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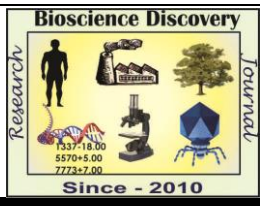

Hepatoprotective and anti-inflammatory activity of marine red algae Gracillaria corticata

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Abstract

Marine organisms offer rich source of natural products. Marine environment can be a source of functional materials including PUFA, polysaccharide, essential minerals, vitamins, antioxidant, enzymes and peptides. Water extract of several species of *Gracillaria* have pronounced hepatoprotective, cardioprotective, antitumour activity etc. Our study aims to find out the hepatoprotective and anti-inflammatory effect of *Gracillaria corticata*. Different types of cultured cell lines were used for our study. Results revealed that there is pronounced anti-inflammatory and hepatoprotective effect in the cell lines that were treated with *Gracillaria corticata*. Studies showed that extract preserved the structural integrity of the hepatocellular membrane and improved metabolic processes because of the anti-inflammatory activity of seaweeds. The restorative effect of *Gracillaria corticata* extract could be attributed to its ability to promote the metabolism of carbon tetrachloride (CCl_4) into more nontoxic metabolite. Present study revealed that the extracts of *G.corticata* increases the viability of Chang liver cell showed that extract could minimize the production of free radicals and boost the activities of their scavengers, reducing hepatocellular injury.

INTRODUCTION

Sea weeds are considered as a novel antioxidant which plays an important role in scavenging resource of important compounds and some of them possess biological activity for potential medicinal purpose. (Konig *et al*., 1994). During cellular metabolism reactive oxygen species are formed that include hydroxyl radical and super oxide anion radical (Badury, 2004). The important anti immatory activity of marine red algae was extensively studied (Smith *et al*., 2004).

Sea weed is also a major source of natural antioxidant compound. The antioxidant property of several red algae and brown algae are extensively studied and reported. (Matsukawa *et al*., 1997). Present study describes the hepatoprotective and anti inflammatory activity of marine red algae, *Gracillaria corticata.*

India ranks the top position among all other countries bordering the Indian Ocean ahead of Australia and South Africa in the number of recorded sea weed including red algae Rhodophyceae, green algae Chlorophyceae and brown algae phaeophyta (Dykens *et al*., 1992). Sea weeds possess different types of antioxidants in order to counteract extreme environmental stress. Different varieties of sea weeds were found to possess useful untapped biochemical compound which might be potential source of drug in the future. The species of genus *Gracillaria* contain abundant aminoacid,fatty acid,vitamins, minerals, polyphenolic compounds and carbohydrates (Kim *et al*., 2010). Supplementation with sea weed may improve the nutritive quality of the diet. Carbon tetra chloride induced hepatotoxicity has been revoked by ethanolic extract of *Gracillaria corticata*.

Hepatoprotective action of number of algae has been reported (Artimesia *et al.*, 1995). Literature survey of marine algae *Gracillaria corticata* revealed that no extensive pharmacological potential studies have been carried out. Generally seaweeds, that produce a wide range of secondary metabolites with broad-spectrum of biological activity and these are reported to have immense biomedical potential (Smith *et al.*, 2004 and Gama *et al.*, 2010) and it has been used in folk medicine for a variety of remedial purposes such as in disease including eczema, gallstone, gout, and renal problems (Hoppe *et al.*, 1979)

Chronic administration of CCl_4 induces liver cirrhosis by multiple step mechanism.CCl_4 biotransformed in the liver to trichloro methyl radicals which react with excess oxygen to form reactive free radicals. These free radicals can promote the peroxidation of membrane polyunsaturated fatty acids and microsomal lipids and proteins forming lipid peroxides that follows the pathological changes and cellular disorders (Shin *et al.*, 2011). It should be scavenged properly in order to prevent and reduce the potential mutation. Anti-inflammatory effect of methanolic extract of red algae has important role in neurological disease including, inhibiting cellular reactive oxygen species generation, H_2O_2 induced lipid peroxidation and inducible nitric oxide synthase. Highest amount of total phenolic content and the highest anti oxidant activity was found in *Gracillaria corticata*. The aim of our study is to evaluate the hepatoprotective (Hwang *et al.*, 2008) and anti-inflammatory effects (Hwang *et al.*, 2011) of *Gracillaria corticata.*

