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Britanin improved high-fat diet-induced obesity by regulating the MAPK signaling pathway

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Abstract: **Background**: The escalating prevalence of obesity has made it a critical public health concern. There is an urgent need to identify naturally derived compounds with anti-obesity potential. Britanin (BRI), a bioactive sesquiterpene lactone derived from Inula species, has shown promise in metabolic disorder management, but its anti-obesity mechanisms remain uncharacterized. **Objectives**: Combining animal experiments and network pharmacology analysis to explore the effect of BRI in high-fat diet-induced obesity. **Methods**: C57BL/6J male mice were used for experiment. A high-fat diet (HFD)-induced obese mouse model was treated with BRI (5/15 mg/kg, i.p.) to validate lipid metabolism and weight loss. Network pharmacology identified potential targets via SwissTargetPrediction, GeneCards and OMIM databases, with molecular docking (CB-DOCK) and PPI network analysis (STRING/Cytoscape). Relevant validations were conducted based on the screened targets. Additionally, a biosafety assessment was performed. **Results**: *In-vivo*, 15 mg/kg BRI reduced body weight by 18%, decreased serum TG (-45%, $p<0.001$), TC (-37%, $p<0.001$) and LDL-C (-32%, $p<0.01$) and reversed adipocyte hypertrophy. Thirty-nine intersection targets were identified, with MAPK1, EGFR, PTGS2, MAP2K1 and MAPK8 as top hubs (degree centrality >15). BRI exhibited strong binding affinity (-7.7 to -10.3 kcal/mol) to these targets. Mechanistically, BRI exerts its anti-obesity effects by regulating key targets within the MAPK signaling pathway, particularly MAPK1 and inhibited the PPARγ, thereby blocking adipogenesis and promoting the transition of adipose tissue. **Conclusion**: BRI may alleviate obesity by regulating the Mitogen-Activated Protein Kinase signaling pathway, providing a rationale for natural compound-based obesity therapy.

Keywords: Britanin; MAPK; Network pharmacology; Obesity

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INTRODUCTION

Globally, the incidence of obesity has risen sharply over the past few decades, leading to its classification as a significant public health burden. (Perdomo *et al.,* 2023). As a complex and highly prevalent chronic disease, obesity is strongly linked to a wide spectrum of comorbidities, including cardiovascular diseases, osteoarthritis, obstructive sleep apnea, type 2 diabetes and various cancers, such as breast, colorectal, endometrial (Lauby-Secretan *et al.,* 2016; Yárnoz-Esquiroz *et al.,* 2022). These comorbidities contribute to significant morbidity and mortality, underscoring the urgent need for effective obesity management strategies. The growing prevalence of obesity has become a major public health concern worldwide. Obesity has emerged as one of the most pressing global health challenges. Future projections are even more concerning. Current estimates indicate that by 2050, around 280 million children worldwide may be living with obesity. Among adults, the number is expected to exceed 4 billion. Such figures illustrate the scale of the epidemic and reinforce the need for new preventive and therapeutic approaches. (Chooi *et al.,*2019; Kivimäki *et al.,* 2022; Jessica A Kerr *et al.,* 2025).

Sustained weight loss of more than 10% of total body weight has been shown to significantly improve obesity-related complications, while also enhancing overall quality of life (Ahern *et al.,* 2017; Lean *et al.,* 2019). Current therapeutic strategies for obesity mainly include lifestyle modification, pharmacological treatment, endoscopic interventions and bariatric surgery. These approaches constitute the mainstay of clinical management. However, pharmacotherapy remains constrained by limited effectiveness. Many anti-obesity drugs provide only modest weight-loss outcomes and are often accompanied by undesirable adverse effects, which restrict their long-term clinical use. (Martel *et al.,* 2017). Notably, several pharmacological agents have been withdrawn from the market due to their associations with severe cardiovascular and neurological complications (Walter *et al.,* 2014). These limitations have led to growing interest in the identification of naturally derived

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compounds as potential therapeutic agents for obesity and its related diseases (Martel *et al.,*2017; Wan *et al.,*2022).

Current pharmacological treatments for obesity, such as Phentermine and Semaglutide, have shown clinical benefits but still face limitations related to efficacy plateaus and potential safety concerns. These challenges have prompted increasing interest in alternative therapeutic sources, particularly bioactive compounds derived from natural products. Among them, Britanin (BRI), a sesquiterpene lactone isolated from plants of the genus Inula, has attracted attention because of its multitarget pharmacological properties and relatively low toxicity. Previous studies have demonstrated that BRI possesses strong antioxidant, antitumor activities and anti-inflammatory. (Wang *et al.,* 2014; Zeng *et al.,* 2020; Kajdanek *et al.,* 2024). Previous studies have demonstrated that BRI exerts its effects through the modulation of cellular proliferation, apoptosis, autophagy, immune responses and metabolic processes (Kajdanek *et al.,*2024). Recent studies suggest that BRI can ameliorate non-alcoholic steatohepatitis induced by high-cholesterol, high-fat and high-fructose diets in mice. (Dou *et al.,*2024). Despite these promising findings, the anti-obesity potential of BRI remains unexplored. This study was designed to evaluate its efficacy and mechanistic pathways, with the ultimate goal of developing BRI as a safe and effective anti-obesity agent.

Network pharmacology is a branch of systems biology and interplay between drugs and biological systems (Hopkins 2008; Zhang GB *et al.,* 2013). In recent years, continuous advancements in network pharmacology have positioned it as a powerful tool in drug discovery and this approach has been particularly valuable in traditional Chinese medicine (TCM) research (Yang *et al.,* 2020; Li *et al.,* 2023). Molecular docking is a computational technique used to predict interactions between proteins and ligands (such as drug molecules). By integrating network pharmacology with molecular docking, potential anti-obesity compounds were pre-screened prior to *in-vivo* experimentation, thereby establishing a reliable theoretical framework for subsequent experimental validation.

This study integrates computational target prediction and *In-vivo* validation to uncover the anti-obesity mechanism of BRI and to verify the potential mechanisms. The objective is to identify a safe and effective anti-obesity agent devoid of toxic side effects.

MATERIALS AND METHODS

Animal experiments

Animals and housing conditions

Ethical approval for all experimental procedures was granted by the Suzhou University Animal Ethics Committee (No. 202408A0490), following guidelines for animal care. The study utilized 24 male C57BL/6J mice (5 weeks old; 18 ± 1 g) sourced from the university's Laboratory Animal Center. Housing was provided in a specific pathogen-free (SPF) facility where environmental factors were tightly controlled, including a 12-h light/dark cycle, temperature set at 25±2 °C and humidity levels of 50±10%.

Experimental design, obesity model induction and drug administration

The animal study followed previously established protocols (Machinal-Quélin *et al.,*2002; Zeng *et al.,*2020; Dou *et al.,*2024). After a 5-day acclimation period, mice were randomly assigned to two dietary groups: a control group (n = 6) fed a standard normal diet and an obesity group (n = 18) fed a high-fat diet (HFD). Body weight was recorded weekly, usually obesity was confirmed when the average weight of the HFD-fed mice exceeded ≥20% of the control group (Zhou *et al.,* 2023), but in this study, the mice whose body weight increased by more than 30% compared to the control group were ultimately selected as the obesity group, because more stringent thresholds improve model robustness and metabolic consistency. The dosage was selected based on literature consultation (Wu *et al.,* 2017) In a related preliminary study, doses exceeding 50 mg/kg were found to result in reduced animal activity; therefore, lower doses of 5 mg/kg and 15 mg/kg were chosen for the current investigation. Mice with significantly lower body weights were excluded from the study.

The remaining obese mice were further randomized into three groups: (1) Obesity group (vehicle-treated), (2) Obesity + BRI 5 mg/kg group and (3) Obesity + BRI 15 mg/kg group. Standard normal diet: 10% fat, 19% protein, 71% carbohydrates, 3.6 kcal/g. HFD: 60% fat, 19.4% protein, 20.6% carbohydrates, 5.0 kcal/g (provided by Wuxi Fanbo Biotechnology Co., Ltd.). BRI was administered via intraperitoneal injection for 7 consecutive days at the designated doses, the 7 days intervention duration was determined based on well-established short-term intervention models for obesity-related metabolic studies in rodents, as widely adopted in peer-reviewed literature (Kern *et al.,* 2024; Lee *et al.,* 2025). Body weight was monitored every three days. BRI (purity ≥ 98%) was purchased from Chengdu Dest Biotech/Lemei Tian Pharmaceuticals (catalog no. 33627-28-0) and was supplied as a colorless, odorless crystalline powder, stored at 4°C under low-temperature conditions.

Blood sample collection and tissue harvesting

Following a 7-day drug withdrawal period, all mice underwent a 12-hour fasting period before anesthesia with sodium pentobarbital. Blood samples were collected via retro-orbital extraction using ophthalmic forceps and transferred into coagulation tubes. After standing at room

temperature for 30 minutes, the samples were centrifuged at 3000 rpm for 15 minutes to separate the serum.

