INHIBITORY EFFECT OF AQUEOUS EXTRACT OF SAFFRON (*CROCUS SATIVUS* L.) ON ADJUVANT-INDUCED ARTHRITIS IN WISTAR RAT

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Summary

Crocus sativus L. (Saffron) is a plant of the iris family (Iridaceae). Saffron flower contains various chemical constituents. Saffron has been long used in folk medicine for treating some disease conditions. Its stigma contains crocin, anthocyanin, carotene and lycopene which are known to have pharmacological effects on various illnesses. In this study we have investigated the effects of saffron stigma aqueous extract (SAE) in complete Freund's adjuvant (CFA) induced arthritis rat model. So, Male Wistar rats weighed 120-150 g were used. Animals were injected with 100 µl of CFA (100 mg/ml) into the right tibiotarsal joint. After complete establishment of arthritis on day 14, animals were placed in 6 groups (five animals in each); groups II, III, IV and V AIA rats were injected intraperitoneally with 100, 200, 400 and 800 mg/kg saffron aqueous extract (SAE), respectively. Groups I and VI of AIA rats were injected intraperitoneally with an equal amount of normal saline and dexamethasone at a dose of 2 mg/kg body weight as a standard reference, respectively. Saffron, normal saline and dexamethasone injections were repeated four more times every other day (days 2, 4, 6 and 8). Measurement of the paws footpad and tibiotarsal joints diameters on the injected and not-injected paws were performed every three days. An arthritis index was assigned to each animal based on footpad paw diameter, ankle joint diameter and hyperalgesia. Our results show that saffron aqueous extract at 400 mg/ml has the best effect on reducing the footpad and tibiotarsal joint diameters, arthritis indexes and limitations of movement in CFA induced arthritis rats compared to non treated group. However this reduction is not as significant as dexamethasone treated group. It seems that aqueous extract of saffron stigma can alleviate the inflammatory condition of CFA induced arthritis. We showed the effect of saffron aqueous extract on improving disease condition in adjuvant-induced arthritis in rats.

Keywords: *Crocus sativus* L., saffron, Adjuvant-induced arthritis, Wistar rat

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease and is associated with disability and premature mortality (1, 2). Although many medicines are prescribed for the treatment of RA, they are known to produce various side effects including gastrointestinal disorders, immunodeficiency and humoral disturbances. So, there is still a need to seek therapeutic agents with lower side effects that can be used for long-term administration (3).

Saffron is produced from dried stigmas of *Crocus sativus* L. which is a bulbous perennial of the iris family (Iridaceae) and is principally grown in Iran. *Crocus sativus* L. is used for its golden-colored, pungent stigmas, which are dried and used to flavor and color foods as well as a dye (4-7). Study on chemical composition of saffron has identified approximately 40–50 constituents including carbohydrates, minerals, mucilage, vitamins and pigments, flavonoids, amino acids, proteins, starch and gums (8-10).

Saffron has several biological activities and is considered as an anodyne, anticonvulsant, antidepressant, respiratory decongestant, antispasmodic, diaphoretic, emmenagogue, expectorant, and sedative. Saffron has also been used in the treatment of scarlet fever, smallpox, colds, asthma, eye and heart diseases, tumor and cancer (11-19). In a recent study, aqueous extract of *C. sativus* stigma (saffron) was used in treatment of acute inflammation in a xylene-induced ear edema in mice and also in chronic inflammation in formaldehyde induced arthritis in hind paw of rats. Their results showed that aqueous extract of saffron stigma which dominantly contains alkaloids and saponins has an antinociceptive effect, as well as activity on acute and chronic inflammation (20).

Adjuvant arthritis in rats is a widely used experimental model of chronic inflammation with manifestations resembling clinical disorders such as RA and ankylosing spondylitis (21). In this study, we assessed the effects of saffron aqueous extract on adjuvant-induced arthritis (AIA) in Wistar rats as a model for rheumatoid arthritis.

Materials and methods

Animals

Male Wistar strain (150–180 g) rats were used in this study. The animals were housed in standard laboratory conditions with 12±1 h day and night rhythm throughout the experimental period. The animals were housed in large spacious polypropylene cages and they were fed according to standard protocols. The rats were kept in our animal house for at least one week before the experiment.

