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Anti-arthritic potential of marine macroalgae *Turbinaria ornata* in Complete Freund's Adjuvant induced rats

S. Ananthi[a], V. Gayathri[a], R. Malarvizhi[b], Meenakshi Bhardwaj[b], Hannah R. Vasanthi[a,b,*]

[a] Herbal and Indian Medicine Research Laboratory, Department of Biochemistry, Sri Ramachandra University, Porur, Chennai 600 116, India
[b] Natural Products Research Laboratory, Department of Biotechnology, Pondicherry University, Puducherry 605014, India

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ABSTRACT

T. ornata a macroalgae rich in bioactive molecules possess various biological activities. Herein, the aim of the study is to evaluate the aqueous extract and the sulphated polysaccharide isolated from *T. ornata* for its anti-arthritic potential in Complete Freund's Adjuvant (CFA) induced arthritis in rats. Anti-arthritic potential of aqueous *T. ornata* (ATO) and *T. ornata* sulphated polysaccharide (TSP) was evidenced by the significant reduction in paw volume and arthritic score. Inflammatory and antioxidant markers were found to be restored in the drug treated groups which was found to be in line with dexamethasone a standard anti-inflammatory drug. The histopathological and radiological examination adds on the support to the above findings confirming the anti-arthritic potential of ATO and TSP. It is interesting to note that the sulphated polysaccharide inhibits inflammation and bone damage at very low dose itself. Hence, TSP could be considered as a better candidate in the management of chronic inflammatory diseases like rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that causes joint destruction and functional disability due to destruction of joints and often delineate by chronic inflammatory responses primarily affecting the synovium of diarthrodial joints (Pan et al., 2010). It is characterized by an infiltration of the affected articulations by blood-derived cells, mainly neutrophils and chronic inflammation of the synovial joints, which leads to progressive destruction of the cartilage and the bone (Nagate et al., 2007). Hence, RA patients have an increased risk of disability leading to various degenerative disorders and finally death. The incidence and prevalence of RA vary across populations, statistical methods, and disease definitions. The prevalence of RA in India is 1.5%–2% of the total population. The average female: male ratio of the incidence is 3:1 and the prevalence is 1% of the world populations (Selvarani and Bai, 2014). In general, RA affects the age group between 30 and 55 years (Reddy et al., 2014). The advent of several new therapeutic agents has played a major role in the management of RA. Essentially, safer Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) or 'Coxibs' have become available followed by interventions designed specifically to target pathogenic cytokines have reached the clinic (Kumar and Marwaha, 2003). The other classes of anti-inflammatory and analgesic agents include glucocorticoids, opiates and diproqualone, respectively. Though these drugs possess an effective remedy for RA, each drug has its own demerits as side effects like ulcerations, renal and hepatic diseases. The NSAIDs provide only a symptomatic relief but has no significant effect on the underlying disease process. Due to several restrictions and risks of prevailing therapy, people are exploring radical measures to treat the disease (Rathore et al., 2007).

Turbinaria ornata a brown algae of the Sargassaceae family is found intertidally on Hawaiian reefs and throughout the Pacific and Indian Ocean (Le Lann et al., 2008). It is commercially used in agro-based industries. It also exhibits cytotoxic activity on murine melanoma and colon cancer cells (Asari et al., 1989) and it is used as antipyretic agents and also in the treatment of goitre, scrofula and stomach ailments (Anggadiredja, 2009). Recently, the fucoidan content of eleven abundantly occurring brown seaweeds of Indian coast was investigated, among them *Turbinaria ornata* showed a high content of fucoidan in it confirming the presence of sulphated polysaccharides (Eluvakkal et al., 2010). Further, we have identified that *Turbinaria ornata* exhibits anti-inflammatory and antioxidant potential (Ananthi et al., 2010) and the crude polysaccharide isolated confirms the presence of sulphated polysaccharide responsible for the anti-inflammatory potential (Subash

