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Danggui-Huangjing Herb Pair Attenuates Simple Obesity via EGFR-Mediated PI3K-Akt Signaling Inhibition

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We use this protocol and it's working

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Abstract

Background: Simple obesity (SO) has become a global public-health challenge, yet available therapies are markedly limited. The medicinal-edible herbs *Angelica sinensis*(Danggui) and *Polygonatum*(Huangjing) both contain lipid-lowering active constituents. Here, we construct the Danggui-Huangjing herbal pair (Danggui-Huangjing Pair,D-H Pair) and systematically investigate its preventive/therapeutic effects on SO and the underlying molecular mechanisms, offering a new avenue for SO management. **Aim:** In this study, a network-pharmacology approach was employed to elucidate the pharmacological mechanisms of the D-H Pair in SO therapy. Guided by the network-analysis findings, the core targets and signaling pathways linked to its lipid-lowering effect were experimentally validated in vivo. **Methods:** The candidate bioactive constituents of the D-H Pair and their putative targets, together with known therapeutic targets of SO, were retrieved from public databases. The “herb-ingredient-target” network for the D-H Pair in SO treatment was constructed. A protein-protein interaction (PPI) network of the potential targets was built to screen the core genes. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were subsequently performed. Beyond biochemical and histopathological indices, the core targets and signaling pathways related to lipid metabolism were further validated at the molecular level in C57BL/6J mice. **Results:** A total of 15 active ingredients in the D-H Pair and 4,238 SO-related targets were retrieved from databases. A high proportion of the core targets and top signaling pathways were involved in the regulation of lipid metabolism and inflammatory suppression. The D-H Pair markedly ameliorated hepatic steatosis and adipocyte hypertrophy and reduced lipid deposition histopathologically. In particular, it reversed body-weight gain, adiposity and lipid-metabolism disorders in obese C57BL/6J mice, significantly suppressed inflammatory cytokines such as interleukin-6 (IL-6) and tumour-necrosis-factor- α (TNF- α), and down-regulated the **EGFR-PI3K/Akt-SREBP-1/FASN/ACC axis** to attenuate hepatic lipid accumulation. **Conclusion:** This study is the first comprehensive investigation of the active constituents, potential targets, and molecular mechanisms underlying the D-H Pair as a therapeutic intervention for SO. The pair exerts its anti-obesity effects by modulating multiple targets and signaling pathways, in particular by suppressing inflammation and reshaping lipid metabolism. The findings indicate that the D-H Pair represents a promising multi-target therapeutic strategy for the treatment of simple obesity.

