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Effects of Prunus Tomentosa Thumb Total Flavones on adjuvant arthritis in rats and regulation of autophagy

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Abstract.
BACKGROUND: Rheumatoid arthritis (RA) is a slow in taking effect systemic autoimmune disease. Prunus Tomentosa Thumb Total Flavones (PTTTF) has anti-inflammatory and antioxidant properties.
OBJECTIVE: The purpose of this study is to the PTTTF on adjuvant arthritis (AA) in rats and to explore the mechanism of autophagy.
METHODS: Adjuvant arthritis model was established in rats. The cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-17 (IL-17), tumor necrosis factor (TNF-α) of rat synovial tissue were determined by RT-PCR. The histopathological varieties of knee joints in AA rats were observed by HE staining. The expressions of autophagy-related proteins ATG5, ATG7, ATG12, Beclin1, Lc3II and Bcl-2 in rat synovial tissue were determined by Western Blotting.
RESULTS: PTTTF (50, 100, 200 mg/kg) significantly inhibited inflammation in rats ($P < 0.01$). PTTTF significantly inhibited inflammatory factor COX in rat synovial tissue. COX-2, IL-1β, IL-6, IL-17, TNF-α expression ($P < 0.05$); PTTTF can significantly improve the pathological damage of rat knee joint PTTTF and can significantly inhibited the expression of autophagy-related proteins in rat synovium ($P < 0.05$).
CONCLUSION: PTTTF can inhibit adjuvant arthritis in rats and can inhibit the expression of autophagy-related proteins ATG5, ATG7, ATG12, Beclin1, Lc3II and Bcl-2.

Keywords: Prunus Tomentosa Thumb Total Flavones, autophagy, inflammatory factors

1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by joint disease. Its main clinical manifestations are joint swelling and pain caused by synovium of facet joint, followed by

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cartilage destruction, joint space narrowing, joint stiffness, deformity, late severe bone destruction and dysfunction caused by absorption. The disease is easy to recur, poor prognosis with and high disability rate. Rheumatoid arthritis is primarily treated with disease-modifying antirheumatic drugs (DMARDs), which are pain relievers used to help relieve pain. There are two main categories of DMARDS: synthetic compounds and biologics [1], which require subcutaneous or intravenous administration, and are toxic and accompanied by many side effects of. There is so far no ideal treatment method [2].

Prunus Tomentosa Thumb can be found in Changbai Mountains, they are the fruits of wild shrubs and are a type of cherry. Prunus Tomentosa is abundant in number in China. However, little has been explored about it, and only few academic documents are found–most of the relative readings for Prunus Tomentosa are unofficial popular articles, saying "Cherry-soaked wine, folk Chinese used it to treat frostbite and rheumatism".

Our research group has been long focusing on the anti-frostbite use of Prunus Tomentosa, because it is a very possible breakthrough for the cure for Rheumatoid arthritis (RA). The total flavonoids in Prunus Tomentosa has shown clear effects in anti-frostbite cases and anti-inflammatory cases. The analgesic and anti-inflammatory characteristics of Prunus Tomentosa may be in connection with the anti-oxidative stress of flavonoids and the inhibition of COX-1. COX-2 generation [3,4]. In recent years, many studies done by worldwide scientists have confirmed that flavonoids have significant anti-inflammatory effects [5], which is of great medicinal development value.

In this paper, to establish the rat arthritis model induced by Freund's adjuvant. The anti-inflammatory action of PTTTF on adjuvant arthritis in rats was observed. The changes of body weight, thymus index, spleen index, and cytokines in synovial membrane were determined. The effect of total flavonoids of Prunus Tomentosa Thumb on autophagy during anti-inflammatory process provides a theoretical basis for the exploitation of anti-inflammatory medicine to Prunus Tomentosa Thumb.

2. Materials and methods

2.1. Experiment animals

SPF grade Wistar rats, 60 body weight 160–180 g, male, bought from Changchun City Yisi Experimental Animal Technology Co., Ltd. Experimental Animal Center, under the SPF experimental conditions of the Experimental Research Center of the College of Pharmacy, Beihua University.

2.2. Instruments and reagents

Prunus Tomentosa Thumb Total Flavones are provided by the Laboratory of Medicinal Chemistry, School of Pharmacy, Beihua University. Freund's complete adjuvant (Beijing Dingguo Changsheng Biotechnology Co., Ltd.), primers IL-6 ,TNF-α, IL-1β, COX-1, COX-2, IL-17 (Beijing Dingguo), Beclin1, Lc3II , β-actin (abcam), ATG5, ATG7, ATG12, Bcl2 (abclonal).

The Bio-Rad 680 microplate reader was purchased from Bio-Rad. The PCR instrument (Mastercycler gradient 5331) was purchased from Eppendorf. The gel imaging analysis system (Tocan 240 type) was purchased from Shanghai Lingcheng Biotechnology Co., Ltd. The UVP chemiluminescence imaging system was purchased from analytikjena. The Model 5810R Multi-Function Desktop Refrigeration Centrifuge was purchased from Eppendorf. The SW-CJ-2 ultra-clean workbench was purchased from Suzhou Purification Equipment Co., Ltd. PV-200 toe volume measuring instrument (Chengdu Qinmeng Technology Co., Ltd.)

