Effects of *Pereskia aculeate* Miller Petroleum Ether Extract on Complete Freund's Adjuvant-Induced Rheumatoid Arthritis in Rats and its Potential Molecular Mechanisms

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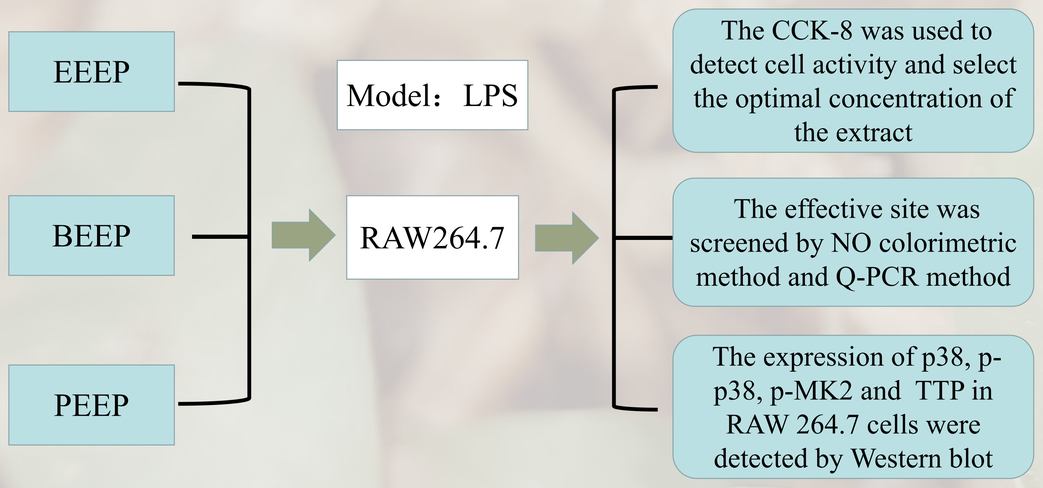
Introduction

Rheumatoid arthritis is an autoimmune disease characterized by polyarthritis, progressive joint damage, and swelling deformities. The pathological features of RA are joint synovial lesions, including excessive synovial cell proliferation, inflammatory cell infiltration, pannus formation, joint bone and cartilage destruction. According to relevant investigations and studies, the incidence of this disease worldwide is as high as 1-2%. There are more than 5 million RA patients in China, most of whom are in the 30-50 years old age group. It is the second largest cause of disability in the Chinese population. It can quickly develop into multi-system inflammation and irreversible joint damage, leading to a decline in quality of life, disability and death without proper treatment. Because the etiology and pathogenesis of RA are not clearly understood. The targets and methods of clinical treatment are not clear, which brings certain difficulties to treatment. There are no highly targeted therapeutic drugs in clinical practice, the treatment of RA still from the aspects of analgesia, anti-inflammatory, preventing or reduce joint deformation and damage and increasing joint mobility. These drugs reduce joint inflammation, inhibit the development of lesions and irreversible bone destruction, but long-term use of these drugs lead to immune system decline, bone marrow suppression, liver and kidney function damage, gastrointestinal function decline, cartilage degeneration, infection. Therefore, it is clinically necessary to find effective drugs with low toxicity to treat RA. In recent years, ethnomedicine and some natural medicine products have attracted more and more researchers' attention because of their unique safe and effective pharmacological activities. Ethnomedicine can be a new resource for the treatment of rheumatoid arthritis with more and more evidences.

Pereskia aculeata Mill is a plant of the genus Pereskia in the Cactaceae family, also known as leaf cactus and tiger thorn. It is mainly produced in Yunnan, Guangdong, Guangxi and other regions south of the Yangtze River. It has a long history of folk medicine, with low side effects, and anti-inflammatory, antioxidant, antibacterial and other pharmacological activities. Its medicinal liquor can treat bruises and rib pain. According to reports in the literature, there are as many as 30 chemical components in the essential oil of *Pereskia aculeata* Mill leaves, among which the higher content includes phytol, palmitic acid, linoleic acid. In addition, the plant essential oil has the highest content of oxidized sesquiterpenes (44.92%), acorus spirenone comes next. The current research on the pharmacological effects of *Pereskia aculeata* Mill mainly focuses on analgesia, cardiovascular protection and other related aspects. There are few studies on its anti-RA effect. The previous research show that *Pereskia aculeata* Mill ethanol extract inhibited acetic acid-induced increase in vascular permeability and writhing behavior of mice in acetic acid-induced writhing reaction experiment, reduce the swelling of the feet of CFA rats and the level of IL-6 and other inflammatory cytokines in the rat serum, inhibit LPS-induced inflammatory response in RAW264.7 macrophages.