Materials

The active chemical ingredients of the two herbs contained in the D-H Pair and the putative targets of these ingredients were retrieved from the Traditional Chinese Medicine Systems Pharmacology (TCMSP, <https://tcmsp-e.com/tcmssp.php>, updated 2023) database by entering “Danggui” and “Huangjing” as search terms. Ingredients were filtered by Absorption, Distribution, Metabolism, and Excretion (ADME) parameters: oral bioavailability (OB) $\geq 30\%$ and drug-likeness (DL) ≥ 0.18 , and the qualified molecules together with their target names were collected. To capture additional actives not listed or below the OB/DL cut-off but supported by literature, the Herbal high-throughput experiment and reference database (HERB, <http://herb.ac.cn>, updated 2020) and China National Knowledge Infrastructure (CNKI) were further searched. The molecular structures and Chemical Abstracts Service (CAS) registry numbers of the collected constituents were verified in the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>); entries with mismatched structures or missing CAS data were discarded, and the remaining set was retained as the final ingredient list. Canonical SMILES strings of these actives were then obtained from PubChem and uploaded to the SwissTargetPrediction server (<https://swisstargetprediction.ch/>, probability $3e0$ for Homo sapiens) to predict putative targets. All target IDs were standardised to UniProt official gene symbols (UniProt, <https://www.uniprot.org/>, updated April 2025), establishing the D-H Pair ingredient library and target library. Known therapeutic targets implicated in simple obesity were searched in GeneCards (<https://www.genecards.org>, updated 16 July 2025), OMIM (<https://www.omim.org>, updated 21 October 2025), the Comparative Toxicogenomics Database (CTD, <https://ctdbase.org/>), and the Therapeutic Target Database (TTD, <https://db.idrblab.net/ttd/>) using the keyword “simple obesity”. Target IDs were unified to UniProt accessions, and the intersection between the D-H Pair putative targets and the obesity-related therapeutic targets yielded the potential treatment targets for subsequent analyses. Granules of Danggui (50 g; Beijing Kangrentang Pharmaceutical Co., Ltd; lot 24022621) equivalent to 75 g crude drug, and wine-processed Huangjing (50 g; lot 24016691) equivalent to 60 g crude drug, were purchased from the Out-patient Pharmacy of the Third Affiliated Hospital of Beijing University of Chinese Medicine. Each week the two granules were dissolved 1:1 (w/w) in pure water, stored at 4°C, and warmed to 40°C in an ultrasonic bath immediately before gavage. Anhydrous ethanol (Sinopharm Chemical Reagent Co., Ltd, China, cat.100092683), xylene (cat.10023418), n-butanol (cat.100052190), eco-friendly dewaxing solution (Wuhan Servicebio Technology Co., Ltd Technology (Servicebio), China, cat.G1128), universal tissue fixative (cat.G1101), haematoxylin (cat.G1004), differentiation solution (cat.G1039), bluing reagent (cat.G1040), Oil Red O stain (cat.G1260), glycerol gelatin mounting medium (cat.G1402), isopropanol (cat.80109218), eosin Y solution (Baso Zhuhai Biotech Co., Ltd, China, cat.BA4022), neutral balsam (cat.10004160), RNA extraction, reverse-transcription and qPCR kits (Hunan Accurate Biotechnology, cat.AG21017, AG11728, AG11739), ELISA kits for mouse interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- α) and interleukin-1 beta (IL-1 β) (Quanzhou Ruixin Biological Technology, China, cat.RX203049M, RX202412M, RX203063M), transforming growth factor beta 1 (TGF- β 1) ELISA kit (Elabscience, cat.E-EL-0162), BCA protein assay kit (Beijing Yamei Biotechnology, China, cat.PC201), primary antibodies against mouse phosphorylated Phosphatidylinositol 3-Kinase (p-PI3K) (Cell Signaling Technology, Inc (CST), cat.4228), Phosphatidylinositol 3-Kinase (PI3K, CST, cat.4257), phosphorylated Protein Kinase B (p-Akt, CST) cat.13038, Protein Kinase B (Akt, CST, cat.4685), Acetyl-CoA Carboxylase (ACC, Abcam, ab72046), Epidermal Growth Factor Receptor (EGFR, Abcam, ab76462), Sterol Regulatory Element-Binding Protein 1 (SREBP-1, Abcam, ab28481) and beta-actin (β -actin, CST, cat.8457T), and colorimetric assay kits for mouse triglyceride (TG), total cholesterol (TC), HDL-cholesterol and LDL-cholesterol (Shenzhen Rayto Life Sciences Co., Ltd, China, cat.S03027, S03042, S03025, S03029) were used. Total protein was extracted from liver tissues with ice-cold RIPA lysis buffer (Solarbio, cat.BC3710). The lysate was centrifuged



at 12 000×g for 30 min at 4°C. Protein concentrations were determined with a BCA Protein Assay Kit (Beijing Yamei Biotechnology, cat.PC201, China). Equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membranes (Millipore, Burlington, MA, USA). Membranes were blocked with protein-free rapid blocking buffer (Beijing Yamei Biotechnology, cat.PS108P, China) for 20 min at room temperature and then incubated overnight at 4°C with primary antibodies against p-PI3K, PI3K, p-AKT, AKT, ACC, EGFR, SREBP-1 and β -actin (all at 1:1000 dilution), followed by washing. After incubation with the appropriate secondary antibodies (1:1000 dilution) for 1h at room temperature, target protein bands were detected with enhanced chemiluminescence substrate (APExBIO Technology LLC, lot K1231171348CAF). Each WB analysis was performed three times. Semiquantitative analysis was carried out using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Biological Function and Pathway Analysis

- 1 Gene Ontology (GO) enrichment analysis mainly describes the biological functions of genes—such as their activity, process, and site of action—and is widely used for gene-function classification. The ClueGO plug-in of Cytoscape was employed to perform enrichment analysis of biological process (BP), cellular component (CC), and molecular function (MF) terms associated with the obtained targets. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was subsequently carried out with the Database for Annotation, Visualization and Integrated Discovery (DAVID, <https://david.ncifcrf.gov/>, updated 17 April 2025) Bioinformatics Resources 6.8 tool to identify signaling pathways related to the target genes, with $P < 0.05$ considered statistically significant.