2.3. Animal grouping and administration

60 male Wistar rats were stochastically separated into 6 groups based on their body weight. There are normal group, model group, model with dexamethasone group (0.125 mg/kg), and the model with cherry extract group. The model with cherry extract group was further divided into low, medium and high doses (50, 100, 200 mg/kg) groups. There are 10 rats in each group, and after 3 days of adaptive feeding, the treated group was prevented from being administered for 7 days, referring to the modeling method [6]. Except for the normal group, rats in the other groups were injected hypodermic injection of 0.2% of Freund's complete adjuvant in the right hind paw of the rats, and the normal rats were injected subcutaneously with the equal amount of physiological saline. During a modeling period, the joint redness, subcutaneous nodules, and erythema of the rats were observed and recorded, and the body mass and the degree of swelling of the rats were measured once every 2 days. After the inflammation, except for the model group, the treatment groups were started to be administered, and the normal group was given the same amount of distilled water for 28 days.

2.4. Measuring rat paw swelling

The instrumental measures of the swelling of rats' left and right paws were done using a PV-200 Toe Volume Measuring Instrument.

Applied Formula: Paw swelling extent = Right paw swelling volume (mL) – Left paw swelling volume (mL)

2.5. Organ index

After 24 hours after the last administration, anesthesia was performed by intraperitoneal injection of 5% chloral hydrate. Then the spleen, thymus and joints were separated, rinsed with iced physiological saline, blotted with filter paper, weighed, and calculated the organ index.

$$\text{Applied Formula: Organ Index} = \frac{\text{Weight of organ (mg)}}{\text{Weight of rat}(g)}$$

2.6. Pathological observation of joints (HE staining) [7]

The pathological changes of joints in each group were watched and took pictures under the microscope.

2.7. PCR detection of inflammatory cytokine expression in the synovium tissue of the tested joint

The synovium tissue of the joint was taken, and total RNA was extracted according to the kit instructions, and reverse transcribed into cDNA by a PCR instrument. The resulting cDNA was amplified by a PCR machine according to the kit operation. The primer sequences of RT-PCR are referred to Table 1.

2.8. Western blotting detection of the expression of autophagic factors in the synovium tissue of the joint

The synovium tissue of the joint was taken, and the total protein was extracted according to the kit operation. The same amount of protein was subjected to SDS-PAGE electrophoresis, and the protein was shifted to PVDF membrane. After blocking with 5% nonfat dried milk, it was incubated with anti-Beclin1, Lc3II, ATG5, ATG7, ATG12, Bcl-2, anti-β-actin antibody, respectively. Incubation of the secondary antibody, addition of ECL reagent, imaging by ultrasensitive chemiluminescence imaging system, analysis of band absorbance values, representative of protein ratios of Beclin1, Lc3II, ATG5, ATG7, ATG12, Bcl-2 and β-actin bands The amount of expression.

Table 1
The primer sequences of RT-PCR

Primer	Forward primer ($5' \rightarrow 3'$)	Reverse primer ($5' \rightarrow 3'$)
COX-1	GCC AGT ATT AGC AGC AGC AGG TATC	GCC GAA GAA TCA GAA TAG GTGT
COX-2	CCC GCC CCA TCT AAC ATC TC	CCC CAC ATG GAG GAA TAGGC
IL-1β	GGA TGA TGA CGA CCT GCT AGT	CAC TTG TTG GCT TAT GTT CTGTC
IL-6	CCA ACT TCC AAT GCT CTC TCC TAAT	CGA GTA GAC CTC ATA GTG ACCTT
IL-17	CGC CGA GGC CAA ATA ACT TTC	GGT TGA GGT AGT CTG AGGGC
TNF-α	ATG TGG AAC TGG CAG AGGAG	AGT AGA CAG AAG AGC GTGTG
GAPDH	GGC AAG TTC AAC GGC ACAG	GCC AGT AGA CTC CAC GACAT

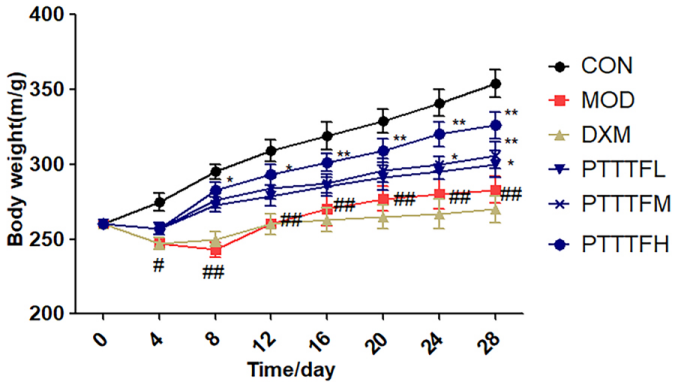

Fig. 1. The effect of PTTTF on weight in rats with adjuvant arthritis. The number of samples in each group was 10. All data represent the mean $\pm$ SD. Compared with control group, # $P < 0.05$, ## $P < 0.01$; compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group, PTTTFL: PTTTF low dose group, PTTTFM: PTTTF medium dose group, PTTTFH: PTTTF high dose group.

