# Study on Anti-Inflammatory Effects and Mechanism of Sojae Semen Praeparatum

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**Abstract: This study explores the anti-inflammatory effects and mechanisms of Sojae Semen Praeparatum (SSP) through network pharmacology and animal experimentation. First, active ingredients and therapeutic targets in SSP were identified using the TCMSP database and literature review. In addition, inflammation-related ingredients, targets, and pathways were sourced from databases such as TTD, KEGG, and Drug Bank. These findings were used to construct and analyze networks of ingredient-target-pathway interactions, target distribution across tissues, and key protein associations. Finally, a rat paw edema test was performed to experimentally confirm SSP's anti-inflammatory effects. The results identified 16 inflammation-related active ingredients in SSP, which act on 33 target proteins across 73 pathways. Furthermore, SSP extracts significantly reduced paw edema in rats, indicating strong anti-inflammatory potential. This study demonstrates that SSP exerts anti-inflammatory effects through multi-ingredient, multi-target, and multi-pathway interactions, providing a scientific basis for its clinical application.**

**Keywords: Sojae Semen Praeparatum; Anti-Inflammatory; Network Pharmacology; Mechanism of Action; Paw Edema**

## 1. Introduction

Sojae Semen Praeparatum (SSP) is made from the mature seeds of the soybean plant, which belongs to the dicotyledonous family. It is prepared by fermenting the seeds with mulberry leaves and wormwood. SSP was first recorded in the ancient Chinese text "Ming Yi Bie Lu" under the name "Chi" and classified as a medium-grade medicinal substance [1]. SSP is bitter in taste, cold in nature, and non-toxic. It has properties that help relieve exterior syndromes, alleviate irritability, and disperse constrained heat. It can be used both as food and medicine [2,3]. SSP is mainly used to treat symptoms such as external wind-cold, headache, irritability, chest tightness, and insomnia. SSP is often combined with other herbs to treat conditions like colds, fever, and dysentery. Notable traditional Chinese medicine formulas include Yin Qiao San (from "Wen Bing Tiao Bian"), Cong Chi Decoction (from "Zhou Hou Fang"), and Zhi Zi Chi Decoction (from "Shang Han Lun"). Additionally, SSP is an ingredient in marketed Chinese patent medicines such as Vitamin C Yin Qiao Tablets, Lingyang Ganmao Soft Capsules, and Yinqiao Detoxification Capsules. SSP also has applications in the treatment of osteoporosis [4].

SSP has diverse medicinal values, including antiviral, antibacterial, and antitumor effects. However, most research on SSP focuses on its fermentation process and the microbial changes during fermentation, as well as its physiological functions like antidepressant, anti-influenza, lipid-lowering, antioxidant, blood sugar-lowering, anticancer, thrombolytic, and estrogen-like effects [5]. Modern medical studies show that these conditions are often associated with inflammation, suggesting that SSP may contain anti-inflammatory ingredients. Currently, there is limited research on SSP's anti-inflammatory activity and its underlying mechanisms. This study aims to verify SSP's anti-inflammatory activity and elucidate its molecular mechanisms using a combination of network pharmacology and pharmacological methods.

## 2. Experimental Materials

Instruments: RE-52A Rotary Evaporator (Shanghai Yarong Biochemical Instrument

 

Factory); Constant Temperature Ultrasonic Instrument (Kunshan Ultrasonic Instrument Co., Ltd.); PV-200 Paw Edema Meter (Chengdu Taimeng Technology Co., Ltd.)

Materials and Reagents: Sojae Semen Praeparatum (Guangxi Tairong Pharmaceutical Co., Ltd., Batch No. 190301); Indomethacin Tablets (Zhejiang Taicang Pharmaceutical Co., Ltd., Batch No. 090807); Carrageenan (Shanghai Sangon Biotech Co., Ltd., Batch No. CN1138)

## 3. Methods and Results

### 3.1 Network Pharmacology Study

3.1.1 Candidate Ingredient Screening

Chemical ingredients of SSP were collected using the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP). Ingredients with oral bioavailability (OB) ≥ 18% and drug-likeness (DL) ≥ 0.1 were selected as candidate active ingredients. Combined with literature review, 25 candidate ingredients were identified (see Table 1).

