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Therapeutic effects of gentiopicroside on adjuvant-induced arthritis by inhibiting inflammation and oxidative stress in rats

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ABSTRACT

The purpose of this research was to evaluate the therapeutic effects of gentiopicroside (GPS) on adjuvant-induced arthritis (AA) rats. Rats were injected with complete Freund's adjuvant (CFA) for 0.1 mL in the right hind paw to induce AA. Thirty rats from three groups were treated with GPS (30, 60, 90 mg/kg) from day 15 to day 26. Arthritis was evaluated by arthritis index, paw volume, paw thickness, and X-ray. The effect of GPS on inflammation was assessed by measuring the levels of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6 and IL-17, as well as related mRNA. Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), glutathione (GSH) protein carbonyl (PCO) and malondialdehyde (MDA) were measured to assess the effect of GPS on oxidative stress. These results indicate that GPS increases the levels of GSH-Px, SOD and GSH, and reduces the levels of MDA and PCO. GPS can significantly down-regulate the levels of IL-1β, TNF-α, IL-6 and IL-17, as well as related mRNA. In addition, X-ray and histopathological results show that GPS has a therapeutic effect on joints in AA rats. In summary, the therapeutic effects of GPS on AA rats are associated with anti-inflammation and antioxidation.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease with chronic inflammation, which is characterized by bone damage, synovial hyperplasia [1] and joint deformity [2,3]. RA affects 0.5%–1% of the world population [4,5], in which woman patients are approximately three times than men [6]. Cardiovascular disease (CVD) which is caused by long-term chronic inflammation is considered as the main reason of mortality in RA patients [6,7], and the risk of CVD in RA patients is around two times than the general population [8,9]. Therefore, the inhibition of chronic inflammation is important in the therapeutic strategies of RA. Proinflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6 and IL-17 [6], stimulate inflammation, which will lead to irreversible cartilage damage [8] and bone erosion. Thus, reducing proinflammatory cytokines and related mRNA are beneficial for inhibiting inflammation, preventing joint damage, attenuating structural damage progression and are disease modifying.

Apart from this, it has been reported that oxidative stress (OS) occurs in RA with excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [10]. In-depth study indicates that ROS is positively correlated with the severity of RA [11]. There are two main categories of ROS in cells [11]. On the one hand, the oxidative metabolism of biological processes such as mitochondrial respiratory chain. On the other hand, immune cells produce highly toxic active oxygen to eliminate pathogens under the stimulation of antigens [12].

Adjuvant-induced arthritis (AA) is a widely used model for studying RA because of the similar pathophysiology to RA patients [13]. In the AA model complete Freund's adjuvant (CFA) contains inactivated *Mycobacterium tuberculosis* which is highly antigenic and functions to activate the immune responses. Like human patients, rats also produce high activity hydroxyl radicals by metabolism and Fenton reaction. Mitochondria are the “energy converter” in eukaryotic cells that convert ADP to ATP by oxidative phosphorylation [10]. However, some electrons leak from the mitochondrial respiratory chain, which undergo reduction reactions with molecular oxygen. Then high activity hydroxyl radicals were produced by metabolism. In neutrophils, the production of superoxide radicals are catalyzed by nicotinamide adenine dinucleotide phosphate (NADPH) oxidases system, and high activity hydroxyl radicals are generated by Haber-Weiss and Fenton reactions

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[10]. Mitochondria are considered to be the major subcellular structure of ROS production, but other organelles such as the endoplasmic reticulum and lysosomes can also produce ROS [11]. Some of these ROS react with lipids to produce lipid peroxidation that cause damage to the cell membrane, react with proteins to produce protein carbonyl compounds, and react with deoxyguanosine to cause DNA damage [15]. In addition, extracellular ROS can activate classically activated macrophages which produce excess pro-inflammatory cytokines leading to inflammation and tissue damage [11]. Normal levels of ROS play an important role in maintaining cell function, regulating signaling pathways and transcription factors, whereas high levels of ROS cause tissue injury in RA. Therefore, we evaluated the effect of GPS on oxidative stress in AA rats.

Currently, Non-steroidal antiinflammatory drugs (NSAIDs), disease-modifying antirheumatic Drugs (DMARDs), biological agents and glucocorticoids [16,17] are widely used to treat RA. NSAIDs have effects on reducing acute pain and stiffness in early diagnosis [18], but have less impact on joint destruction and are not disease modifying [19]. DMARDs inhibit immunomodulation and prevent joint destruction, but are associated with liver and kidney damage, allergic reactions and myelosuppression. Glucocorticoids provide rapid treatment for RA, which ultimately lead to many side effects [19] including osteoporosis, hypertension and gastric ulcer. Biological agents are mainly including TNF inhibitors, anti-cytokines, Janus kinase inhibitors, but increase the risk of CVD. Due to these disadvantages [20], novel high-efficiency and low-toxic anti-RA drugs are urgently needed.

In China, traditional Chinese medicine *Gentianae Macrophyllae Radix* has been commonly used to treat RA for thousands of years in clinical. Gentiopicroside (GPS, Fig. 1) [21] is one of the main active components of *Gentianae Macrophyllae Radix*. It has been reported that GPS has effects on protecting liver [22], immunomodulation [23], reducing pain [24] and inhibiting RANKL-induced osteoclastogenesis [25]. These studies indicate that GPS has the potential to treat RA. However, the underlying mechanisms of GPS on AA rats were still unclear. The proposal of this research was to evaluate the effects of GPS on AA rats and to study the underlying molecular mechanisms.