2.9. Statistical analysis

The data obtained are expressed as mean $\pm$ SD. All statistical analyses were carried out adopting SPSS 17.0 software. The t test was adopted to compare the significant differences between the experimental group and the control group. Significant differences between the two groups were analyzed by one-way analysis of variance.

3. Results

3.1. The influence of prunus tomentosa thumb total flavones on body weight of rats

Compared with the blank group, there was a significant difference in the model group on the 4th day ($P < 0.05$), and on days 8, 12, 16, 20, 24, 28, the difference was very significant ($P < 0.01$). Compared with the model group, PTTTF (50, 100, 200 mg/kg) can up-regulate the body weight of adjuvant arthritis rats, showing a dose-effect relationship. It indicated that the total flavonoids of cherries had a certain regulatory effect on the normal growth of rats. The results are shown in Fig. 1.

3.2. The effect of prunus tomentosa thumb total flavones on paw swelling of rat

An increase in paw swelling after one week of inflammation may be a secondary inflammatory reaction caused by inflammation [8]. PTTTF (50, 100, 200 mg/kg) significantly inhibited the swelling of the foot

Table 2
The influence of PTTTF on paw swelling in rats with adjuvant arthritis

Groups	n	2 days	7 days	14 days	21 days	28 days
Control group	10	0.02± 0.01	0.01± 0.01	0.01± 0.01	0.03± 0.02	0.03± 0.02
Model group	10	$0.72\pm 0.19^{\#\#}$	$0.84\pm 0.23^{\#\#}$	$0.77\pm 0.23^{\#\#}$	$0.76\pm 0.16^{\#\#}$	$0.73\pm 0.15^{\#\#}$
Positive group	10	0.30± 0.14	0.59± 0.22	0.39± 0.08	0.18± 0.11	0.14± 0.08
Low dose group	10	0.59± 0.17	0.69± 0.13	$0.54\pm 0.12^{*}$	$0.53\pm 0.11^{**}$	$0.49\pm 0.09^{**}$
Medium dose group	10	$0.54\pm 0.17^{*}$	$0.63\pm 0.12^{*}$	$0.54\pm 0.07^{**}$	$0.48\pm 0.12^{**}$	$0.47\pm 0.12^{**}$
High dose group	10	$0.48\pm 0.10^{**}$	$0.53\pm 0.14^{**}$	$0.49\pm 0.07^{**}$	$0.46\pm 0.12^{**}$	$0.36\pm 0.17^{**}$

The number of samples in each group was 10. All data represent the mean ± SD. Compared with control group, # $P < 0.05$, ## $P < 0.01$; Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$.

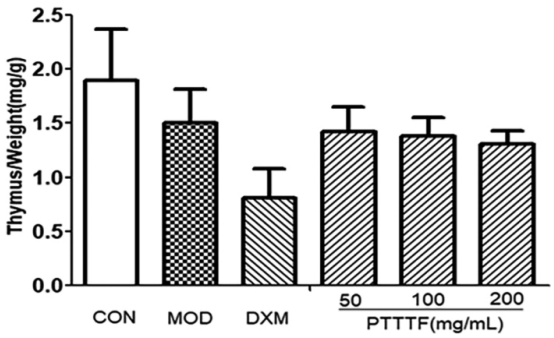

Fig. 2. The influence of PTTTF on thymus index in rats with adjuvant arthritis. The number of samples in each group was 10. All data stand for the mean ± SD. Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

in rats with adjuvant arthritis. PTTTF (200 mg/kg) remarkably restrained the swelling of the foot in rats with adjuvant arthritis ($P < 0.01$). The results are referred to Table 2.

3.3. The influence of prunus tomentosa thumb total flavones on immune organ index

The thymus and splenic organ are the main immune organs of human body, and changes in their relative quality play a significant role in immune evaluation. They have been widely used to evaluate the immune level of the body. The effect of drugs on the quality of thymus and spleen of animals can be used as an immunological pharmacological mechanism. Preliminary indicator [9]. PTTTF (50, 100, 200mg/kg) had a certain inhibitory effect on the spleen. PTTTF (200 mg/kg) significantly inhibited the growth of spleen ($P < 0.05$), showing a dose-effect relationship. The results are referred to Figs 2 to 3.

3.4. The effect of prunus tomentosa thumb total flavones on pathological changes of joints

Pathological changes [10] are an important feature of RA, often manifested as joint effusion, synovial inflammation such as lining cell layer thickening, massive cell infiltration in the interstitial layer, microvascular neovascularization, synovial cell surface expression activation of antigen, taking shape of angiospasm and destruction of cartilage tissue. Pathological sections preserved pathological changes in the pathogenesis of RA in the form of sections. Compared with the pathological sections of the synovial cartilage in the treatment group, the blank group and the treatment group can objectively understand the progression of RA and the changes of histopathological structure before and after treatment.