**Table 1. 25 Candidate Ingredients of SSP**

| MOL ID | Molecule Name/Compound | OB | DL |
|---|---|---|---|
| M1 | 6"-O-Acetyldaidzin | 19.73 | 0.83 |
| M2 | 6"-O-Acetylgenistin | 22.10 | 0.84 |
| M3 | 6"-O-Acetylglycitin | 27.06 | 0.85 |
| M4 | 6"-O-malonyldaidzin | 27.13 | 0.84 |
| M6 | 6"-O-Malonylglycitin | 30.40 | 0.81 |
| M7 | Glycitin | 22.48 | 0.78 |
| M8 | Glycitein | 50.48 | 0.24 |
| M9 | Daidzin | 14.32 | 0.73 |
| M10 | Daidzein | 19.44 | 0.19 |
| M11 | Genistin | 13.35 | 0.75 |
| M12 | Genistein | 17.93 | 0.21 |
| M13 | Biochanin A | 25.21 | 0.24 |
| M14 | Tetramethylpyrazine | 20.01 | 0.03 |
| M15 | Isoflavone | 49.03 | 0.13 |
| M16 | Sitogluside | 20.63 | 0.62 |
| M17 | beta-Sitosterol | 36.91 | 0.75 |
| M18 | Thymine | 74.20 | 0.02 |
| M19 | Adenine | 62.81 | 0.03 |
| M20 | Uracil | 42.53 | 0.02 |
| M21 | Uridine | 23.40 | 0.11 |
| M22 | Apigenin | 23.06 | 0.21 |
| M23 | Stigmasterol | 43.83 | 0.76 |
| M24 | Campesterol | 37.58 | 0.71 |
| M25 | Syringaldehyde | 67.06 | 0.71 |
| M26 | Syringic acid | 47.78 | 0.5 |

3.1.2 Inflammation-Related Targets and Pathways

The targets of the 25 candidate ingredients were obtained from the TCMSP database and normalized to gene names using the Uniprot database [6]. Candidate targets were identified. Inflammation-related targets were collected from the TTD, KEGG, and Drug Bank databases [7-9], resulting in 33 targets (see Table 2), corresponding to 16 active ingredients, mainly flavonoids and sterols. The results indicate that the anti-inflammatory effects of SSP are primarily achieved by regulating target proteins such as PTGS2, PTGS1, and HSP90AA1. Potential targets were then mapped to the KEGG pathways, identifying 73 inflammation-related pathways.

**Table 2. Inflammation-Related Targets of SSP**

| Gene Name | Uniprot ID | Degree | BC |
|---|---|---|---|
| PTGS2 | P35354 | 13 | 0.2435 |
| PTGS1 | P23219 | 10 | 0.1311 |
| HSP90AA1 | P07900 | 7 | 0.0661 |
| PPARG | P37231 | 7 | 0.0165 |
| ESR1 | P03372 | 6 | 0.0234 |
| RXRA | P19793 | 6 | 0.0288 |
| AR | P10275 | 5 | 0.0259 |
| CASP3 | P42574 | 4 | 0.0241 |
| NOS2 | P35228 | 4 | 0.0105 |
| PGR | P06401 | 4 | 0.0493 |
| BCL2 | P10415 | 3 | 0.0116 |
| NOS3 | P29474 | 3 | 0.0086 |
| TNF | P01375 | 3 | 0.0148 |
| TP53 | P04637 | 3 | 0.0148 |
| AKR1B1 | P15121 | 2 | 0.0076 |
| AKT1 | P31749 | 2 | 0.0052 |
| AHR | P35869 | 1 | 0.0000 |
| AKR1C3 | P42330 | 1 | 0.0000 |
| APP | P05067 | 1 | 0.0000 |
| CCND1 | P24385 | 1 | 0.0000 |
| CFTR | P13569 | 1 | 0.0000 |
| CYP3A4 | P08684 | 1 | 0.0000 |
| HDAC6 | Q9UBN7 | 1 | 0.0000 |
| HSD3B1 | P14060 | 1 | 0.0000 |
| HTR3A | P46098 | 1 | 0.0000 |
| IFNG | P01579 | 1 | 0.0000 |
| IL2 | P60568 | 1 | 0.0000 |
| MAPK3 | P27361 | 1 | 0.0000 |
| NFKBIA | P25963 | 1 | 0.0000 |
| NR3C2 | P08235 | 1 | 0.0000 |
| PLA2G2A | P14555 | 1 | 0.0000 |
| PPARA | Q07869 | 1 | 0.0000 |
| TBXAS1 | P24557 | 1 | 0.0000 |