Materials and Methods

For studying the anti-inflammatory and hepatoprotective effects (Hwang, HJ et al 2011). We have used cell lines. The cell lines we used for our study are Chang Liver, THP1 cell lines. THP 1 cell lines (Human monocytic cell lines) was cultured in RPMI 1640 [HIMEDIA] media, supplemented with 10% heat inactivated FBS, antibiotics (Penicillin and Streptomycin) and 1.5% sodium bicarbonate. The media was filtered using 0.2µm pore sized cellulose acetate filter (Sartorius) in completely aseptic conditions. The cells were then grown till 60% confluence followed by activation with 1µl LPS (1µ/ml). LPS stimulated THP 1 cells were exposed with different concentrations of samples such as 100µg/ml, 500µg/ml and1000µg/ml from a stock of 100mg/ml dissolved in 1% DMSO and incubated for 24 hours. The anti inflammatory effects of samples were determined by assessing the inhibition of COX, LOX, and Myleoperoxidase and nitrate levels spectrophotometrically. The isolation was done by spinning at 6000 rpm for 10 minutes. Supernatant was discarded and 200µl of cell lysis buffer (1MTris Hcl, 0.25M EDTA, 2M Nacl, 0.5% Triton) was added. The incubation was done for 30 minutes at 4°C and enzymes assay was done in pellet suspended in a small amount of supernatant. Marine algal natural products are rich source of antioxidants. Nowadays searches of natural first response of the immune system to infection and plays a pivotal role in many diseases (Kim *et al.*, 2012). NO is one of the major factors relevant to inflammation macrophages and is generated from L-arginine by NO synthase, a three-member enzyme family that includes medicinal herbs against inflammatory disease especially from marine algae with certain advantages are attracting the attention of many scientists around the world (*Moncada et al.*, 1991), NO plays an important role in various body functions but its overproduction in macrophages in particular can lead to inflammation and development of auto immune disorders (Liu and Hotchkiss *et al.*, 1995). Additionally Cox-2 is an important mediator of inflammatory mediators has been shown to be important in the treatment of inflammation.

COX determined by method of Walker and Girerse (Giersa *et al.*, 2010). Lox estimated by method of Cellular nitrite is measured by method of (Lepoivre M *et al.*,1990). Myeloperoxide assayed by method of. Suzuki K et al (Suzuki K *et al.*,1983). For the study of hepatoprotective effect chang liver cell lines were used. Several studies revealed the hepatoprotective effect of aqueous extract of algae Gracillaria corticata (Salunkhae *et al.*, 2017**)**. Hepatocytes make up 70-80% of the cytoplasmic mass of the liver. These cells are involved in protein synthesis; protein storage and transformation of carbohydrates. Other roles for these cells are synthesis of cholesterol, bile salts and phospholipids as well as detoxification, modification and excretion of exogenous and endogenous substance. Chronic liver disease is characterized by excessive deposition of collagen and other extracellular matrix and protein within the liver.

RESULTS AND DISCUSSION

Ant inflammatory effect of ethanolic extract of Gracillaria corticata includes inhibition of cellular reactive oxygen species and induction of cellular reactive oxygen species and induction of nitric oxide synthase. Administration of Gracillaria corticata inhibited the production of inflammatory markers like Nitric oxide, cyclooxygenase, Lipooxygenase etc. The algae Gracillaria corticata possess intense anti inflammatory effect as it was evidenced by the activities of LOX, COX, Myeloperoxidase and level of cellular nitrate as compared to that of the control group. The % of inhibition of cox is relevant when moderate amount of Gracillaria corticata extract was added.The % of inhibition of Lox was very high when 100µgm/ml of Gracillaria corticata extract were added. The % of inhibition of Lox was increased to 22%, 29%,53% when G .cortiata extracts were added at a concentration of10µgm,50µgm and 100µgm . It was found that by increasing the concentration of the extract the activity of Lox was also inhibited. It was found that activity of Myeloperoxidase was very much decreased when extracts of Gracillaria corticata extract added. The level of cellular nitrite was 752.2 µgm when 10µgm /ml G.corticata extract were added. The level of cellular nitrite was decreased to 519.75µgm when 50µgm/ml extract were added. It was found that drastically lowered to 491.53µgm when100µgm /ml Gracillaria corticata extract were added. There was dramatical difference was seen when compared to the control group, the amount of cellular nitrite was 828µgm.This is in connection with the previous reports on several marine algae possessing anti inflammatory activity

Oxidative stress plays important role in endothelial dysfunction, lung disease, gastrointestinal dysfunction, atherosclerosis all of which involve inflammatory reaction. Many marine natural products that contain anti oxidants which are known to have anti inflammatory effect. (Abad MJ, et al 2012 and Wang W. et al 2012), Aqueous extract of algae produces anti-inflammatory effects. Sea weed is one of the potential objects for all extraction of anti-inflammatory agents. The anti-inflammatory activity of w-3-PUFAS in vivo and invitro had been obtained. Anti inflammatory substance with different nature have been separated from Marine algae (sterol glycoside from Undaria pinnatifida and enteromorphic linza