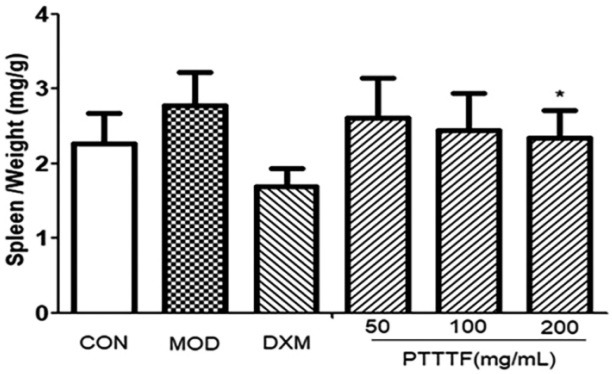

Fig. 3. The effect of PTTTF on spleen index in rats with adjuvant arthritis. The number of samples in each group was 10. All data stand for the mean ± SD.Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

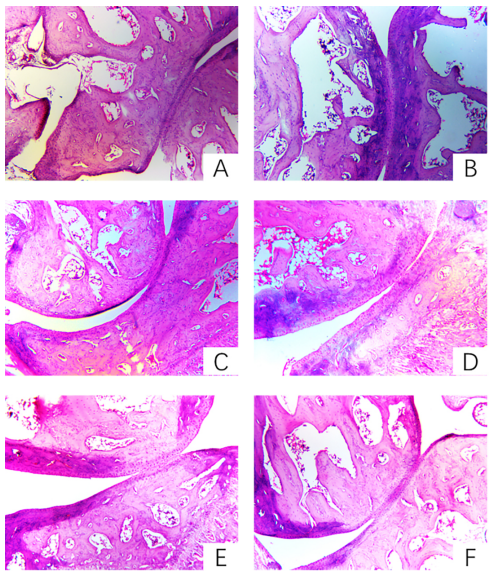

Fig. 4. The influence of PTTTF on knee joint histopathology in rats with adjuvant arthritis(HE stain, × 200) A: Control knee joint histopathology B: Histopathological manifestations of knee joints in AA rats C: Histopathological manifestations of knee joints in AA rats treated with DXM (0.125 mg/kg) D: Histopathological manifestations of knee joints in AA rats treated with PTTTF (50 mg/mL) E: Histopathological manifestations of knee joints in AA rats treated with PTTTF (100 mg/mL) F: Histopathological manifestations of knee joints in AA rats treated with PTTTF (200 mg/mL)

In the model group, the synovial membrane of the synovium of the model group was thickened and hypertrophied, tissue vasodilation, fibrosis, and a large number of chronic inflammatory cells. PTTTF (50, 100, 200 mg/kg) can alleviate synovial cell and tissue proliferation of knee joints in AA rats and reduce inflammatory cell infiltration. The results are shown in Fig. 4.

3.5. The effect of prunus tomentosa thumb total flavones on synovial tissue inflammatory factors

PTTTF (50, 100, 200 mg/kg) remarkably decreased the expression of inflammatory factors, and PTTTF (50 mg/kg) significantly decreased IL-1β ($P < 0.05$), IL-6 ($P < 0.05$), IL-17 ($P < 0.05$) and COX2

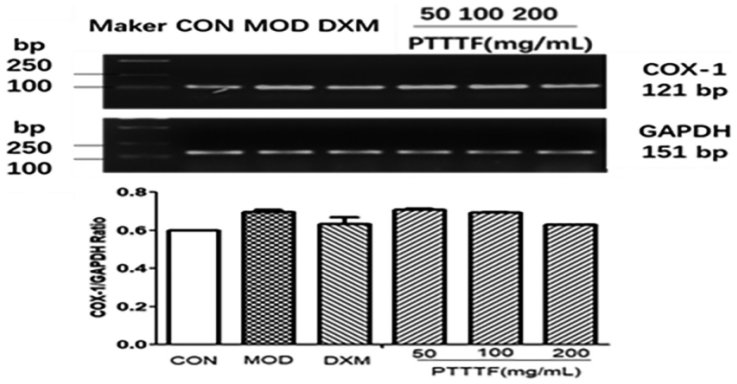

Fig. 5. The influence of PTTTF on the expression of COX-1 in synovial tissue of rats with adjuvant arthritis. The number of samples in each group was 10. All data stand for the mean ± SD. Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

