Differential Brain Tissue Proteins in Ovariectomized Hyperlipemia Rats After Intervention Using Erxian Extract

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Research

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Abstract

Background: Erxiantang (EXT) is a popular traditional Chinese medicine formula that has phytoestrogens with protective effects on the nervous system, hypertension, menopausal syndrome. We aimed to explore the effects of EXT on differential brain tissue proteins in ovariectomized (OVX) hyperlipemia rats using label-free high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

Methods: We randomly divided 40 eight-week-old female Sprague-Dawley rats into four groups: (1) SHAM (sham-operation, equal volume ddH₂O); (2) OVX (ovariectomized, equal volume ddH₂O); (3) EXT (ovariectomized, Erxian extract: 5.46 g/kg body weight); and (4) EV groups (ovariectomized, estradiol valerate: 0.1 mg/kg body weight). After administration for 6 weeks, we measured body weight, serum lipid, and estrogen. Rat brain tissues were analyzed using HPLC-MS/MS. Subsequently, we performed bioinformatics analysis and screened differential proteins for detection and verification through western blot analysis.

Results: We identified 4678 proteins. There were 87 differential proteins in the SHAM and OVX group; 50 were up-regulated and 37 were down-regulated. There were 242 differential proteins in the EXT and OVX groups; 95 were up-regulated and 147 were down-regulated. Proteins in the EXT, EV, and SHAM groups that showed a similar trend to the OVX group were ApoE, slc6a1, gpd1, eno3, hmgcs1, u2af2, march5, mcam, actl6b, tmc2, coq3, dctn5, seleno, tmem109, derl1, syvn1, rbm39, cnpy2, bcl-2, acsf2, dc42ep4, and mapk8. These proteins were mainly involved in lipid metabolism, biological oxidation, signal transduction, cholesterol metabolism, immune response, stress response, and cell apoptosis. Western blot analysis of ApoE, GAT1, JNK, and p-JNK proteins showed that their expression in the EXT group was lower than in the OVX group, which was consistent with the proteomics analysis results.

Conclusions: EXT may protect the OVX hyperlipemia rats by exerting an effect on lipid metabolism, immune response, stress response, and apoptosis. Moreover, the identified differential proteins may be related to the occurrence and development of hyperlipemia-related diseases.

Full Text

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Figures

Figure 1

HPLC-MS mass spectrometry analysis of the EXT extracts. A) First-order spectrogram positive ion mode. B) Second-order spectrogram positive ion mode. C) Mass spectrometry of the main components; 1. curculigoside; 2. icaridisid I; 3 mangiferin; 4 sarsasapogenin; 5 jatrorrhizine.

Figure 2

Representative 12% SDS-PAGE gels showing the total proteins purified from brain samples. A1, A2, A3 (EXT group); B1, B2, B3 (EV group); C1, C2, C3 (SHAM group); D1, D2, D3 (OVX group).

Figure 3

Body weight gain (A) and levels of serum E₂, FSH, and LH (B, C, D). Values are presented as means $\pm$ Standard Error of Mean (SEM), n=10 per group; #P<0.05 versus SHAM group; *P<0.05 versus OVX group.

Figure 4

Levels of serum TC, TG, HDL-c, and LDL-c. Values are expressed as means $\pm$ Standard Error of Mean (SEM), n=10 per group; #P<0.05 versus SHAM group; *P<0.05 versus OVX group.

Figure 5

LC-MS/MS quality control and functional annotation and enrichment analysis of identified proteins. A): Repeatability Coefficient of Variation (CV) analyses. B): Gene ontology (GO) annotation of indicated proteins. The annotated amino acid sequences were classified and grouped into different functional subcategories: cellular component (CC), molecular function (MF), and biological process (BF). C): Gene ontology (GO) functional classification of differentially expressed proteins between EXT group and OVX group (A vs P-value ≤ 0.05). D: KEGG Pathway annotation of indicated proteins. E: KEGG Pathway enrichment top20 bubble diagrams between EXT group and OVX group (A vs D).

Figure 6

Differential proteomics analysis in each group. A), B), C): Volcano map of significant differential proteins between groups. The gray is non-differential proteins, the red is up-regulated significant difference proteins, the green is down-regulated significant difference proteins. D), E), F): Heat map of hierarchical clustering of proteins between groups. The up-or down-regulated proteins are indicated in red and green, respectively. G): Interaction network of the differential proteins. The underlined proteins were from the identified protein list. The other proteins were obtained from the extensive interactome feature of the network Analyst software. [C vs D (SHAM group vs. OVX group); B vs D (EV group vs. OVX group); A vs D (EXT group vs OVX group)].

Figure 7

Proteins expression verification of ApoE, GAT1, JNK and p-JNK by western blot. A), D) Gene levels of ApoE, GAT1, JNK and p-JNK in Hypothalamus and Hippocampus were analyzed by western blot. B), C), E), F) Semi-quantitative analysis of ApoE, GAT1, p-JNK and JNK proteins expression compared with tubulin & GAPDH in Hypothalamus and Hippocampus. Values are presented as the means $\pm$ Standard Error of Mean (SEM), $n=3$ per group. $\#P<0.05$, compared to the SHAM group; $*P<0.05$, compared to the OVX group