



Received on 28 March 2014; received in revised form, 17 April 2014; accepted, 30 April 2014; published 01 May 2014

DETERMINATION OF ANTIOXIDANT ACTIVITY OF *CROCUS SATIVUS* IN ADJUVANT INDUCED ARTHRITIC MICE

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Keywords:

Rheumatoid arthritis, Prevalence, AIA, Oxidative stress, Herbal remedy, *Crocus sativus*

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ABSTRACT: Rheumatoid arthritis (RA) is a chronic systemic disease which results in pain, swelling, and stiffness in joints. It affects about 1% of the total world's population. RA begins with an edematous thickening of the synovia which latter converts into inflammatory synovium called pannus which erodes the surrounding cartilage. Conventional therapy exists to overcome illness but prolonged uses of drugs cause various side effects. Several reports demonstrate herbal remedy for RA treatment. Saffron is the natural yellow spice derived from the flower of *Crocus sativus*, possess antioxidant, anti-inflammatory activities. Therefore, in this study role of saffron for its treatment has been explored in AIA mice. The effect of three doses of ethanolic extracts of *Crocus sativus* (Family: Iridaceae) on adjuvant-induced arthritic mice was examined and evaluated. FCA injection caused a significant increase in joint inflammation. A daily oral dose of *Crocus sativus* extract (CSE) for 47 days resulted in a significant decrease in Lipid peroxides level as compared to AIA mice, while on the other hand the significant increase in Catalase and Glutathione peroxidase activity was recorded. From the present study, it may be concluded that CSE is effective in scavenging the free radical and hence capable of reducing oxidative stress during disease.

INTRODUCTION: Rheumatoid arthritis (RA) is a systemic inflammatory^{1, 2} autoimmune disease^{3, 4} which mainly affects the diarthrodial joint^{5, 6, 7}. It is an inflammatory disease of the synovium that results in pain, stiffness, swelling, deformity and at last involve the loss of function in the joints⁸.

There are many factors which may cause arthritis including environmental factors, genetic factors⁹, as well as nongenetic host factors. About 1% of the world's population is afflicted by rheumatoid arthritis¹⁰, in which women are three times more often affected than men^{11, 12}. The onset is most frequent between the ages of 40 and 70, but people of any age can be affected¹³. This can be a disabling¹⁴ and painful condition, which may lead to substantial loss of functioning and mobility if not adequately treated.

The site of location of RA is the synovial lining, a highly vascularized tissue which surrounds the



bones of the joint¹⁵. The inflammatory cells like the neutrophils and macrophages attack the affected joint and experience a "respiratory burst" which is characterized by an increased generation & release of reactive oxygen species. Excessive production of ROS leads to oxidative stress, loss of cell function, and ultimately apoptosis or necrosis.

The level of ROS is also a function of the antioxidants such as vitamins A, C, E and of metabolites like uric acid, bilirubin, etc. that are capable of either scavenging these reactive oxygen species directly or indirectly. However, a fine balance exists that may be disrupted in favor of oxidants (oxidative stress), leading to cellular damage, which in turn may play a role in major diseases such as cancer, rheumatoid arthritis, and atherosclerosis¹⁶.

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 administration¹⁷.

Crocus sativus Linn. (Family: Iridaceae) is a flowering plant in the crocus family and is commonly known as saffron¹⁸. It is native to Iran and Greece. It is now cultivated largely in Southern Europe, Tibet, and other countries. In India, it is mainly cultivated in Kashmir and Uttaranchal. The stigmas of the plant are mainly used for therapeutic purposes. The medicinal properties attributed to saffron are extensive. Its stigma contains crocin, anthocyanin, carotene and lycopene which are known to have pharmacological effects on various illnesses. We described herein the antioxidant effect of saffron on adjuvant-induced arthritic mice.

MATERIAL AND METHODS:

Preparation of Aqueous Saffron Extract: *Crocus sativus* L. stigmas were procured from Hi-media Ltd. Bombay. In the maceration method, 4 g of stigmas were macerated in 70% ethanol for three days. The mixture was subsequently filtered and concentrated under reduced pressure at 50 °C. The remainder liquid extract was dried at room temperature. As a result, the extract yield was 48.2%.