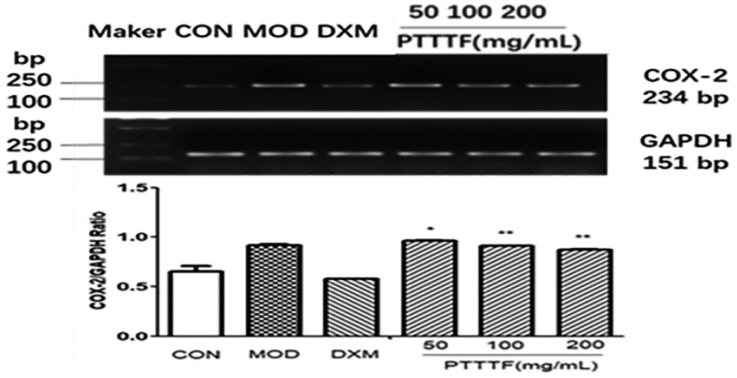

Fig. 6. The influence of PTTTF on COX-2 expression in synovium of adjuvant arthritis rats. The number of samples in each group was 10. All data stand for the mean ± SD. Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

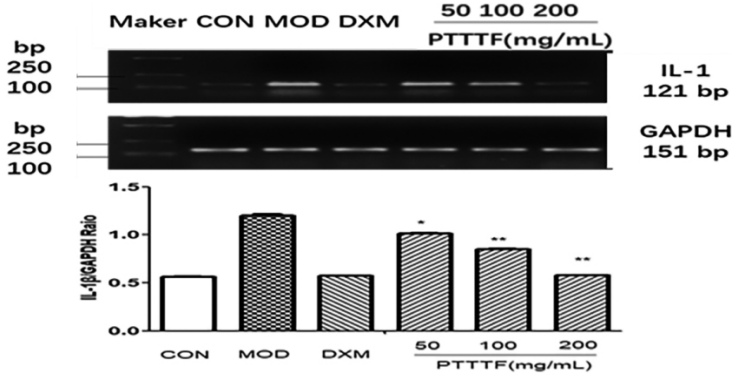

Fig. 7. The influence of PTTTF on IL-1β expression in synovium of adjuvant arthritis rats. The number of samples in each group was 10. All data stand for the mean ± SD. Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

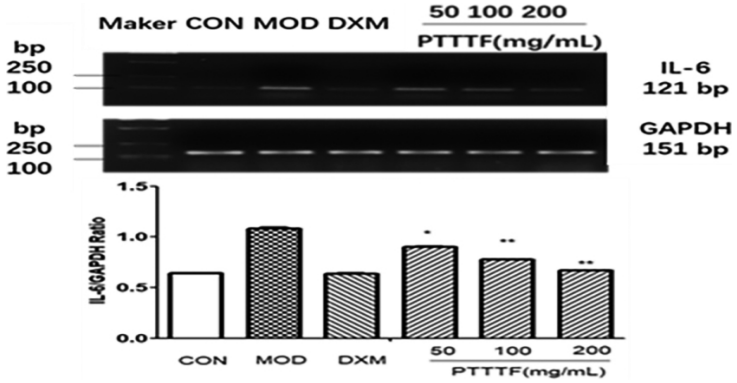

Fig. 8. The influence of PTTTF on IL-6 expression in synovium of adjuvant arthritis rats. The number of samples in each group was 10. All data stand for the mean ± SD. Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

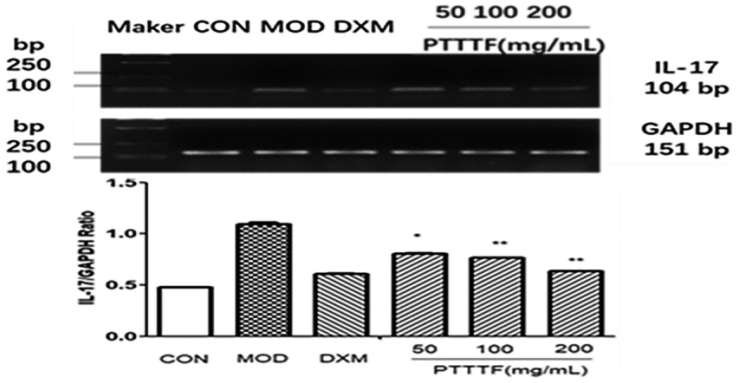

Fig. 9. The influence of PTTTF on IL-17 expression in synovium of adjuvant arthritis rats. The number of samples in each group was 10. All data stand for the mean ± SD. Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

($P < 0.05$); PTTTF (100, 200 mg/kg) significantly inhibited IL-1β ($P < 0.01$), IL-6 ($P < 0.01$), IL-17 ($P < 0.01$).) and COX2 ($P < 0.01$), the inhibition effect on COX1 is not obvious, consistent with the results [11]. The results are referred to Figs 5–10.

3.6. The effect of prunus tomentosa thumb total flavones on autophagy related factors in synovial tissue

PTTTF (50, 100, 200 mg/kg) significantly decreased the expression of autophagy-related proteins, PTTTF (50 mg/kg) significantly decreased ATG5 ($P < 0.05$), ATG7 ($P < 0.05$), ATG12 ($P < 0.05$). Beclin1 ($P < 0.05$), LC3II ($P < 0.05$) and Bcl-2 ($P < 0.05$); PTTTF (100, 200 mg/kg) significantly inhibited ATG5 ($P < 0.01$) and ATG7 ($P < 0.01$). ATG12 ($P < 0.01$), Beclin1 ($P < 0.01$), LC3II ($P < 0.01$) and Bcl-2 ($P < 0.01$).The results are referred to Figs 11–16.