2. Materials and methods

2.1. Reagents

CFA was supplied from Sigma Company (United States). Methotrexate (MTX) of 99% purity was obtained from Dalian Meilun Biotechnology Co., Ltd. (China). GPS (HPLC purity ≥99%) was purchased from Dister Biotechnology Co., Ltd. (Sichuan, China). TNF-α, IL-1β, IL-17 and IL-6 enzyme-linked immunosorbent assay (ELISA) kits for rats were supplied from R&D Systems (USA). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione (GSH) malondialdehyde (MDA) and protein carbonyl (PCO) biochemical kits for rats were provided by Jiancheng Co., Ltd. (Nanjing, China). Pentobarbital sodium salt was used as the anesthetic, which was provided by Beijing Solarbio Science & Technology Co., Ltd. (China). Ultrapure RNA, Super RT cDNA Synthesis, and Ultra SYBR Mixture kits were provided by CoWin Biosciences Co., Ltd. (Jiangsu, China). Primers were obtained from Ruiyou Biotechnology Co., Ltd. (Zhengzhou, China).

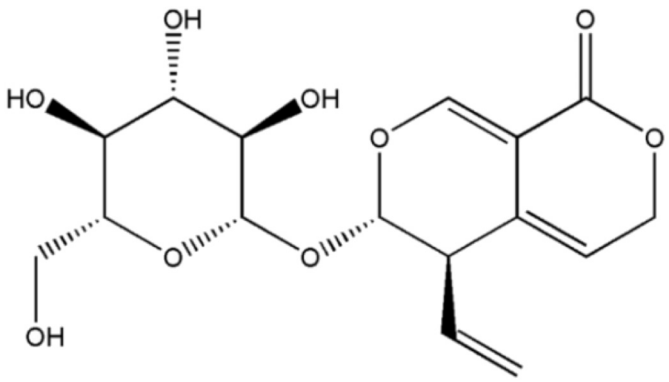

Fig. 1. The chemical structure of GPS.

2.2. Animals

Sixty male Sprague-Dawley (SD) rats (200 g ± 20 g) were obtained from Henan province Laboratory Animal center of China. Before the experiment, all animals were placed in the Laboratory for System of Individually Ventilated Cages (IVC) for seven days at 25 ± 3 °C with a 12-hour dark/light cycle. Moreover, the cages of rats received frequent and routine cleaning. All animals and procedures were approved by the School of Pharmaceutical Sciences, Zhengzhou University (No. 2017-0005).

2.3. Experimental design

There were six groups, the Normal group, the AA group, GPS (30, 60, 90 mg/kg) group and the MTX group, with ten rats for each group. MTX is the first-line medication recommended in early diagnosis of RA [26]. Thus, the MTX group was used as the positive control group in this research.

Normal group: on day 0, each rat was injected with normal saline for 0.1 mL in the right hind paw, and received normal saline (1 mL/100 g) by intragastric administration from day 15 to day 26.

AA group: on day 0, each rat was injected with CFA (1 mg/mL) [14] [27] for 0.1 mL in the right hind paw, and received normal saline (1 mL/100 g) by intragastric administration from day 15 to day 26.

GPS (30, 60, 90 mg/kg) group: on day 0, each rat was injected with CFA for 0.1 mL in the right hind paw and received GPS solution (dose: 30,60,90 mg/kg) by intragastric administration from day 15 to day 26.

MTX group: on day 0, each rat was injected with CFA for 0.1 mL in the right hind paw, and received MTX solution (0.2 mg/kg) by intraperitoneal injection from day 15 to day 26.

Ten days after CFA injection, AA rats developed secondary swelling and peaked at 17–19 days. All rats were sacrificed after anesthesia on day 26. Immune organs and synovial tissues of each rat were collected and weighed. Hind paws samples were collected and then stored at 10% paraformaldehyde (PF).

2.4. Evaluation of arthritis

Arthritis in each rat was evaluated by arthritis index, paw thickness and paw swelling. The arthritis index was defined as five grades, 0: normal, 1: slight paw swelling, 2: slight swelling of paw and ankle, 3: moderate swelling with erythema of paw and ankle joint, 4: severe swelling with erythema of paw and ankle joint [3,16,28]. The maximum score for each rat was 8 points (4 points × 2 hind paws). To avoid observer bias, arthritis index was assessed by using a double-blind trial [17]. In addition, paw swelling was detected by the YLS-7B toe volume measuring instrument (Jinan Technology Development Co., Ltd., China), and paw thickness was detected with a digital vernier caliper (The Great Wall Precision Industry Co., Ltd., China). Each paw was measured three times.

2.5. Indexes of immune organs and synovial hyperplasia

To assess the effect of GPS on immune organs in AA rats, the indexes of thymus and spleen were measured in this research. Immune organ index was expressed as the organ weight (mg) versus body weight (g) [17]. Synovial hyperplasia contributes to joint deformity and bone erosion. Therefore, we measured the weight of synovial tissues to assess

