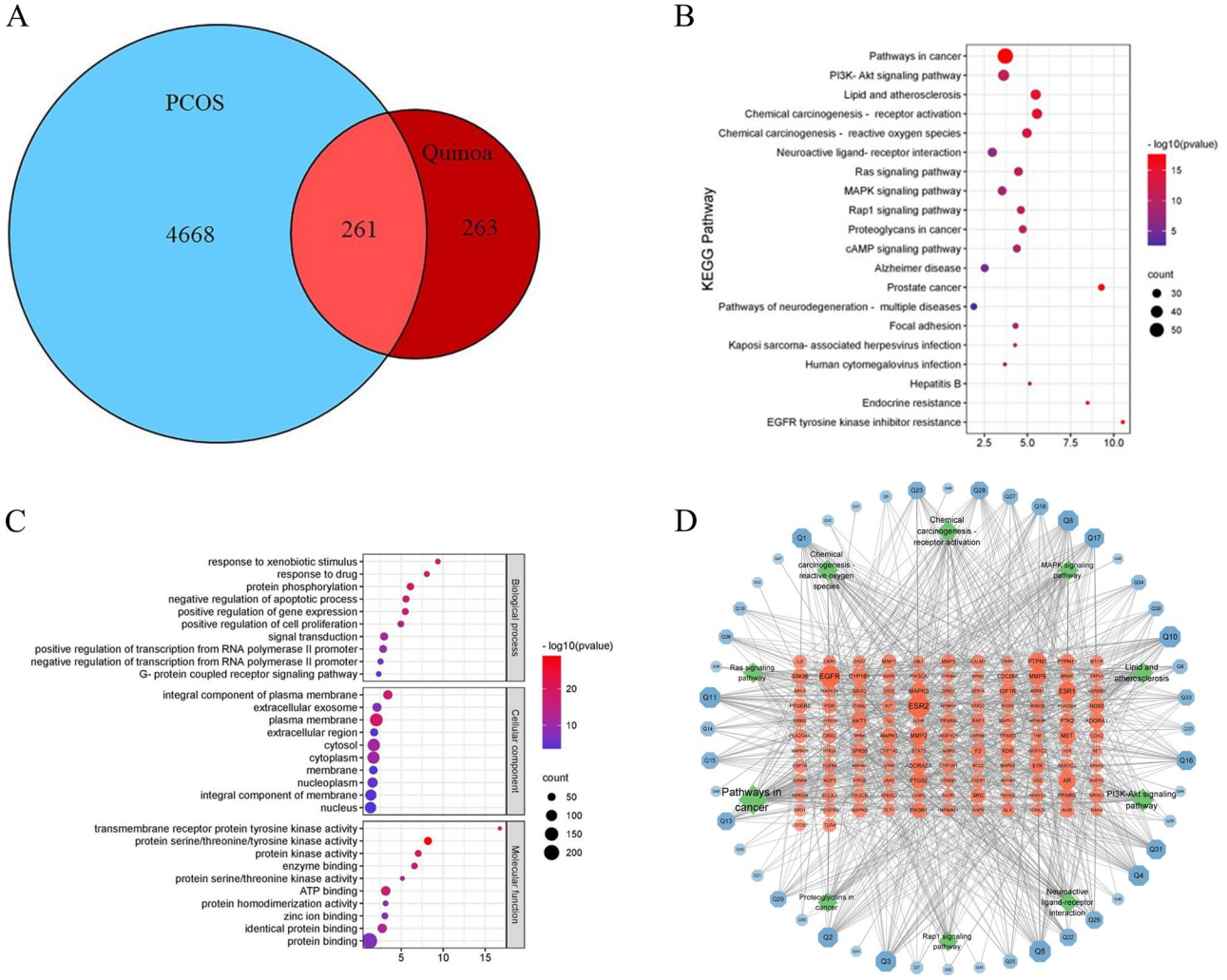


**Fig. 2** Network pharmacology analysis of quinoa on PCOS. (**A**) Venn analysis diagram of quinoa with PCOS. (**B**) KEGG pathway enrichment analysis. (**C**) GO pathway enrichment analysis. (**D**) Component-target-pathway diagram of quinoa in the treatment of polycystic ovary syndrome. (Q stands for quinoa)

binding, ATP binding, protein phosphorylation, enzyme binding, positive regulation of cell proliferation, and et al.

Using Cytoscape 3.9.1 to construct the quinoa component-target-pathway network diagram, and through its built-in tools to analyze the topologic parameters of quinoa intervention in polycystic ovary syndrome target network, get the core components. As shown in Fig. 2D, this network is composed of 181 nodes and 974 edges. The red nodes represent potential targets, the blue nodes represent quinoa active components, the green nodes represent potential signal pathways, and the connecting lines represent the interaction between the three. The larger the node area and the darker the color in the figure, the greater the impact on polycystic ovary syndrome. In cytoscape network analysis, the degree value of Q5 (Flavonol) is 32, the betweenness centrality is 0.0315, and the closeness centrality is 0.419. It is predicted that Flavonol is the main component of quinoa in the intervention of polycystic ovary syndrome, followed by Q10 (apigenin-7-methylether) with the degree value of 31, the betweenness centrality of 0.0389, and the closeness centrality is 0.417, and Q11 (Acacetin) with the degree value of 30, the betweenness centrality of 0.027, and the closeness centrality is 0.415.

### Molecular docking

Seven core potential compounds were docking with eight core targets EGFR (PDBID:3IKA), MET (PDBID:4R1V), MAPK3(PDBID:), AKT1(PDBID:1UNQ), IGF1R (PDBID:1IGR), PIK3R1 (PDBID:2IUG), MAPK1 (PDBID:6SLG), GSK3B (PDBID:1GNG) on the PI3K-AKT signaling pathway, and finally 56 groups of receptor ligand docking results were obtained. Among the 56 groups of receptor ligand results, 30 groups with affinity < -5 kcal · $mol^{-1}$ and affinity < -7.9 kcal · $mol^{-1}$ accounted for more than 50%. The highest docking score is MET-Quercetin, with a score of -9.2 kcal · $mol^{-1}$, and the lowest docking score is AKT1-apigenin-7-methyl, with a score of -5.7 kcal · $mol^{-1}$, indicating that the screened core compounds may have good binding activity with the core targets on the PI3K-AKT signaling pathway. See Fig. 3 for molecular docking results.

