

Anti-inflammatory Effects of *Kelussia odoratissima* in Rats Model of Rheumatoid Arthritis

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ABSTRACT

Background and Purpose

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease of the joints and other body organs, which 1 % of the human population is affected. RA induced in the fourth and fifth decades of life and in women is more common. *Kelussia odoratissima* contains compounds such as flavonoids, mainly aggregated in the inflorescence and stems of *Kelussia*, has anti-inflammatory effects. Present study aimed to investigate the anti-inflammatory effect of *Kelussia odoratissima* on rheumatoid arthritis induced in Wistar rats.

Materials and Methods

A total of 30 female Wistar rats using subcutaneous injection of complete Freund's adjuvant has been induced and were randomly divided into five groups containing negative control (no treatment), positive control (receiving indomethacin (3mg/ Kg) and three rheumatoid arthritis group receiving three different doses (100,200and 300mg/kg) of hydroalcoholic *Kelussia odoratissima* extract. material injection were tested in the animals for 10 days.

The symptoms of Rheumatoid arthritis were evaluated according to standardized scoring method at paw and double-blind for the different categories daily. CRP levels were measured and Data were analyzed using the SPSS statistical program (version 17 for Windows). In all the cases for comparison between different groups, Mann-Whitney U-test was used.

Results

The symptoms indicating disease severity was observed in the treated group compared to the negative control so that decreasing trend in disease severity in the group receiving 300 mg / kg of *Kelussia* extract was significant. However these severity of the disease was increasingly in the negative control. Serum CRP levels in groups () were significantly decreased.

Conclusions

Kelussia odoratissima has a anti-inflammatory effects and can reduce the inflammation and morbidity in rheumatoid arthritis. Thus it can be used as a drug for reducing and controlling inflammation in Rheumatoid arthritis.

Keywords- Rheumatoid arthritis, *Kelussia odoratissima*, Inflammation, Rat.

INTRODUCTION

Rheumatoid arthritis is a chronic, systemic autoimmune disease, which causes inflammation of the synovial lining and subsequent joint destruction and deformity of the bones and cartilage in joints¹. RA caused by number of pro-inflammatory molecules released by macrophages². These include that reactive oxygen species and eicosanoids such as prostaglandins, leukotrienes and cytokines (IL- β 1, IL-6, and TNF α)^{3,4}. The regulation of these mediators secreted by macrophages and other immune cells therefore may control the chronic inflammatory conditions^{4,5}. The nuclear factor kappa B (NF-kB) and activator protein-1 (AP-1) mediated cytokine pathways and the cyclooxygenase-2 (COX-2) prostaglandin cascade are the most well studied pathways⁶. Action pathway has been well studied in mediated cytokine, cyclooxygenase-2 and in cascade pathway of prostaglandin in this regard⁶. Today several classes of drugs are used for the treatment of RA, including modifying disease drugs, non-steroidal, anti-inflammatory drugs, immune suppressants corticosteroids or biological agents⁷.

Since no special medicine for cure of disease has not been considered and more corticosteroid and analgesics are used in this context however these drugs have adverse effects.

Today, through the use of alternative medicine therapies such as acupuncture⁸, herbal medicine⁹ and the effects of aerobic exercises¹⁰. Use of medicinal plants¹¹ for the treatment of rheumatoid arthritis have attracted many research assemblies in recent years¹¹. Recent evidence indicates that various herbal extracts including *Kelussia* has potent anti-inflammatory activity in a variety of inflammatory¹². *Kelussia odoratissima* (kind of umbelliferus) in certain native areas of Iran such as Chahar Mahal and Bakhtiari, Isfahan, Kohgiluyeh

and Boyer Ahmad and so far there has not been reported in other parts of the world¹³. The first scientific identification name of this plant is *Kelussia odoratissima Mozaffarian* in Iran^{14,15}. This plant has Fibrinolytic properties , analgesic , anti-inflammatory, anti-anxiety, sleep hypoglycemic¹⁵⁻¹⁷, As well as antioxidant and sedative effects¹⁸ and this herb contains flavonoids and compounds such as “Linamarose” and” vitamin A”¹⁹. This study aimed to evaluated the anti-inflammatory effects of different doses of *Kelussia odoratissima* on inflammation rheumatoid arthritis induction duration and severity of symptoms by Freund's complete adjuvant arthritis in female Wistar rats used.