After euthanasia via thoracotomy and laparotomy, systemic perfusion was performed by opening the right atrium and perfusing with PBS. The liver, kidneys, epididymal white adipose tissue (WAT) and interscapular brown adipose tissue (BAT) were harvested, washed with PBS to remove residual debris and weighed using an analytical balance. Representative tissue samples were fixed for histological analysis and the remaining portions were stored at −80°C for subsequent molecular experiments.

Serum lipid and glucose analysis

To assess metabolic profiles, serum levels of VLDL, HDL-C, TC, TG, LDL-C and glucose were measured via a Mindray BS-420 automated biochemical system. The protocol involved analyzing 300μL aliquots of each serum sample in duplicate. Final datasets were then compiled for statistical assessment.

Histological analysis

Paraffin sectioning and hematoxylin & eosin (H&E) staining

Standard histological processing was performed on fresh tissues, beginning with fixation in 10% formalin (≥24 h), followed by graded ethanol dehydration and paraffin infiltration. Sections obtained by microtomy were floated on a 42°C water bath and affixed to slides, which were then dried at 65°C. Subsequent steps included deparaffinization, rehydration and hematoxylin and eosin (H&E) staining. To finalize preparation for microscopic examination, sections were dehydrated, cleared in xylene and sealed with neutral resin.

Frozen sectioning and oil red O staining

For lipid accumulation analysis, fresh tissues were fixed in 4% paraformaldehyde for at least 24 hours, then cryoprotected in 15% and 30% sucrose solutions at 4°C. Dehydrated tissues were embedded in OCT compound and rapidly frozen. Cryosections (8–10μm) were mounted on glass slides and stored at -20°C until staining. Sections were thawed, air-dried and fixed in fixative for 15 minutes. After washing with tap water, the slides were stained with Oil Red O for 8–10 minutes in the dark. Excess stain was removed with 75% ethanol, followed by hematoxylin counterstaining. After differentiation in acid alcohol and bluing in ammonium hydroxide solution, sections were mounted with glycerol gel and examined under a microscope (Ramírez-Zacarías *et al.,*1992).

Quantitative real-time PCR (qPCR) analysis

Total RNA was extracted from adipose tissue using TRIzol reagent. RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer. cDNA synthesis was performed using reverse transcription kits. Gene expression levels were quantified on a QuantStudio 5 real-time PCR system using SuperReal PreMix Plus SYBR Green. GAPDH was used as an internal reference and relative gene expression was calculated using the 2^−ΔΔCt method. Primer sequences are listed in table S1(Livak and Schmittgen 2001).

Western blot analysis

Protein extraction from 60 mg adipose tissue was conducted using a tissue-specific kit, followed by concentration assessment via BCA assay. Separated proteins (SDS-PAGE) were blotted onto PVDF membranes and subjected to blocking. Primary antibody incubation (1:1000 for MAPK1, MAP2K1, p-MAPK1 and p-MAP2K1) was carried out overnight at 4°C. Subsequent washing involved three cycles of 15 min in TBST (0.05% Tween-20), after which membranes were exposed to HRP-conjugated anti-rabbit IgG (1:1000) for 2 h at ambient temperature. Chemiluminescent signals were generated using ECL Plus and imaged accordingly. Densitometric analysis was executed in ImageJ, normalizing target band densities to the loading control(Liu *et al.,*2014).

Drug toxicity assessment

To assess the safety of BRI, serum biochemical parameters were analyzed using an automated biochemical analyzer, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Meanwhile, liver and kidney samples were collected for hematoxylin and eosin (H&E) staining to evaluate potential histopathological changes induced by different BRI doses.

Network pharmacology

Prediction of drug targets for BRI

The molecular structure and formula of Britanin (BRI) were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) using its PubChem CID 14466541. The molecular formula was subsequently imported into the Swiss Target Prediction database (http://swisstargetprediction.ch/) to predict potential drug targets. Data retrieval was limited to Homo sapiens, selecting only those predicted targets with a probability score > 0. These candidates underwent verification against the UniProt database (https://www.uniprot.org/). Concurrently, to compile a list of obesity-related targets, the keyword 'obesity' was employed to mine relevant gene entries from both the GeneCards (https://www.genecards.org/) and OMIM (https://www.omim.org/) repositories.

PPI network construction and core target screening

Intersection analysis was conducted on the Bioinformatics platform (https://www.bioinformatics.com.cn/) to converge predicted drug targets from Swiss Target Prediction with obesity-related targets curated from GeneCards and OMIM. The identified common genes served as input for the STRING database (https://cn.string-db.org/) to establish a protein-protein

interaction (PPI) network. Following the export of the network topology and TSV data, further topological analysis was executed in Cytoscape (v3.9.0). Specifically, the CytoNCA algorithm was applied to identify hub targets, prioritizing nodes based on the metric of degree centrality.

Target-pathway network construction and visualization

To understand how BRI alleviates obesity, the target-pathway network using Cytoscape (v3.9.0) was constructed. This network visualized interactions between BRI-related genes and signaling pathways, providing a molecular map of potential therapeutic effects.

Functional and enrichment analysis of BRI-associated targets

Functional annotation of the overlapping target set was performed via the Metascape portal, leveraging both Gene Ontology (GO) and KEGG databases. The GO spectrum was dissected across three primary ontologies—Biological Process (BP), Cellular Component (CC) and Molecular Function (MF)—to gain insights into the biological functions underpinning the anti-obesity potential of BRI-related targets.

Molecular docking and binding energy calculation

Utilizing the CB-Dock interface, molecular docking and evaluated binding energetics were conducted. The study utilized Britanin, with its structural data obtained from PubChem, as the small-molecule ligand. Structural templates for the key protein targets—specifically MAPK1, EGFR, MAPK8, PTGS2 and MAP2K1 (corresponding to Mitogen-Activated Protein Kinase 1, Epidermal Growth Factor Receptor, Mitogen-Activated Protein Kinase 8, Prostaglandin-Endoperoxide Synthase 2 and Mitogen-Activated Protein Kinase Kinase 1)—were fetched from the Protein Data Bank to serve as receptors. Both BRI and the selected protein structures were uploaded to CB-DOCK and docking was conducted using the blind docking mode to predict potential binding sites. Following docking, the binding energy and docking scores were analyzed to assess the strength of BRI-protein interactions. To evaluate the stability of BRI-protein interactions, the root mean square deviation (RMSD) values for each docking complex were calculated using PyMOL software. Lower RMSD values indicate higher docking stability, providing additional validation of the molecular interactions.

Data processing and statistical analysis

For data analysis, all results are expressed as mean ±SD. One-way ANOVA was used to assess differences between groups. GraphPad Prism 8.0.1 was used for visualization. Statistical significance was set at $P<0.05$, ensuring rigorous evaluation of experimental effects.

RESULTS

Animal experiment results

Body weight changes and fat mass analysis

After being fed a high-fat diet, the mice exhibited a gradual increase in body weight. By week 12, the average body weight of the obesity model group was 1.4 times that of the control group, confirming successful model establishment. Three mice with body weights below 32 grams were excluded, leaving 15 obese mice for subsequent intervention. These were randomly assigned into three groups: Obesity group, Obesity + BRI 5 mg/kg group, Obesity + BRI 15 mg/kg group. In the normal diet control group, one mouse was excluded due to a lower limb injury during fighting. The grouping and intervention conditions are illustrated in Fig. 1A, with the appearance of the mice presented in Fig. 1B. The body weight change curve is shown in Fig. 1C. By week 12, a statistical difference was observed between the high-fat fed obesity group and the control group. Nevertheless, baseline comparisons revealed no statistically significant disparities among the three experimental groups prior to treatment initiation (Fig. 1E). Following a 7-day therapeutic regimen, a marked reduction in body weight was observed in the treated cohorts. Notably, the group receiving 15 mg/kg demonstrated the most substantial weight loss compared to the other groups (Fig. 1D). 15 mg/kg BRI reduced body weight by 18%. Fig. 1F and 1G show that body weight gain after 7 days of treatment was dose-dependent, with statistically significant differences between groups. The liver weight in obesity notably raised, but after BRI 15mg/kg treatment, the weight of liver was closely to control group (Fig. 1H). Obesity remarkably increased white adipose tissue (WAT) mass compared with the control group, while BRI treatment markedly reduced WAT mass in obese mice (Fig. 1I). In contrast, brown adipose tissue (BAT) mass was decreased in obese mice, whereas BRI administration partially restored BAT mass (Fig. 1J). No clear differences in food intake were observed among groups before treatment (Fig. 1K). However, after BRI treatment, food intake was significantly reduced in a dose-dependent manner (Fig. 1L).