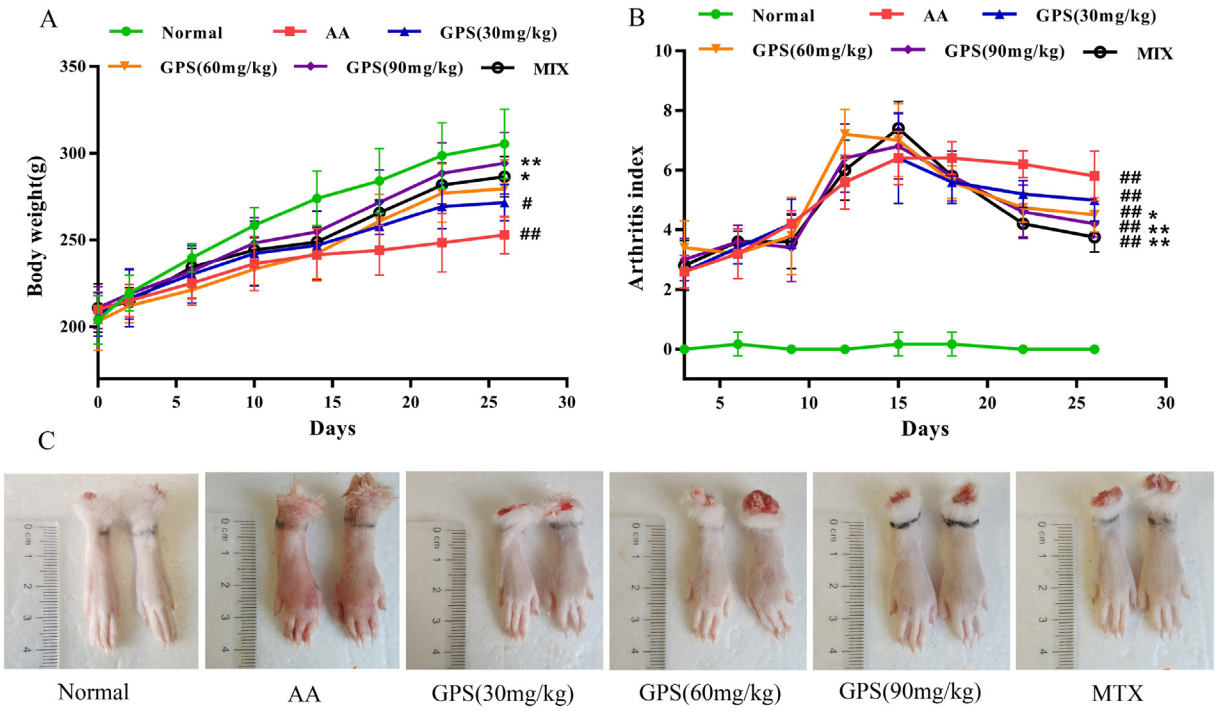

Fig. 2. Effects of GPS on body weight and arthritis index. (A) The body weight of rats. (B) Arthritis index. (C) The observation of hind paw images. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs the Normal group, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs the AA group.

the effect of GPS on synovial hyperplasia.

2.6. Measurement of plasma biochemical factors

On day 26, blood samples were obtained from abdominal aorta after anesthesia, then collected in EDTA coated tubes. Blood samples were separated at 2500 rpm for 25 min to obtain the plasma. IL-1β, TNF-α, IL-6 and IL-17 levels of plasma were detected by ELISA kits to evaluate effects of GPS on proinflammatory cytokines. First, plasma samples were taken from the freezer and thawed for 30 min at 25 °C. According to the instructions from the manufacturer, the standard curve was established and $R^2 > 0.95$ was ensured, then the absorbance of samples which was detected at 450 nm was substituted into the equation to calculate concentrations (ng/L). Each plasma sample was measured three times.

AST and ALT levels were measured by biochemical kits to assess liver function parameters in the plasma of rats. Effect of GPS on antioxidant was measured by MDA, PCO, SOD, GSH and GSH-Px biochemical kits.

2.7. Quantitative real-time PCR studies

All instruments were treated for ribonuclease (RNase)-Free, and all procedures followed the instructions of kits. In quantitative real-time PCR (qPCR), mRNA levels (TNF-α, IL-1β, IL-6 and IL-17) of synovial tissues were measured by Ultra-pure RNA, Super RT cDNA Synthesis, and Ultra SYBR Mixture kits. RNA was extracted by the Ultra-pure RNA kit, and the concentration was determined by a Microvolume UV–Vis Spectrophotometer (Thermo Fisher Scientific, USA) with OD_{260}/OD_{280} from 1.8 to 2.2 [29]. Each 20 μL reverse transcription system contains 2000 ng of total RNA. Procedures of qPCR were preincubation at 95 °C for 600 s, 2 step Amplification including denaturation (95 °C, 15 s) and annealing (60 °C, 60 s) for 40 cycles. The experimental results were analyzed by $2^{-\Delta\Delta Ct}$ method with the software of Roche Light Cycler 96 system (Roche, Switzerland).

Forward and reverse primer sequences (from 5′ to 3′) for qPCR were TNF-α (AGAT GTGGAACTGGCAGAGG and GAGCCCATTTGGGAACT TCT), IL-6 (CCGGAG AGGAGACTTCACAG and ACAGTGCATCATCGCT GTTC), IL-1β (GCTACCTA TGTCTTGCCCGT and CAGGTCGTCATCAT CCCACG), IL-17 (TGAGAGTTGA CATTCGCATCTT and GATACAGCCT GAGTGTCTGCAC) and β-actin (GTCGT ACCACTGGCATTGTG and TCTCAGCTGTGGTGGTGAAG) [30]. In the q-PCR study, β-actin is the housekeeping gene and is tested as normalization.

2.8. X-ray and histopathological studies

On day 26, rats were anesthetized with 1% pentobarbital sodium solution (0.3 mL/100 g) and placed on the sample plate. The assessment of X-Ray was performed by using the small animal living imaging instrument (Bruker Imaging Station, USA) [31]. The exposure time of samples was 10 s and X-ray images were displayed by Bruker MI software. X-Ray was used to assess the effects of GPS on ankle joint deformities and paw swelling in AA rats.

Hind paw samples were fixed in 10% PF solution for 48 h, then each sample was dehydrated and embedded in paraffin at 50–60 °C [28]. Each pathological section was stained with hematoxylin and eosin (H&E), and images were viewed by software K-Viewer (KFBIO technology for health Co., Ltd., China).

2.9. Statistical analysis

Data were shown as mean ± standard deviation (SD). In this study, data was analyzed by using SPSS 22.0 (SPSS Software, USA). Multiple group comparisons were performed by one-way ANOVA with Bonferroni test and Dunnett's test. When $P < 0.05$ were considered statistically significant.