The intensity of the degenerative and necrotic changes of hepatocytes in Chang liver cell lines treated with extract of *Gracillaria corticata* was mild when compared with that of CCl_4 treated liver cell lines. This emphasized that the treatment with *Gracillaria corticata* considerably prevented the alterations in the liver cells, structural integrity triggered by CCl_4.The % of viability was14.5 only when ccl4 for added chang liver cell. The % of viability was increased to 28.19%, 48.3%, 52.8%, 57.3%, 62.74% when 6.25µgm, 12.5µgm, 25µgm, 50µgm, 100µgm added respectively. The percentage of viability is increased to 48 % while the addition of 100µg/ml of ethanolic extract of Gracillaria corticata. This is in relation with the work done by (Fedekar F et al, 2012) on the protective effect of ethanol extract of Sargassum dentifolium (Phaeophycea) in carbon tetrachloride induced hepatitis in rats.

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Table 1: Hepatoprotective effect of Gracillaria corticata (chang liver cell)

Sample concentration	Absorbance at 540 nm	% of viability
Control	0.3589	100.00
CCl4	0.0522	14.5444
6.25	0.1012	28.1972
12.5	0.1735	48.34216
25	0.18	52.82809
50	0.2057	57.31402
100	0.2252	62.74728

Table 2. Assay of Cox

Sample Concentration (µg/ml)	OD(nm)	Percentage inhibition (%)
Control	0.096	100
10	0.034	64
50	0.022	77
100	0.007	92

Table 3. Assay of Lox

Sample Concentration (µg/ml)	OD(nm)	Percentage inhibition (%)
Control	0.522	100
10	0.406	22
50	0.369	29
100	0.241	53

Table 4. Assay of Myeloperoxidase

Sample Concentration (µg/ml)	OD	Enzyme Activity (U/ml)
Control	0.789	0.26004± 0.005
10	0.041	0.0028±0.0003
50	0.024	0.00792 ±0.0003
100	0.003	0.00099±0.000039

Table 5. Estimation of Cellular Nitrite Levels

Sample Concentration (µg/ml)	OD	Concentration (µg)
Control	0.1673	828 ±16.56
10	0.1521	752.2 ± 15.04
50	0.105	519.75 ±10.39
100	0.0993	491.53 ± 9.83

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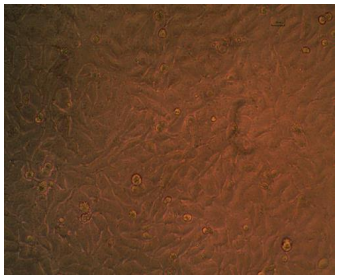
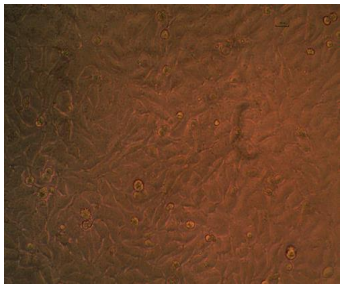
Plate 1: flourescent microscopic observation of chang liver cell (Different concentration of ethanolic algal extract added)

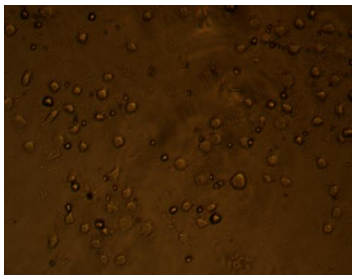
Control



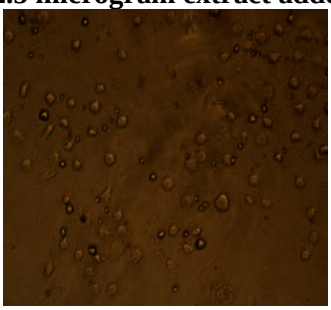
Ccl4 added



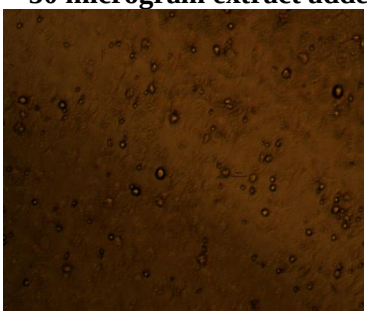
6.25microgram extract added



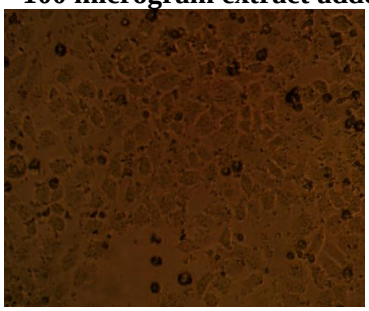
12.5 microgram extract added



50 microgram extract added



100 microgram extract added



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