Blood lipid and blood glucose analysis

Blood glucose and lipid analyses revealed notable changes in the obesity group compared to the control group. Significantly, the obesity group exhibited elevated levels of TC, LDLC, HDLC and blood glucose ($P<0.001$), while VLDL was significantly reduced ($P<0.0001$). Following BRI treatment, a dose-dependent decrease in blood glucose and lipid levels was observed. Specifically, at 5 mg/kg, TG ($P<0.0001$), TC ($P<0.01$), LDLC ($P<0.05$) and blood glucose ($P<0.001$) were significantly reduced, while VLDL and HDLC did not exhibit significant changes.

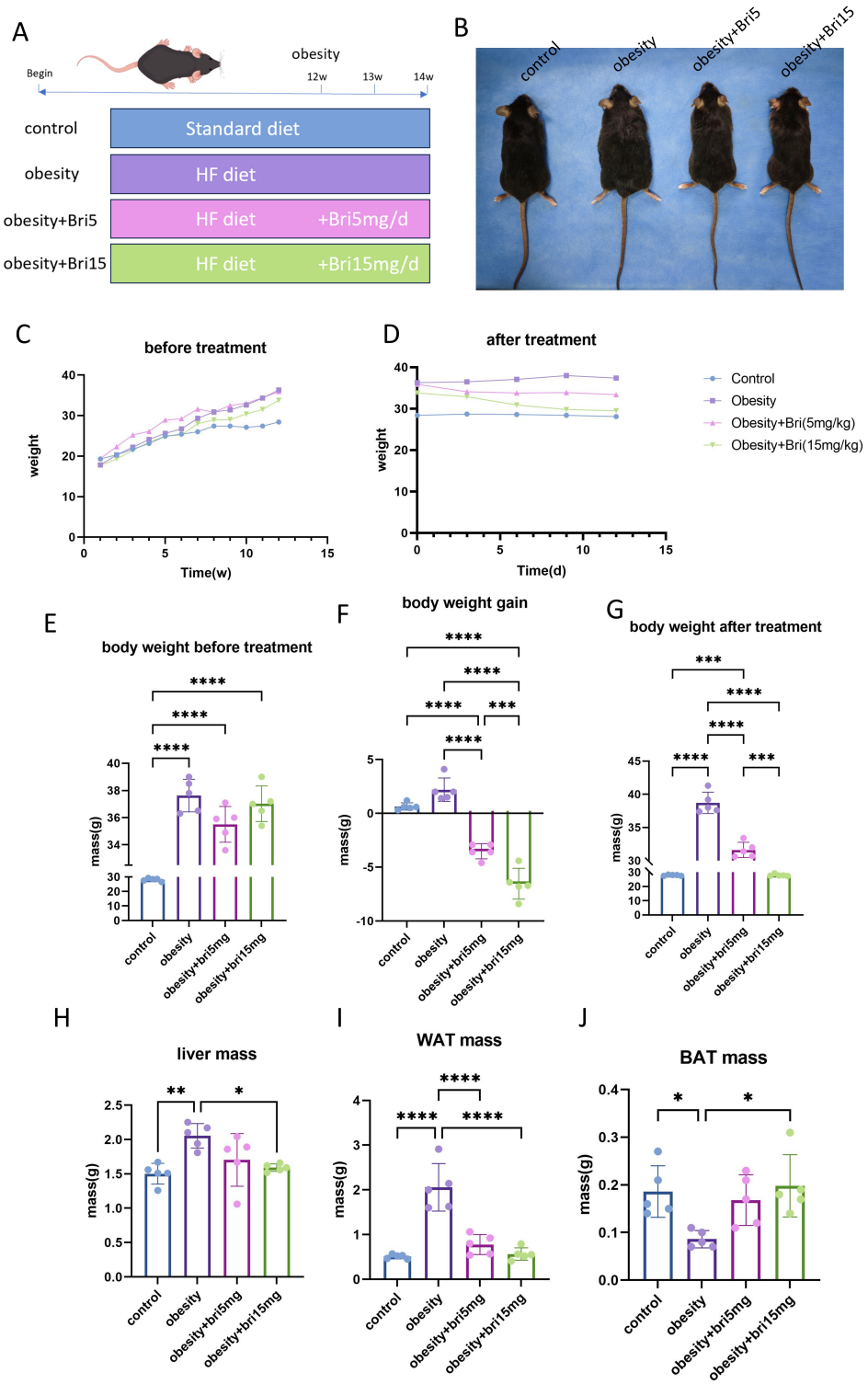

Fig. 1: Effect of BRI on body weight in high-fat-diet mice. (A) Schematic illustration of the experimental design for administering BRI to C57BL/6J mice; (B) Representative photographs of mice body shape; (C) Dynamic changes in body weight during feeding before treatment; (D) Body weight gain in each group after treatment; (E) Relative body weight of mice before treatment; (F) Relative body weight gain of mice during treatment; (G) Relative body weight gain of mice at termination of study; (H) Liver weight at termination of study; (I) epididymal white adipose tissue (WAT) weight at termination of study; (J). brown adipose tissue weights at termination of study; (K) Daily food intake per mouse before treatment; (L) Daily food intake per mouse after treatment. (n=5) ∗P< 0.05, ∗∗P< 0.01, ∗∗∗P< 0.001

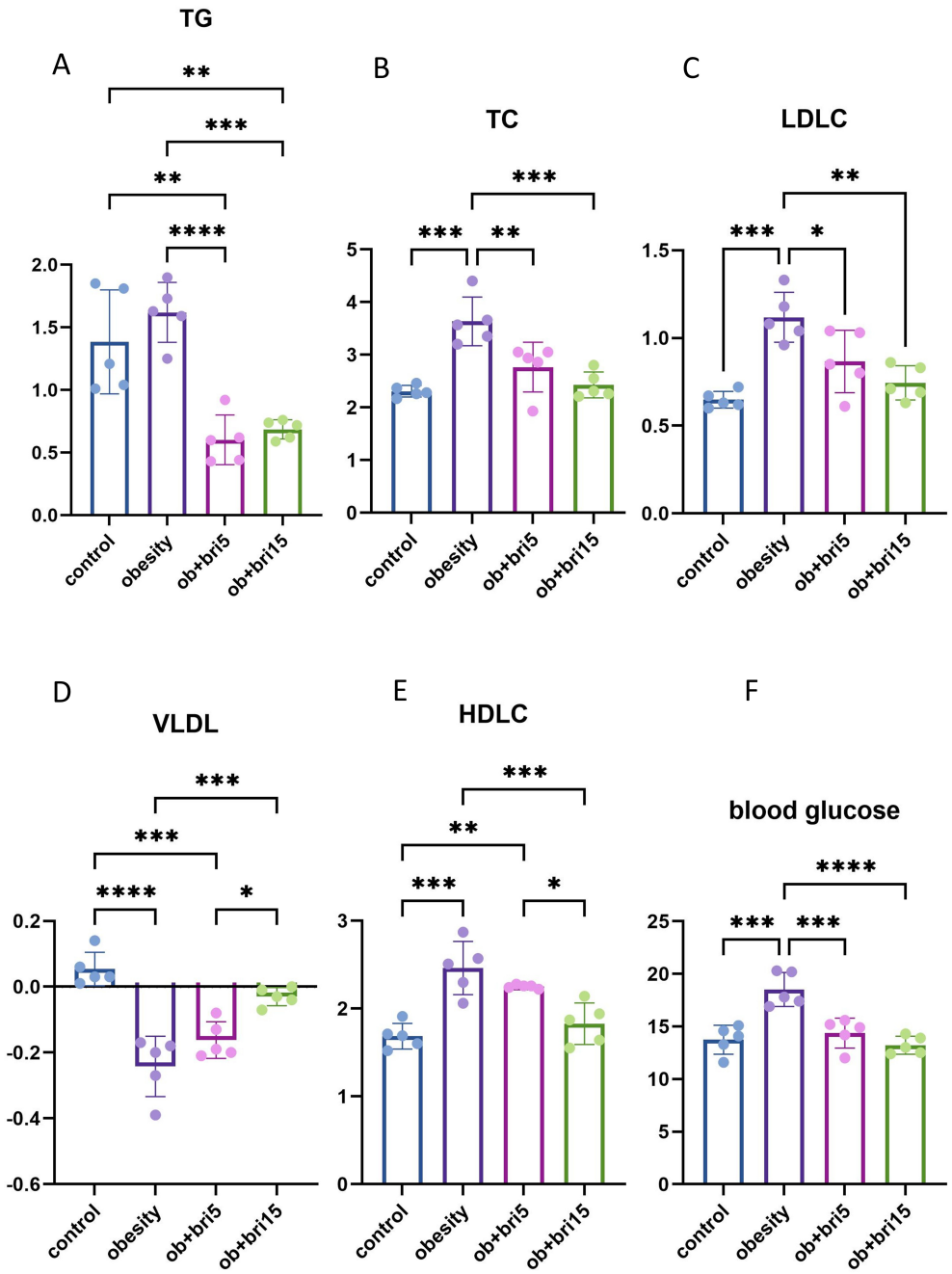

Fig. 2: Blood lipid and blood glucose analysis. (A) TG; (B) TC; (C) LDL-C; (D) VLDL; (E) HDLC; (F) GLU. (n=5) ∗P< 0.05, ∗∗P< 0.01, ∗∗∗P< 0.001

At 15 mg/kg, a more pronounced effect was observed, BRI decreased serum TG (-45%, P<0.001), TC (-37%, P<0.001) and LDL-C (-32%, P<0.01), HDLC (P<0.001) and blood glucose (P<0.0001) all significantly reduced and VLDL (P<0.001) significantly increased. These results confirm that BRI significantly improves glucose and lipid metabolism in a dose-dependent manner (Fig. 2).

