Journal of Traditional and Complementary Medicine xxx (2017) 1–10

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original Article

Anti-rheumatoid activity of ethanolic extract of *Sesamum indicum* seed extract in Freund's complete adjuvant induced arthritis in Wistar albino rats

A. Ruckmani [a, *], Vinayak Meti [a], R. Vijayashree [b], R. Arunkumar [a], Venugopala Rao Konda [a], Lakshmipathy Prabhu [a], E. Madhavi [a], Sobita Devi [c]

[a] Department of Pharmacology, Chettinad Hospital & Research Institute, Kelambakkam, Chennai, 603103, Tamil Nadu, India
[b] Department of Pathology, Chettinad Hospital & Research Institute, Kelambakkam, Chennai, 603103, Tamil Nadu, India
[c] Central Animal House, Chettinad Hospital & Research Institute, Kelambakkam, Chennai, 603103, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 10 March 2017
Received in revised form
23 May 2017
Accepted 13 June 2017
Available online xxx

Keywords:
Sesamum indicum
Rheumatoid arthritis
Freund's complete adjuvant
Interleukin-6
Tumor necrosis factor-α

ABSTRACT

Sesamum indicum, one of the first recorded plants used for its seeds, is reported to have analgesic, antioxidant, anticancer, anti-obesity as well as hepato and nephro protective activities. The current study evaluated the effects of two doses (400 and 800 mg/kg) of ethanolic extract of *S. indicum* seeds in Freund's complete adjuvant induced arthritis in rats in comparison with diclofenac and methotrexate by the changes produced in body weight, body temperature, paw volume and spontaneous activity, hemoglobin, erythrocyte sedimentation rate, total white blood cells, red blood cells, Interleukin-6 and Tumor necrosis factor-α as well as joint changes in X-ray and histological changes in joint tissue. Unlike the untreated group, the groups treated with *S. indicum* showed significant decrease in paw volume, body weight, white blood cell count, erythrocyte sedimentation rate, Interleukin-6 and Tumor necrosis factor-α and an increase in body weight, spontaneous activity, hemoglobin level, and red blood cell count. Histopathological examination showed gross reduction in synovial inflammation and cartilage damage. X-ray revealed significant improvement in joint space. The effect of ethanolic extract of *S. indicum* was found to be equivalent to methotrexate and greater than diclofenac.

1. Introduction

Rheumatoid arthritis (RA) is a chronic multi system disease characterized by persistent inflammatory synovitis involving peripheral joints leading to progressive functional impairment.[1] About 1 % of the world's population and 0.7 % (88 lakhs) of Indian population are afflicted with RA. It is more common in women and generally occurs between 40 and 60 years of age.[2,3]

A panel of drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, disease modifying anti rheumatoid drugs (DMARDs), biologicals such as TNF-α and IL antagonists and JANUS kinase inhibitors (JKIs) have been used to relieve pain and reduce immunological reaction mediated inflammation and joint damage.[4,5] A step wise approach starting with NSAIDs followed by glucocorticoids and DMARDs, either alone or in combinations are used in the current management of RA.[6] However these drugs neither offer a complete cure nor are free from adverse effects. Hence finding out new drugs is essential.

In this context, a well known plant from indigenous system, *Sesamum indicum* (SI) has been chosen for the current study.

SI, an ancient spice and one of the first recorded plants belongs to the family Pedaliaceae. It is used for its seeds for thousands of years and is still an oil seed of worldwide significance. Sesame oil is commonly used in margarine production and cooking.[7] SI contains sesamin 31.3 %, Sesamol 34.2 % sesamolin 46.7 % per 100 g and also contain oleic acid, α-tocopherol, γ-tocophereol, palmitic acid, stearic acid and linoleic acid, α-linolenic acid.[8–10] In addition SI contains vitamin B1: 0.28 mg, 1.48 mg of copper, 0.88 mg of manganese, 120 mg of tryptophan, 351.00 mg of calcium, 126.36 mg of magnesium, 5.24 mg of iron, 226.44 mg of phosphorus, 2.80 mg of

* Corresponding author.
E-mail address: ruckmani.nirmal@gmail.com (A. Ruckmani).
Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2017.06.003

Please cite this article in press as: Ruckmani A, et al., Anti-rheumatoid activity of ethanolic extract of *Sesamum indicum* seed extract in Freund's complete adjuvant induced arthritis in Wistar albino rats, Journal of Traditional and Complementary Medicine (2017), http://dx.doi.org/10.1016/j.jtcme.2017.06.003

zinc and dietary fiber.[11] The pharmacological activities reported include analgesic, antioxidant, anticancer, anti-obesity as well as hepato and nephro protective activities.[12–16]

Considering its traditional use, the phytochemical constituents and the reported activities the present study was undertaken to evaluate the effects of ethanolic extract of SI seed in Freund's complete adjuvant-induced rheumatoid arthritis in rats.

2. Materials and methods

2.1. Extract preparation

The black seeds of SI were collected from Chennai (Tamil Nadu state, India) and authenticated by Dr. Narasimhan, Associate Professor of Botany, Madras Christian College, Tambaram, Chennai, Tamil Nadu.