METHODS

In methodology of the study were used 30 female Wistar rats weighing 170-200 gr. Animals were obtained from the ESFAHAN University and were kept in PNU animal care center for examination. The animals used in this research were kept under standard conditions and fed with water and food ad libitum. The experimental procedures were done in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Academy Press, which was accepted by the ethnic committee of the AUSR in Iran (Washington, D.C. 1996).

Extraction method

Kelussia odoratissima were obtained of Shahr-e-Kord (Kohrange). After removing the redundant parts of the plant, processes of washing, drying and powdering was applied on it.

The resulting powder inonetotwo were mixed with70% sweet ethanol a solvent. Solution was left at room temperature for 48 hours. The solution with aid of Büchner and funnel filter was smooth and the solvent was re added one at one and

incubated for 24 hours. The filtrate was centrifuged at 4000 rpm for 10 min, and finally was kept in an oven at 50° C for 48 hours and was completely separated from the solvent and the desired extract was obtained.

Disease induction

Animals were temporarily anesthetized using ketamine and xylazine and after rheumatoid arthritis was induced with subcutaneous 0.05ml complete Freund's adjuvant (Mycobacterium tuberculosis in mineral oil) in rats^{1,7,20}.

Drug therapy

Animals were randomly divided into five groups:

Negative control group was given received normal saline daily (intraperitoneally).

Positive controls received the amount of 3 mg/kg indomethacin daily (intraperitoneal).

Group exp. 1: received 100 mg/kg of *Kelussia* extract daily (intraperitoneal).

Group exp. 2: received 200 mg/kg *Kelussia* extract Daily (intraperitoneally).

Group exp. 3: 300 mg/kg of *Kelussia* extract Daily (intraperitoneally).

Evaluation of disease severity

The severity and symptoms was performed of injection by a person who was unaware of the amount of drugs from the second day and was scored the animal according to the following procedure: Asymptomatic tillers score of zero, to deal with the symptoms of swelling and inflammation around the injection point only one, Mild swelling and inflammation in the knee and paw points in two, Swelling and inflammation in the knee, paw and fingers warm up with a little more extreme three points, Severe inflammation and swelling of the fingers and paw with plus deformity and loss of motion and failure to use four point.

After the end of the experiment, blood samples were taken from rats and CRP levels in serum were examined and it was used for comparison of data from SPSS software. Surface $P < 0.05$ was considered as significant level.

RESULTS

Figure 1 shows the Average clinical conditions in different groups of rats. As can be seen in the negative control group gradually increased in severity. Surveillance in the treatment group showed a decrease in the average amount of clinical conditions so that was significant the process of reducing disease severity in group EXP 2,3 compared to the negative control group ($P < 0.05$).

Serum CRP levels is (12.4) in the negative control group. However CRP levels is (2) in the positive control group.

The serum CRP levels was reduced in groups treated with different concentrations of *Kelussia* extract compared to the negative control group so that were significant the amount in group EXP 2,3 ($P < 0.05$) The results are shown in Figure 2.

DISCUSSION

The study of rheumatoid arthritis is greatly facilitated by animal models that enable investigation of a complex system involving inflammation, immunological tolerance, and autoimmunity. Although the models cover several species and pathogenetic mechanisms and can be classified as induced or spontaneous, all converge on arthritis. The different methods are used to create animal models of rheumatoid arthritis. These methods include Adjuvant Arthritis, Rat type II collagen, mouse type II collagen arthritis, antigen arthritis and etc¹. Freund's complete adjuvant is generally used induce arthritis in animal models. Adjuvant arthritis in rat (AA

rat) shows several clinical and histological similarities to human RA¹⁴.

In the present study, following a single injection of FCA at the plantar surface, rats developed pronounced arthritis in the paws, showing 100% incidence. In this AA model, CRP was found to be markedly associated with the development of the disease, and significantly elevated CRP levels was noted throughout the course of the experiment as compared to control rats.

In this study, after injection of complete Freund's adjuvant and control rats were signs of the disease. In addition to changing clinical conditions, there was a increase in serum CRP levels. The results of this study agree with the results of other work in the field which is to create models of rheumatoid arthritis. *Kelussia odoratissima* extract caused a reduced rate of adverse clinical conditions in the treatment groups.

Kelussia odoratissima containing compounds such as flavonoids have anti-inflammatory and analgesic properties¹¹. According to a study undertaken on the properties of this plant such as sedative, antitussive¹². Reduction in cardiovascular disease, cerebral vascular¹³ anticonvulsants¹⁴. It has been attributed to all of the diseases, an evidence for anti-inflammatory properties of this plant¹⁵.