### Quinoa may regulate PI3K/AKT/mTOR signaling pathway and autophagy in ovary of PCOS

In order to gain insights into the underlying molecular mechanism of PCOS, we conducted further validation in animal model according to results of network pharmacology. We detected the expression proteins associated with PI3K/AKT/mTOR pathway and autophagy in the ovary by immunohistochemistry and western blot. Results indicated that there was a significantly decrease of PI3K, AKT, mTOR, Bcl-2 and p62 in the PCOS-like rats than the control group. Following quinoa supplementation, importantly increasing trends were shown in the quinoa group compared with PCOS group (Fig. 4A, B, C, D, I, J, K, L, M, O and Q). Meanwhile, there was an obvious increase of Beclin 1, ULK1 and LC3B in the PCOS group than the control group, while there were importantly upregulating trends in the quinoa group versus the PCOS group (Fig. 4A, E, F, J, N, P and R). The results demonstrated that quinoa had the effect on PCOS, which may

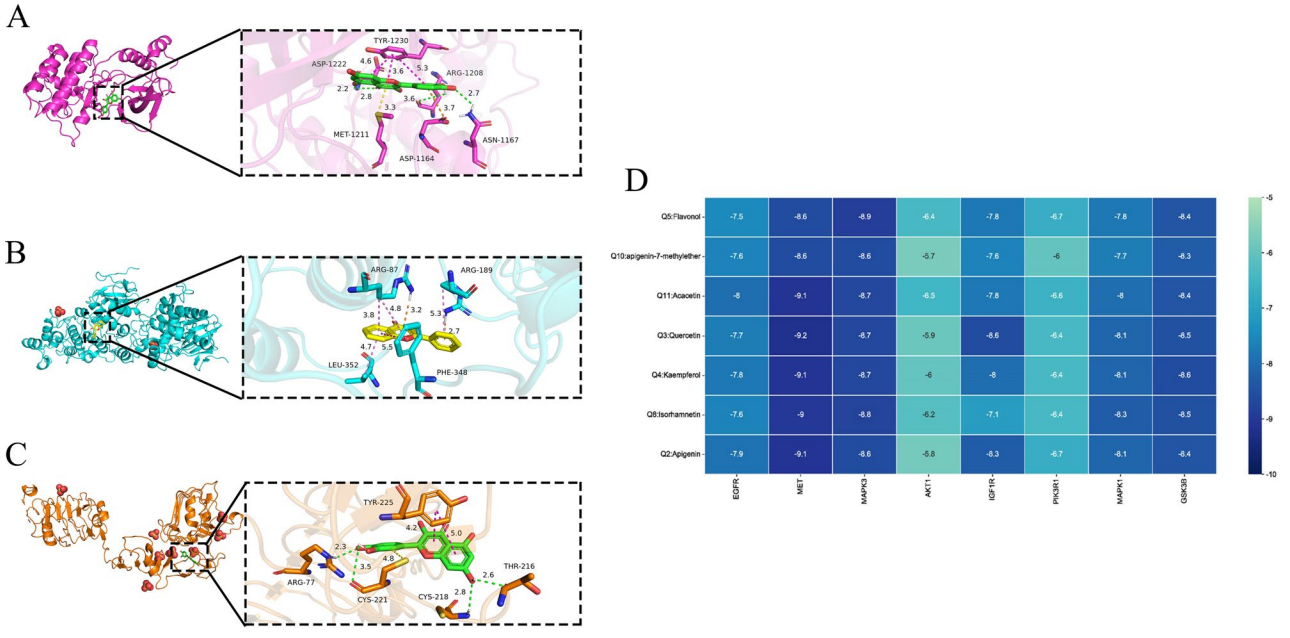


**Fig. 3** Molecular docking of some core compounds in quinoa. (**A**) MET interacts with Quercetin. (**B**) MAPK3 interacts with Flavonol. (**C**) IGF1R interacts with Quercetin. (**D**) Molecular docking results. Unit: Affinity (kcal · $mol^{-1}$)

