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Original Article

EFFECT OF CRUDE SULPHATED POLYSACCHARIDE FROM MARINE BROWN ALGAE IN TPA INDUCED INFLAMMATION ON POLY MORPHONUCLEAR LEUKOCYTES

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ABSTRACT

Objective: The present study aims to evaluate the anti-inflammatory activity of crude sulphated polysaccharide from marine brown algae *sargassum ilicifolium*

Methods: The present study involves the investigation of crude sulphated polysaccharide from marine brown algae *sargassum ilicifolium* for anti-inflammatory activity by TPA [12- O- Techanoyl 13 – Myristate] induced inflammation in Polymorphonuclear leukocytes [PMNL]. T.h.e cell viability by Trypan blue dye exclusion assay, MTT assay, release of Cathepsin D, Nitrite, TNF-α levels was assessed. Diclofenac was used as the standard drug for comparison.

Results: Crude sulphated polysaccharide were isolated from brown seaweed *sargassum ilicifolium*. Crude sulphated polysaccharide showed the significant increase in the number of viable PMNL cells. It has further reduced the release of Cathepsin D, Nitrite and TNF-α. The levels of which are increased during inflammatory conditions.

Conclusion: Therefore our studies support the isolation and the use of sulphated polysaccharide in treating inflammation and rheumatism.

Keywords: Sulphated polysaccharide, PMNL cells, TNF-α, TPA.

INTRODUCTION

Seaweeds, the marine macro-algae has been recommended in early Chinese pharmacopoeia and Materia Medica [Sher- Nung Ben Cao Jing 200 BC and Ben Cao Gang Mu c 1518-1593 AD] for diverse maladies as antigoitre, antioedema, antipyretic, antitumor and diuretic agents and were regarded as valuable drugs for their expectorant function. Sulphated polysaccharides from sargassum species was found to possess wide pharmacological actions, especially as a potent antioxidant [1,2] hepatoprotective [3,4] analgesic, anti-inflammatory, antipyretic, immunomodulatory, and anticancer property. Inflammation is the organ initial response to tissue damage. Neutrophils and macrophages are known to recruit and produce an oxidative burst at the site of inflammation and thus play pivotal roles in acute and chronic inflammation respectively [5]. The increase of leukocytes into the site of inflammation is critical to the pathogenesis on inflammatory conditions. The ability of the bioactive agent from marine source to inhibit tumor necrosis factor [TNF-α] which are pro-inflammatory cytokines, involved in fever, inflammation, tissue destruction, determines the mechanism of action for its anti inflammatory potential. Therefore, inhibition of the cellular reactions in one of these targets are typically used as an *in vitro* model for evaluation of anti-inflammatory activity. Hence the present study is focused to validate a scientific approach. The modulation of inflammatory marker by crude sulphated polysaccharide from *Sargassum ilicifolium*.

MATERIALS AND METHODS

Collection of seaweed

The marine brown algae *Sargassum ilicifolium* was collected from Mandapam, Gulf of Mannar region, Rameswaram, India. Identified and authenticated by Dr. V. Krishnamurthy, Director Krishnamurthy Institute of Algology, Chennai. It was washed in seawater and then with fresh water thoroughly to remove the contaminants. The sample was subsequently air dried in shade, powdered coarsely and used.

Extraction of crude sulphated polysaccharide

The extraction of polysaccharide from the marine brown algae was carried out according to the method described by Tako et al [6] with some modifications. 10 g of dry algae was suspended in 200 mL of 0.05 M Hcl and stirred at room temperature for 2 h. Then centrifuged at 3575 rpm for 20 min and the supernatantfiltered. The filtered fraction was then neutralized with 0.5 M NaOH and the crude polysaccharide precipitated in two volumes of ethanol. After concentration in a rotoevaporator, the crude Sulphated polysaccharide was freeze-dried.