Abbreviations: RA, rheumatoid arthritis; CFA, complete Freund's adjuvant; ATO, aqueous extract of *T. ornata*; TSP, *Turbinaria ornata* Sulphated Polysaccharide; NSAIDs, non steroidal anti-inflammatory drugs; CRP,C, reactive protein; CMC, carboxymethylcellulose; CPCSEA, The Committee for the Purpose of Control and Supervision of Experiments on Animals; SOD, superoxide dismutase; GPx, glutathione peroxidase; GSH, reduced glutathione; LPO, lipid Peroxide

* Corresponding author at: Natural Products Research Laboratory, Department of Biotechnology, Pondicherry University, Puducherry, Puducherry-605014, India.
E-mail address: hrvasanthi@gmail.com (H.R. Vasanthi).

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et al., 2016). Based on these beneficial aspects the present study aimed to study the anti-arthritic potential of the aqueous extract and the sulphated polysaccharide isolated from *Turbinaria ornata*.

2. Materials and methods

2.1. Materials

Dexamethasone tablets were purchased from Sri Ramachandra pharmacy, Chennai. The standard normal diet pellet for the animals were purchased commercially from Tetrachem chemie Pvt limited, Bangalore. A biochemical kit for the assay of CRP (C – reactive protein) was purchased from Spin React, Spain and Accurex Biomedical Pvt Ltd, Thane, India. The TNFα and IL6 assay was carried out using Thermo Scientific Kit (ER3TNFA and ER2IL6) USA. The prostaglandin E_2 levels in plasma samples were estimated using Prostaglandin E_2 Express EIA kit – Monoclonal (500141) – Cayman Chemical Company USA. Complete Freund's adjuvant (CFA) was purchased from Sigma Chemical Co., St. Louis, MO, USA. All solvents, concentrated acids and all other reagents are of analytical grade and they were purchased from SRL, SISCO laboratories, Mumbai.

2.2. Collection of Turbinaria ornata

The brown algae *Turbinaria ornata* was collected by hand picking from the intertidal waters of the Mandapam coast (Longitude 780 8'E, Latitude 90 17' N) in the Gulf of Mannar in the early sunrise period. The algal material was identified and authenticated by an eminent algologist Professor V.Krishnamurthy, Director, Krishnamurthy Institute of Algology, Chennai, Tamilnadu and a voucher specimen is maintained in the Herbal and Indian Medicine Research Laboratory, Sri Ramachandra University, Chennai. The collected algal material was washed with sea water and then with fresh water and freed from sand, salts and epiphytes, shade dried and powdered.

2.3. Extraction of ATO and TSP

About 100 g of powdered algae was extracted sequentially with 500 ml of water by hot percolation method using a soxhlet apparatus. The algal filtrate was concentrated under reduced pressure and aqueous extract of *T. ornata* (ATO) was obtained. The percentage yield of ATO was 12%.

Extraction of sulphated polysaccharide from *Turbinaria ornata* was done according to the method of Zhu et al., 2003. Briefly 300 g of the dried seaweed powder was depigmented with acetone followed by hot water extraction at 90–95°C for 3–4 h. The brown coloured syrup was then filtered through Whatmann No.3 filter paper, concentrated to 1/4th of the original volume, cooled, and precipitated with three volumes of ethanol overnight at 4°C. The precipitate was collected by centrifugation and dehydrated with diethyl ether to get a dried *Turbinaria* Suphated Polysaccharide (TSP-10% yield). TSP was further subjected to GC(MS) and LC(MS) analysis and was identified to be fucoidan like sulphated polysaccharide (Subash et al., 2016).

2.4. Experimental animals

Sprague–Dawley rats (180–200 g) were obtained and maintained in the Central Animal Facility of Sri Ramachandra University, Porur, Chennai. Animals were housed in polypropylene cages at a room temperature of 21 ± 2 °C with12 h light/12 h dark cycles and had free access to standard pellets and water *ad libitum*. Approval from the Institutional Animal Ethical Committee (IAEC/SRU/90/2008) for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) was obtained prior to the experiment.