HE and oil red O staining of adipose tissue

Histological analysis of WAT by H&E and Oil Red O staining showed that in the obesity group, adipocytes were significantly enlarged, with visible cell swelling and cytoplasmic rupture, indicative of cellular damage. After BRI treatment, the adipocytes in the treatment groups showed a reversal of this enlargement, with cell size reduced, more notably in the 15 mg/kg group (Fig. 3A). Subsequently, a pathological statistical analysis was performed, the specific results of which are shown in figure 3 B.C.D.E. (P<0.0001). In BAT, while the mass exhibited significant differences between groups, no pathological changes were observed in the cells (Fig. 3).

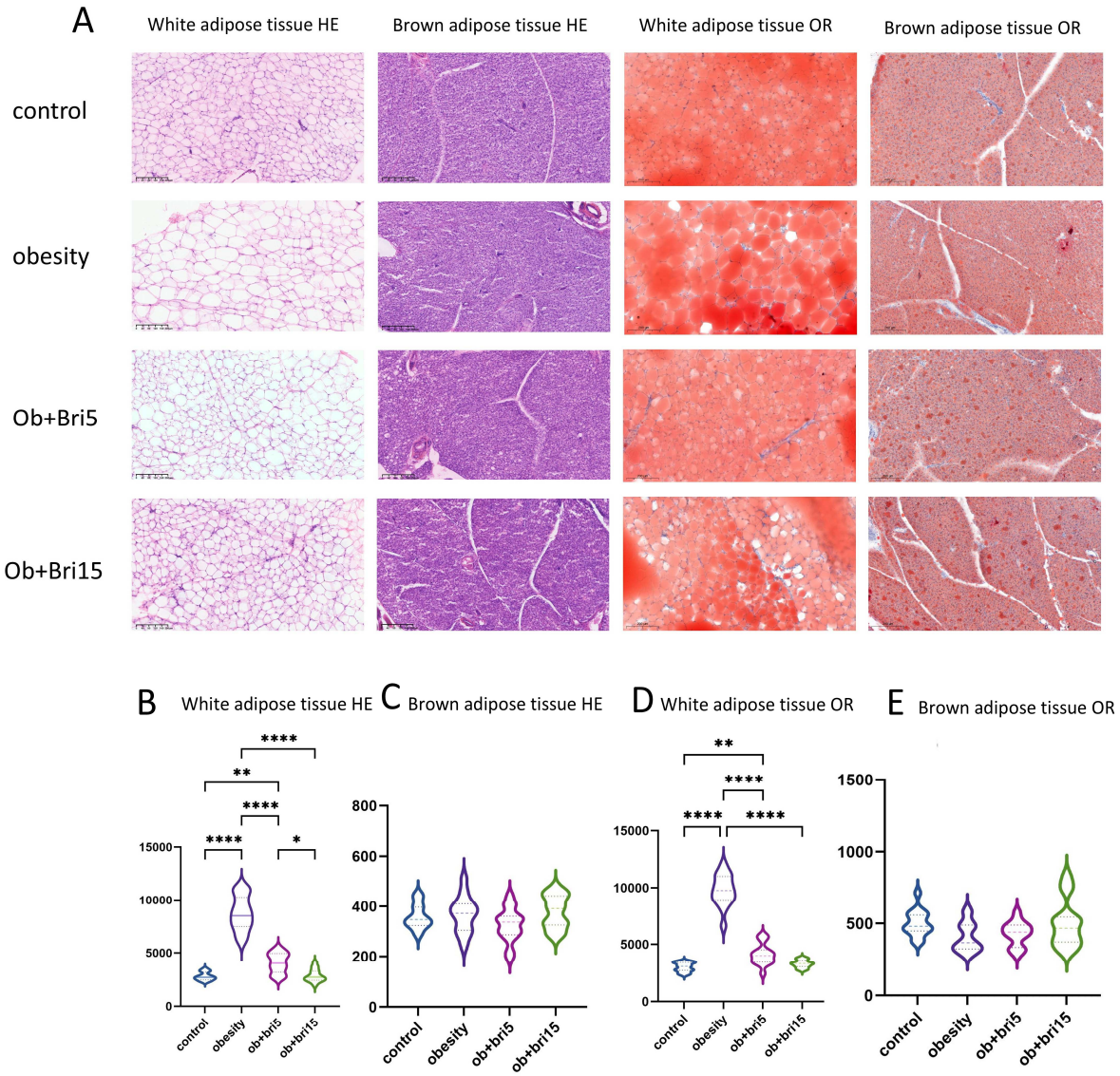

Fig. 3: (A) Representative HE and oil-red staining of WAT and BAT from mice. Scale bars, 200 μm. (n=5). The specific pathological statistical analysis shows in (B). (C). (D). (E).

Network pharmacology analysis

Inula helenium, a member of the Asteraceae family, is widely distributed in nature, typically growing along slopes, riverbanks and field embankments. After harvesting and drying, BRI can be extracted from dried Inula flowers (Fig. 4A). BRI is a flavonoid compound that contributes to the plant's primary therapeutic effects, with its chemical structure depicted in figure. 4B. The Swiss Target Prediction database identified 97 potential drug targets for BRI. Meanwhile, a search of the Gene Cards and OMIM databases retrieved 2,211 disease-related gene targets associated with obesity. After eliminating duplicates, 39 overlapping genes were identified, visualized through a Venn diagram. (Fig. 4C) These 39 intersecting genes were uploaded to the STRING database to construct a PPI network (Fig. 4D), which consisted of 39 nodes and 104 edges. The data were further analyzed using Cytoscape (v3.9.0) to construct a target-pathway network of BRI in obesity treatment (Figs. 4E, 4F). Using the degree algorithm, the core genes were ranked, with the top five being: EGFR, MAPK1, MAPK8, PTGS2, MAP2K1. To further investigate the biological significance of these genes, GO enrichment analysis was performed using R software (v4.3.2), identifying 208 enriched terms, categorized as follows:151 terms related to biological processes (BP),20 terms associated with cellular components (CC),37 terms linked to molecular functions (MF). Visualization of the top ten ranked GO terms was performed to present the most significant enrichment results (Fig. 4G). Additionally, KEGG pathway enrichment analysis identified 104 pathways relevant to BRI's anti-obesity effects (Fig. 4H). These findings suggest that BRI may alleviate obesity by regulating multiple signaling pathways.