Plant material

Saffron was purchased from Novin Saffron Co. (Mashhad, Iran).

Extraction procedure

Aqueous extract of stigma was prepared as described previously (20). Briefly, the powder of stigma was extracted using maceration with distilled water. The powdered plant was macerated in water for 3 days and subsequently, the mixture was filtered and concentrated under reduced pressure at 45°C. The yield (w/w) of aqueous extract of stigma was 34.3%. The aqueous extract was dissolved in normal saline.

Induction of adjuvant-induced arthritis in rats

For induction of experimental arthritis, the plantar surface of the right hind paw of each rat was injected with 0.1 ml of Freund's complete adjuvant (containing 10 mg of heat killed mycobacterium tuberculosis per ml of paraffin oil). Left hind paw (not-injected) was assumed as control (21, 22).

Herbal treatment

Fourteen days after Freund's complete adjuvant (CFA) injection (Day 0), the rats were divided into 6 groups, each containing five rats. Group II, III, IV and V AIA rats were injected intraperitoneally with 100, 200, 400 and 800 mg/kg saffron aqueous extract (SAE), respectively. Groups I and VI of AIA rats were injected intraperitoneally with an equal amount of normal saline and dexamethasone at a dose of 2 mg/kg body weight as a standard reference, respectively (23). Saffron, normal saline and dexamethasone injections were repeated four more times every other day (days 2, 4, 6 and 8).

Assessment of adjuvant-induced arthritis

The rats were assessed on days 3, 6 and 9 for signs of arthritis by means of a well-established, widely used scoring system developed to evaluate the severity of AIA (24-27). Briefly, the physical symptoms of arthritis were judged by the following grading system: 0=normal paws; 1=erythema of toes; 2=erythema and swelling of paws; 3=swelling of ankles; 4=complete swelling of the whole leg and inability to bend. Arthritis index for each group was calculated by adding the scores for each rat. The maximum arthritic score per group was 20 (4 points× 5 paws). The clinical severity of arthritis was also determined by measuring the footpad and tibiotarsal joint diameters (as an indicator of edema) of right (CFA injected) and left (control) paws using a digimatic caliper (Mitutoyo Co., Japan).

Statistical analysis of data

Student's paired *t*-test was used to calculate the statistical significance of difference between groups. The difference between groups at the $P \leq 0.05$ levels was considered statistically significant. The related data were presented as mean ± S.D. (standard deviation).

Results

Effect of SAE administration on adjuvant-induced rat paws edema

Arthritis was induced in all animals after injection of CFA. The first manifestation of disease was erythema of ankle joints, followed by the involvement of metatarsal and interphalangeal joints. Diameter values of the right and left footpad paws and tibiotarsal joint were increased gradually and reached to peak at 14 days after injection of CFA. Treatment with SAE (100, 200, 400 & 800 mg/kg) and dexamethasone (2 mg/kg) decreased the paw footpad and tibiotarsal joint CFA induced swelling from day 14 to 23.

Physical symptom of arthritis was recorded using assessment the arthritis scores of right paws (Figure 1). In comparison with day 3, arthritis scores of group IV (p=0.016) and group VI (p=0.0015) were significantly decreased after ninth days.

