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Study on Antirheumatoid Arthritis Activity

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Abstract: In recent years much attention was paid on role of polysaccharides isolated from natural sources. This study was carried out to investigate the effects of polysaccharide extracted from the green seaweed *Cladophora indica Marten* (Ci-ps) on collagen induced arthritis. To investigate these effects of Ci-ps of 2 varying test doses (test-200mg/kg , test-400mg/kg) in comparision to standard dexamethasone (0.1 mg/kg/day/po) ,assessment of various parameters such as body weight changes, arthritis index, paw volume and haematological parameters were performed. The body weight was fallen for all the arthritis induced groups, but. Ci-ps of Test -400mg/kg treated group had shown steady increase in bwt throughout the study period. Assessment of arthritis score revealed that high dose of Ci-ps extract has shown remarkable reduction in score when compared to arthritic control group. Test-400mg had shown remarkable decrease in paw volume on day 5 7, and 9 respectively. Haematological results revealed that there is a significant reduction in elevated WBC count, CRP & RF values. The study shows that Ci-ps extract (preferentially test-400mg/kg bwt) could effectively ameliorate CIA and significantly suppress the immune response against Collagen. Hence it can be concluded the polysaccharides of this green seaweed has potential antirhuematoid arthritic activity as they had shown promising results in ameliorating the disease associated altered parameters.

Keywords: anti-rheumatoid, seaweeds, Green algae, Collagen induced arthritis and polysaccharides

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I. INTRODUCTION

Rheumatoid arthritis is a condition defined with imprecise etiology characterized by progressive joint destruction, bone deformities and can lead to premature death. The consequent morbidity and mortality have a substantial socioeconomic impact. The pathological conditions of RA are well known such as the leucocyte infiltration, chronic inflammation, pannus formation and extensive destruction of the articular cartilage and bone.

In particular it was reported that the inflammatory cytokines, such as TNF-α,IL-1β and IL-6,play key roles in inflammation and joint damages during the development of RA. Epidemilogy of RA in female to male is 3:1 and the prevalence is 1% of the world population. Drug therapy used in most cases is represented by: adrenocorticoid hormones, anti-rheumatic and immunosuppressive agents that can cause severe adverse effects such as liver and renal dysfunction, cortisol dependence, stomach problems. In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, It has been demonstrated by several research workers that the seaweed extracts exhibited anti-inflammatory, antioxidant, antiarthritic activity [1]. Green seaweeds have been repeatedly used as natural materials from which to extract bioactive substances over the past 20 years because of their widespread distribution and large biomass. They are usually grown or collected for food consumption and especially known for their high nutritional value and health benefits. Marine green algae remain largely unexploited among the three main divisions of macroalgae (*i.e.*, Chlorophyta, Phaeophyta, and Rhodophyta). Interest in utilizing green seaweeds as natural resources has recently increased because of their many active ingredients, particularly those that may be used for medical purpose [2]. Green seaweeds reportedly contain lipid fractions, proteins, peptides, polysaccharide, carotenoids, phenolic compounds, alkaloids, thallus, holdfast, mucilaginous, and whole plants .Among all these active ingredients, polysaccharides are the components most intensively investigated for medical purposes[3].Considering the features described above the production and application of original polysaccharides as therapeutic agents have become increasingly important topics of research.in this context our study focuses on antirhematoid arthritis activity of green seaweed belonging to the order *Cladophorales*

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II. MATERIALS AND METHODS

2.1 Collection and purification of seaweed

The green seaweed were collected from suryalanka beach (province with coordinates of 15^0 53′20" N in latitude and 80^0 28′12"E in longitude) in Bapatla. It is washed thoroughly with sea water followed by fresh water to remove the soils, epiphytic forms, salts and other suspended materials. The cleaned algae were air dried in shade for 4-5 days and powered with electrical blender.. The algae was identified as *cladophora indica Martens* belonging to Cladophorales, Chlorophyta. The herbarium specimen with the accession number 20150724001 has been deposited at National Facility for Marine Algae Herbarium, Marine Algal Research Station(CSIR), Mandapam camp[4].