Experimental Validation Animals

- 2 Sixty male C57BL/6J SPF-grade mice, 8 weeks old and weighing 18 ± 2 g, were supplied by Beijing Spehpharm Biotechnology Co., Ltd. (License No. SYXK [Beijing] 2023-0011). Animals were housed in the animal facility of Liangxiang Campus, Beijing University of Chinese Medicine, under standard conditions: 12 h light/dark cycle, temperature $24 \pm 2^\circ\text{C}$, and relative humidity $50 \pm 5\%$. After a 1-week acclimatization period, mice were fed ad libitum either a 45 % high-fat diet (TP23100) or a 10 % fat control chow (TP23104) produced by Jiangsu Trophic Animal Feed High-Tech Co., Ltd. All experimental procedures were approved by the Animal Ethics Committee of Beijing University of Chinese Medicine (Ethics No. BUCM-2024030403-1123) and were conducted in strict accordance with the committee guidelines.

Drugs and Reagents

- 3 Granules of Danggui (50 g; Beijing Kangrentang Pharmaceutical Co., Ltd; lot 24022621) equivalent to 75 g crude drug, and wine-processed Huangjing (50 g; lot 24016691) equivalent to 60 g crude drug, were purchased from the Out-patient Pharmacy of the Third Affiliated Hospital of Beijing University of Chinese Medicine. Each week the two granules were dissolved 1:1 (w/w) in pure water, stored at 4°C , and warmed to 40°C in an ultrasonic bath immediately before gavage.
- 4 Anhydrous ethanol (Sinopharm Chemical Reagent Co., Ltd, China, cat.100092683), xylene (cat.10023418), n-butanol (cat.100052190), eco-friendly dewaxing solution (Wuhan Servicebio Technology Co., Ltd Technology(Servicebio), China, cat.G1128), universal tissue fixative (cat.G1101), haematoxylin (cat.G1004), differentiation solution (cat.G1039), bluing reagent (cat.G1040), Oil Red O stain (cat.G1260), glycerol gelatin mounting medium (cat.G1402), isopropanol (cat.80109218), eosin Y solution (Baso Zhuhai Biotech Co., Ltd, China, cat.BA4022), neutral balsam (cat.10004160), RNA

extraction, reverse-transcription and qPCR kits (Hunan Accurate Biotechnology, cat.AG21017, AG11728, AG11739), ELISA kits for mouse interleukin-6(IL-6), tumor necrosis factor alpha (TNF- α) and interleukin-1 beta (IL-1 β)(Quanzhou Ruixin Biological Technology, China, cat.RX203049M, RX202412M, RX203063M), transforming growth factor beta 1 (TGF- β 1) ELISA kit (Elabscience, cat.E-EL-0162), BCA protein assay kit (Beijing Yamei Biotechnology, China, cat.PC201), primary antibodies against mouse phosphorylated Phosphatidylinositol 3-Kinase (p-PI3K)(Cell Signaling Technology, Inc(CST), cat.4228), Phosphatidylinositol 3-Kinase (PI3K,CST, cat.4257), phosphorylated Protein Kinase B (p-Akt ,CST)cat.13038), Protein Kinase B (Akt ,CST, cat.4685), Acetyl-CoA Carboxylase (ACC,Abcam, ab72046), Epidermal Growth Factor Receptor (EGFR,Abcam, ab76462), Sterol Regulatory Element-Binding Protein 1(SREBP-1,Abcam, ab28481) and beta-actin (β -actin,CST,cat.8457T), and colorimetric assay kits for mouse triglyceride (TG), total cholesterol (TC), HDL-cholesterol and LDL-cholesterol (Shenzhen Rayto Life Sciences Co., Ltd, China, cat.S03027, S03042, S03025, S03029) were used.