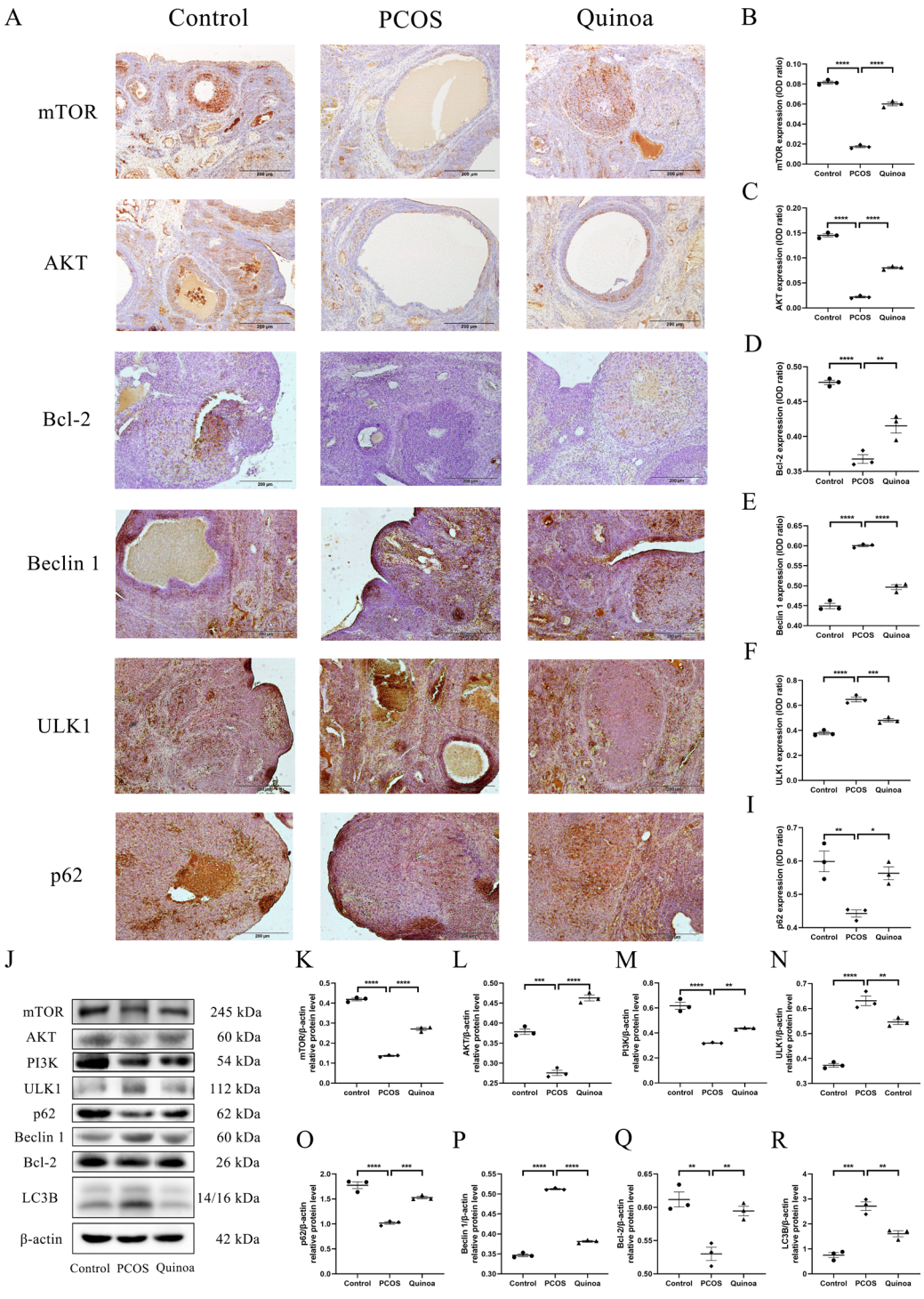


**Fig. 4** Quinoa may regulate PI3K pathway and autophagy in the ovary of PCOS rats. (**A**) IHC of mTOR, AKT, Bcl-2, Beclin 1, ULK1 and p62 in the ovary. (**B**-**I**) Quantification average optical density (AOD) values of mTOR, AKT, Bcl-2, Beclin 1, ULK1 and p62 in the ovary by Image-Pro Plus. (**J**) Representative Western blot of PI3K, AKT, mTOR, ULK1, p62, Beclin 1, Bcl-2 and LC3B in the ovary. (**K**-**R**) Quantitative assessment of the Western blot analysis results of PI3K, AKT, mTOR, ULK1, p62, Beclin 1, Bcl-2 and LC3B by Image J. Values are expressed as mean ± SD ($n = 3$). *$P < 0.05$.** $P < 0.01$. *** $P < 0.001$. **** $P < 0.0001$

associate with PI3K/AKT/mTOR signaling pathway and autophagy.

### Quinoa improved intestinal permeability and autophagy in colon of PCOS rats

To explore the influence of quinoa on duodenal and colonic pathological changes, we examined duodenum and colon tissue sections by HE staining. As shown in Fig. 5A, in the duodenum of PCOS rats, the submucosa was involved, and the villi became blunt. Following the quinoa supplementation, the duodenum was improved compared with the PCOS group. Analogously, the results of colon tissues (Fig. 5B) indicated that the structure of lamina propria, mucous layer and muscle layer of colon in PCOS group were seriously damaged, while that in quinoa group were alleviated, with only slight epithelial damage.

As shown in Fig. 5C-F, a significant reduction in the expression of tight junction protein such as Claudin 5 and Occludin, was observed in the colon of the PCOS group compared to the control group ($P<0.0001$, $P<0.01$). After supplementation with quinoa, Claudin 5 showed an increase compared with the expression in the PCOS group.

In addition, the results of IHC (Fig. 5G-L) showed that the expression of Beclin 1 and ULK1 increased, and the expression of p62 decreased in the PCOS group compared with control group ($P<0.0001$), while Quinoa reversed these trends compared with PCOS group ($P<0.001$, $P<0.001$, $P<0.01$).

### Quinoa regulated the intestinal microflora of PCOS-like rats

We performed 16s rRNA sequence analysis to explore the role of intestinal microflora in PCOS and the therapeutic mechanism of quinoa. The α-diversity was used to examine the differences in intestinal microflora richness and diversity among the control group, PCOS group and quinoa group (Fig. 6A). Although Chao1 index, Shannon index and Simpson index indicated that the gut microbial diversity of PCOS-like rats was lower than control group, the quinoa intervention didn't change the richness and diversity of the microbial community significantly. The β-diversity analysis was provided to assess the differences between microbial communities. As shown in Fig. 6B, principal coordinates analysis (PCoA) indicated that a more difference of intestinal flora composition between control group and PCOS group, similar composition between PCOS group and quinoa group.

Our study also explores the specific changes in fecal microbiota, the related level of microbial community was evaluated at the phylum and genus levels. In control rats, the gut flora was mainly composed of *Firmicutes* (72.67%), *Bacteroides* (24.94%), *Proteobacteria* (1.58%), and *Tenericutes* (1.48%) at phylum levels. However, the abundance of *Firmicutes* was increased and *Bacteroides* and *Tenericutes* were decreased in the model group, following the supplementation of quinoa reversed the variation (Fig. 6C). Also worthy of mention was the ratio of *Firmicutes* to *Bacteroidetes* (*F/B* ratio), and they together accounted for approximately 96% of gut microbiota, which increased in the PCOS group than the control group. Interestingly, the ratio level was upregulated with quinoa supplementation (Fig. 6D). These results suggested that quinoa could regulated the dysbacteriosis in PCOS-like rats. At the genus level, the abundance of *Lactobacillu* was downregulated in the PCOS group than the control group, while *Prevotella* was increased. Compared to the PCOS group, *Lactobacillu* abundance increased in the quinoa group, whereas the *Prevotella* decreased (Fig. 6E). As shown in Fig. 6F-G, we further analyze the abundance on the phylum and the genus. Compared to the PCOS group, quinoa supplementations increased of *Lactobacillu*, *Bacteroides* and *Coprococcus*, while decreased the level of *Blautia*.

LEfSe (LDA Effect Size) analysis was used to evaluate fecal microbiota with a statistically significant difference at the Phylum, Class, Order, Family, and Genus levels. In total, 28 biomarkers were observed from Phylum level to Species level among the control group, PCOS group, and quinoa group, respectively 5, 4, 4, 9 and 6 (Fig. 6H). The purpose of the heat map is to further compare the differences in species composition and to explore the distribution trend of species abundance among groups (Fig. 6I). The results showed that the genus of *Alistipes*, *Desulfovibrio*, *Coprococcus*, *Pseudoxanthomonas*, *Bosea*, *Dehalobacterium*, *Bacillus*, *Helicobacter*, *Pseudobutyrivibrio* were higher in control group than PCOS group and quinoa group. The genus of *p−75−a5*, *Anaerostipes*, *Mycoplasma*, *Clostridium*, *Adlercreutzia*, *Porphyromonas*, *Ethanoligenens*, *Caloramator*, *rc4−4*, *SMB53* increased in PCOS group compared to control group and quinoa group. Interestingly, *Lactobacillus*, *Paraprevotella*, *Roseburia*, *Bacteroides*, *Parabacteroides*, *Rothia*, *Anaerofustis*, *Lachnospira*, *Butyricicoccus*, *Bifidobacterium*, *Streptococcus*, *Faecalibacterium* higher in the quinoa group than PCOS group. Collectively, our results suggested that quinoa supplementation could effectively regulate the intestinal microflora structure in PCOS-like rats.