4. Discussion

AA rat is one of the widely used rheumatoid arthritis models. It is a commonly used animal model for rheumatoid arthritis, and its pathogenesis is basically consistent with rheumatoid arthritis. The

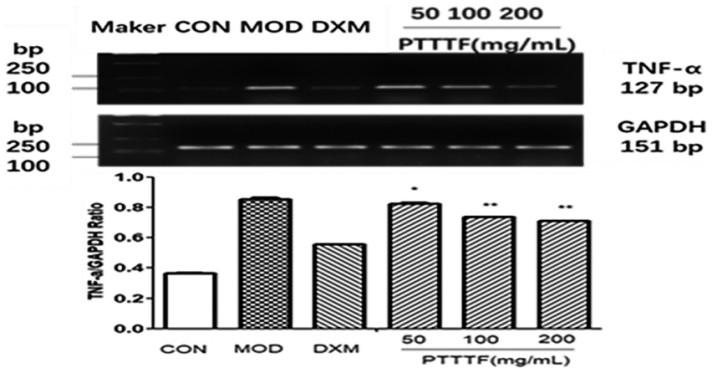

Fig. 10. The influence of PTTTF on TNF-α expression in synovium of adjuvant arthritis rats. The number of samples in each group was 10. All data stand for the mean $\pm$ SD. Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

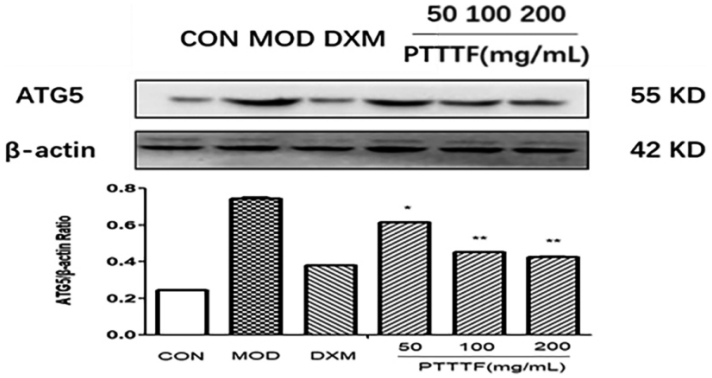

Fig. 11. The influence of PTTTF on ATG5 expression in synovium of adjuvant arthritis rats. The number of samples in each group was 10. All data stand for the mean $\pm$ SD. Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

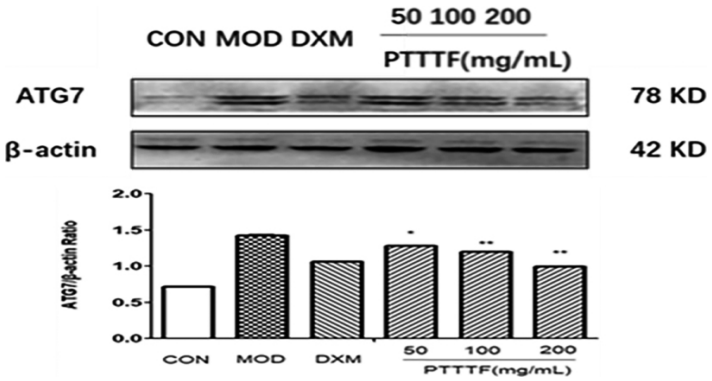

Fig. 12. The influence of PTTTF on ATG7 i expression in synovium of adjuvant arthritis rats. The number of samples in each group was 10. All data stand for the mean $\pm$ SD. Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

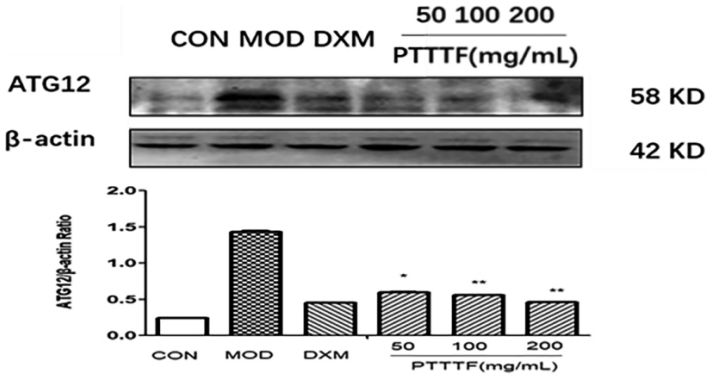

Fig. 13. The influence of PTTTF on ATG12 expression in synovium of adjuvant arthritis rats. The number of samples in each group was 10. All data stand for the mean ± SD. Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

