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Molecular targets and mechanism of Longji Xiaozhong ointment in treating acute ankle sprain: A network pharmacology based analysis

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Acute ankle sprain is one of the most common lower limb injuries particularly in athletes and it accounts for 16-40% of all sports-related injuries. A significant proportion of patients experience persistent residual symptoms and recurrence. Traditional Chinese medicine (TCM) offers effective treatment in terms of pain relief, swelling reduction, functional improvement, and shorter disease duration. This study provides a comprehensive analysis of the active ingredients, molecular targets, signaling pathways, and Longji Xiaozhong ointment's clinical efficacy for treating acute ankle sprains. We followed network pharmacology approach to identify the molecular targets and mechanism of action of Longji Xiaozhong ointment in treating acute ankle sprain and to validate its clinical value using relevant clinical data. Initially, Longji Xiaozhong Ointment's active ingredients were screened using the TCMSP platform based on drug properties similar to DLP ≥0.18 and oral bioavailability OB ≥30%. The molecular targets were identified from the ETCM database, followed by constructing protein-protein interaction (PPI) network diagrams and GO and pathway enrichment analysis on key genes. Furthermore, the UniProt database was used to investigate the 3D structures of these key genes. Finally, the clinical data of 111 patients with acute ankle sprains were retrospectively analyzed and further explored by comparing the different curative effects of Longji Xiaozhong ointment with Celebrex and Shexiang Analgesic ointment to check the clinical value of Longji Xiaozhong ointment in treating acute ankle sprain. In the TCMSP database, we found that there were 42 active ingredients in Longji Xiaozhong ointment, including 13 kinds of *Strychnosnux-vomica* L., 7 kinds of *Ligusticum chuanxiong*, and 22 kinds of Safflowers (*Carthamus tinctorius*), and a total of 3206 target genes were obtained. After applying the OB ≥50% criteriaand DL ≥0.5, 19 key target genes were selected based on their correspondence to the active ingredients. Protein mutual aid network construction and module analysis yielded two high-scoring Clusters and identified seven key proteins, including SLC6A4, ADRB2, ADRA1B, CHRM1, F2, OPRM1, and OPRD1. Functional enrichment analysis (FEA) of candidate target genes of *Strychnosnux-vomica*, *Ligusticum chuanxiong*and Safflowers in Longji Xiaozhong ointment in ETCM database showed SUMOylation of intracellular receptors, blockade of NMDA receptors, and activation of GABA A receptors, lipid metabolism regulationby peroxisome proliferator-activated receptor alpha (PPARα). PPARA activates aspects of gene expression and transcriptional activation of mitochondrial biogenesis. On retrospective analysis, all patients were divided into Group A: Celebrex combined with brace immobilization group (36 cases); Group B: Shexiang Jietong ointment combined with brace immobilization group (37 cases); and Group C: Longji Xiaozhong ointment combined with brace immobilization group (38 cases). After treatment, the VAS score, swelling degree and scope score of Longji Xiaozhong ointment combined with brace immobilization group were significantly lower than those of the other two groups, and the effect was significantly better in terms of daily activities, sports, quality of life and occurrence of re-sprain than the other two groups. Our findings, supported by clinical data, demonstrate that Longji Xiaozhong ointment effectively alleviates swelling and pain, accelerates the repair of ankle ligaments, enhances ankle joint function, and improves ankle joint stability.

Keywords: *Carthamus tinctorius*, Gene expression map, *Ligusticum chuanxiong*, Nux vomica, Quaker buttons, Safflowers, Semen strychnos, Strychnine tree, *Strychnosnux-vomica*, Traditional Chinese medicine