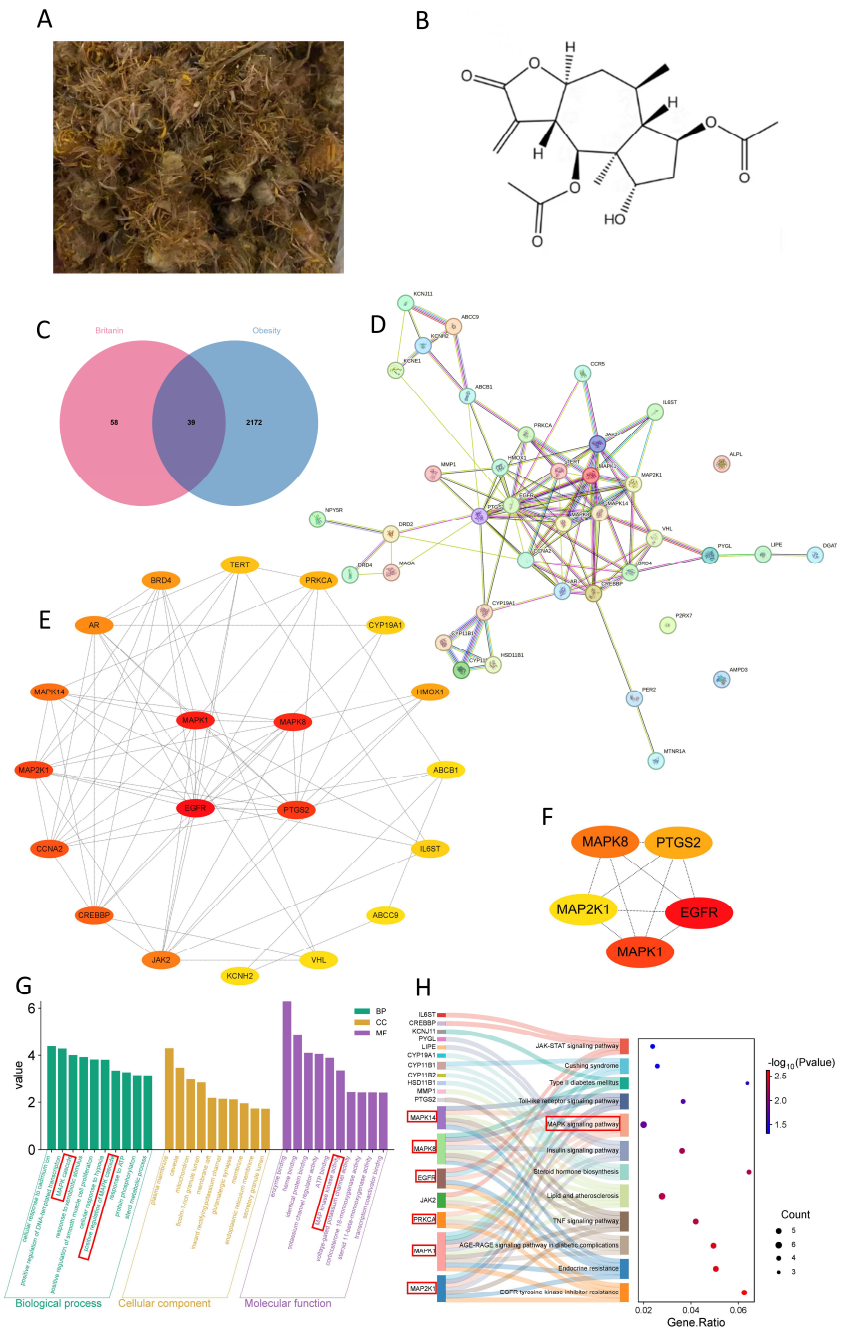

Fig. 4: Analysis of targets associated with BRI and obesity. (A) BRI can be extracted from dried Inula flowers; (B) Chemical structures of BRI; (C) Venn diagram of BRI and obesity-related targets; (D) PPI network of BRI anti-obesity targets; (E) Target-pathway network of BRI in treating obesity; (F) Top five targets were selected from E; (G) GO enrichment analysis; (H) KEGG pathway enrichment analysis based on the DAVID database.

Molecular docking and binding energy analysis

Molecular docking of BRI with obesity-related core target proteins were performed. Prior to formal docking, all macromolecular structures were re-docked to ensure accuracy. The binding conformations of BRI with key proteins are illustrated in figure. 5. Molecular docking was conducted using the CB-DOCK online platform, targeting EGFR (A), MAPK1 (B), MAPK8 (C), PTGS2 (D) and MAP2K1 (E). The docking results were analyzed at both macro and micro levels. A binding energy lower than -1.2 kcal/mol (or -5 kJ/mol) is generally considered indicative of a favorable ligand-protein interaction (Wagoner *et al.,*2006). To evaluate the stability of BRI-protein interactions, the root mean square deviation (RMSD) values for each docking complex were calculated using PyMOL software. Lower RMSD values indicate higher docking stability, providing additional validation of the molecular interactions.

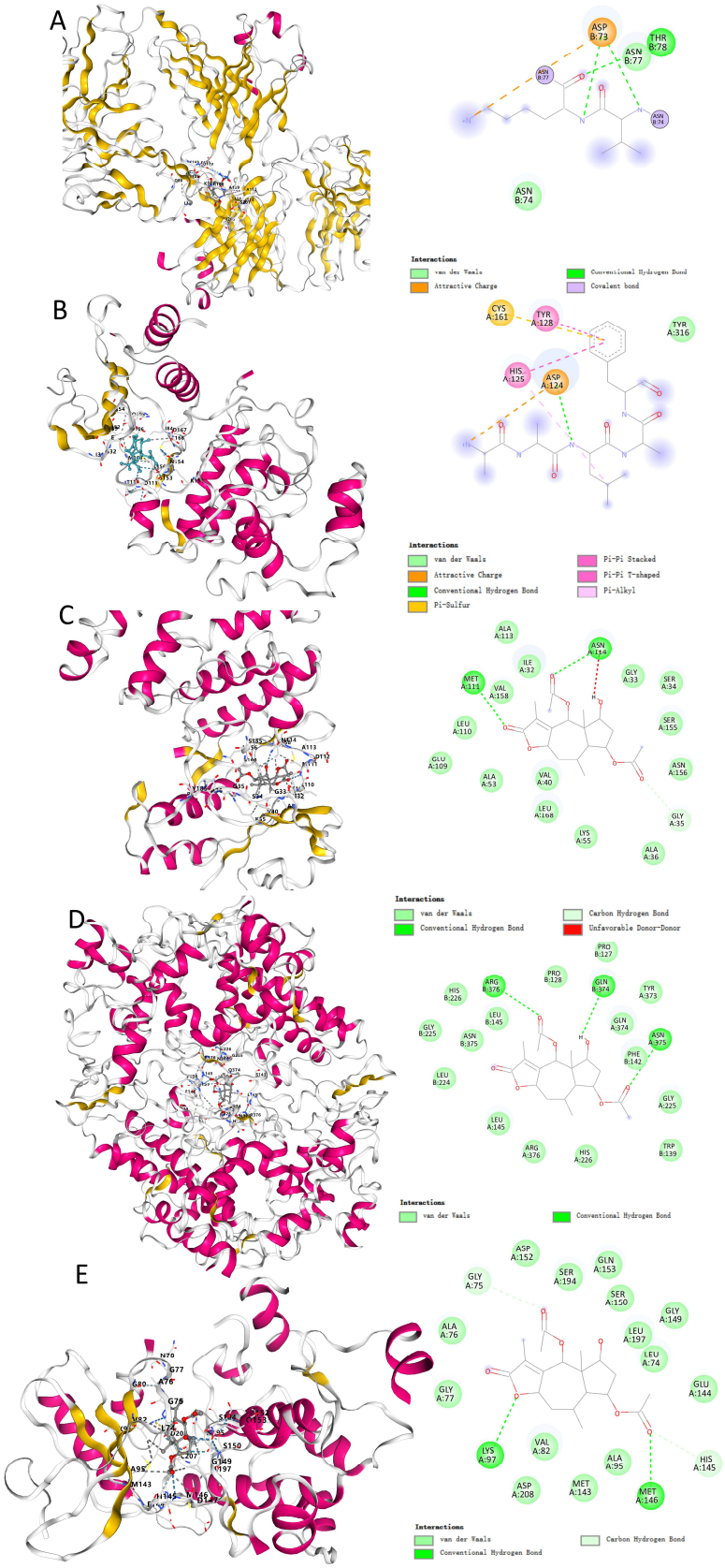

Fig. 5: Molecular docking models of BRI with possible core anti-obesity targets. (A) MAPK1; (B) EGFR; (C) MAPK8; (D) PTGS2; (E) MAP2K1

Table 1: Results of the molecular dynamics binding energies and analyzed the RMSD values of core target structures

Structural domain	Compound	Binding energies(kcal/mol)	RMSD
MAPK1 (6SLG)	Britanin	-7.7	0.233
EGFR (2XKN)	Britanin	-7.9	0.233
MAPK8 (3ELJ)	Britanin	-8.9	0.230
PTGS2 (5F19)	Britanin	-9	0.231
MAP2K1 (3VD3)	Britanin	-10.3	-

Table 2: The results of the molecule docking specific binding bond of core target structures.

Structural domain	Hydrogen bonds	Salt bridges	Pi-Charge	Pi-Sulfur
MAPK1 (6SLG)	ASP A:124	ASP A:124	TYR A: 128, HIS A:125	CYS A:161
EGFR (2XKN)	ASP B:73; ASN B:77	ASP B:73	-	-
MAPK8 (3ELJ)	ASN A:114	-	-	-
PTGS2 (5F19)	ARG B:376; GLN B:374; ASN A:375	-	-	-
MAP2K1 (3VD3)	LYS A:91; MET A:146	-	-	-

As summarized in table 1, BRI exhibited strong binding affinities with all five target proteins, indicating that EGFR, MAPK1, MAPK8, PTGS2 and MAP2K1 serve as primary direct targets for BRI's anti-obesity effects. At the atomic level, BRI binds to these proteins through various non-covalent interactions, including: salt bridges, hydrogen bonds and π-π stacking, the specific results are shown in table 2. These binding interactions enhance the stability of the ligand-protein complexes, reinforcing the notion that BRI exerts its pharmacological effects by engaging in direct molecular interactions with obesity-related targets.