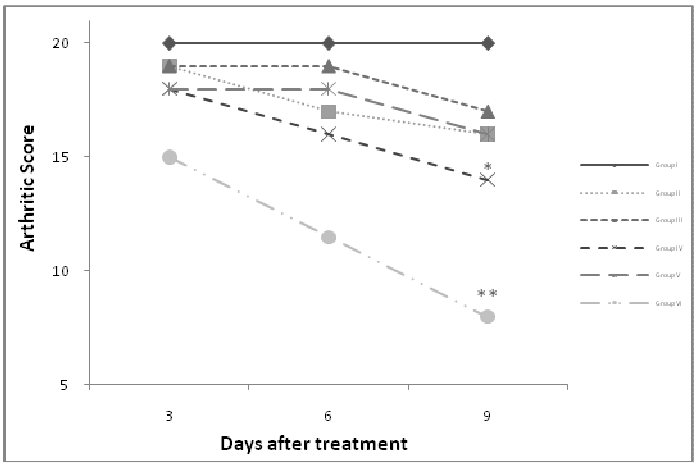
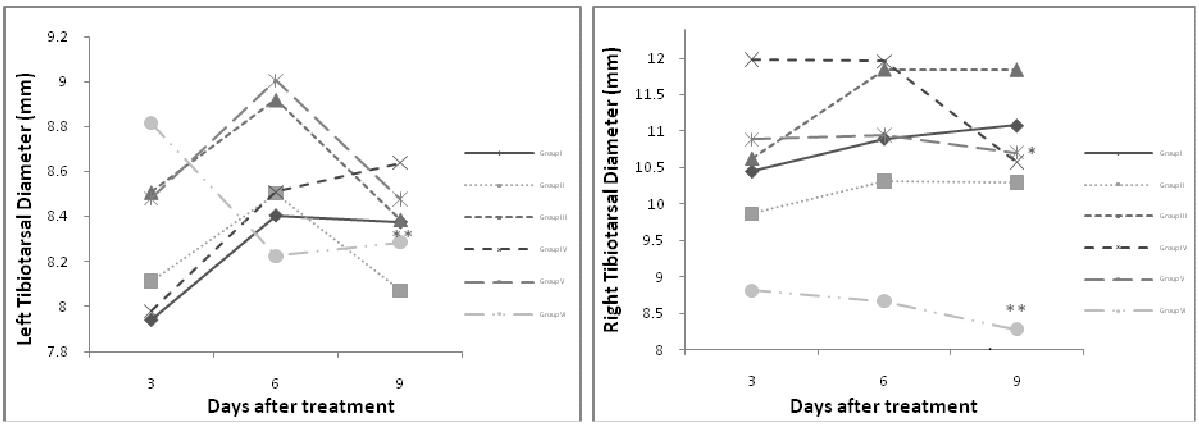


Figure1. Arthritic scores (arthritis index) of male Wistar rats treated with saffron aqueous extract (SAE) and dexamethasone in male Wistar rats with established adjuvant-induced arthritis on days 3, 6 and 9 by a set of visual criterion. Data were expressed as mean ± S.D.; n = 5 rats for each group. * $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$ *vs*. day 3, analyzed with paired *t*-Test.

Clinical severity of arthritis was determined using measuring of the right (injected) and left (not-injected) paw footpad and tibiotarsal joint diameters (Figure 2).

Right (injected) paw edema was increased in group I from day 3 to day 9. Right paw diameters of groups III and VI (P=0.477 and P=0.311, respectively) were decreased in the end of experiment (day 9) in comparison of day 3. Right (injected) tibiotarsal joint diameters of groups IV and VI (P=0.05 and P=0.003, respectively) were significantly decreased at day 9 in comparison with day 3. Although left paw (not-injected) diameters in group IV (p=0.1) was decreased, however only in group VI (P=0.036), there was a significant decrease in left paw diameters after 9 days. Left (not-injected) tibiotarsal joint diameters in ninth day decreased in group VI (P=0.003) in comparing with day 3.