This study aimed to screen effective sites by establishing an LPS-induced RAW264.7 macrophage inflammation model, and through the CFA rat model to evaluate the therapeutic effect and possible mechanism of petroleum ether extract of *Pereskia aculeate* Miller (PEEP), provide a more adequate theoretical basis for the development and utilization of PEEP.

Methods



Results

In vitro: PEEP, Ethyl Acetate Extract of *Pereskia aculeate* Miller (EEEP), N-butanol Extract of *Pereskia aculeate* Miller (BEEP) have no toxic effects on RAW264.7 macrophages. PEEP, EEEP and BEEP reduce the secretion of NO in RAW264.7 cells induced by lipopolysaccharide (LPS), only PEEP significantly inhibited the mRNA expression levels of inflammatory factors TNF-α and IL-6; PEEP-dependently reduce the secretion of TNF-α and IL-6, decrease the expression of p-p38 and p-MK2, and the level of TTP phosphorylation in LPS-induced RAW264.7 cells. In vivo: PEEP improve the living conditions of CFA rats, reduce foot swelling, spleen index, bone surface erosion and joint space narrowing; reduce the formation of synovial cells, inflammatory cells and pannus in the foot and ankle joints. PEEP reduce the secretion of TNF-α, IL-6, PGE2 in rat serum, down-regulate the expression of p-p38 and p-MK2 in the ankle joint, and reduce the phosphorylation of TTP.

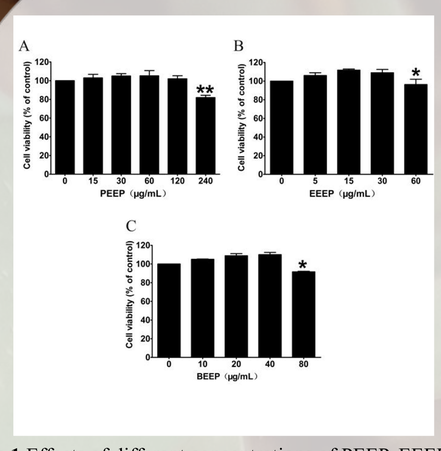


Figure 1 Effects of different concentrations of PEEP, EEEP and BEEP on cell viability in RAW264.7 cells. Results were exhibited as the means ± S.E.M. of three independent experiments. $^{*}P<0.05$, $^{**}P<0.01$ vs. Normal group.

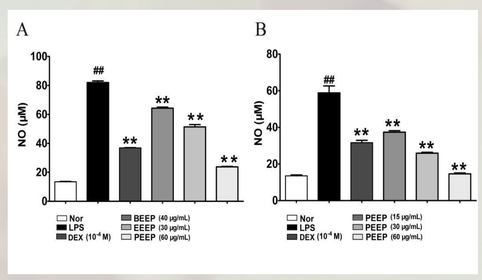


Figure 2 Effects of different concentrations of PEEP, EEEP and BEEP on cell viability in RAW264.7 macrophages. (A) Effect of PEEP (60 μg/mL)、EEEP (30 μg/mL) and BEEP (40 μg/mL) on the content of NO induced by LPS in RAW264.7 macrophages. (B) Effect of PEEP (15，30，60 μg/mL) on the content of NO induced by LPS in RAW264.7 macrophages. Results were exhibited as the means ± S.E.M. of three independent experiments. $^{\#\#}P<0.01$ vs. Normal; $^{*}P<0.05$, $^{**}P<0.01$ vs. LPS group.