BRI alleviates obesity by activating the MAPK signaling pathways

After the HFD induction, leptin levels were extremely reduced in WAT of the obesity group. However, after BRI treatment, leptin levels were restored in a dose-dependent manner, as shown in figure. 6A. Further analysis of mRNA levels revealed that MAPK1 levels were significantly increased after BRI treatment ($P<0.05$) compared to the obesity group (Fig. 6B). In contrast, MAPK8 levels were decreased in the obesity group, however, after treatment, these levels were restored, with a statistically notable difference happened in the high-dose group ($P<0.01$) (Fig. 6C). MAP2K1 expression levels were markedly suppressed in the obesity-induced group; however, therapeutic intervention successfully restored these levels to baseline. Notably, the high-dose treatment group exhibited a statistically significant recovery compared to the model group ($P<0.05$) (Fig. 6D). In addition, a significant decrease in peroxisome proliferator-activated receptor γ (PPARγ) levels was observed following the BRI intervention, indicating reduced lipogenesis. ($P<0.01$) (Fig.6E). The Western blot data indicated that phosphorylation of MAPK1 and MAP2K1 was severely attenuated in the obese cohort ($P<0.001$). Notably, BRI intervention ameliorated this deficit, driving a statistically significant reactivation of MAPK1 and MAP2K1 phosphorylation ($P< 0.01$ or $P < 0.001$) (Fig 6F). Taken together, the present data suggest that BRI promotes lipolysis in adipose tissue by activating the MAPK signaling pathway, thereby improving the obesity phenotype.

Assessment of BRI toxicity in obese mouse models

Histological examination of the kidneys using H&E staining showed no significant inflammation or histopathological changes among the groups (Fig. 7A). In the liver, compared with the normal group H&E staining revealed that the obese group exhibited macrovascular steatosis in hepatocytes, whereas BRI treatment reversed the hepatic steatosis (Fig. 7B). Furthermore, obesity significantly increased serum ALT (Fig. 7C) and AST (Fig. 7D) levels, while administration of the treatment markedly reduced ALT and AST, the results are consistent with the pathological findings.

DISCUSSION

Obesity has become a global health crisis, with its incidence rising steadily in recent years. Recent developments in biomedical technology have fostered significant progress in this area, particularly in exploring the potential of TCM for managing metabolic diseases. Inula helenium, a plant in the Asteraceae family, has long been used in TCM for treating ailments such as cough, sputum and edema. A key bioactive compound in this plant, Britanin (BRI), a natural sesquiterpene lactone, has demonstrated promising properties such as anti-inflammatory, antibacterial, antidiabetic and anticancer effects (Lu *et al.,* 2022; Shao *et al.,* 2024). This study investigates the potential therapeutic effects of BRI in treating obesity. Using network pharmacology, molecular docking and animal experiments, BRI exerts its anti-obesity effects by regulating key targets within the MAPK signaling pathway, particularly MAPK1, MAPK8 and MAP2K1 and inhibited the PPARγ, thereby blocking adipogenesis and promoting the transition of adipose tissue from a 'storage mode' to a 'breakdown/remodeling mode'. The experiments suggest that BRI may serve as a promising lead compound for obesity and other metabolic diseases.

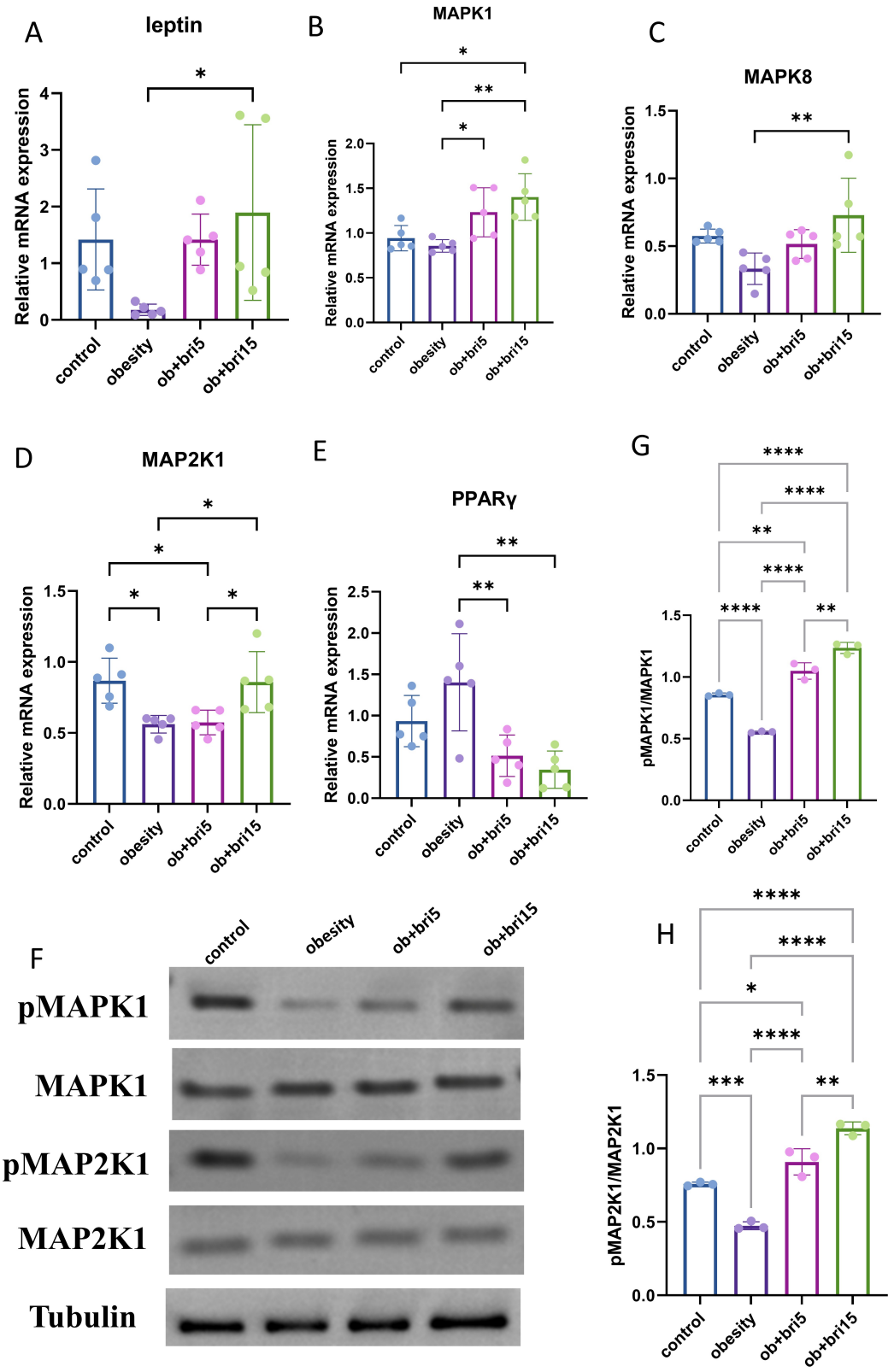

Fig. 6: Relative mRNA levels in adipose tissue(n=5). (A) leptin; (B) MAPK1; (C) MAPK8; (D) MAP2K1; (E) PPARγ; (F) BRI increased the protein levels of p-MAPK1 and p-MAP2K1, while the protein levels of MAPK1 and MAP2K1 was no changed in adipose tissue (n=3); (G). Relative protein levels of p-MAPK1/MAPK1. (H) Relative protein levels of p-MAP2K1/MAP2K1. ∗P< 0.05, ∗∗P< 0.01, ∗∗∗P< 0.001