The seeds were washed thoroughly, dried under shade and powdered. 1.5 kg of the air-dried seeds was subsequently pulverized to uniform powder using an electric blender (25–28 °C). Pulverized seed (1.5 kg) was then defatted by mixing with n-hexane (3000 ml) using a magnetic stirrer at room temperature for 6 h. The resultant slurry was filtered and the residue was air dried for 24 h. The dried defatted residue (1000×*g* obtained from 1.5 kg) was then subjected to continuous extraction with 5 L of 95% v/v ethanol using Soxhlet apparatus at a temperature of (60–70° C) for 15 cycles. This process was repeated for 3 times. The extract thus obtained was dried by using rotary evaporator. 1000 g of dried defatted residue yielded 113.4 g of extract and the percentage of extraction was 11.34 %. The extract was brown in colour and it was transferred to a clean bottle and stored at 4 °C in a refrigerator until further use.

2.2. Drugs and chemicals

Freund's Complete Adjuvant (FCA) was procured from Sigma chemicals Co. ELISA kits of TNF-α and IL-6 were purchased from Ray Biotech, diclofenac and methotrexate from M/S Alkem laboratories ltd. All other chemicals were of the highest purity and analytical grade.

2.3. Animals

Wistar albino rats were obtained from the animal house of Chettiand Hospital and Research Institute. The study was initiated after obtaining approval from the Institutional Animal Ethics Committee (IAEC2/Desp.No.49/Dt.29.07.2013).

Rats were used according to the guidelines given by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in India. They were housed in clean poly propylene cages at 23–25 °C with relative humidity of 50–60 % in natural 12 h light-dark cycle with food and water.

2.4. Experimental design

A total of 36 male Wistar albino rats weighing 250–300 g were selected and allocated to 6 groups of 6 rats in each group. Group I was used as normal control. Group 2 to 6 were RA-induced and treated as given in Table 1.

2.4.1. Dose selection

Acute toxicity studies were not conducted as its safety up to 2000 mg/kg has been reported in an earlier study.[13] The two doses 400 and 800 mg/kg were selected based on earlier studies which have reported better response for doses above 400 mg/kg for analgesic, hepato and nephro protective activities.[12–14]

Table 1
Experimental design.

Group	Treatment
Group 1	Normal control (NC)
Group 2	RA control (RAC)
Group 3	FCA + Diclofenac (D) 25 mg/kg
Group 4	FCA + Methotrexate (M) 50 μg/kg/week
Group 5	FCA + EESI 400 mg/kg
Group 6	FCA + EESI 800 mg/kg

Drugs were administered daily orally from day 8–28.

2.5. Evaluation of anti-rheumatoid activity

2.5.1. Freund's complete adjuvant-induced arthritis

Freund's complete adjuvant contains heat-killed dead mycobacterium tuberculosis bacteria in liquid paraffin in the concentration of 10 mg/ml. All the rats, except those in the normal control group, were injected intradermally with 0.1 ml of FCA into the left hind paw on day '0'. An interval of 7 days was given for arthritis to develop. All the animals developed the signs of arthritis such as swelling, redness and restricted movement during this period.[17] On day 8, 3 ml of blood samples were collected by retro-orbital puncture for baseline biochemical assays and treatment was started. The treatment was ended on day 28. Body weight, temperature, spontaneous activity and paw volume of rats were measured once in 7 days from day '0' to '28'. On day 28, X-ray of the hind paw was taken and blood sample was again collected for biochemical assays. The rats were then sacrificed by administering high dose of halothane and the ankle joints were dissected for histological studies.

2.6. Determination of serum IL-6 and TNF-α levels

Serum was separated from blood samples by centrifugation (3000 rpm for 10 min) and stored at -20 °C. IL-6 and TNF-α were determined by ELISA kits according to the manufacturer's protocol (Ray Biotech, USA). Optical density (OD) was measured by Bio-Rad ELISA reader at 450 nm.

2.7. Radiological changes

The x-ray of lower limbs were taken with Siemens, Heliphos D X-ray machine and joint changes were assessed based on joint space and soft tissue swelling.

2.8. Histopathological study of joints

Rats were sacrificed by administering high dose of halothane. Ankle joints were removed and fixed in 10 % buffered formalin. The bones were decalcified in 5 % formic acid, processed for paraffin embedding, sectioned at 5 μm thickness and subsequently stained with haemotoxylin-eosin for examination under a light microscope for the presence of changes in synovium, cartilage and joint space.

2.9. In-vivo anti oxidant activity

The antioxidant activity was assessed using joint tissue homogenate by the following assays.

200 mg of joint tissue was cut into small pieces, crushed using mortar and pestle and homogenized at 4 °C in 1.5 ml of 0.1 M phosphate buffer (pH 7.2) to prepare joint homogenate. The

Please cite this article in press as: Ruckmani A, et al., Anti-rheumatoid activity of ethanolic extract of *Sesamum indicum* seed extract in Freund's complete adjuvant induced arthritis in Wistar albino rats, Journal of Traditional and Complementary Medicine (2017), http://dx.doi.org/10.1016/j.jtcme.2017.06.003