3. Results

3.1. Effects of GPS on arthritis index and body weight

Results of body weight and arthritis index are shown in Fig. 2, which are used to evaluate the effects of GPS on AA rats. AA rats were induced at day 0 and treated drugs from day 15, then photographic

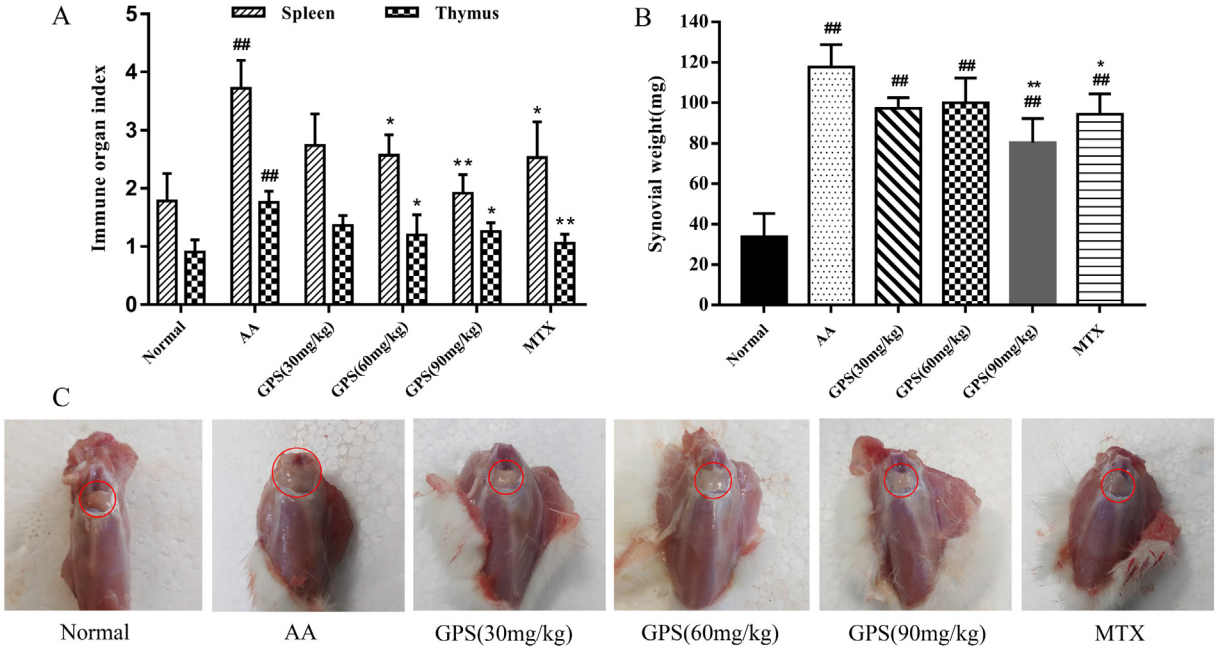

Fig. 3. Effects of GPS on immune organ index and synovial hyperplasia. (A) Indexes of the spleen and thymus. (B) The synovial weight of knee joints. (C) Images of synovial hyperplasia in AA rats. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs the Normal group, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs the AA group.

images (Fig. 2C) were taken on day 26. As shown in Fig. 2A, the AA group inhibited the increase of body weight compared with the other groups. The body weight of the GPS (90 mg/kg) group and the MTX group showed a significant increase ($P < 0.01$ and $P < 0.05$) compared with the AA group. As shown in Fig. 2B, The Normal group showed a lower arthritis index than the other groups ($P < 0.01$). Moreover, compared with the AA group, the GPS (60, 90 mg/kg) group and the MTX group showed a significant difference ($P < 0.05$, $P < 0.01$ and $P < 0.01$). These results indicate that GPS can reduce arthritis index and increase body weight in AA rats, which are dose-dependent.

3.2. Effects of GPS on immune organ index and synovial hyperplasia

In this experiment, we measured the immune organ index, and the result was shown in Fig. 3A. Compared with rats in the AA group, the spleen index of the GPS (60, 90 mg/kg) group and the MTX group have a significant decreased ($P < 0.05$, $P < 0.01$ and $P < 0.05$). The thymus index of the GPS (60, 90 mg/kg) group and the MTX group showed a significant reduction ($P < 0.05$, $P < 0.05$ and $P < 0.01$).

In addition, synovial hyperplasia was assessed by weight in this study and the result was shown in Fig. 3B. The synovial weight of the GPS (90 mg/kg) group and the MTX group showed a significant decrease ($P < 0.01$ and $P < 0.05$). Furthermore, images were taken on day 26, and the area in the red circle was to assess the effect of GPS on synovial hyperplasia (Fig. 3C).

3.3. Effects of GPS on paw swelling and paw thickness

Results of paw swelling and paw thickness are shown in Fig. 4. As shown in Fig. 4A, we found that GPS could alleviate paw swelling from day 15. On day 26, compared with the AA group, there was an obvious decrease of the GPS (30, 60, 90 mg/kg) group and the MTX group ($P < 0.05$, $P < 0.01$, $P < 0.01$ and $P < 0.01$). The details of paw thickness are shown in Fig. 4B, it is obvious that GPS can reduce paw thickness from day15. These results strongly confirmed that GPS could relieve paw swelling and thickness.

3.4. Effects of GPS on AST and ALT levels

AST and ALT levels of plasma in AA rats were measured to assess liver function parameters, and results are shown in Fig. 5A. It is obvious that the GPS (90 mg/kg) group significantly decreased AST and ALT levels. As expected, there is no liver damage in AA rats after treated with GPS for 26 days. From these results, we can infer that GPS of 90 mg/kg has a protective effect on the liver.

3.5. Effect of GPS on antioxidant

GSH-Px widely exists in the body and protects cells from peroxide damage. SOD can eliminate harmful substances produced by organisms during metabolism. GSH is an antioxidant. MDA and PCO are markers for the evaluation of oxidative damage. The results are shown in Fig. 5B, C, D, E and F compared with the AA group, in the GPS (30, 60, 90 mg/kg) and MTX groups, the levels of PCO and MDA were decreased and the levels of GSH, SOD, GSH-Px were increased.