2.2 Preparation of Polysaccharide Extract.

It was washed thoroughly with sea water followed by freshwater to remove the soils, epiphytic forms, salts and other suspended materials. The cleaned seaweed were air dried in shade for 4-5 days and powered with electrical blender. Dried CI powder (20 g) was steeped in 500 ml of distilled water for 3 h at 80 ^{0}C. The aqueous extract was then clarified by centrifugation and subsequent filtration through whatmann No. 3 filter paper to remove insoluble materials. The PS in the extract were precipitated with two volumes of ethanol and recovered by filtration. The recovered PS precipitate (2.3 g) was used as the crude *Ci-ps* preparation [5].

2.3 Materials required

IFA(in complete freunds adjuvant) and Mycobacterium tuberculosis H37 Ra were obtained as a gift sample from DIFCO , where as type-II collagen from chondrex Inc. immunization Grade, dexamethasone injection and other chemicals are used are of analytical quality.

2.4 Experimental animals

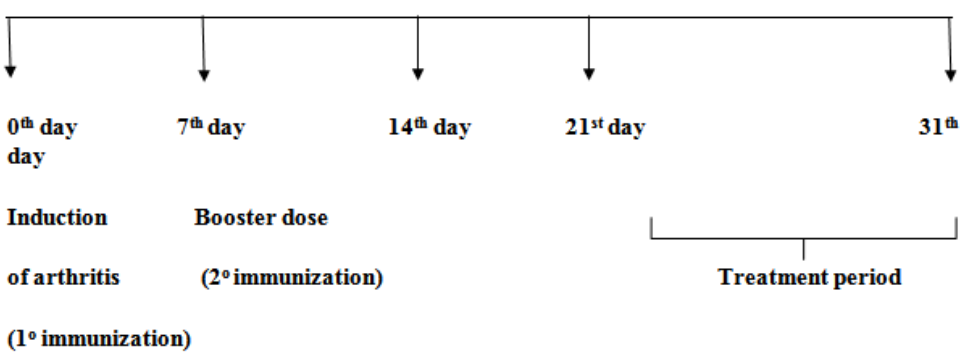
Adult male wistar rats, weighing between 200-250 gms were procured from animal house of Bapatla college of Pharmacy. The animals were starved overnight and deprived of water only during the experiment. The experimental protocol was duly approved by IAEC of Bapatla College of Pharmacy with reg no 1320.

2.5 Collagen induced arthritis in rats

Each treatment group contained six wistar rats. The rats were randomly divided into 5 groups Group 1 : normal control
Group 2 : arthritic control
Group 3 : standard ie (dexamethsone 0.1 mg/kg/day/po)
Group 4 : test sample ie *Ci-ps* (200 mg/kg/day/po)
Group 5 : test sample ie *Ci-ps* (400mg/kg/day/po)

On day 0 all the rats were immunized subcutaneously at the base of the tail with 100 µl of Bovine type II collagen(4mg/ml)-CFA(4mg/ml) emulsion ,later a booster dose 100µl of Bovine type II collagen(4mg/ml)-IFA emulsion was given on 7th day . All the rats were allowed to have free access for food and water for 20 days. On day 21 after the primary immunization. the rats were carefully monitored for onset of early signs of arthiritis,i.e.,redness/deformities/swelling in the joints and/or toes, etc. After 15 days of secondary immunization, test sample (*Ci-ps*)at the doses of 200mg/kg and 400mg/kg were admimistered orally to the test groups and dexamethasone (0.1mg/kg/po) to standard group,daily for 10 days. Once treatment was started arthritis score was taken for every 2 days and paw volume was measured using plethysmometer on alternate days and body weight once in every 3 days. On 31st day the blood samples were collected from retro- orbital plexus for estimation of haematological parameters

SCHEMATIC REPRESENTATION OF INDUCTION OF ARTHRITIS



2.6 Statistical analysis

The results were expressed as Mean ± SEM and analysis was carried out by one-way ANOVA followed with Post-hock analysis by Dunnett's multiple comparison tests to estimate the significance of difference between various individual groups. $P < 0.05$ was considered to statistically significant.