### Correlations of the key gut microbiota with hormone levels of PCOS rats

In order to investigate the relationships between gut microbiota and PCOS-related hormon, we used Spearman correlation analysis and generated a heatmap to visualize these associations. We selected the top 20 most abundant bacteria based on their relative abundance at both the phylum and genus levels for further analysis. At

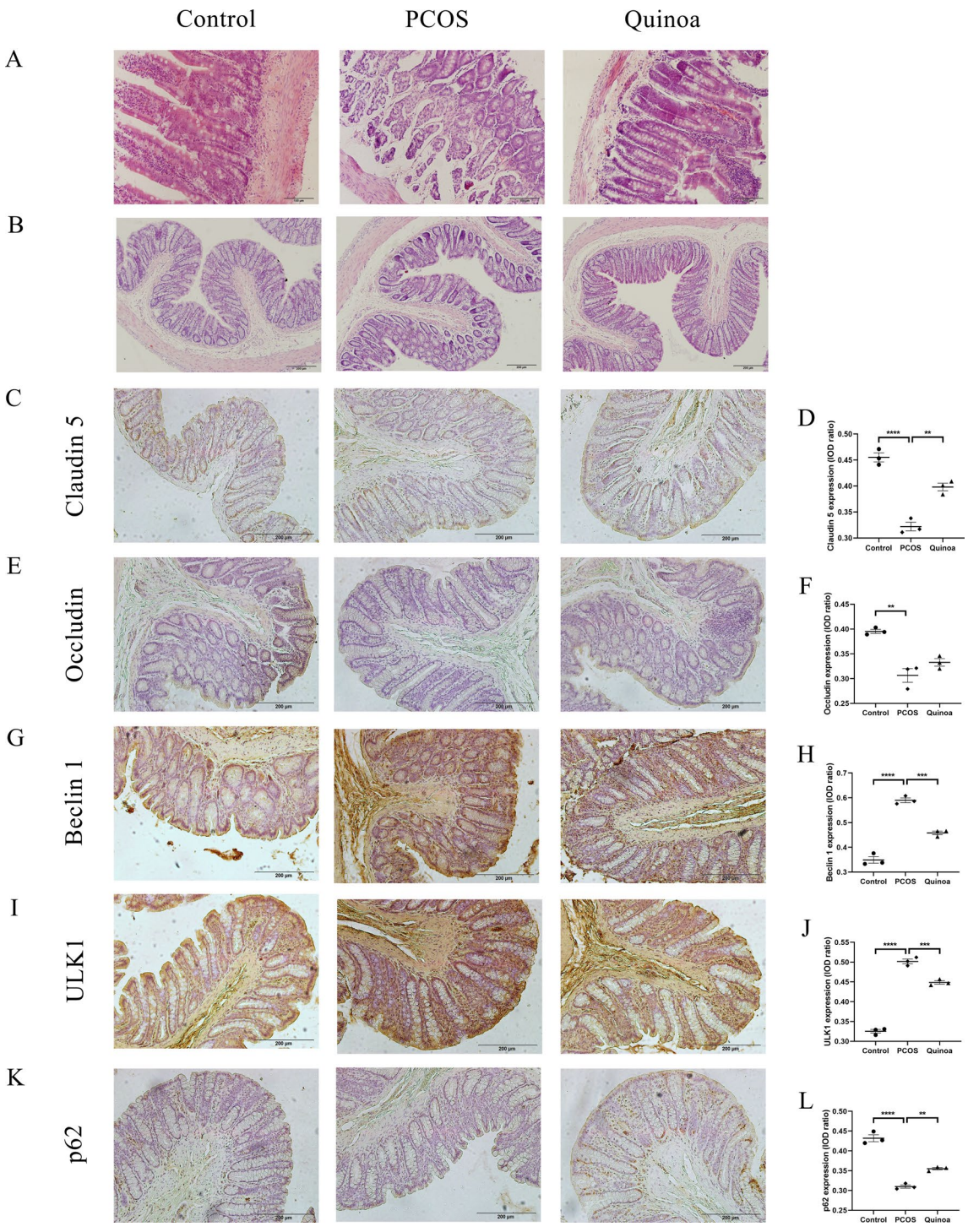


**Fig. 5** Quinoa may improve intestinal permeability and regulate autophagy in the colon of PCOS rats. (**A**) HE staining of duodenum tissue. (**B**) HE staining of colon tissue. (**C**-**L**) IHC and quantification average optical density (AOD) values by Image-Pro Plus of Claudin 5, Occludin, Beclin 1, ULK1 and p62 in the colon Values are expressed as mean ± SD ($n = 3$). *$P < 0.05$.** $P < 0.01$. *** $P < 0.001$. **** $P < 0.0001$

the phylum level (Fig. 7A), *Tenericutes* displayed a strong negative correlation with T levels ($P<0.05$). *Elusimicrobia* showed a negative association with LH ($P<0.05$). Conversely, *Fusobacteria* exhibited positive correlations with LH and FINS. At the genus level (Fig. 7B), *Prevotella* had negative correlations with FSH ($P<0.05$), but positive correlations with LH ($P<0.05$) and LH/FSH ($P<0.01$). *Unclassified_Prevotellaceae* exhibited positive associations with LH/FSH ($P<0.05$), while negative correlations with FSH ($P<0.01$). *Parabacteroides* and