3.6. Effects of GPS on proinflammatory cytokines and related mRNA

In this research, in order to assess the anti-inflammation effect of GPS, we measured levels of TNF-α, IL-1β, IL-6 and IL-17. The results are shown in Fig. 6A, compared with the AA group, there was an obvious decrease of TNF-α, IL-17, IL-1β and IL-6 in the GPS (30, 60, 90 mg/kg) group and the MTX group. Therefore, it is obvious that GPS can inhibit inflammatory cytokines in the plasma of AA rats. Furthermore, mRNA levels of TNF-α, IL-6, IL-1β and IL-17 in synovial tissues were measured by qPCR to evaluate effects of GPS at the transcriptional level, which are shown in Fig. 6B. From the qPCR results, it is clear that GPS has effects on reducing the mRNA levels of IL-1β, TNF-α, IL-17 and IL-6 in synovial tissues. IL-6 and TNF-α are considered as the major cytokines leading to chronic inflammatory, bone destruction and synovitis in RA. Therefore, the underlying mechanisms of GPS on anti-inflammatory is to down-regulate mRNA levels of proinflammatory cytokines and then reduce the levels of IL-1β, TNF-α, IL-6 and IL-17.

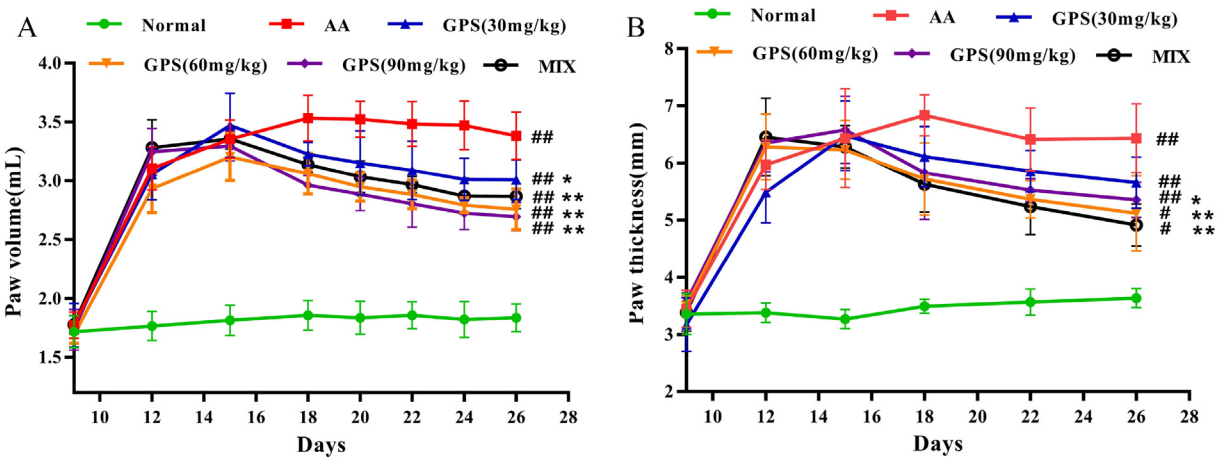

Fig. 4. Effects of GPS on paw swelling and paw thickness. (A) Hind paw volume. (B) Hind paw thickness. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs the Normal group, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs the AA group.

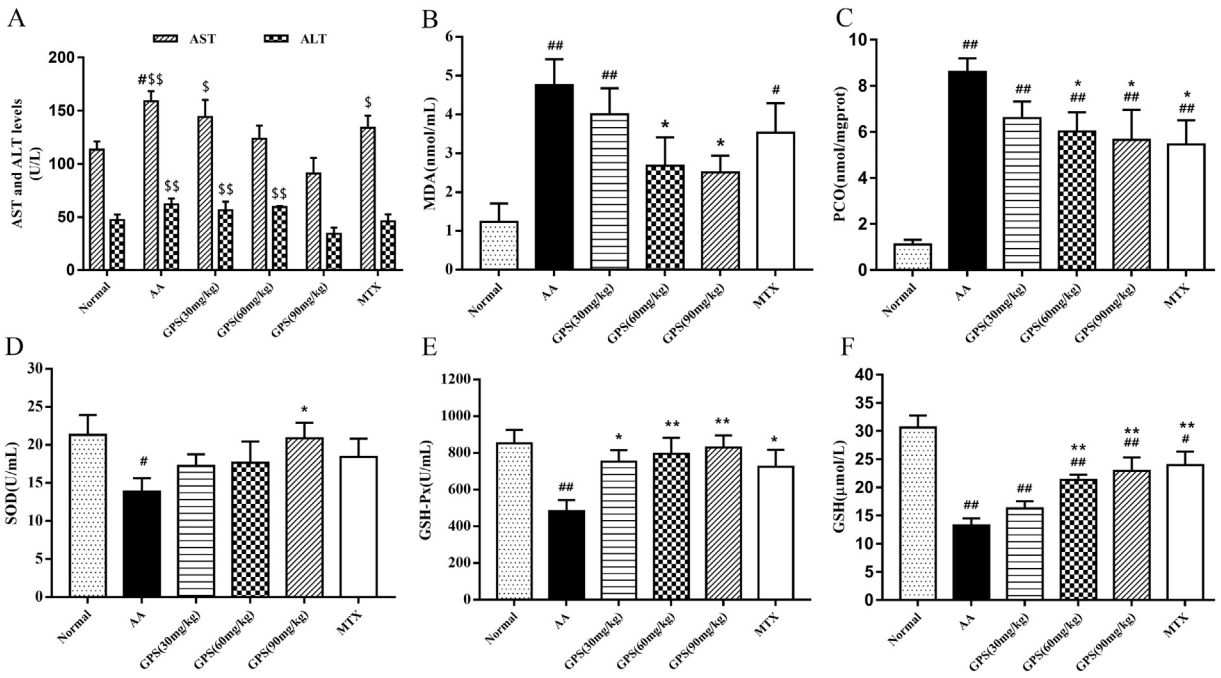

Fig. 5. Effects of GPS on AST, ALT (A), MDA (B), PCO (C), SOD (D), GSH-Px (E) and GSH (F) levels. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs the Normal group, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs the AA group, $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ vs the GPS (90 mg/kg) group.