3.1.3 Construction of Key Target Protein Interaction Network

To clarify the interactions among potential anti-inflammatory targets in SSP, the inflammation-related targets were imported into the String network platform [10]. The

confidence level was set to 0.9 to construct the key target protein-protein interaction (PPI) network (see Figure 1). The results identified 7 core targets: HSP90AA1, AKT1, MAPK3, TP53, ESR1, RXRA, and TNF. These core targets play significant roles in the network, acting as key nodes that interact with other proteins.

3.1.4 Target Tissue Distribution

The tissue distribution of the targets was determined based on microarray analysis data from different tissue types in the BioGPS database. The results are shown in Figure 2. The findings indicate that out of 84 normal tissues, the 33 anti-inflammatory targets of SSP are highly distributed in 9 tissues and organs, including the heart (19 targets), liver (17 targets), and lungs (16 targets). This suggests that SSP has significant therapeutic effects on inflammation in these tissues, such as cardiovascular diseases, liver inflammation, and pneumonia.

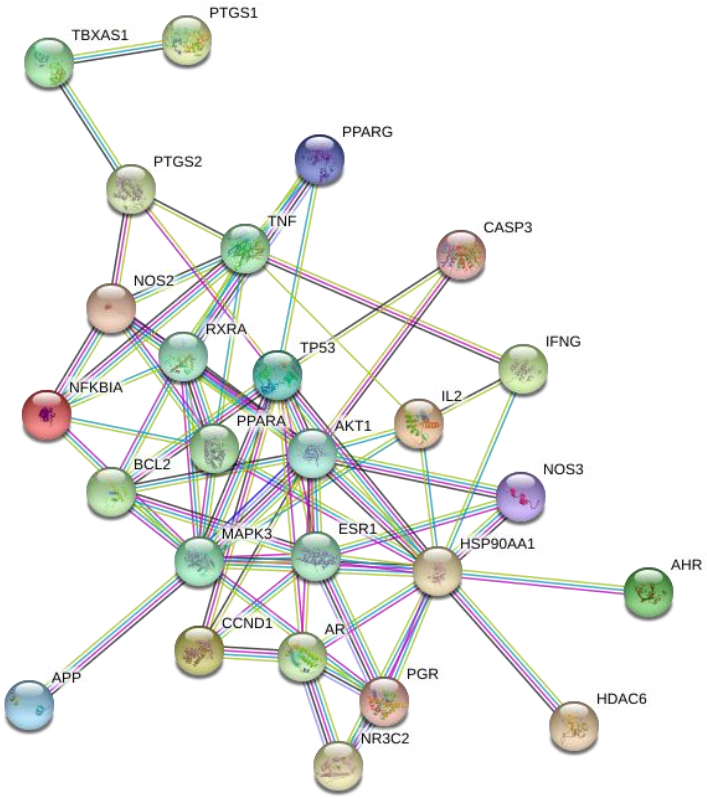


**Figure 1. Target Protein PPI Network**

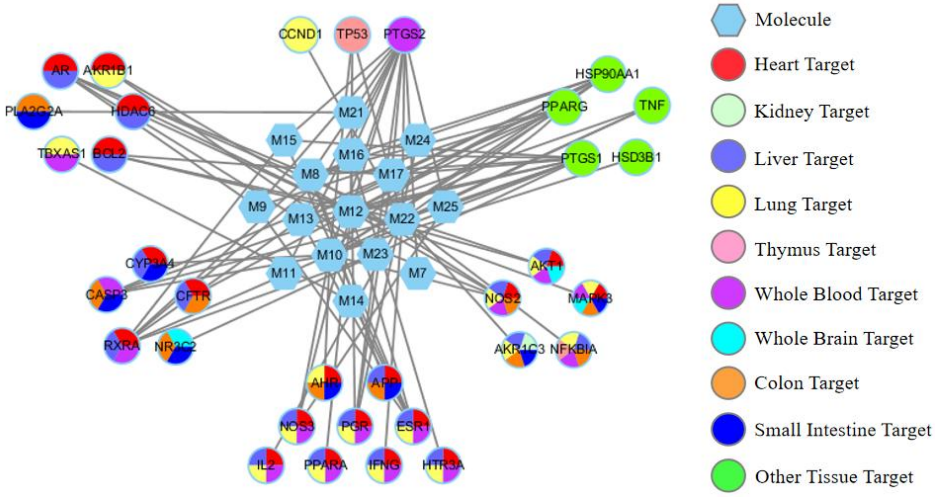


**Figure 2. Target-Tissue Network for Anti-Inflammatory Effects of SSP**

3.1.5 Construction and Topological Analysis of the Ingredient-Target-Pathway Network

Using Cytoscape software, networks for inflammation-related active ingredients to

 

targets and targets to pathways were constructed. These networks were analyzed using two key topological parameters: Degree and Betweenness Centrality (BC). The results are shown in Figure 3 and Figure 4. In the active ingredient-target network (Figure 3), the ingredient with the highest Degree is M12 (Genistein, Degree=19), followed by M22 (Apigenin, Degree=14). The target with the highest Degree is PTGS2 (Degree=13), followed by PTGS1 (Degree=10). In the target-pathway network (Figure 4), the target with the highest Degree is AKT1 (Degree=48), followed by MAPK3 (Degree=47), with an average Degree of 12.07 for targets. The pathway with the highest Degree is the "Pathway in cancer" (Degree=13), followed by the "PI3K-Akt signaling pathway" (Degree=9), with an average Degree of 4.89 for pathways.