III. RESULTS ANALYSIS

3.1 Effects on body weight

In the arthritis control group, there was a significant decrease in the body weight ($P < 0.05$) probably after secondary immunization. Arthritis rats that had oral intake of test -400 mg had showed a steady increase in the body weight throughout the study period and a significant difference w. r to arthritic control group at the end of study . Even though there was an increase in the body weight with test -400 mg treatment, the weight change did not increase significantly compared to normal control rats (Figure 1).

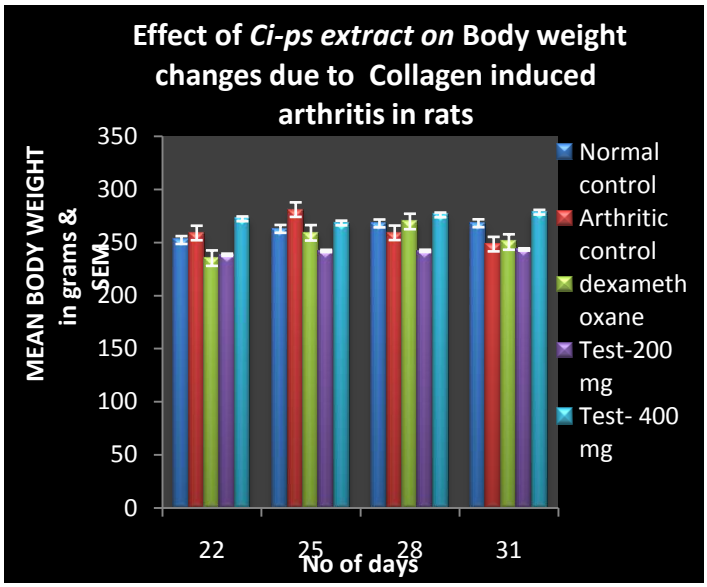
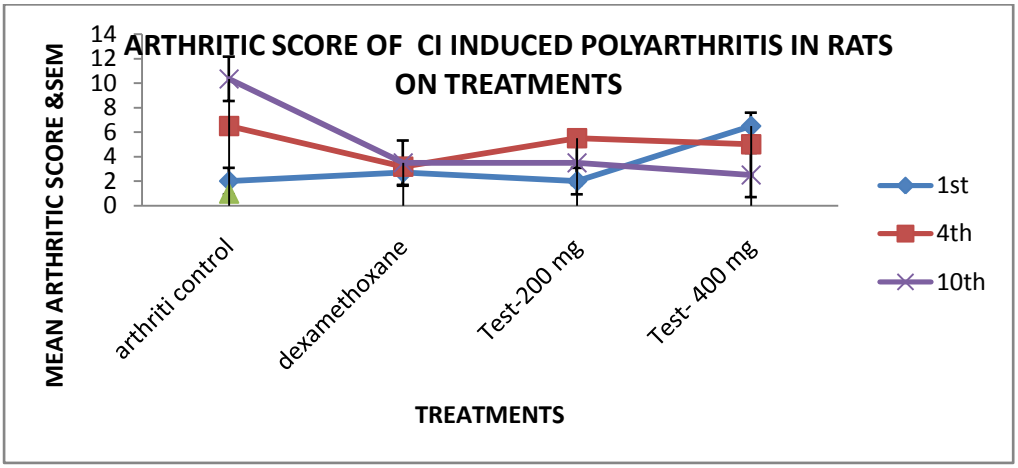


Fig 1: *Changes in body weight in the experimental groups. The values were expressed in mean±SEM*

3.2 Effect of CI induced arthritis on arthritic score

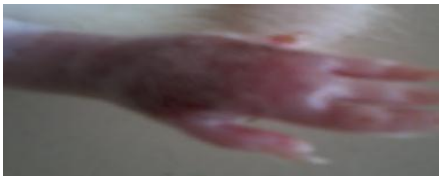
Administration of CI resulted in significant rise in arthritic index in all CI treated rats as compared to standard and this had shown a biphasic response. Rats treated with test-400 mg had shown remarkable decrease in the score when compared to arthritic group where as test-200 mg and standard were akin to each other.(Fig 2(A))