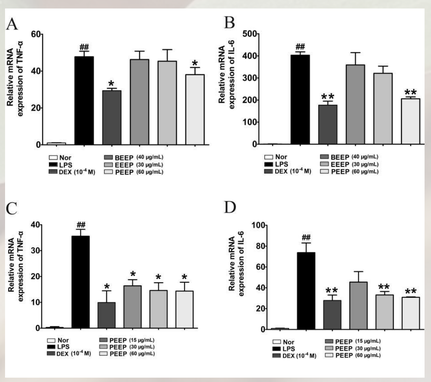


Figure 3 Effect of PEEP、EEEP and BEEP on the expressions of inflammatory factors in RAW264.7 macrophages induced by LPS. (A) The effect of PEEP (60 μg/mL)、EEEP (30 μg/mL) or BEEP (40 μg/mL) on mRNA expressions of TNF-α were detected using Q-PCR assays. (B) The effect of PEEP (60 μg/mL)、EEEP (30 μg/mL) or BEEP (40 μg/mL) on mRNA expressions of IL-6 were detected using Q-PCR assays. (C) The effect of PEEP（15, 30, 60 μg/mL）on mRNA expressions of TNF-α were detected using Q-PCR assays. (D) The effect of PEEP （15, 30, 60 μg/mL）on mRNA expressions of IL-6 were detected using Q-PCR assays. The data were expressed as the means ± S.E.M. of three independent experiments. $^{\#\#}P<0.01$ vs. Normal group; $^{*}P<0.05$, $^{**}P<0.01$ vs. LPS group.

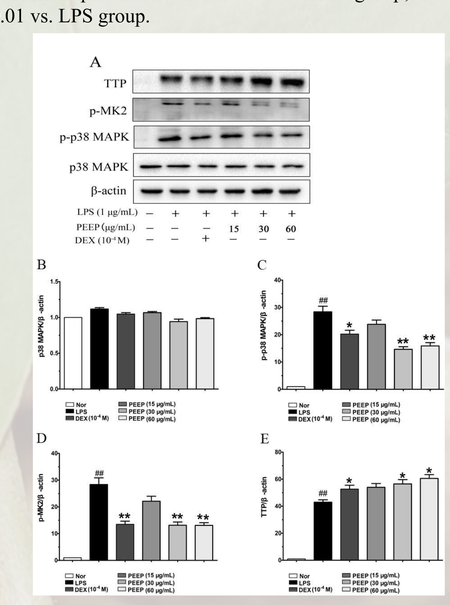


Figure 4 Effect of PEEP on p38、p-p38、p-MK2 and TTP expressions induced by LPS in RAW264.7 macrophages. (A) The protein expressions of p38、p-p38、p-MK2 and TTP were detected by using western blot analysis. (B) Quantitative analysis of gray value of p38 was performed in several groups with β-actin as loading control. (C) Quantitative analysis of gray value of p-p38 was performed in several groups with β-actin as loading control. (D) Quantitative analysis of gray value of p-MK2 was performed in several groups with β-actin as loading control. (E) Quantitative analysis of gray value of TTP was performed in several groups 27with β-actin as loading control. Data represent the mean ± S.E.M. of three independent experiments. $^{\#\#}P<0.01$ vs. Normal group; $^{*}P<0.05$, $^{**}P<0.01$. vs. LPS group.

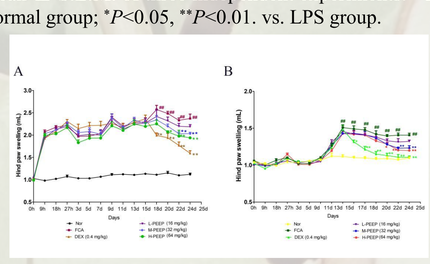


Figure 5 Effects of different doses of PEEP on swelling of paw in CFA rats. (A)Effects of different doses of PEEP on swelling of right hind paw in CFA rats. (B) Effects of different doses of PEEP on swelling of left hind paw in CFA rats. $^{\#\#}P<0.01$ vs. Normal group; $^{*}P<0.05$, $^{**}P<0.01$ vs.CFA group.

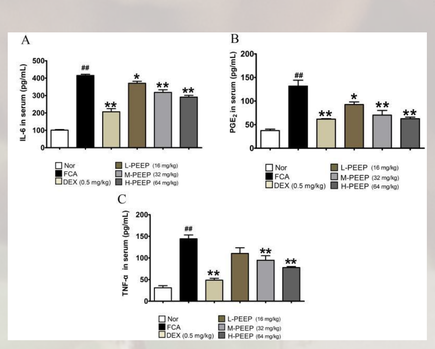


Figure 6 Effects of different doses of PEEP on the expression of inflammatory cytokines in CFA rat serum. (A) The concentrations of IL-6 in serum were determined by ELISA. (B) The concentrations of PGE2 in serum were determined by ELISA. (C) The concentrations of TNF-α in serum were determined by ELISA. The data were expressed as the means ± S.E.M. (n=5). $^{\#\#}P<0.05$ vs. Normal group; $^{*}P<0.05$, $^{**}P<0.01$ vs. CFA group.