3.7. Effects of GPS on ankle joints in AA rats

Effects of GPS on ankle joints were determined by X-Ray (Fig. 7). Images of rats in the Normal group showed normal joints and no joint deformities. Soft tissue swelling and wide gaps of the metatarsus and phalange were observed in the picture of the AA group. Mild swelling of the soft tissue was observed around the ankle joint in the picture of the MTX group. In GPS groups, gaps of the metatarsus were narrowed and the swelling of soft tissues around the ankle joints was reduced.

3.8. Effects of GPS on histopathology

Effects of GPS on cartilage damage and synovial hyperplasia in the ankle joint of AA rats were evaluated by histopathological studies. The results are shown in Fig. 8, synovial hyperplasia and bone damage were not observed in the articular cavity of rats in the Normal group. Pathological images of rats in the AA group showed that synovial hyperplasia, bone erosion, as well as a narrow articular cavity. Mild bone erosion was observed in the pathological images of the MTX group and the GPS (30 mg/kg) group. Histological studies in the GPS (30, 60 mg/kg) group showed the normal joint cavities without bone erosion and synovial proliferation.

4. Discussion

It has been reported that there is oxidative stress in RA patients with the increase of MDA and the decrease of antioxidant such as GSH-Px, SOD and GSH [32]. Moreover, there are synovitis, elevated levels of inflammatory cytokines, bone erosion, joint deformities and the enlargement of immune organs in RA patients. MTX is a commonly used drug for the treatment of RA [26]. AA is induced by CFA which stimulates immune responses and the effect continues for 28 days. The study of these parameters in AA is reasonable because of the similar pathophysiology between AA and RA [12,33,34].

As is known that immune disorders and oxidative stress are the main factors contribute to the pathogenesis of RA [35,36]. On the one

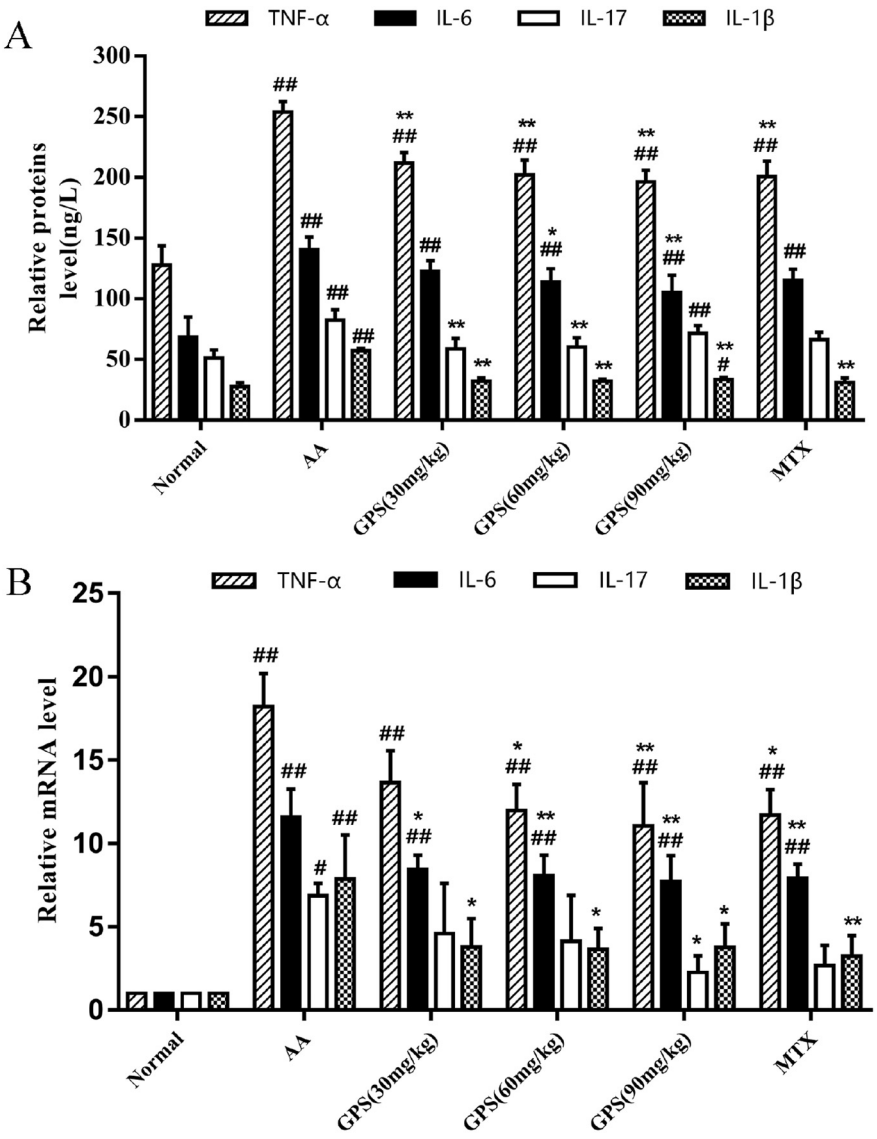

Fig. 6. Effects of GPS on proinflammatory cytokines and related mRNA levels. (A) Levels of proinflammatory cytokines in plasma. (B) Related mRNA levels of synovial tissues. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs the Normal group, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs the AA group.

hand, the body has reactive oxygen species (ROS) including hydroxyl and superoxide, as well as antioxidant systems such as SOD, catalase (CAT) and GSH-Px [34]. Oxidative stress occurs when ROS levels are higher than antioxidants [37]. ROS are considered as important contributors to autoimmune diseases [38]. ROS activates MAPK, NF-KB signaling pathways which mediate inflammatory responses and increase levels of proinflammatory cytokines in cells of RA [11]. What's more, oxidative stress is responsible for high cardiovascular disease (CVD) risk in RA patients [39]. Because oxidative stress plays an important role in RA, MDA and PCO were detected as indicators of oxidative damage, while SOD, GSH-Px and GSH were measured as antioxidant factors in this research.