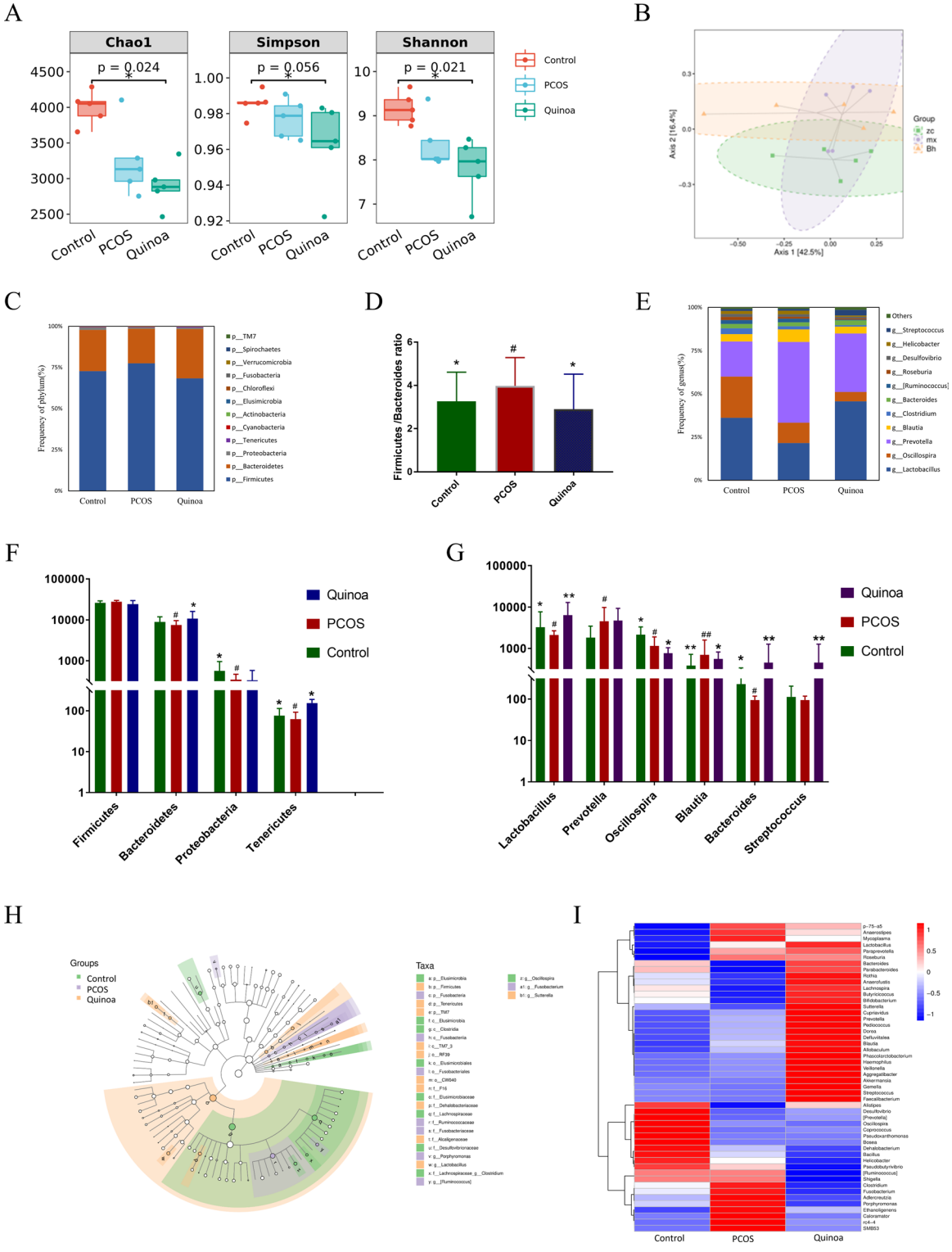


**Fig. 6** Quinoa regulated the gut microbiota composition in PCOS rats. (**A**) Analyses of alpha-diversity (Chao1, Simpson and Shannon indices) in each group. (**B**) Principal coordinates analysis (PCoA) analysis. (**C**) Gut microbiota composition at the phylum level among groups. (**D**) The ratio of *Firmicutes/Bacteroidetes* (*F/B*) in three groups. (**E**) Gut microbiota composition at the genus level among groups. (**F**) The relative abundances of *Firmicutes*, *Bacteroides*, *Proteobacteria and Tenericutes*. (**G**) The relative abundances of *Lactobacillu*, *Bacteroides*, *Oscillospira*, *Blautia*, *Prevotella* and *Streptococcus* in three groups. (**H**) LEfSe analysis. (**I**) Heat map among groups. The red color indicates that the abundance of the genus higher than other, while the blue color showed that lower than other

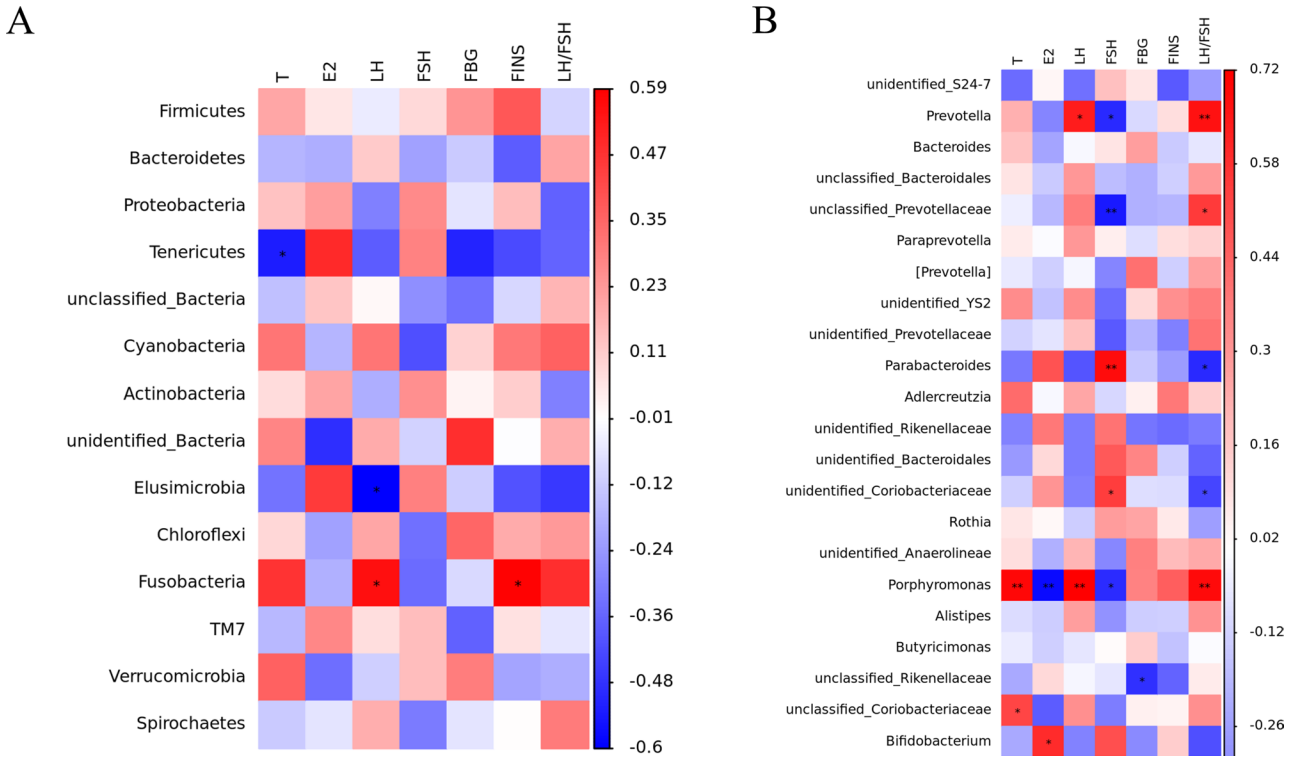


**Fig. 7** Spearman's relationships between the most important gut bacteria and hormone levels of PCOS rats. (**A**) Heatmap of the relationship between the key gut microbiota and hormone levels at the phylum level. (**B**) Heatmap of the relationship between the key gut microbiota and hormone levels at the genus level (|r| > 0.5, FDR adjusted *$P < 0.05$, **$P < 0.01$)

*unidentified_Coriobacteriaeae* showed negative correlations with LH/FSH ($P<0.05$), while positively correlated with FSH ($P<0.01$, $P<0.05$). *Porphyromonas* was significantly positively correlated with T, LH and LH/FSH ($P<0.01$), while negatively correlated with E2 and FSH ($P<0.01$, $P<0.05$). FBG was significantly negatively correlated with *unclassified_ Rikenellaceae* ($P<0.05$). *Unclassified_Coriobacteriaceae* and T, *Bifidobacterium* and E2 exhibited positive correlations ($P<0.05$).