According to this few studies we expect that *Kelussia odoratissima* may be controlling inflammation can reduce adverse clinical conditions.

The results of this study confirmed the anti-inflammatory properties of the plant.

For more effective anti-inflammatory properties of *Kelussia* in this study, CRP levels in different groups were evaluated. CRP levels in patients with inflammation and tissue destruction, especially in patients with rheumatoid arthritis is increased^{16,17}.

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During the study HOJATI and colleagues in 1390 reported that *Kelussia odoratissima* can be due to its anti-inflammatory properties inhibited ileum contractions²². According to this few studies we expect that *Kelussia odoratissima* by controlling inflammation can reduce adverse clinical conditions. The results of this study confirmed the anti-inflammatory properties of the plant.

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Due to the significant reduction of serum CRP levels in groups *Kelussia* extract can be treated as potential anti-inflammatory effects of *Kelussia odoratissima* and phytochemical elements that can be identified and further investigation underlying the use of this plant in the treatment of rheumatoid arthritis provided.

CONCLUSIONS

Kelussia odoratissima has a anti-inflammatory effects and can reduce the inflammation and morbidity in rheumatoid arthritis. Thus it can be used as a drug for reducing and controlling inflammation in Rheumatoid arthritis.

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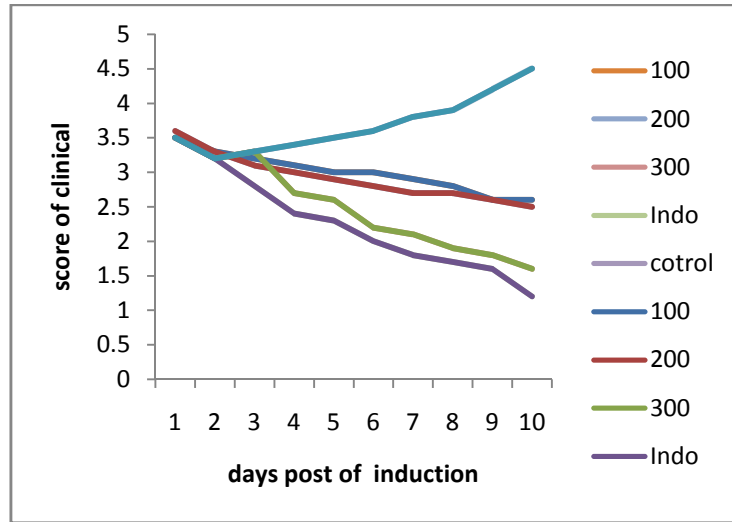


Figure 1. The mean clinical disease severity and treatment groups on different days

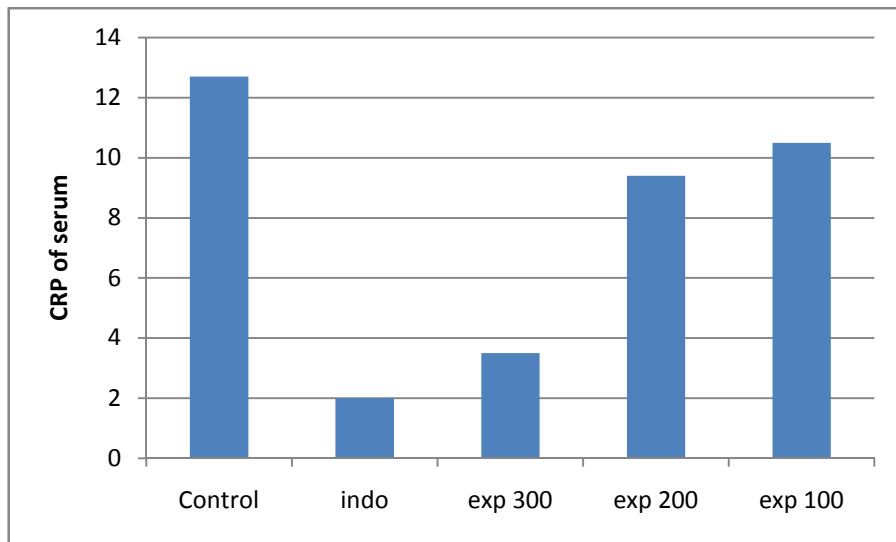


Figure 2. Serum CRP levels in different groups of rats