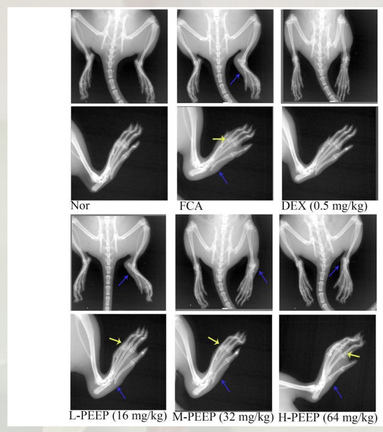


Figure 7 X-ray evaluation of joint injury in CFA rats. X-rays showed the effects of different doses of PEEP on hind paw and right paw of CFA rats. Blue arrow, swelling of soft tissue; yellow arrow, narrow joint space, blurred joint surface.

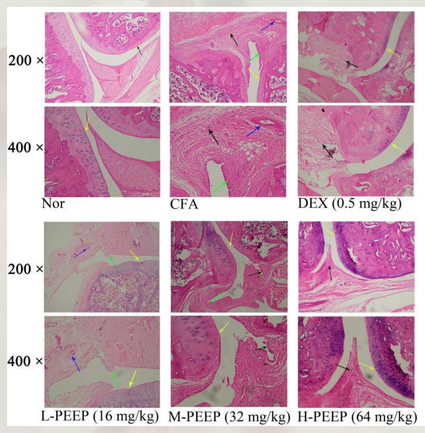


Figure 8 The pathologial slice pictures of the ankle joint of CFA rats treated with various doses of PEEP. Red arrow, articular cartilage; black arrow, synovial hyperplasia; green arrow, inflammatory cell infiltration; blue arrow, pannus.

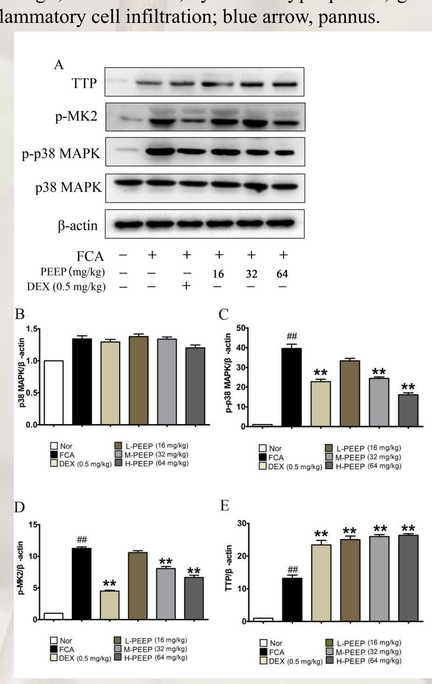


Figure 9 Effects of different doses of BGE on the p38/MAPK signaling pathway. (A) The expression of p-38, p-p38, p-MK2, and TTP obtained from ankle joints of several groups was detected by western blot analysis. (B) Quantitative analysis of gray value of p38 was performed in several groups with β-actin as loading control. (C) Quantitative analysis of gray value of p-p38 was performed in several groups with β-actin as loading control. (D) Quantitative analysis of gray value of p-MK2 was performed in several groups with β-actin as loading control. (E) Quantitative analysis of gray value of TTP was performed in several groups with β-actin as loading control. Data represent the mean ± S.E.M. of three independent experiments. $^{\#\#}P<0.01$ vs. Normal group; $^{**}P<0.01$ vs. CFA group.

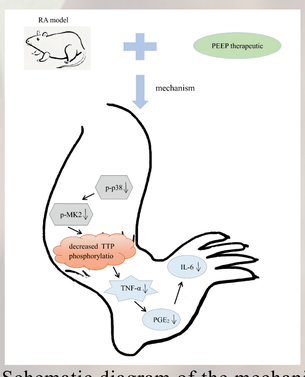


Figure 10 Schematic diagram of the mechanism of action of PEEP in vivo.

Conclusions

PEEP improve the living conditions of CFA rats, reduce the degree of foot swelling, protect immune organs, reduce inflammatory cell infiltration, cartilage damage, pannus formation, reduce inflammation and RA damage. The mechanism through regulating the signal pathway of p38 mitogen-activated protein kinase (p38/MAPK), which reduces the release of TNF-α, IL-6, and PGE2 in the serum.

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