## Discussion

PCOS is a prevalent endocrine and metabolic disorder in premenopausal women, and it is frequently associated with risk factors for abdominal fat, IR, obesity, and metabolic disorders [30, 31]. Hyperandrogenism and IR are important pathological features of PCOS. The level of T was affected by P450arom. Hyperandrogenism results in antral follicle accumulation, decreased FSH and decreased function. FSH-dependent P450arom synthesis is inhibited, and the transformation of ovarian T to E2 slowed down, resulting in an increase in T and a decrease in E2. High LH / FSH ratio is considered to be a diagnostic marker of PCOS, and increasing FSH levels can promote follicular growth [32]. This is consistent with the changes of hormone levels in PCOS rats in our study. And we found that quinoa obviously alleviated symptoms of letrozole-induced PCOS and correlated indices, and reduced the body weight, regulated hormone level, and improved IR in PCOS-like rat. These results implied that quinoa has a positive effect on the treatment of PCOS. However, the mechanism is not yet clear.

To explore the potential mechanism of quinoa on improving PCOS, we performed network pharmacology analysis. Results of KEGG and GO enrichment analysis on key targets showed that quinoa's effect was highly enriched in PI3K-AKT signaling pathway. On this basis, we constructed the component target pathway map based on the relevant information on the first ten pathways enriched by KEGG, so as to predict the core components of quinoa in the treatment of PCOS. Molecular docking was performed between the core components and the targets on the PI3K-Akt signaling pathway, and the docking results of 56 groups were < -5 kcal · $mol^{-1}$, indicating that there was a good binding between the core components of quinoa and the targets on the PI3K/Akt signaling pathway. We verified the above prediction results in PCOS rats and found that PI3K/Akt/mTOR signaling pathway was down-regulated in PCOS rats, which was reversed by quinoa. Studies have shown that androgen testosterone and luteinizing hormone concentration abnormally increase in patients with PCOS, which inhibits the development of early follicles and leads to

the accumulation of a large number of early follicles in the ovary, unable to form mature follicles and dominant follicles, resulting in anovulation [33]. Yan's study showed that modulating the PI3K/AKT/mTOR signal pathway could protect rat ovarian granulosa cell [34]. Meanwhile, the PI3K/Akt/mTOR pathway is another well-known insulin effector pathway that increases insulin sensitivity by activating the PI3K-Akt insulin signaling pathway [35].

Additionally, obesity is known to increase release of some growth factors and inflammatory factors, which may cause the ovaries to produce excess androgens and inhibit the aromatization of androgens into estrogens [36]. The maintenance of adipose tissue metabolic function requires the coordination of many pathways, which autophagy regulation is considered to be an important one. Studies have shown that adipocyte autophagy markers LC3-II and ATG5 protein expression are higher in obese people, while mTOR protein expression is significantly decreased [37]. The quinoa secondary metabolites, 20HE and epicatechin epigallocatechin-3-gallate, may be directly or indirectly involved in PI3K/Akt/mTOR signal pathway [38]. In our study, quinoa could upregulate the expression of PI3K, AKT, mTOR and downregulate autophagy-related proteins in the ovary. We speculate that quinoa improves PCOS, including decreased body weight, the insulin and testosterone levels, associated with its some related components, which could directly or indirectly regulate PI3K/AKT/mTOR signal pathways and autophagy.

Host-microbe symbiosis is critical in maintaining intestinal homeostasis. Studies using in vitro and in vivo models as well as human clinical studies have shown that autophagy is essential for the maintenance of intestinal homeostasis, regulation of intestinal ecology, proper intestinal immune response, and antimicrobial protection [39]. Defective autophagy has been reported to strongly influence the course of PCOS by disrupting intestinal homeostasis, affecting gut microbiota composition, impairing intracellular bacterial clearance, and amplifying intestinal inflammation [40].

The intestine is widely considered to be the most important organ in digestion, endocrine function and immune response [41]. Studies have shown that PCOS patients exist increased intestinal epithelial permeability [42], which is correlated with IR and severity of menstrual disorders. It suggests that alterations in gut permeability may play a role in the pathophysiology of PCOS [43]. Tight junction proteins between epithelial cells (such as Claudin protein family, ZO-1 and Occludin) form most of the intestinal mucosal barrier to prevent toxins and antigens from passing through the mucosa. In this study, a significant decrease in the expression of Claudin 5 and Occludin was observed in the colon of PCOS rats, accompanied by a substantial increase in intestinal permeability, suggesting a compromised intestinal barrier function. This finding is consistent with previous literature [44], and it suggests that changes in hormone levels may be partially attributed to the increased permeability of the intestines.We found it noteworthy that quinoa increased the expression of tight junction protein that protects the intestinal mucosal barrier, leading to a decrease in intestinal permeability. This also serves as the basis for quinoa's protective effects on PCOS mediated through gut microbiota.

More and more evidence show that gut microbes and their metabolites are closely related to the occurrence and development of PCOS [45, 46]. Studies have reported that compared with healthy people, PCOS intestinal microbial β diversity is reduced, and there is a negative correlation between hyperandrogenism and β diversity [47]. Consistent with previous research results, our results show that quinoa does not change the intestinal microbial species richness of PCOS rats, but could significantly change the composition of the intestinal microbial community.