A



(B)

1. Normal control



2. Arthritic control .

3. Dexamethsone (.1 mg/kg)

4. *Ci-ps extract(*Test-200mg/kg)

5. *Ci-ps extract(*Test-400 mg/kg)

Fig 2: *(A)Effect Ci-ps on arthritic index in collagen induced arthritic rats. Results were presented as mean ± SEM (n = 6).(B) Hind paw images of representative rats of each group.*

3.3 Paw volume

One of the most important physiological parameter in case of assessment of RA is paw volume From the day of induction of arthritis , arthritic control group has shown raise in paw volume.Test-400mg had shown remarkable decrease in paw volume on day 5 7, and 9 respectively.

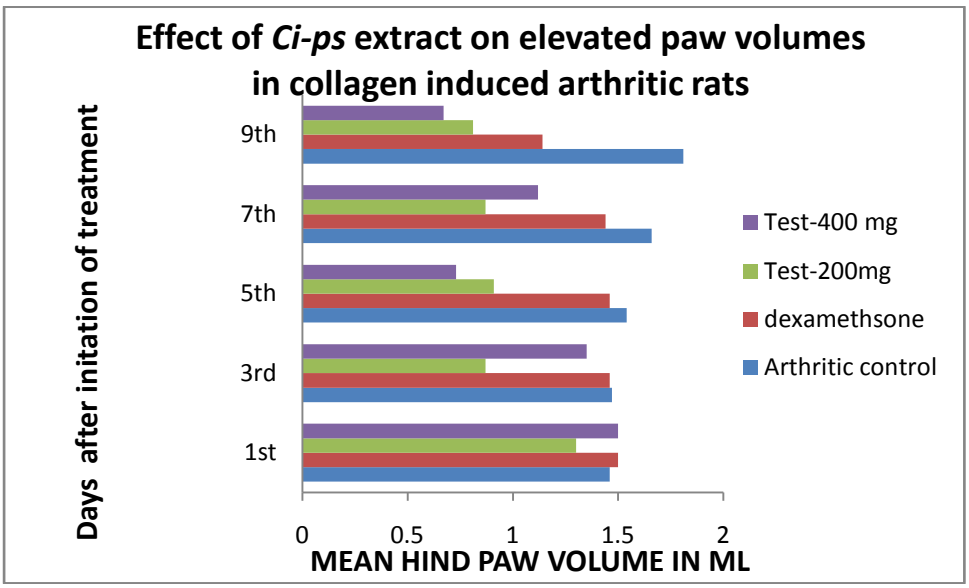


Fig 3: *Effect of Ci-ps on Paw edema. Values are expressed as mean ± SEM. n = 6.*

3.4 Haematological parameters (Table 1)

3.4.1 WBC count: Both standard and test-400mg have shown more ao less equal effect on reduction of raise d WBC count w.r.to arthritic control group.

RBC count: test -400mg had significantly increased RBC count.

CRP: Both test groups had significantly decreased the elevated CRP levels when compared to the arthritic control group.

Table 1.Haematological data of effect of *Ci-ps* on collagen induced arthritis in rats.

Experimental group	Total WBC cells/mm3	Total RBC millons/ mm^3	CRP mg/L
Normal control	4566.67± 95.43	5.97±.1	0.92±.13
Arthritic control	6,642±195	3.5±.13	11.08±.49
dexamethasone	5,467±84*	5.21±.22	1.9±.17**
Test-200mg	6,910±34	4.4±.1	5.07±.09**
Test-400 mg	5,600±37*	6.05±.04*	5.22±0.07**

Table.2: Results are presented as mean ± SEM(n = 6). The data was analysed using one-way analysis of variance (ANOVA) followed by Dunnett test. **P < 0.05, and*P <0.01 when compared with arthritic control group.