Anti-inflammatory activity

The extracted crude sulphated polysaccharide was subjected to *in vitro* anti-inflammatory activity using TPA [12- O- Techanoyl 13 – Myristate] induced inflammation in PMNL cells [7,8]. Polymorphonuclear leukocytes [PMNLs] were isolated from healthy individuals by standard procedure, cultured in RPMI-1650 media and was maintained at 37^0C and in 5% co_2 humidified co_2 incubator in animal tissue culture lab [9]. The approval for the collection of blood was given by Institutional Ethics Committee [IEC], Sri Ramachandra University, Porur, Chennai. IEC reference number: IEC-NI/12/MAR/27/18.

Experimental design

Group 1: Control [PMNL cells without drug]

Group 2: Cells + DMSO

Group 3: Cells+ TPA [4ng]

Group 4: Cells +crude sulphated polysaccharide extract [135µg]

Group 5: Cells+TPA**+**crude sulphated polysaccharide extract [135µg]

Group 6: Cells + Diclofenac sodium [185µg]

Group 7: Cells + TPA + Diclofenac sodium [185µg]

The parameters such as IC_{50} of TPA [12- O- Techanoyl 13 – Myristate], Diclofenac Sodium, Crude Sulphated Polysaccharide were determined. Trypan blue exclusion assay [10], MTT assay [11,12], Cathepsin D assay [13], Nitric oxide synthase activity assay [14,15], Assay of TNF-α using ELISA kit [Diaclone SAS, France] [16] were carried out and the results tabulated.

RESULTS AND DISCUSSION

TPA mimics the action of diacyl glycerol. Activates protein kinase C, causes the subsequent rise in cellular calcium level ultimately leading to inflammation. The results shown in fig.1 indicate that increasing concentration of TPA had cytotoxic effects which resulted in a decrease in cell number. The cell number was maximum in control and a gradual decrease in cell number was seen with increasing concentrations of TPA. This is caused by the induction of inflammation in Polymorphonuclear Leukocytes. The toxicity was started observing at a concentration of 4ng and hence this concentration of TPA was fixed at 50% inhibition in PMNL cells and 4ng was considered as IC_{50} value for TPA and it was used as minimum inhibitory concentration of TPA for the study DMSO did not show any decrease in cell number and hence the false interpretation of the results by dissolving the drug in DMSO was eliminated. The cytotoxicity was totally due to the action of TPA on leukocytes.

In the present study, the cells were post-treated with various concentrations (50-300µg/ml) of crude sulphated polysaccharide and Diclofenac Sodium after induction of inflammation by adding minimal inhibitory concentration of TPA to the respective cells and incubated. The increase in concentration of Crude sulphated polysaccharide and Diclofenac was inversely proportional to the cell numbers to a certain extent after which increase in drug concentration would cause toxicity to the cell. The IC_{50} value of Crude sulphated polysaccharide from *sargassum ilicifolium* and Diclofenac sodium was found to be 135µg and 185µg respectively. The test and standard have dose dependent protective action on cells.

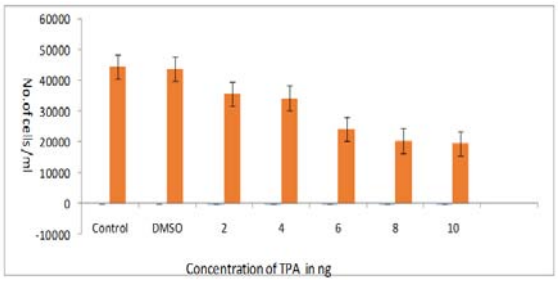

Fig. 1: Effect of various concentrations of TPA on cells viability (n=3)

Table 1: Cell viability, cathepsin D and Nitrite levels in inflammation induced PMNL cells