For intestinal microbiota, quinoa promoted the relative abundance of *Bactreoides*, *Lactobacillu* and repressed the level of Gram-negative bacteria, especially, *Prevotella*. This is consistent with previous report [48]. Short-chain fatty acids (SCFAs) are well known candidates that produced by gut bacteria such as Bacteroides. Previous studied showed that the contents of SCFAs may associated with the role of preventing inflammation and improving insulin sensitivity in mice [49]. A growing body of literature highlight the contribution of probiotics and PI3K/Akt/mTOR signaling pathway in metabolic syndrome, gastrointestinal disorders [50]. *Lactobacillus* strains have long been thought to have the ability to prevent many human diseases and enhance the production of anti-inflammatory cytokines involved in immunity by modulating the PI3K/Akt/mTOR pathway [51]. Notably, following our experiment, the abundance of *Bactreoides* and *Lactobacillu* was increased in quinoa group rats. In addition, *Prevotella* is a potential proinflammatory bacterium that is strongly related to low-grade inflammation and IR [52, 53]. Interestingly, our results showed that quinoa reduced the abundance of *Prevotella*. Therefore, we speculate that quinoa may improve PCOS by increasing the abundance of *Bactreoides*, *Lactobacillu*, and reduce *Prevotella* to affect the insulin and anti-inflammation, which may be linked with PI3K/AKT/mTOR. In addition, *Bacteroidetes* and *Firmicutes* were the dominant bacteria in the control, PCOS, and quinoa groups. Human and animal studies have shown that higher ratios of *F/B* make the gut microbiota more efficient at extracting energy from the diet, which may contribute to adiposity [54, 55]. Evidence from recent study suggested that the *F/B* ratio, considered a known marker of bacterial disease, is

positively associated with enhanced expression of inflammatory cytokines and obesity [56]. A lower *F/B* ratio is thought to be beneficial in improving obesity and inflammation. Our work demonstrated the above and observed that quinoa can reduce *F/B* ratio and the *Blautia* level, thus reducing body weight, which could be a potential factor to improve PCOS by reducing obesity through quinoa.

## Conclusions

In summary, we found that the PI3K/AKT pathway was closely associated with the effect of quinoa in the treatment of PCOS by network pharmacology. Further study proved that quinoa supplementation modulated sex hormone levels, alleviated insulin resistance, reversed the high intestinal permeability and intestinal microflora dysbiosis, which may be related to PI3K/AKT/mTOR signaling pathway and autophagy.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12986-024-00855-3.

Supplementary Material 1

**Acknowledgements**
Not applicable.

**Author contributions**
GJ and RZ designed the experiments; YW wrote the manuscript; JD and RH revised the manuscript; JD, YZ, YW, JL, and XZ performed the experiments; TW, CZ, and YL analyzed the data. All authors read and approved the final manuscript.

**Funding**
This work was supported by Grants from the National Natural Science Foundation of China (82260931).

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

## Declarations

**Ethics approval and consent to participate**
The study protocol was approved by the Animal Care and Management Committee of the Beijing University of Chinese Medicine. All manipulations were at the request of the guidelines of the Animal Care Committee.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

**Author details**
[1]School of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing 100029, China
[2]Affiliated Hospital of Youjiang Medical University for Nationalities, Key Laboratory of Research on Clinical Molecular Diagnosis for High Incidence Diseases in Western Guangxi of Guangxi Higher Education Institutions, Key Laboratory of Research and Development on Clinical Molecular Diagnosis for High-Incidence Diseases of Baise, Guangxi, China
[3]Beijing Zhongli Biological Technology Co., Ltd, Beijing, China
[4]Gansu Chunjie Plateau Agricultural Technology Co., Ltd, Wuwei, China
[5]Institute of Materia Medica, Xinjiang Uyghur Autonomous Region, Urumqi 830004, China

Received: 21 June 2024 / Accepted: 20 September 2024
Published online: 11 October 2024

**References**

1. Stańczak NA, Grywalska E, Dudzińska E. The latest reports and treatment methods on polycystic ovary syndrome. Ann Med. 2024;56(1):2357737.
2. Lizneva D, et al. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. Fertil Steril. 2016;106(1):6–15.
3. Wekker V, et al. Long-term cardiometabolic disease risk in women with PCOS: a systematic review and meta-analysis. Hum Reprod Update. 2020;26(6):942–60.
4. Shahid R, et al. Diet and lifestyle modifications for effective management of polycystic ovarian syndrome (PCOS). J Food Biochem. 2022;46(7):e14117.
5. Zhang H, et al. Obesity is associated with lower levels of negative emotions in polycystic ovary syndrome in clinical and animal studies. Ann Med. 2024;56(1):2373199.
6. Pace L et al. *Economic Burden of Endometrial Cancer Associated With Polycystic Ovary Syndrome.* J Clin Endocrinol Metab, 2024. null.
7. Glintborg D, et al. Prospective risk of type 2 diabetes in 99 892 nordic women with polycystic ovary syndrome and 446 055 controls: national cohort study from Denmark, Finland, and Sweden. Hum Reprod. 2024;39(8):1823–34.
8. Tay CT et al. 2023 *International Evidence-Based Polycystic Ovary Syndrome Guideline Update: Insights From a Systematic Review and Meta-Analysis on Elevated Clinical Cardiovascular Disease in Polycystic Ovary Syndrome.* J Am Heart Assoc, 2024. 13(16): p. e033572.
9. Lin M et al. Quinoa secondary metabolites and their Biological activities or functions. Molecules. 2019;24(13).
10. Graf BL, et al. Quinoa seeds leach phytoecdysteroids and other compounds with anti-diabetic properties. Food Chem. 2014;163:178–85.
11. Noratto GD, Murphy K, Chew BP. Quinoa intake reduces plasma and liver cholesterol, lessens obesity-associated inflammation, and helps to prevent hepatic steatosis in obese db/db mouse. Food Chem. 2019;287:107–14.
12. Zheng CY, et al. Revealing the mechanism of quinoa on type 2 diabetes based on intestinal flora and taste pathways. Food Sci Nutr. 2023;11(12):7930–45.
13. An T, et al. Supplementation of quinoa regulates glycolipid metabolism and endoplasmic reticulum stress in the high-fat diet-induced female obese mice. Volume 18. Nutrition & Metabolism; 2021. 1.
14. Dou J et al. Quinoa alleviates osteoporosis in ovariectomized rats by regulating gut microbiota imbalance. J Sci Food Agric. 2024.
15. Wang T-Y et al. Quinoa reduces high-fat diet-induced obesity in mice via potential Microbiota-gut-brain-liver interaction mechanisms. Microbiol Spectr. 2022;10(3).
16. Bo X et al. Metformin ameliorates polycystic ovary syndrome in a rat model by decreasing excessive autophagy in ovarian granulosa cells via the PI3K/AKT/mTOR pathway. Endocr J, 2022.
17. Jiang X, et al. Bu-shen-zhu-Yun decoction inhibits granulosa cell apoptosis in rat polycystic ovary syndrome through estrogen receptor α-mediated PI3K/AKT/mTOR pathway. J Ethnopharmacol. 2022;288:114862.
18. Meihe L et al. *Quercitrin alleviates lipid metabolism disorder in polycystic ovary syndrome-insulin resistance by upregulating PM20D1 in the PI3K/Akt pathway.* Phytomedicine, 2023.
19. Li D, et al. Autophagy is activated in the ovarian tissue of polycystic ovary syndrome. Reproduction. 2018;155(1):85–92.
20. Li X, et al. The role of androgen in autophagy of granulosa cells from PCOS. Gynecol Endocrinol. 2019;35(8):669–72.
21. Kumariya S, et al. Autophagy in ovary and polycystic ovary syndrome: role, dispute and future perspective. Autophagy. 2021;17(10):2706–33.
22. Yilu Z, Jie H. Gut microbiota: guardians of the female gut health. Cancer Cell. 2023.
23. He Y, et al. The emerging role of the gut microbiome in polycystic ovary syndrome. Volume 2. F&S Reviews; 2021. pp. 214–26. 3.
24. Jobira B, et al. Obese adolescents with PCOS have altered Biodiversity and relative abundance in gastrointestinal microbiota. J Clin Endocrinol Metab. 2020;105(6):e2134–44.