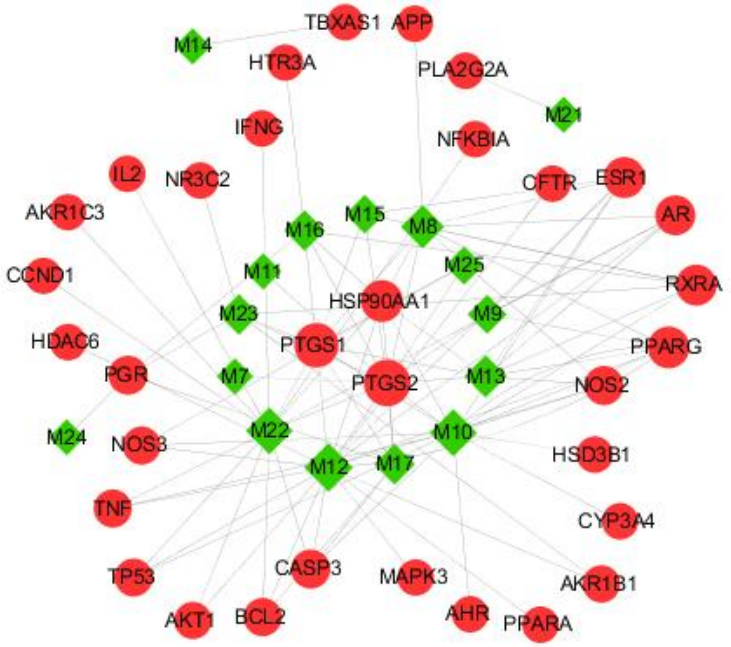


**Figure 3. Network of Inflammation-Related Active Ingredients and Targets in SSP**

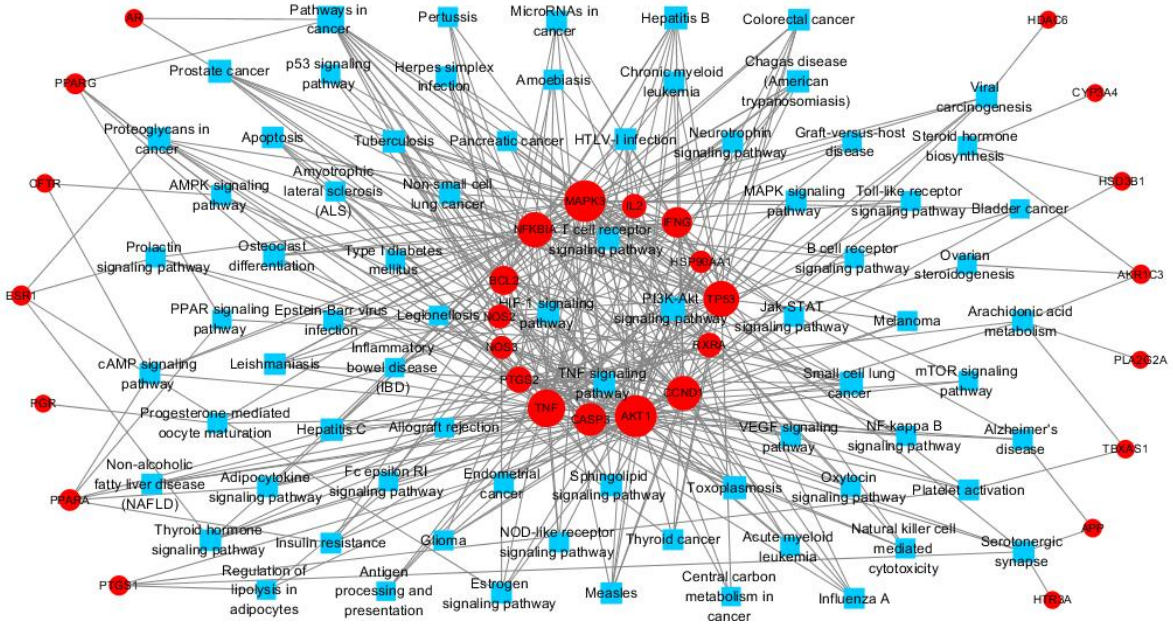


**Figure 4. Network of Potential Targets (T, red circles) and Pathways (P, blue squares)**

3.1.6 Systematic Analysis of the Anti-Inflammatory Mechanism of SSP

To further understand the molecular mechanisms of SSP's anti-inflammatory effects, focus was put on key pathways closely related to inflammation. These pathways include the PI3K-Akt signaling pathway, HIF-1 signaling pathway, T Cell Receptor

signaling pathway, and TNF signaling pathway. These pathways were integrated into a comprehensive "SSP Inflammation-Related Pathway", as shown in Figure 5. The results indicate that inflammatory infections can trigger responses in multiple pathways within the human immune system. This activation initiates widespread defense mechanisms, leading to the production of cytokines and chemokines, among other responses.