3.5 ESR and RF

A slight increase in ESR rate was noted in arthritic control, and a significant reduction caused by *Ci-ps* (test-200mg) and standard w.r.to arthritic control group in Rf values observed.

Table: 2 ESR (erythrocyte sedimentation rate) and RF(Rheumatoid factor)

Experimental groups	ESR(mm/hr)	RF IU/L
Arthritic control	6.8±.21	31.58±2.2
Dexamethasone	2.8±.45	13.34±.67**
Test-200mg	4.08±.1	15.5±1.2**
Test-400 mg	5.08±.3*	24.67±.5*

Table.3: Results are presented as mean ± SEM(n = 6). The data was analysed using one-way analysis of variance (ANOVA) followed by Dunnett test. **P < 0.05, and*P <0.01 when compared with arthritic control group.

IV. DISCUSSION

At present, many people with RA turn to alternative medicine including natural products based remedies or the functional food, because most of current treatments are in adequate and drug induced severe side effects occur. As a kind of marine natural products derived from the green seaweed, some polysaccharides from seaweeds have proven by many researcher as a potential chemicals in the treatment of many inflammatory diseases such as IBD, arthritis, etc although little is known about the exact effect of these products[7] In the present study an attempt was made to prove the significant role of polysachharides derived from seaweed in combating the commonly occurring effects of arthritis in arthritis induced rats. Rats were selected to induce arthritis because rats develop a chronic swelling in multiple joints with influence of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid disease [1]. Changes in body weight have also been used to assess the course of the disease and the response to therapy of anti-inflammatory drugs and test sample of this study.

During experimental period significant changes in body weight was observed. Body weight, food intake and metabolism are affected by immunity and inflammation and they are regulated by a cytokine-like hormone known as Leptin. It has been previously reported that T cell proliferation to promote Th1 responses in autoimmunity is stimulated by leptin[8,9] . In Collagen induced arthritis, within 24 h. of the administration of Collagen the plasma leptin levels were rapidly increased which led to anorexia and loss of body weight. As the severity of disease increased, the body weight also reduced significantly.During experiment, it was observed that the normal rats gain body weight, whereas arthritic rats reduce their body weight. *CI-PS* treated rats (Test-400mg)significantly improved the body weight and in standard group rats, dexamethasone shows weight increment initially but reduction on 31 st day (Fig 1).

The CI induced arthritis is an established model for evaluating anti-arthritis activity of drugs, which has been used frequently to assess anti-edematous effect of natural products . The collagen preparation used in this study contains both collagen solution and Freund ‘s complete adjuvant. Freund’s Complete Adjuvant(CFA) contains IFA with an inactivated and dried mycobacteria which are mainly responsible for stimulation of cell-mediated immunity which ultimately increased the production of certain immunoglobulin’s. Immunological hypersensitivity to collagen the major structural component of connective tissue , could explain both the systemic nature and chronicity of the inflammation occurring in rheumatoid arthritis . thus an immune response to the cartilage type of collagen could explain the predilection of rheumatoid arthritis to involve diarthodial joints Recent demonstration of antibodies to collagen is seen from patients with rheumatoid arthritis supports this premises[10].

After twice immunization with CII with an interval of 1 week,the arthritis symptoms (edema and arythema in one or both hind limbs) appeared on day 10 after first immunization.Fully developed arthritis ,red and swollen paws ,was observed 7 to 9 days after onset of inflammation.The clinical score in arthritic control group reached a peak approximately 15 days after the first immunization.The effects of different dose of *Ci-ps extracts* indicated that treatment with *Ci-ps* (Test-400mg/kg) had markedly reduced arthritic score when compared to standard(Fig 2A). In Fig 2B both immunological and inflammatory changes were demonstrated clearly through representative rats of each group.