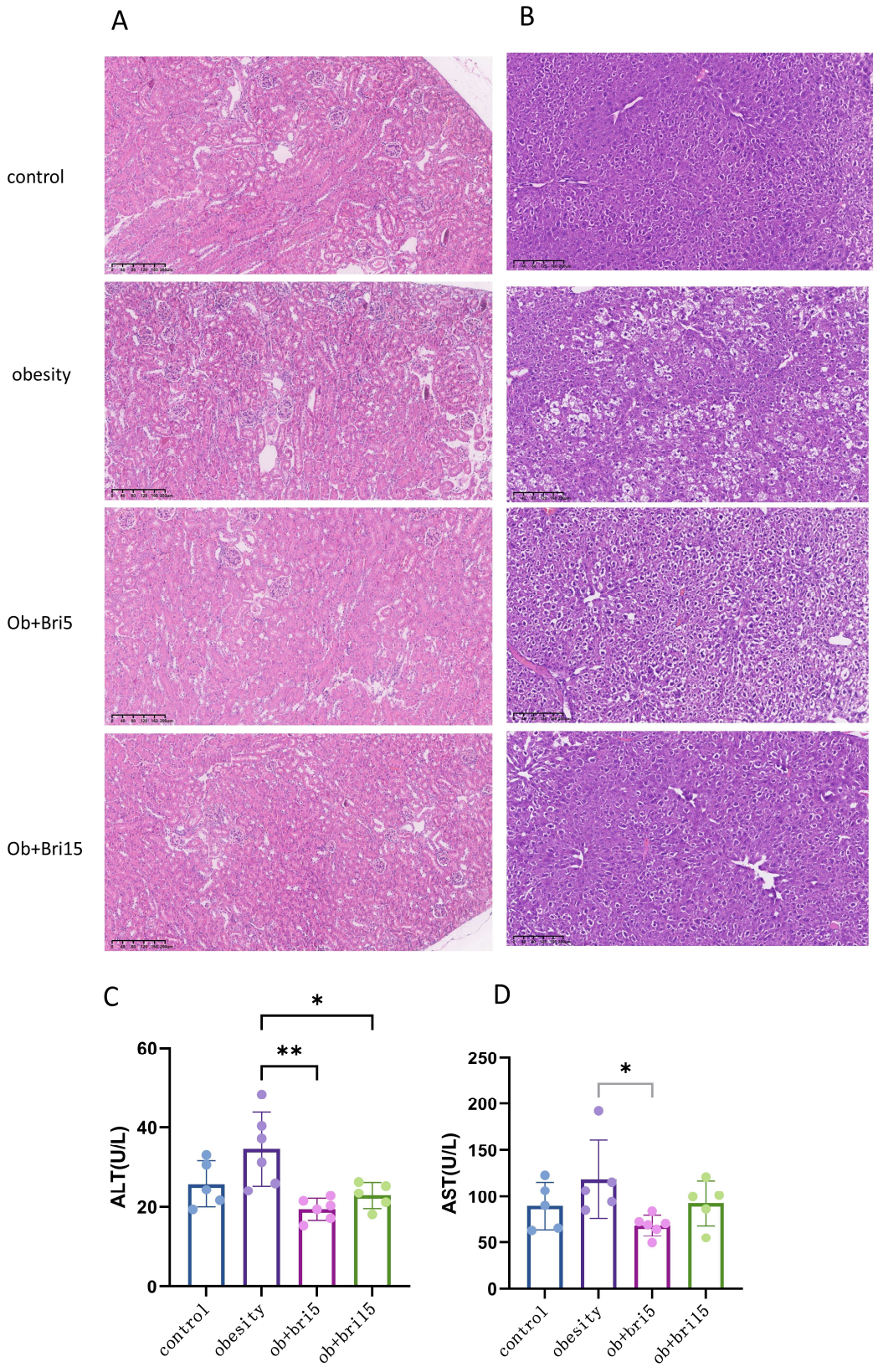

Fig. 7: (A) Representative HE staining of kidney from mice. Scale bars, 200 μm; (B) Representative HE staining of liver from mice. Scale bars, 200 μm; (C) ALT levels in serum (n=5); (D) AST levels in serum (n=5). ∗P< 0.05, ∗∗P< 0.01

Network pharmacology has proven to be an invaluable tool for identifying the mechanism (Li *et al.,* 2023). Moreover, network pharmacology supports TCM in areas such as disease phenotyping, target prediction, active compound screening and elucidating disease-treatment mechanisms (Löscher and Klein 2022). Through network pharmacology analysis, several potential targets underlying the anti-obesity effects of BRI were identified. These targets were primarily enriched in the MAPK signaling pathway, which has been extensively reported to participate in obesity-related metabolic regulation (Fang *et al.*, 2019; Lan *et al.*, 2022; Wu *et al.*, 2021). Among them, Mitogen-Activated Protein Kinase 1 (MAPK1) functions as a key serine/threonine kinase involved in various biological processes, including cellular proliferation, survival, metabolic regulation, migration, growth and differentiation (Lavoie *et al.*, 2020). It has been implicated in disorders of liver lipid metabolism and cardiac metabolic disturbances(Kwong *et al.,*2015; Hara *et al.,*2023). Specifically, p38 MAPK plays a critical role in inflammatory activation in metabolic liver diseases. (Zhang X *et al.,*2019). In the context of high-fat diet-induced obesity, p38 MAPK contributes to endothelial dysfunction and insulin resistance, linking obesity-related metabolic disturbances to cardiovascular disease (Yu *et al.,*2014). The network pharmacology analysis aligns with these findings, providing strong evidence that BRI's anti-obesity effects may be mediated through the MAPK signaling pathway.

This work utilized a HFD to construct a robust murine model of obesity, serving as a platform to scrutinize the intervention effects of BRI. Network pharmacology analysis identified key targets of BRI, including MAPK1, MAPK8 and MAP2K1, which were significantly elevated after BRI treatment compared to the obesity group. As a key adipokine derived mainly from fat tissue, leptin orchestrates body mass control by conveying crucial information about systemic energy storage to neural centers in the brain. Leptin acts centrally to suppress appetite and peripherally to promote lipolysis and fatty acid oxidation (Pico *et al.,* 2022). In obesity, leptin initially compensates for excess fat by increasing its secretion, but over time, leptin resistance develops, leading to a decrease in leptin levels and exacerbating obesity. Studies on preadipocytes have shown that low concentrations（8, 20, 80 ng/ml） of leptin can promote their proliferation and differentiation, while high concentrations（250, 500 ng/ml） can inhibit this process (Machinal-Quelin *et al.,* 2002; Wagoner *et al.,* 2006; Palhinha *et al.,* 2019). Our findings suggest that after BRI treatment, leptin levels were restored in obese mice, which likely activated the MAPK signaling pathway, leading to increased expression of MAPK1, MAPK8 and MAP2K1. The p38 MAPK pathway, a critical component of the MAPK signaling cascade, is closely associated with obesity. In obesity, the reduction of leptin levels leads to inhibition of the p38 MAPK pathway, resulting in decreased fat breakdown, lipid accumulation and increased expression of adipogenesis and lipid synthesis-related proteins like PPARγ, which exacerbates obesity (Pico *et al.,* 2022). However, after BRI intervention, activation of the MAPK pathway promoted fat breakdown and suppressed PPARγ expression, effectively reversing obesity. Notably, the restoration of leptin levels in BRI-treated mice correlates with p38 MAPK activation, suggesting a feedback loop between adipokine signaling and lipid metabolism. Notably, our findings align with a study by Li *et al.*, (2021) who reported that a human milk-derived peptide inhibits adipogenesis by maintaining ERK activity and downregulating PPARγ.

Several limitations should be considered: a substantial part of the mechanistic insight was derived from computational approaches, including network pharmacology, molecular docking and molecular dynamics simulations. Furthermore, while the network pharmacology analysis suggests that BRI's effect on obesity involves multi-component, multi-target and multi-pathway actions, The primary focus has been on the MAPK signaling pathway. Limitations included the inability to experimentally confirm all predicted targets and to integrate animal and cellular studies with clinical validation, thereby hindering a comprehensive understanding of the anti-obesity mechanisms of BRI. While this study focuses on MAPK signaling, BRI's multitarget profile (e.g., EGFR, PTGS2) warrants investigation in cellular models. Future work should explore BRI's efficacy in human adipocytes and long-term safety in preclinical models. Besides, intraperitoneal administration serves as a validation strategy, while future translational efforts should prioritize oral formulation optimization to achieve clinically relevant delivery of BRI. Besides, proteomic and biochemical studies are needed to elucidate BRI's upstream binding partners. Additionally, the long-term safety and efficacy of britanin in chronic obesity treatment require further investigation, including pharmacokinetic and toxicological analyses. Furthermore, Following BRI treatment, a significant reduction in food intake was observed, and weight loss was found to be strongly associated with this decrease. However, further investigation into these mechanisms—potentially involving the nervous system and taste perception-related pathways—remains to be conducted. Additionally, due to the unavailability of an animal energy metabolism monitoring system, data regarding energy metabolism consumption could not be provided but are recommended for inclusion in future experimental designs.

CONCLUSION

The anti-obesity effects of BRI appear to be associated with modulation of the Mitogen-Activated Protein Kinase signaling pathway, suggesting its potential value as a

natural compound for obesity management and BRI is safe with no significant toxic side effects. In the future, the anti-obesity effect of BRI can be explored through metabolomics sequencing, drug target screening, proteomic and biochemical studies.

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Author's contributions
YiQing Zhang: Conceptualization, Methodology, Software, Investigation, Formal Analysis, Writing - Original Draft; Zhikang Wu: Conceptualization, Methodology, Software; Xian Li: Data Curation, Software, Investigation, Formal Analysis; HeZi Jiang: Visualization, Investigation; Xinyu Zhou: Resources, Supervision; JinSheng Shen: Software, Validation; Xiangyu Wang: Visualization, Writing - Review & Editing; GuoXing Zhang: Conceptualization, Resources, Supervision, Writing - Review & Editing; Yafeng Zhou: (Corresponding Author): Conceptualization, Funding Acquisition, Resources, Supervision, Writing - Review & Editing.