These results were used to evaluate the effects of GPS on antioxidant in the plasma of AA rats. We found that GPS significantly increased levels of SOD, GSH-Px and GSH and significantly reduced the levels of MDA and PCO compared to AA rats. Therefore, we conclude that the antioxidant effects of GPS are achieved by increasing the levels of antioxidant enzymes, reducing oxidative stress, and reducing tissue degradation in AA rats.

On the other hand, immune disorders also contribute to the development of RA. Proinflammatory cytokines are produced by immune cells, which are responsible for persistent chronic inflammation and drive the progression of RA [40,41]. TNF-α is produced by monocytes, T cells and macrophages, which leads to inflammation and bone erosion by activating leukocytes, synovial fibroblasts, osteoclasts, endothelial cells and inhibiting the function of regulatory T cells. Therefore, TNF-α plays a dominant role in the inflammatory response [42]. IL-6 is also produced by many kinds of immune cells, which activates leukocytes, osteoclasts and endothelial cells, promotes the proliferation of synovial fibroblasts, and is similar to TNF-α in some respects. IL-17 is mainly produced by helper T cells and other T cells, leading to the proliferation of synovial fibroblasts, the activation of osteoclasts and the increased production of other cytokines [43,44]. IL-1β contributes to the differentiation of T cells and also activates chondrocytes and osteoclasts. However, pro-IL-1β is useless and cleaved by caspase-1 to produce mature IL-1β in Nucleotide-binding, oligomerization Domain (NOD)-like Receptor family, pyrin domain containing 3 (NLRP3) inflammasome [45]. What's worse, there is a synergy between these cytokines which lead to persistent inflammation. The Janus Kinase/Signal transducers and activators of transcription (JAK/STAT) signaling pathway is the junction of these cytokines [46], and other RA-related signaling pathways are Mitogen-activated protein kinase (MAPK), Nuclear Factor

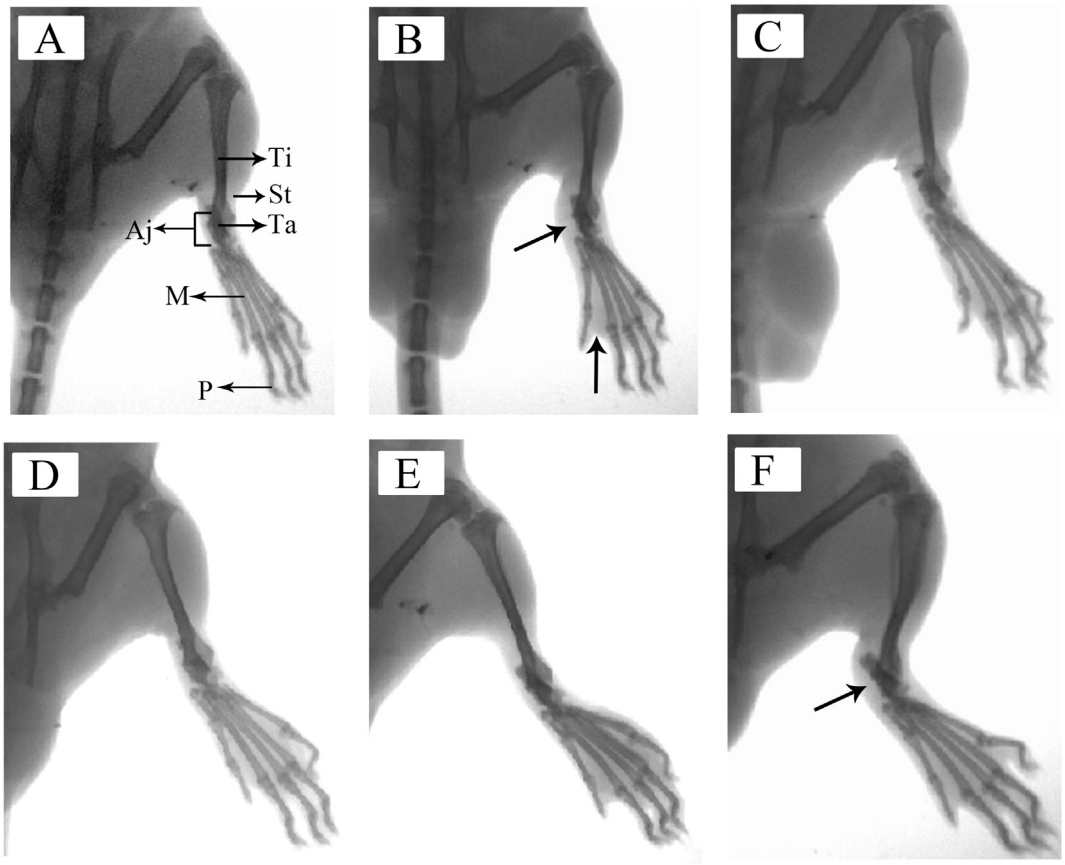

Fig. 7. Effects of GPS on ankle joints in AA rats. Radiographic examinations of the Normal group (A), the AA group (B), the GPS (30 mg/kg) group (C), the GPS (60 mg/kg) group (D), the GPS (90 mg/kg) group (E), and the MTX group (F). Aj: ankle joint, Ti: tibia, M: metatarsus, Ta: talus, P: phalange, St: soft tissue.