Animal Model and Treatments

- 5 After a 1-week acclimation, C57BL/6J mice were randomly assigned to five groups (n = 12 each): Control, Model, D-H Pair-Low (1.76 g kg⁻¹ day⁻¹), D-H Pair-Medium (3.52 g kg⁻¹ day⁻¹) and D-H Pair-High (7.04 g kg⁻¹ day⁻¹). Control mice received a 10 % kcal fat standard chow throughout the 18-week study; Model and D-H Pair groups were fed a 45 % kcal high-fat diet. Mice whose body weight exceeded that of Control by ≥ 20 % ($P \leq 0.05$) at week 18 were considered to exhibit simple obesity. Dosing was based on Research Methodology of Traditional Chinese Medicine Pharmacology: the human adult male dose (10 g herb per day) was converted to the mouse equivalent (12.33-fold) via body-surface-area normalization; Medium dose = 3.52 g kg⁻¹ day⁻¹, Low = $\frac{1}{2}$ ×Medium, High = 2×Medium. D-H Pair solutions were administered by gavage every second day for 18 weeks, while Model animals received an equal volume of de-ionised water. Food intake and body weight were recorded every 3 days.

Sample Collection

- 6 At the end of the 18th week, after the final gavage, mice were fasted for 12 h with free access to water. Animals were anesthetized with isoflurane in an animal gas anesthesia machine, blood was rapidly collected by enucleation, kept at room temperature for 3 h, then centrifuged at 4°C, 5 000 rpm for 15 min. Serum was aspirated, aliquoted and stored at -20°C. Liver, epididymal white adipose tissue (eWAT) and brown adipose tissue (BAT) were dissected; liver and eWAT were weighed. Neat edge slices were fixed in 4 % paraformaldehyde, remaining tissue was snap-frozen in liquid nitrogen and transferred to -80°C until further analysis.

Body Composition and Tissue Weight Assessment

- 7 Body mass was recorded twice weekly. Before necropsy, total body fat was quantified in conscious mice by EchoMRI (Shanghai Niumag Electronic Technology Co., Ltd, China, model EchoMRI). After dissection, liver and eWAT were excised and weighed on an electronic balance; data were collated for statistical analysis.

Serum Lipid Biochemistry Analysis

- 8 For serum chemistry, mice were fasted 12 h with free access to water. Blood obtained by enucleation was kept at room temperature for 3 h, centrifuged, and the resulting serum was analyzed on an automatic biochemical analyzer for triglyceride (TG), total cholesterol (TC), HDL and low-density lipoprotein (LDL) concentrations.

Histopathological Analysis of Liver and Adipose Tissue

- 9 Paraffin sections were deparaffinized in xylene I and II for 20 min each, rehydrated through absolute ethanol I and II and 75 % ethanol for 5 min each, rinsed in running water, and subjected to Hematoxylin and Eosin (HE) staining: hematoxylin for 3–5 min (nuclei blue-purple), water wash, instantaneous differentiation, second water wash, bluing solution, extensive rinse; sections were then pre-dehydrated through 85 % and 95 % ethanol for 5 min each, immersed in eosin for 5 min (cytoplasm red), dehydrated through absolute ethanol I–III for 5 min each, cleared in xylene I and II for 5 min each, and mounted with neutral balsam. Images were captured at 200× magnification to evaluate hepatic steatosis, inflammatory infiltration and adipocyte size. For hepatic lipid-droplet quantification, fresh liver was fixed in 4 % paraformaldehyde for 24 h, cryoprotected in 15 % and 30 % sucrose at 4°C, blotted dry, embedded in Optimal Cutting Temperature compound (OCT) and snap-frozen. Cryosections (8–10 µm) were cut, mounted on slides and stored at –20°C. After equilibration to room temperature and 15 min fixation, sections were rinsed briefly in 70 % ethanol, stained with Oil-Red-O working solution (stock : water = 6 : 4, upper phase) for 5–10 min, rapidly differentiated in 70 % ethanol, washed in running water for 30 s, counter-stained with hematoxylin for 1–2 min, sequentially immersed in three changes of pure water (5 s, 10 s, 30 s), 60 % ethanol differentiation solution for 1–2 s, two changes of double-distilled water (10 s each), bluing solution for 5–10 s, running water (5 s, 10 s), and mounted with glycerol gelatin. Images were acquired at 200× magnification.