Paw swelling is an index of measuring the antiarthritic activity of various drugs. The determination of paw swelling is simple, sensitive, and quick procedure for evaluating and assessing the degree of inflammation and thetherapeutic and curative effects of drugs . There is edema of periarticular tissues such as ligaments and joint capsules. The swelling increases in the initial phase of inflammation and then becomes constant in two weeks. These changes in paw volume are associated with increase in granulocytes and monocytes . In chronic inflammation activation of macrophages results in the production of several cytokines including IL-1, IL-6, interferon-γ, and TNF-α which have been implicated in immune arthritis . IL-6 is considered to play a central role in chronic inflammation and is expressed in excess at sites of inflammation. Like IL-1 and TNF, IL-6 stimulates acute phase protein production. It also elicits the development of specific cellular and humoral immune responses such as B cell differentiation and T cell activation . TNF-α is mainly involved in the perpetuation of the inflammatory cascades in autoimmune diseases, which affect connective tissues where the connective tissues become hypercontracted due to inflammation [11] .In the present study, the standard drug Dexamethasone and Test -400mg significantly suppressed the paw edema swelling (Fig 3). This indicates the anti-inflammatory activity of *Ci-ps* in rheumatoid arthritis. In arthritic condition there is a mild to moderate rise in WBC count due to release of IL-Ib inflammatory response. IL-Ib increases the production of both granulocyte and macrophages colony stimulating factor. In the present study, the migration of leucocytes into the inflamed area was significantly inhibited by the standard drug and Test -400mg. Also from the haematological data it was observed raise in Crp levels in arthritis control group but test- 400mg showed significant fall in crp levels indicating(Table 1) , the *Ci-ps* efficiency in combating both primary as well as secondary inflammatory lesions associated with collagen . Crp(c-reactive protein) is an important marker of systemic inflammation. It is secreted from liver cells upon stimulation with cytokine s released from activated macrophages like Il-6 [12].

Anemia is commonly noted in patients with chronic arthritis [13]. The two most common explanations are gastrointestinal blood loss due to arthritis medications and bone marrow changes in patients with inflammatory arthritis, which prevents the release of iron for incorporation into red Incollagen induced arthritis model, arthritic control rats showed reduced RBC count when compared to normal rats but which the count was elevated to double the reduced value by the effect of *Ci-ps.(Table 1).*

An increase in the ESR is attributed to the accelerated formation of endogenous proteins such as fibrinogen and α/β globulin, and such a rise in the ESR indicates an active but obscure disease process . The acute phase proteins in ESR share the property of showing elevations in the concentration in response to stress or inflammations. A slight increase in ESR rate was noted in arthritic control group(Table 2).

Prominent immunologic abnormalities that may be important in pathogenesis of RA include immune complexes that are found in joint fluid cells and in vasculitis. Plasma cells produce antibodies (e.g., IgM) that contribute to these complexes. Serum RF measures the amount of antibody IgM titre present in the serum . RF is the immunological expression of an individual's immune system reaction to thepresence of an immunoglobulin molecule that is recognized as nonself. This response to the nonself immunoglobulin results in the presence of immune complexes; these in turn bind to the complement and may eventually lead to destruction of synovium, cartilage, and bone.The higher the levels of serum RF are, the higher the development of inflammations . Determination of serum RF levels in rheumatoid arthritis is essential to understand and measure the disease progression and to facilitate the development of novel treatments for rheumatoid arthritis. Serum RF is a marker of systemic inflammation and antibody production against the injected adjuvant. In collagen -induced arthritic rats, activated

CD4+ T cells stimulate B cells to produce immunoglobulins, which are associated with increase in the plasma levels of serumRF . The standard and *Ci-ps* treated groups showed a significant recovery from the elevated serum RF level.(Table 2) this indicates that the imunnological process can be slowed down with this treatments.

V. CONCLUSION

Marine algae contain huge amount of polysaccharides mainly cell wall structure and also mycopolysaccharides and storage polysaccharides. Mainly the seaweeds contain polysaccharide concentration in the range from 4% - 76%.. *Chlorophyceae* or green algae contain sulphated galactans, sulphuric acid polysaccharide . In this study,the induction of arthritis using collagen showed marked inflammatory and

immunological effects, and the results showed that polysachharide extract ie *Ci-ps* had significantly improved these effects. However further implication is drawn towards extension of the study to explore the specific group of polysachharides.

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