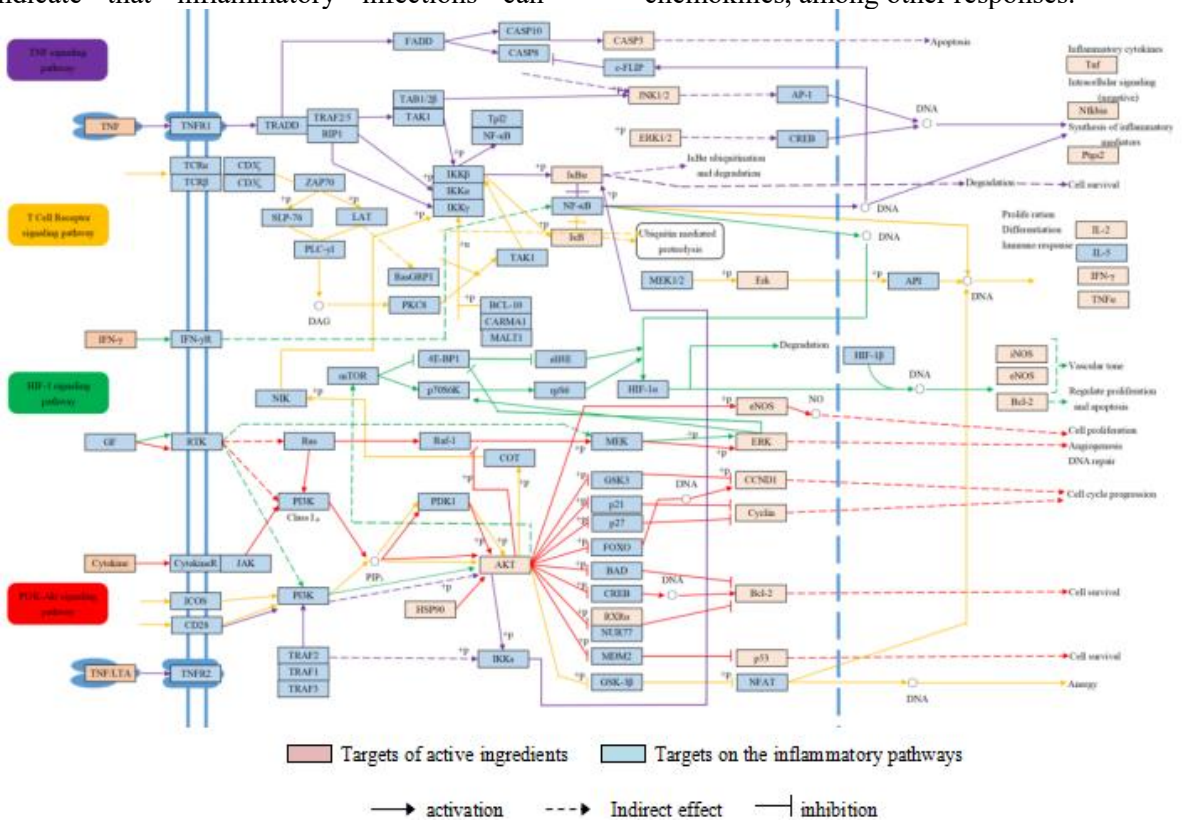


**Figure 5. Systematic Analysis of the Anti-Inflammatory Mechanism of SSP**

### 3.2 Study on the Anti-Inflammatory Effect Using the Paw Edema Method

3.2.1 Extraction of Active Ingredients

SSP was ground into a fine powder and passed through a 20-mesh sieve. The powder was defatted using petroleum ether and then dried. The dried material was soaked in 80% ethanol at a ratio of 1:10 (material to liquid) for 24 hours at room temperature. Ultrasonic extraction was performed three times at 60°C for 30 minutes each time. The extracts were filtered, and the filtrate was collected and concentrated using a rotary evaporator to obtain a thick extract (4 g of raw herb per mL). Before use, the extract was diluted to the required concentration with distilled water.

3.2.2 Animal Grouping and Administration

Thirty SD rats were randomly divided into five groups, with six rats in each group. The groups were set as model control group (normal saline); positive drug group (indomethacin 25 mg/kg); SSP extract high-dose group (20 g/kg); SSP extract medium-dose group (10 g/kg); SSP extract low-dose group (5 g/kg). Each group received their respective treatments via gavage for five days, while the model control group received an equal volume of normal saline. Thirty minutes before the last administration, each rat was placed in a restraining box, and a mark was made on the outer ankle of the hind paws using a marker. The paws were then straightened and placed in a glass cylinder of a paw edema meter to record the baseline paw volume (mL). One hour after the last administration, 0.1 mL of 1% carrageenan was injected subcutaneously into the right hind paw of each rat to induce inflammation. The volume of the swollen right hind paw was measured every hour. The results are shown in Figure 6. The results indicated that the degree of paw edema in the model control group peaked at 3 hours after inflammation induction and then gradually decreased. Compared to the model control group, the positive drug group showed a significant reduction in paw edema from 2 to 5 hours after inflammation ($P < 0.01$ or $P <$

 

0.05). The high-dose SSP group showed a significant reduction in paw edema at 2, 3, and 4 hours, and the medium-dose group at 3 and 4 hours. The low-dose group did not show a significant inhibitory effect at any time point.