ELISA Analysis

- 10 Plates and reagents for IL-6, TNF- α , IL-1 β and TGF- β 1 were brought to room temperature for 1 h; the concentrated wash buffer was dissolved in a 37°C water-bath and diluted 1:20. Substrates A and B were mixed in equal volumes and left standing for 15 min. Required strips were removed, the remainder sealed and refrigerated. Standard, zero, blank and sample wells were set up in sequence, receiving 50 µL of gradient standards, diluent, no fluid or test sample, respectively. Except for blanks, 100 µL Horseradish

Peroxidase(HRP)-conjugated detection antibody was added to each well, the plate was covered and incubated at 37°C in the dark for 60 min. After aspiration and blotting, wells were filled with wash buffer for 20 s, flicked dry and washed five times; an automatic plate washer was used for a 30s soak, with final thorough blotting on absorbent paper. One hundred microlitres of substrate mixture was then added to each well, the plate was covered and incubated at 37°C in the dark for 15 min, followed by 50 µL stop solution. Absorbance at 450 nm was read immediately on a microplate reader for quantitative analysis.

RT-qPCR Analysis

- 11 Following the manufacturer's workflow, 20 mg liver tissue was placed in a 2 mL tube, 1 mL lysis buffer added, and the sample homogenized at 55 Hz for 15 s followed by a 10 s pause for 1 min in a pre-cooled grinder. Total RNA was then purified with an RNA extraction kit (Hunan AccoBio Biotechnology Co., Ltd, China, cat.AG21017). Purity was assessed by measuring A260/280 with 2µL on a nucleic-acid/protein analyzer; ratios of 1.9-2.2 indicated high purity. Samples were kept on ice and stored at -80°C. Reverse transcription was performed at 37 °C for 15 min and 85 °C for 5 s to synthesize cDNA. For RT-qPCR, 10 µL SYBR Mix was supplemented with 0.04 µL each of forward and reverse primers, 2 µL cDNA and 7.92 µL ddH₂O. Reactions were run on a StepOne Plus system: 95°C for 30 s pre-denaturation; 40 cycles of 95°C for 5 s and 60°C for 30 s; followed by a melt-curve step (95°C for 15 s→60°C for 60 s). β-Actin was amplified in parallel as an internal control, and relative target mRNA levels were calculated by the 2^{-ΔΔCt} method. Each group was analyzed in triplicate; primer sequences are listed in Table 9.

WB Analysis

- 12 Total protein was extracted from liver tissues with ice-cold RIPA lysis buffer (Solarbio, cat.BC3710). The lysate was centrifuged at 12 000×g for 30 min at 4°C. Protein concentrations were determined with a BCA Protein Assay Kit (Beijing Yamei Biotechnology, cat.PC201, China). Equal amounts of protein were separated by SDS-PAGE and transferred to PVDF membranes (Millipore, Burlington, MA, USA). Membranes were blocked with protein-free rapid blocking buffer (Beijing Yamei Biotechnology, cat.PS108P, China) for 20 min at room temperature and then incubated overnight at 4°C with primary antibodies against p-PI3K, PI3K, p-AKT, AKT, ACC, EGFR, SREBP-1 and β-actin (all at 1:1000 dilution), followed by washing. After incubation with the appropriate secondary antibodies (1:1000 dilution) for 1h at room temperature, target protein bands were detected with enhanced chemiluminescence substrate (APExBio Technology LLC, lot K1231171348CAF). Each WB analysis was performed three times. Semiquantitative analysis was carried out using ImageJ software (National Institutes of Health, Bethesda, MD, USA).



Statistical Analysis

- 13 All data are presented as mean $\pm$ SD. Normality was examined with the Shapiro-Wilk test. Parametric data were analysed by one-way ANOVA; when variances were homogeneous, group differences were evaluated with the least-significant-difference (LSD) test, otherwise Tamhane's T2 test was used. Non-parametric data were analysed with the Kruskal-Wallis test. P $\leq$ 0.05 was considered statistically significant and P $\leq$ 0.01 highly significant.