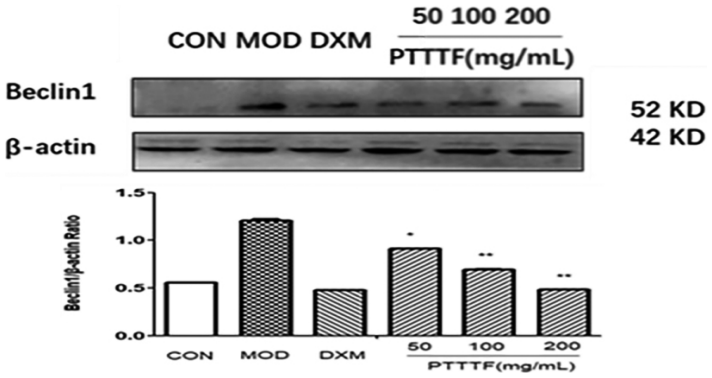

Fig. 14. The influence of PTTTF on Becinl1 expression in synovium of adjuvant arthritis rats. The number of samples in each group was 10. All data stand for the mean ± SD. Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

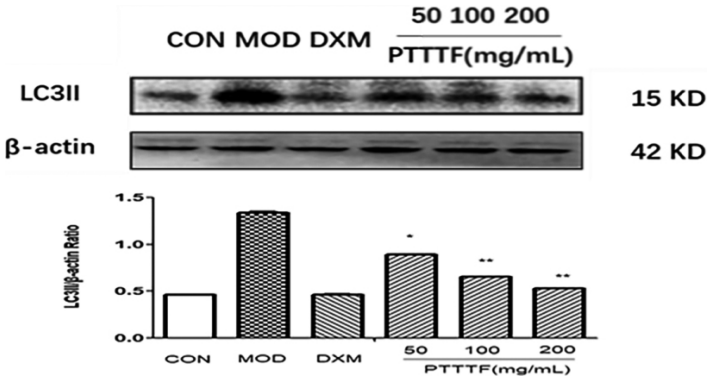

Fig. 15. The influence of PTTTF on LC3II expression in synovium of adjuvant arthritis rats. The number of samples in each group was 10. All data stand for the mean ± SD. Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

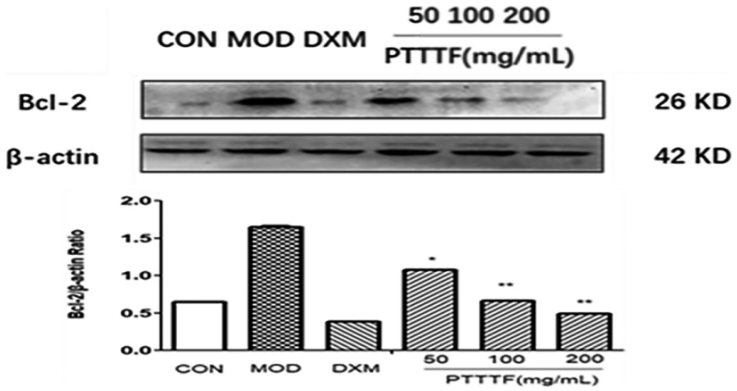

Fig. 16. The Effect of PTTTF on Bcl-2 expression in synovium of adjuvant arthritis rats. The number of samples in each group was 10. All data stand for the mean ± SD. Compared with model group, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: Control group, MOD: Model group, DXM: Positive group.

model is characterized by multiple arthritis. The primary lesions are mainly local acute inflammation, and secondary lesions appear one week later, and immune dysfunction mainly due to cellular immune changes [12].

Autophagy is a transversion of phagocytizing its own cytoplasmic protein or organelle and coating it into vesicles, and fuses with lysosomes to form autophagosomes, which degrade the contents of the cells, thereby realizing the metabolism of the cells themselves. And the renewal of certain organelles, but this process is also used to degrade pathogenic microorganisms (such as viruses, bacteria and protozoa) [13,14]. According to the way cell functions and the way cytoplasmic transports to lysosomes, it can be divided into three distinct modalities of autophagy: macroautophagy, microautophagy and molecular chaperone-mediated autophagy, respectively [15].

Under physiological conditions, moderate autophagy can clean up damaged organelles and intracellular macromolecules and recycle them, thereby maintaining intracellular homeostasis. Under pathological conditions, excessive cellular autophagy can cause damage to cells and tissues.

Beclin1 is an autophagy marker gene that adjusts the orientation of Atg protein in autophagy precursor structure and strengthens autophagy activity. Atg5 and Atg7 are important molecules in BeKlin1 affected by Beclin1 in the precursor structure, which is decisive for the activity of autophagy [16]. The autophagy-accelerating function of Beclin1 is effected by the anti-apoptotic protein Bcl-2; as a matter of fact, when Beclin1 binds to Bcl-2, autophagy is limited; by contrast; Bcl-2 disaggregation makes Beclin-1 and PI3K-III compound interactions and activation of autophagy [17]. Atg12-Atg5-Atg16L and microtubule-associated protein 1 light chain 3 (LC3)-phosphatidylethanolamine (PE) mediate autophagy and autophagosome expansion and closure [18]. LC3 is splitted by the cysteine protease atg4 to produce a cytosolic form of LC3-I. After being activated by Atg7, it is transferred to ATG3 and changed in a form coupled with PE, named LC3-II. Therefore, LC3-II is the most common marker of detecting autophagy activity, followed by the only protein associated with autophagosome stability during steps to ripeness [19].