S. No.	Groups	Trypan blue exclusion assay No. of cells/ml	Cathepsin D assay units/ml	MTT assay % viability	No S assay µM of nitrite release
1.	Control	75000±4998	0.07±0.01	100±5.24	16.26±3.44
2.	DMSO	72733±2369	0.08±0.01	97.25±2.71	16.92±4.13
3.	Cells+ TPA	29926±5927	2.71±0.38	52.58±6.30	49.15±3.22
4.	Cells+ Diclofenac	69350±4075	0.09±0.01	96.9±2.97	17.34±2.57
5.	Cells+ Crude Sulphated Polysaccharide	66683±5195	0.14±0.01	94.65±4.57	19.47±5.59
6.	Cells+ TPA+ Diclofenac	62300±6199	0.12±0.01	95.57±5.26	21.39±2.72
7.	Cells+ TPA+ Crude Sulphated Polysaccharide	61300±6011	0.34±0.09	88.75±6.51	24.72±1.59

Each value represents mean± SD of three observations

Table 2: Cytokine levels in inflammation induced PMNL cells

S. No.	Groups	TNFα (pg/ml)
1.	Control	170.33±10.69
2.	DMSO	172.23±15.58
3.	Cells+ TPA	1665.66±66.71
4.	Cells+ TPA+ Diclofenac	394±32.42
5.	Cells+ TPA+ Crude Sulphated Polysaccharide	453.67±31.72

Each value represents mean ±SD of three observations.

Table 1 shows the effect of standard Diclofenac and crude sulphated polysaccharide on TPA induced inflammation on PMNL cells. Trypan blue stains only the dead cells and the live cells remain transparent. Hence the dead and live cells can be distinguished by the Trypan blue dye and the live cells are counted in the Neubauer chamber. The Trypan blue exclusion assay showed a significant increase in cell number in cells treated with crude sulphated polysaccharide and Diclofenac when compared to TPA alone treated cells.

Cathepsin D is a lysosomal enzyme released during an inflammatory condition. The concentration of Cathepsin D is precisely proportional to the severity of the inflammation. Minimal amount of Cathepsin D is always present in the cells. This enzyme is released more in case of inflammation. A significant decrease in the release of Cathepsin D was observed in Crude sulphated polysaccharide and Diclofenac treated groups compared to TPA induced group which indicates no lysosomal swelling of the cells.

MTT cleaves tetrazolium rings in the presence of mitochondrial dehydrogenase enzyme and forms formazan crystal that accumulates in viable cells. The number of viable cells is directly proportional to the level of formazan product formed. The cell viability showed a maximum in Diclofenac and Crude sulphated polysaccharide treated groups.

Nitric oxide is a molecular mediator of inflammation. The reduction in the release of nitric oxide shown by crude sulphated polysaccharide may be due to their interference in the synthesis of L-Arginine. This may be explained by the protective action of the test and the standard. TNF-α is a multifunctional cytokine implicated in many different pathways in homeostasis and pathophysiology of mammals. The TNF-α assay was performed on Human TNF-α ELISA kit by using culture supernatant. The result was calculated by extrapolating the absorbance value obtained using the TNF-α standard curve. TNF-α levels (Table-2) were markedly reduced in Crude sulphated polysaccharide and Diclofenac treated groups. This indicates that Crude sulphated polysaccharide extract from marine brown algae *sargassum ilicifolium* might inhibit the synthesis of prostaglandins besides reducing the release of bradykinins, histamine, leukotrienes and interleukins. In cells treated with the drug alone showed no significant change in the values of the parameters analyzed in this study when compared to control. In drug alone, [Crude sulphated polysaccharide and Diclofenac] treated groups all the parameters were noted to be near to normal groups.

CONCLUSION

The anti-inflammatory activity of crude sulphated polysaccharide extract from *sargassum ilicifolium* shows their protective role in inflammation through cytokine mediated pathway and may be due

to the presence of sulphated polysaccharide present in it. Further purification and characterization are needed in order to identify the structure and for the future exploitation of seaweed resources.

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CONFLICT OF INTEREST

Declared None

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