Table 1
Experimental Design.

Groups	Treatment
I	Normal
II	Vehicle Control (0.5% CMC)
III	Dexamethasone (0.1 mg/kg, po)
IV	Aqueous extract of *Turbinaria ornata*- ATO(30 mg/kg, po)
V	Aqueous extract of *Turbinaria ornata*- ATO (100 mg/kg, po)
VI	Aqueous extract of *Turbinaria ornata*- ATO (300 mg/kg, po)
VII	*Turbinaria ornata* Sulphated Polysaccharide- TSP (2.5 mg/kg, po)
VIII	*Turbinaria ornata* Sulphated Polysaccharide- TSP (5 mg/kg, po)
IX	*Turbinaria ornata* Sulphated Polysaccharide- TSP (10 mg/kg, po)

2.5. Experimental protocol and design

Freud's adjuvant induced arthritis model was used to assess the anti-arthritic activity in Sprague–Dawley rats. Rats were divided into nine groups with six animals per group as outlined in Table 1. Adjuvant arthritis was induced in rats by injecting 100 µl Complete Freund's Adjuvant (CFA) emulsion, which is heat-killed *M. tuberculosis* (strain *H37Ra*) suspended in mineral oil (Sigma, 5 mg/ml), sub-plantarly at the right hind paw to all groups, except group I. Rats were pre-treated with test drugs/vehicle for a period of one week, twice a day before the induction of arthritis and thereafter CFA was administered and considered as day- 0 of the experiment. Paw volume was measured at Day 0, 4, 8, 12, 16, 20 following CFA injection using a Plethysmometer (UGO Basile, Italy). Clinical scores of inflammation of the limbs were recorded in accordance to criteria described previously (Yeom et al., 2006). In brief, each limb was scored according to a 5- point scoring system (0: no swelling or erythema, 1: slight swelling and/or erythema, 2: low to moderate edema, 3: pronounced edema with limited joint usage, and 4: excess edema with joint rigidity).

2.6. Biochemical analysis

2.6.1. Estimation of bone markers in plasma (Calcium, Phosphorous and Alkaline Phosphatase)

The level of calcium in plasma was determined using colorimetric Arsenazo III method using Greiner Kit. Likewise, the phosphorus content in plasma was determined using UV − End point method. The results of Calcium and Phosphorous in plasma are expressed as mg/dl. The activity of Alkaline Phosphatase (ALP) in plasma was determined based on UV kinetic method using Accurex kit. The readings were noted using a semi-automated biochemical analyzer (Star 21 Plus auto-analyser, Rapid Diagnostics) and the results are expressed as U/L.

2.6.2. Estimation of inflammatory markers in plasma

The level of inflammatory marker C − reactive protein (CRP) in plasma was determined using Pureauto S CRP latex (SS-type) Accurex kit using semi-automated biochemical analyzer (Star 21 Plus auto-analyser, Rapid Diagnostics). The results are expressed as mg/dl. The TNFα assay was carried out using Thermo Scientific Kit (ER3TNFA). A calibration curve was plotted using the standard absorbance and TNF-α concentration in plasma was determined by interpolation from the calibration curve. The results are expressed as pg/ml plasma.

Likewise, the inflammatory cytokine IL-6 in rat plasma was measured using ER2IL6 kit. A calibration curve was plotted using the standard absorbance and IL-6 concentration in plasma was determined by interpolation from the calibration curve. The results obtained are expressed as ng/dl plasma. The prostaglandin E_2 levels in plasma samples were estimated using Prostaglandin E_2 Express EIA kit. The developed color was measured at 405 nm using an ELISA plate reader. The calculation was performed using Cayman log-logit curve fit analysis. The results are expressed as ng/ml plasma.