Autophagy is a cellular catabolic process, which is extensively deemed as a reaction to various excitment, for instance, starvation, hypoxia and abnormal protein accumulation. More and more testimony shows that autophagy is involved in the pathological process of rheumatic diseases, ankylosing spondylitis, Crohn's disease, and systemic lupus erythematosus [20]. As the whole world incidence rate of RA is 0.5–1.0%, and the mortality rate is increasing, the gravity of RA has aroused great concern [21]. Rheumatoid

elements can lead to joint destruction, vasculitis and rheumatoid pleurisy, all of the above that will cause a serious economic and social burden. Autophagy is an important regulator of persistent inflammation in joints [22], but the link between autophagy and inflammation in RA is not clear. Studies have shown that LC3-II protein expression (an indicator of autophagosome formation) in RA patients is elevated, indicating an increase in autophagy activity in RA patients, suggesting that autophagy activation may participate in the pathogenesis of RA. Autophagy and inflammation interact in autoimmune diseases, and future studies should investigate the regulation of autophagy in inflammatory activity in RA [23]. The results of this study showed that the total flavonoids of cherries inhibited the expression of autophagy-related proteins ATG5, ATG7, ATG12, Beclin1, and LC3II. The therapeutic effect of total flavonoids in cherries may be at least partially concerned with the down-regulation of autophagy expression. Studying the pathogenic role of autophagy in RA may open up new ways for the exploitation of new drugs in the next. The relationship between autophagy and inflammation is intricate, and autophagy is participated in the induction and inhibition of inflammation [24]. Proinflammatory cytokines such as tumor necrosis factor (TNF-α) and interleukin (IL-6) have been certificated to provoke autophagy, which can also promote the excretion of these cytokines [25]. Due to the multi-faceted effects of autophagy in the inflammatory response, autophagy has a certain correlation with the onset of autoimmune diseases [26].

Recent research shows that autophagy regulates metabolism is involved in the pathogenesis of RA, synovial fibroblasts, osteoclasts, chondrocytes, T lymphocytes and B lymphocytes, citrullin peptide presentation and proinflammatory cytokine release [27]. Our results showed that in the synovial tissue, the patient's autophagy level was significantly reduced, while the expression level of inflammatory factors was significantly reduced. These results indicate a link between autophagy and RA-associated inflammation, which is consistent with other studies [28]. Resveratrol (an antioxidant) inhibits inflammation by reducing autophagy expression in AA rats [29]. Studies have found that levels of inflammatory factors in synovial tissue are positively correlated with autophagosome levels. Proinflammatory cytokines, for instance IL-1 and TNF-α, reflect chronic joint inflammation and cartilage and bone destruction in RA patients [30]. The study also showed that autophagy expression was significantly reduced in RA patients after 6 months of anti-IL-6R treatment, and autophagy can reduce osteoclast formation and avoid structural damage. In animal models of RA, suppression of autophagy can also reduce synovial inflammation [31]. The complex action of immune cells and inflammatory cytokines (Th17 cells and IL-17) play a significant role in the etiopathogenesis of RA. A recent research showed that IL-17 levels in serum, synovial fluid and synovium were higher in RA patients [32]. There is sufficient evidence that IL-17 plays a decisive role in inflammatory cytokines and can lead to the combined destruction in RA. Our results indicate that total flavonoids in cherries reduce COX-2, IL-1β, IL-6, IL-17 and TNF-α in synovial tissues, and total flavonoids in cherries reduce the expression of autophagy-related proteins, inflammatory factors. Decreased levels, autophagy may participate in the progression of RA by promoting inflammation and survival of self-reactive cells, thereby affecting the response to treatment.

5. Conclusion

This study illustrates that the total flavonoid in cherries inhibited the inflammation of adjuvant arthritis, reduced the expression of inflammatory factors and autophagy in adjuvant arthritis synovial tissue, and its anti-inflammatory and inhibitory effects reduced the expression of autophagy. No significant side effects were found in the treatment of adjuvant arthritis. Inflammation of synovial tissue, inhibition of autophagy may be benefit the curing of RA. This study provides a theoretical basis for studying the anti-inflammatory mechanism of total flavonoids in cherries, and the study also lays the theoretical foundation

for the exploitation of new anti-inflammatory drugs before clinical development. Nevertheless, thinks to time and funding constraints, we have only explored how PTTTF regulates cell proliferation, apoptosis and autophagy in AA rats by inhibiting the expression of autophagy related proteins ATG5, ATG7, ATG12, Beclin1, Lc3II and Bcl-2.Further studies focusing on other signaling pathways are required to further corroborate our findings.

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Conflict of interest

None to report.

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