25. Yushan L, et al. Depletion of gut microbiota influents glucose metabolism and hyperandrogenism traits of mice with PCOS induced by letrozole. Frontiers in Endocrinology; 2023.
26. Yue-lian Y et al. *Intestinal Flora is a Key Factor in Insulin Resistance and Contributes to the Development of Polycystic Ovary Syndrome.* Endocrinology, 2021.
27. Li Y, et al. Depletion of gut microbiota influents glucose metabolism and hyperandrogenism traits of mice with PCOS induced by letrozole. Front Endocrinol (Lausanne). 2023;14:1265152.
28. Wu Y-X, et al. Naringenin regulates gut microbiota and SIRT1/ PGC-1α signaling pathway in rats with letrozole-induced polycystic ovary syndrome. Volume 153. Biomedicine & Pharmacotherapy; 2022. p. 113286.
29. Wang R, et al. Effect of the ketogenic diet on gut microbiome composition and metabolomics in polycystic ovarian syndrome rats induced by letrozole and a high-fat diet. Nutrition. 2023;114:112127.
30. Escobar-Morreale HF. Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. Nat Rev Endocrinol. 2018;14(5):270–84.
31. Jeanes YM, Reeves S. Metabolic consequences of obesity and insulin resistance in polycystic ovary syndrome: diagnostic and methodological challenges. Nutr Res Rev. 2017;30(1):97–105.
32. Yang J, Chen C. Hormonal changes in PCOS. J Endocrinol, 2024;261(1).
33. Franks S, Stark J, Hardy K. Follicle dynamics and anovulation in polycystic ovary syndrome. Hum Reprod Update. 2008;14(4):367–78.
34. Yan J et al. Catalpol protects rat ovarian granulosa cells against oxidative stress and apoptosis through modulating the PI3K/Akt/mTOR signaling pathway. Biosci Rep. 2020;40(4).
35. Wu C, et al. Exercise activates the PI3K-AKT signal pathway by decreasing the expression of 5α-reductase type 1 in PCOS rats. Sci Rep. 2018;8(1):7982.
36. Wang F, et al. Analyses of optimal body mass index for infertile patients with either polycystic or non-polycystic ovary syndrome during assisted reproductive treatment in China. Sci Rep. 2016;6:34538.
37. Chen W, Pang Y. Metabolic syndrome and PCOS: pathogenesis and the role of metabolites. Metabolites. 2021;11(12).
38. Jiao W, et al. Integrated network pharmacology and cellular assay for the investigation of an anti-obesity effect of 6-shogaol. Food Chem. 2022;374:131755.
39. Foerster EG, et al. How autophagy controls the intestinal epithelial barrier. Autophagy. 2022;18(1):86–103.
40. Larabi A, Barnich N, Nguyen HTT. New insights into the interplay between autophagy, gut microbiota and inflammatory responses in IBD. Autophagy. 2020;16(1):38–51.
41. Rastelli M, Cani PD, Knauf C. Gut Microbiome Influences Host Endocr Funct Endocr Rev. 2019;40(5):1271–84.
42. Ibrahim M, Ahmeid M. Metformin effects on zonulin level in polycystic ovarian women. Admet Dmpk. 2021;9(1):49–55.
43. Lingaiah S, et al. Markers of gastrointestinal permeability and dysbiosis in premenopausal women with PCOS: a case-control study. BMJ Open. 2021;11(7):e045324.
44. Rizk FH, et al. Ameliorating effects of adropin on letrozole-induced polycystic ovary syndrome via regulating steroidogenesis and the microbiota inflammatory axis in rats. J Physiol. 2024;602(15):3621–39.
45. Yurtdaş G, Akdevelioğlu Y. A New Approach to Polycystic Ovary Syndrome: the gut microbiota. J Am Coll Nutr. 2019;39(4):371–82.
46. Li T et al. Yogurt enriched with inulin ameliorated reproductive functions and regulated gut microbiota in dehydroepiandrosterone-induced polycystic ovary syndrome mice. Nutrients. 2022;14(2).
47. Le Chatelier E, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013;500(7464):541–6.
48. Yanjie G, et al. Association between Polycystic Ovary Syndrome and Gut Microbiota. Plos One; 2016.
49. Zhao Y, et al. Gut microbiota composition modifies fecal metabolic profiles in mice. J Proteome Res. 2013;12(6):2987–99.
50. Mohseni AH, et al. Modulation of the PI3K/Akt/mTOR signaling pathway by probiotics as a fruitful target for orchestrating the immune response. Gut Microbes. 2021;13(1):1–17.
51. Dai C, et al. VSL#3 probiotics exerts the anti-inflammatory activity via PI3k/Akt and NF-κB pathway in rat model of DSS-induced colitis. Mol Cell Biochem. 2013;374(1–2):1–11.
52. Larsen JM. The immune response to Prevotella bacteria in chronic inflammatory disease. Immunology. 2017;151(4):363–74.
53. Pedersen HK, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature. 2016;535(7612):376–81.
54. Yang JY, et al. Gut commensal Bacteroides acidifaciens prevents obesity and improves insulin sensitivity in mice. Mucosal Immunol. 2017;10(1):104–16.
55. Turnbaugh PJ, et al. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe. 2008;3(4):213–23.
56. Chu W, et al. Continuous light-Induced PCOS-Like Changes in Reproduction, Metabolism, and gut microbiota in Sprague-Dawley rats. Front Microbiol. 2019;10:3145.
57. Feng, Xueping et al. Dendrobium officinale polysaccharide ameliorates polycystic ovary syndrome via regulating butyrate dependent gut-brain-ovary axis mechanism. Front Endocrinol. 2022;13:962775. https://doi.org/10.3389/fendo.2022.962775
58. He, Hefan et al. Sevoflurane post-conditioning attenuates traumatic brain injury-induced neuronal apoptosis by promoting autophagy via the PI3K/AKT signaling pathway. Drug Design Develop Ther. 2018;12:629–638. https://doi.org/10.2147/DDDT.S158313


## Publisher's note