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Data availability statement
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval
In this study, the animal experiments were approved by the Ethics Committee of Soochow University. Ethics number is 202408A0490.The study was conducted in accordance with the principles of good scientific practice and adhered to the guidelines for research integrity and data handling established by Soochow University.

Conflicts of interest
The authors have no conflicts of interest to declare.

Consent for publication
All the authors agree to the publication of the article.

Supplementary data
https://pjps.pk/uploads/2026/08/SUP1787645089.pdf

REFERENCES

Ahern AL, Wheeler GM, Aveyard P, Boyland EJ, Halford JCG, Mander AP, Woolston J, Thomson AM, Tsiountsioura M, Cole D (2017). Extended and standard duration weight-loss programme referrals for adults in primary care (WRAP): A randomised controlled trial. *Lancet*, **389**(10085): 2214-2225.

Chooi YC, Ding C and Magkos F (2019). The epidemiology of obesity. *Metabolism*., **92**: 6-10.

Dou C, Zhu H, Xie X, Huang C and Cao C (2024). Integrated pharmaco-bioinformatics approaches and experimental verification to explore the effect of britanin on nonalcoholic fatty liver disease. *ACS Omega*., **9**(7):8274-8286.

GBD 2021 Adolescent BMI Collaborators (2025).Global, regional, and national prevalence of child and adolescent overweight and obesity, 1990-2021, with forecasts to 2050: A forecasting study for the global burden of disease study 2021. *Lancet*, **405**(10481): 785-812.

Hara T, Sata M and Fukuda D (2023). Emerging roles of protease-activated receptors in cardiometabolic disorders. *J. Cardiol.,* **81**(4): 337-346.

Hopkins AL (2008). Network pharmacology: The next paradigm in drug discovery. *Nat. Chem. Biol*., **4**(11): 682-690.

Kajdanek A, Kolat D, Zhao LY, Kciuk M, Pasieka Z and Kaluzinska-Kolat Z (2024). Britanin - A beacon of hope against gastrointestinal tumors? *World J. Clin. Oncol.*, **15**(4):523-530.

Kivimaki M, Strandberg T, Pentti J, Nyberg ST, Frank P, Jokela M, Ervasti J, Suominen SB, Vahtera J and Sipila PN (2022). Body-mass index and risk of obesity-related complex multimorbidity: An observational multicohort study. *Lancet Diabetes Endocrinol.,* **10**(4): 253-263.

Kwong E, Li Y, Hylemon PB and Zhou H (2015). Bile acids and sphingosine-1-phosphate receptor 2 in hepatic lipid metabolism. *Acta. Pharm. Sin. B*., **5**(2): 151-157.

Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F and Straif K (2016). Body fatness and cancer--viewpoint of the IARC working group. *N. Engl. J. Med.,* **375**(8): 794-798.

Lean MEJ, Leslie WS, Barnes AC, Brosnahan N, Thom G, McCombie L, Peters C, Zhyzhneuskaya S, Al-Mrabeh A and Hollingsworth KG (2019). Durability of a primary care-led weight-management intervention for remission of type 2 diabetes: 2-year results of the DiRECT open-label, cluster-randomised trial. *Lancet Diabetes Endocrinol.,* **7**(5):344-355.

Li L, Yang L, Yang L, He C, He Y, Chen L, Dong Q, Zhang H, Chen S and Li P (2023). Network pharmacology: A bright guiding light on the way to explore the personalized precise medication of traditional Chinese medicine. *Chin Med.,* **18**(1):146.

Liu ZQ, Mahmood T and Yang PC (2014). Western blot: Technique, theory and trouble shooting. *N. Am. J. Med. Sci.,* **6**(3): 160.

Livak KJ and Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods,* **25**(4):402-408.

Loscher W and Klein P (2022). New approaches for developing multi-targeted drug combinations for disease modification of complex brain disorders. Does epilepsy prevention become a realistic goal? *Pharmacol. Ther.,* **229**:107934.

Lu H, Wu Z, Wang Y, Zhao D, Zhang B and Hong M (2022). Study on inhibition of Britannin on triple-negative breast carcinoma through degrading ZEB1 proteins. *Phytomedicine.,* **104**: 154291.

Machinal-Quelin F, Dieudonne MN, Leneveu MC, Pecquery R and Giudicelli Y (2002). Proadipogenic effect of leptin on rat preadipocytes *in-vitro*: Activation of MAPK and STAT3 signaling pathways. *Am. J. Physiol. Cell Physiol.,* **282**(4):C853-863.

Martel J, Ojcius DM, Chang CJ, Lin CS, Lu CC, Ko YF, Tseng SF, Lai HC and Young JD (2017). Anti-obesogenic and antidiabetic effects of plants and mushrooms. *Nat. Rev. Endocrinol.,* **13**(3): 149-160.

Palhinha L, Liechocki S, Hottz ED, Pereira J, de Almeida CJ, Moraes-Vieira PMM, Bozza PT and Maya-Monteiro CM (2019). Leptin induces proadipogenic and proinflammatory signaling in adipocytes. *Front. Endocrinol (Lausanne).,* **10**:841.

Perdomo CM, Cohen RV, Sumithran P, Clement K and Frühbeck G (2023). Contemporary medical, device, and surgical therapies for obesity in adults. *Lancet,* **401**(10382): 1116-1130.

Pico C, Palou M, Pomar CA, Rodriguez AM and Palou A (2022). Leptin as a key regulator of the adipose organ. *Rev. Endocr. Metab. Disord.,* **23**(1):13-30.

Ramirez-Zacarias JL, Castro-Munozledo F and Kuri-Harcuch W (1992). Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. *Histochemistry.,* **97**(6): 493-497.

Shao JJ, Li WF, Sun JF, Zhuang ZS, Min JL, Long XH, Wu GJ, Xu HW and Liang G (2024). Britannin as a novel NLRP3 inhibitor, suppresses inflammasome activation in macrophages and alleviates NLRP3-related diseases in mice. *Acta. Pharmacol. Sin.,* **45**(4): 803-814.

Wagoner B, Hausman DB and Harris RB (2006). Direct and indirect effects of leptin on preadipocyte proliferation and differentiation. *Am. J. Physiol. Regul. Integr. Comp. Physiol*., **290**(6): R1557-1564.

Walter CP, Bleske BE and Dorsch MP (2014). Pharmacotherapy for weight loss: The cardiovascular effects of the old and new agents. *J. Clin. Pharm. Ther*., **39**(5): 475-484.

Wan Y, Xia J, Xu JF, Chen L, Yang Y, Wu JJ, Tang F, Ao H and Peng C (2022). Nuciferine, an active ingredient derived from lotus leaf, lights up the way for the potential treatment of obesity and obesity-related diseases. *Pharmacol. Res.,* **175**: 106002.

Wang GW, Qin JJ, Cheng XR, Shen YH, Shan L, Jin HZ and Zhang WD (2014). Inula sesquiterpenoids: Structural diversity, cytotoxicity and anti-tumor activity. *Expert Opin. Investig. Drugs,* **23**(3): 317-345.

Yang B, Yang N, Chen Y, Zhu M, Lian Y, Xiong Z, Wang B, Feng L and Jia X (2020). An integrated strategy for effective-component discovery of astragali radix in the treatment of lung cancer. *Front. Pharmacol.,* **11**: 580978.

Yárnoz-Esquiroz P, Olazaran L, Aguas-Ayesa M, Perdomo CM, Garcia-Goni M, Silva C, Fernandez-Formoso JA, Escalada J, Montecucco F and Portincasa P (2022). 'Obesities': Position statement on a complex disease entity with multifaceted drivers. *Eur. J. Clin. Invest*., **52**(7): e13811.

Yu Y, Rajapakse AG, Montani JP, Yang Z and Ming XF (2014). p38 mitogen-activated protein kinase is involved in arginase-II-mediated eNOS-uncoupling in obesity. *Cardiovasc. Diabetol.,* **13**: 113.

Zeng Q, Zeng Y, Nie X, Guo Y and Zhan Y (2020). Britanin exhibits potential inhibitory activity on human prostate cancer cell lines through PI3K/Akt/NF-κB signaling pathways. *Planta Med*., **86**(18): 1401-1410.

Zhang GB, Li QY, Chen QL and Su SB (2013). Network pharmacology: A new approach for chinese herbal medicine research. *Evid. Based Complement. Alternat. Med*., **2013**: 621423.

Zhang X, Fan L, Wu J, Xu H, Leung WY, Fu K, Wu J, Liu K, Man K and Yang X (2019). Macrophage p38α promotes nutritional steatohepatitis through M1 polarization. *J. Hepatol*., **71**(1): 163-174.

Zhou M, Huang J, Zhou J, Zhi C, Bai Y, Che Q, Cao H, Guo J and Su Z (2023). Anti-obesity effect and mechanism of chitooligosaccharides were revealed based on lipidomics in diet-induced obese mice. *Molecules.,* **28**(14): 5595.