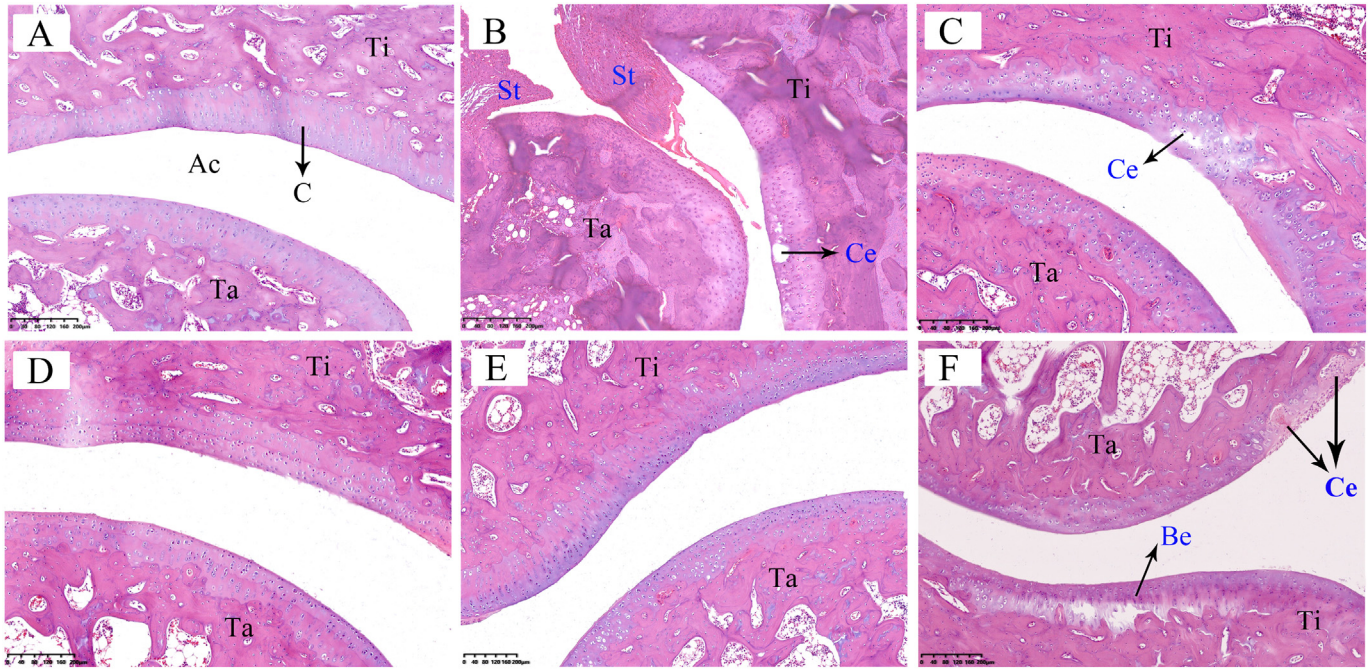

Fig. 8. Effects of GPS on histopathology in AA rats. Histopathological analysis of the Normal group (A), the AA group (B), the GPS (30 mg/kg) group (C), the GPS (60 mg/kg) group (D), the GPS (90 mg/kg) group (E), and the MTX group (F). St: synovial tissue, Ac: articular cavity, Ce: cartilage erosion, Be: bone erosion, Ti: tibia, Ta: talus, C: cartilage. Histopathological changes are marked with blue letters. (Scale bar: 200 μm. H&E staining, magnification 100×.) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

kappa B (NF-κB), phosphatidylinositol- 3-kinase (PI3k), Spleen tyrosine kinase (Syk), Bruton's tyrosine kinase (BTK), Toll-like receptors (TLRs) [47,48] and Wingless/integrated (Wnt) [49]. JAK/STAT, MAPK, NF-κB, TLRs, NLRP3, Syk, and BTK signaling pathways are associated with inflammation, and the PI3k signaling pathway promotes the proliferation of synovial fibroblasts, all of which contribute to the more severe conditions in RA. For example, the activation of the NF-κB signaling pathway: antigens (such as adjuvants) activate immune cells to produce pro-inflammatory cytokines that promote the phosphorylation, ubiquitination and degradation of I kappa B (IκB), and then the nuclear localization sequence of NF-kB is exposed and enters the nucleus to initiate transcription [11]. On the contrary, the Wnt signaling pathway facilitates the differentiation of osteoblasts and promotes the formation of bones to protect the joint cavity.

Due to the cytokine-induced inflammation and bone destruction of RA, we have found that GPS can significantly reduce proinflammatory cytokines in the plasma of AA rats by reducing levels of TNF-α, IL-1β, IL-17 and IL-6 in this research. Furthermore, we also found that GPS inhibits mRNA levels of related cytokines. However, GPS requires more studies on the underlying mechanisms of signaling pathways.

In addition, X-ray and arthritis index are important references for judging the severity of RA patients [40]. These same parameters were evaluated in AA rats in this study. The results showed that GPS significantly reduced hind paw swelling in AA rats, as well as arthritis index. The results of the immune organ index show that GPS can reduce the indexes of the thymus and spleen. X-Ray (Fig. 7) results showed obvious soft tissue swelling in the MTX group compared to the GPS (90 mg/kg) group. Compared to the GPS (90 mg/kg) group, histopathological studies (Fig. 8) showed obvious bone erosion and cartilage damage in the MTX group. Therefore, these results indicate that GPS (90 mg/kg) has good therapeutic effects on AA rats.

5. Conclusion

Oxidative imbalance and the activation of inflammation have been demonstrated to be causative factors in human rheumatoid arthritis (RA). In our adjuvant-induced arthritis rat model, we found evidence for both and showed that GPS ameliorates the arthritis symptoms by interfering with both mechanisms. GPS increases the levels of antioxidant enzymes and antioxidant and reduces tissue damage. Furthermore, GPS down-regulated the levels of TNF-α, IL-1β, IL-17 and IL-6, as well as related mRNA. In addition, X-ray and histopathological results indicate that GPS protects the ankle joint and reduces paw swelling in this research. The results of AST and ALT levels show that GPS of 90 mg/kg may have a protective effect on the liver. All results indicate that GPS has a therapeutic effect on AA rats, and more studies should be done to explore the underlying molecular mechanisms.

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Declaration of competing interest

All authors indicate that there is no conflict of interest in this research.

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