Animals: The experiments were carried out with female Swiss albino mice (12-14 weeks old) weighing 28-30 g. These animals were procured from NABL-compliant Institutional Animal facilities and maintained in the Era's Medical College and Hospital, Lucknow Animal House on the standard mouse diet fed pellet diet (Lipton, India) and water *ad libitum*. The animals used in the present study were maintained by the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), India and approved by the Institute's ethical committee.

Induction of Arthritis: Arthritis was induced by injecting Freund's Complete Adjuvant (FCA) in the right foot pad of mice.

Schedule of Treatment: Following adjuvant injection, AIA mice were divided into five groups of 6 animals each, *i.e.* arthritic, arthritic + *Crocus sativus* extract (CSE) (25, 50, 100 mg/kg bw), arthritic + acetylsalicylic acid (200 mg/kg bw).

Quantitative Determination of Activity and Levels of Antioxidant Enzymes: The CSE was administered orally for 47 days. After the duration of 47 days, Blood was drawn from the retro-orbital complex for biochemical analysis. The enzymatic activity of Lipid peroxidase (LPO), Catalase (CAT), and Glutathione peroxidase (GPO) was estimated as per the conventional methods¹⁹⁻²¹.

Statistical Analysis: The data from the controls and treated were compared by using the Student's t-test. The values were expressed as mean \pm standard deviation (S.D). Microsoft Excel for Windows 2007 was used for statistical analysis. P-values <0.01 (<0.05) were considered to indicate statistical significance.

RESULTS AND DISCUSSION: The data shown in **Table 1** shows the influence of pretreatment with ethanolic crude extract of saffron on rheumatoid arthritis in joints of mice influenced by FCA. A significant reduction was observed in the level of rheumatoid arthritis induced by FCA, following the administration of 25, 50 and 100 mg *Crocus sativus* extract/kg body weight. However, all the three doses were effective in reducing the level of disease.

TABLE 1: PARAMETERS OF OXIDATIVE STRESS IN ADJUVANT INDUCED ARTHRITIC MICE AS COMPARED TO HEALTHY AND STANDARD MICE

	Control	Arthritic 25 mg/kg	CSE I 50 mg/kg	CSE II 100 mg/kg	CSE III 200 mg/kg	ASA
LPO [nmol MDA/ml serum]	8.44 ± 0.78	16.2 ± 5.3	13.23 ± 1.64	10.90 ± 1.36	9.81 ± 1.25	10.69 ± 2.40
CAT [units/mg protein]	78.02 ± 0.56	40.76 ± 0.28	43.86 ± 3.1	46.07 ± 4.54	49.78 ± 7.56	57.87 ± 12.76
GPO [nmol of NADPH oxidized/min/mg protein]	0.68 ± 0.23	0.34 ± 0.12	5.1 ± 1.34	8.5 ± 1.93	11.9 ± 3.98	15.3 ± 6.78

*CSE, *Crocus sativus* extracts; ASA, Acetylsalicylic acid treated

FCA injection caused a significant increase in joint inflammation. A daily oral dose of CSE for 47 days resulted in a significant decrease in Lipid peroxidase (LPO) level activity as compared to AIA mice, while on the other hand; a significant increase in Catalase (CAT) and Glutathione peroxidase (GPO) activity was recorded.

CONCLUSION: Herbal remedies involve pharmacological interventions with naturally occurring agents to suppress the progression of the disease and prevent Rheumatoid arthritis. Saffron is one among and plays a key role in the fight against this terrible disease. The present results have indicated that different oral doses of ethanolic extract of *Crocus sativus* are capable of balancing the antioxidant status in adjuvant-induced arthritic mice and hence is capable of curing the disease before it progresses to its advance stage.

Therefore, it can be concluded that ethanolic extract of *Crocus sativus* is capable of curing rheumatoid arthritis. The study thus concluded that the extract is effective in scavenging the free radical and hence capable of reducing oxidative stress during the progression of the disease.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

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How to cite this article:

Jaggi K, Thakur SK, Rathore B, Chander R, Mahdi F and Mathur A: determination of antioxidant activity of *Crocus sativus* in adjuvant induced arthritic mice. Int J Pharmacognosy 2014; 1(5): 317-20. doi: 10.13040/IJPSR.0975-8232.1(5).317-20.

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