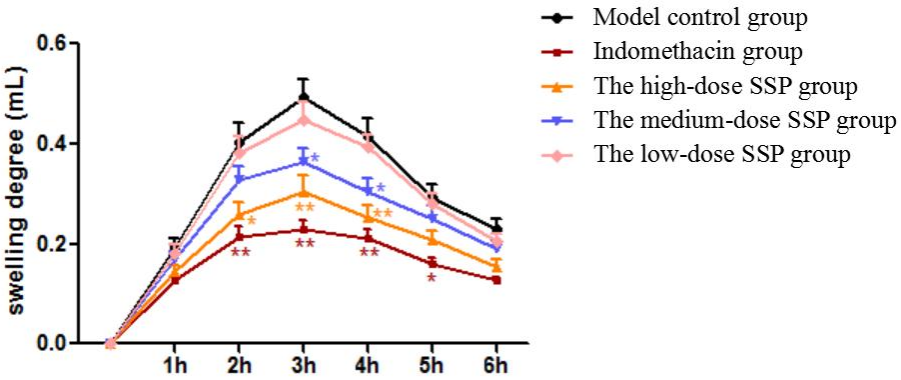


**Figure 6. Effect of SSP Extract on Rat Paw Edema**

## 4. Discussion

PTGS (Prostaglandin-Endoperoxide Synthase) is closely related to the body's synthesis of prostaglandins. There are two isoenzymes, PTGS1 and PTGS2, which play different roles in the body. PTGS1, known as a "housekeeping gene", helps maintain the normal environment of tissues and blood vessels, with an expression range of about 2-4 times. PTGS2, on the other hand, is an "inflammatory response gene" that participates in inflammation by inducing prostaglandin production and promoting cell growth, with expression levels that can increase by 10-80 times [11]. HSP90a is an inducible subtype of heat shock proteins (HSPs). HSPs are a group of highly conserved cytoplasmic proteins with complex internal functions, including protein synthesis and secretion. In both cancerous and adjacent normal tissues, HSP90a shows high inducible and sustained expression. Additionally, HSP90AAI protein is persistently overexpressed in many chronic inflammatory diseases, such as rheumatoid arthritis [12].

Akt is a crucial downstream molecule in the PI3K pathway and can directly or indirectly inhibit various protein genes. Studies have shown that the activation of Akt can suppress the inflammatory response induced by LPS (lipopolysaccharide) in sepsis. The HIF-1 signaling pathway plays a vital regulatory role in the hypoxic environment caused by inflammation. Research confirms that hypoxic conditions and HIF activation are present in many inflammatory sites [13]. The TNF signaling pathway is involved in the signaling and regulation of cell survival, death, and differentiation, interacting with multiple other pathways. TNF receptors can regulate inflammatory cytokines like IL-6 and TNF-α during the acute and early phases of inflammation, contributing to the inflammatory response in various diseases such as atherosclerosis and rheumatoid arthritis [14].

Pharmacological studies have shown that SSP extracts significantly reduce paw edema in rats. The degree of inhibition increases with higher doses, indicating that SSP has notable anti-inflammatory properties. When SSP is extracted with 80% ethanol, eight compounds are obtained, including genistein, genistin, daidzin, and daidzein. Both pharmacological studies and network pharmacology analyses confirm that genistein and daidzein are the primary ingredients responsible for the anti-inflammatory effects of SSP. Additionally, existing research suggests that SSP can be used to treat early-stage atherosclerosis and effectively improve osteoporosis. However, the potential applications of SSP in treating chronic inflammatory diseases of the cardiovascular, immune, and nervous systems remain to be further explored.

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