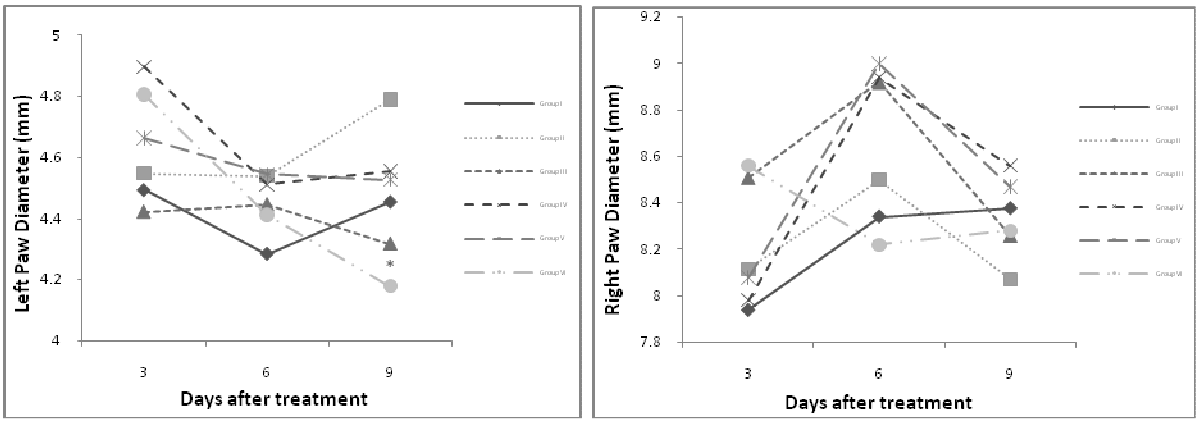


Figure2. Effects of saffron aqueous extract (SAE) and dexamethasone on paw and tibiotarsal joint diameters in male Wistar rats with established adjuvant-induced arthritis. Data were expressed as mean; $n = 5$ rats for each group. * $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$ *vs*. day 3, tested by paired *t*-Test. Right paw edema progression (lower right), left paw edema progression (lower left), right tibiotarsal joint edema progression (upper right) and left tibiotarsal joint edema progression (upper left).

Discussion

Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting about 1% of the population in developed countries. Treatment strategies in rheumatoid arthritis largely depend on the disease activity at the time of presentation. However, therapeutic effects of many drugs that are clinically used for the treatment of RA are restricted because of their side effects (28, 29). Therefore new treatments particularly of herbal sources with lesser side effects are still of research interest in rheumatoid arthritis.

Saffron is used for its golden-colored, pungent stigmas, which are dried and used to flavor and color foods as well as a dye (4-7). It contains about 40-50 different compounds (8-10) with several biological activities (11-19). Also, its stigma aqueous extract was showed to have inhibitory activities against acute and chronic inflammation in animal models (20). The rat AIA model is widely used for the evaluation of candidate anti-inflammatory compounds. This model has many features that are common to rheumatoid arthritis (30).

In the current study, we used AIA model for investigating the therapeutic effect of SAE on the course of arthritis. Administration of different concentrations of SAE or dexamethasone every other day intraperitoneally initiated after the development of the disease. Dexamethasone was used as a control since it has long been used in the treatment of RA. The progression of arthritis is characterized by increase of the paw footpad and tibiotarsal joint diameters. Rats with AIA are often relatively immobile due to the severity of their paws swelling (29).

Our results revealed that treatment with saffron aqueous extract (SAE) and dexamethasone diminished the right paw footpad and tibiotarsal joint swelling from day 14 to 23 induced by CFA. Some concentrations of SAE exhibited anti-inflammatory activity which was maintained until the experiment was terminated on day 23 (Figure 2).

Maximum inhibitory rates in the SAE-treated groups were SAE at concentration of 400 mg/kg for right tibiotarsal joint edema and dexamethasone (2 mg/kg) for right and left tibiotarsal joint edema. These *in vivo* results demonstrated that SAE was effective in diminishing the symptoms of AIA in

rats. We observed that some concentrations of SAE (400 mg/kg) decreased inflammatory processes in the right (injected) and left (not-injected) paws of Wistar rats from day 14 to day 23.
In a previous study aqueous extract of *C. sativus* stigma (saffron) was tested against acute and chronic inflammations. In the xylene-induced ear edema as an acute inflammation model, the 560 and 800 mg/kg concentrations of SAE showed significant inhibitory activity (44.1% and 32.6%, respectively) against the acute inflammation. In the formaldehyde induced arthritis in hind paw of rats as a chronic inflammation model, the 560 and 800 mg/kg concentrations of SAE showed significant anti-inflammatory activity. Diclofenac and SAE (800 mg/kg) showed anti-inflammatory effect on day one. In diclofenac (5 mg/kg) but not SAE-treated group, the hind paw edema of rat disappeared after 6 days (20).
In this study we showed that SAE at concentration of 400 mg/kg can reduce the chronic inflammatory responses associated with induction of arthritis by adjuvant.
This study is the first to demonstrate that *Crocus sativus* L. stigma (saffron) displays anti-inflammatory effect in comparison with dexamethasone and could be administered to treat arthritic inflammation and swelling without any obvious side effects. Possibly its anti-inflammatory activity is mostly mediated by its alkaloids and saponins contents. So, we suggest that saffron may be a safe and effective choice in the treatment of arthritis and related disorders.

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