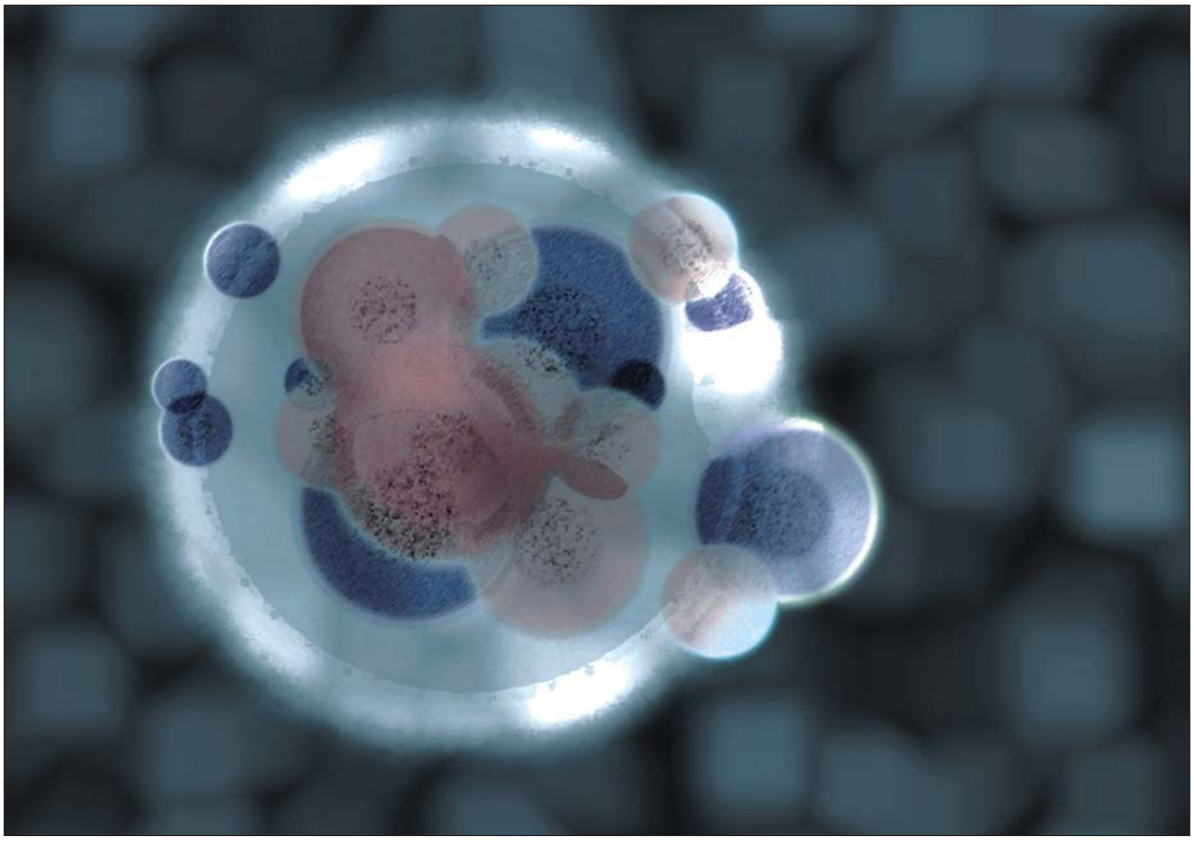

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Potential Hepatoprotective Effect of Aqueous Extract of *Gracilaria corticata* in AFB_1 Induced Hepatotoxicity in Wistar Rats

[1]N. Manoharan, [2]P. Sampathkumar, [2]B. Dheeba, [2]S. Sheikabdulla, [2]G. Vinothprasanna, [2]R. Vinothkannan, [3]S. Kalavathy, [4]A. Vijayaanand and [5]A. Shanmugasundaram
[1]Department of Marine Sciences, Bharathidasan University, Trichy, Tamil Nadu, 620 024, India
[2]Srinivasa Ramanujan Centre, Department of Chemistry and Biosciences, SASTRA University, Kumbakonam, Tamil Nadu, 612 001, India
[3]Department of Botany, Bishop Heber College, Trichy, Tamil Nadu, 620 017, India
[4]Department of Biochemistry, MIET Arts and Science College, Trichy, Tamil Nadu, 620 007, India
[5]Department of Microbiology, Urumu Dhanalakshmi College, Trichy, Tamil Nadu, 620 019, India

Abstract: The hepatoprotective activity of the marine algae *Gracilaria corticata* was investigated against aflatoxin B_1 (AFB_1) induced hepatotoxicity. AFB_1 (1 ppm) induced hepatic damage was studied by assessing parameters such as body weight gain, liver weight, total protein, albumin and enzyme markers viz., transaminases (SGOT and SGPT), Lactate dehydrogenase (LDH) and alkaline phosphatase (ALP). The effect of co-administration of aqueous extract of *G. corticata* on the above parameter was further investigated. Hepatic damage was evidenced by significant ($p<0.05$) decreased level of body weight gain, liver weight, total protein and albumin. Co administration of rats with the aqueous extract of *G. corticata* showed a tendency to attain near normalcy. Elevated levels of total bilirubin, SGOT, SGPT, LDH and ALP in aflatoxin B_1 intoxicated rats were restored to normal level in the rats treated with aqueous extract (250 mg kg^{-1} body weight) and aflatoxin B_1. Thus the study substantiates the hepatoprotective potential of aqueous extract of *G. corticata.*

Key words: Hepatoprotection, aflatoxin, marine algae, *Gracilaria corticata*, liver marker enzymes

INTRODUCTION

Liver is the key organ responsible for drug metabolism and appears to be sensitive target site for substance modulating biotransformation (Gupta *et al*., 2007). Hepatitis associated liver cirrhosis even hepatocellular carcinoma, which has become one of the most prevalent disease in the world induced by virus, alcohol or other toxic chemicals (Pattanayak and Priyashree, 2008). Aflatoxins are probably the best known and most intensively researched mycotoxin produced by the fungi *Aspergillus* on foods and feeds. Aflatoxin, a potent hepatotoxic and hepatocarcinogenic mycotoxin, induce lipid peroxidation in rat liver and associated with various diseases such as aflatoxicosis and hepatocellular carcinoma (Premalatha and Sachdanandam, 1999). Epidemiological survey indicates that occurrence of hepatic and kidney disorders are increasing as life style changes causing serious problem in the area of public health. The British Liver Trust (BLT) estimates that the disease kills more than 600,000 people in the next decade (Rameshvar *et al*., 2008).

The seaweeds were one of the first group of marine organisms whose natural products chemistry was studied extensively. There are historical records available on marine algae from the Indian Ocean in the 17th and 18th centuries. Seaweed could be a potentially valuable alternative source of feed additive for livestock in future (Applegate and Gray, 1995). It is rich in minerals and contains reasonably high amount of Vitamins B and D (Gosh, 2004). Therefore, it can be assumed that supplementation of seaweed may improve the nutritive quality of diet. Carbon tetrachloride induced hepatotoxicity has been revoked by aqueous extracts of seaweeds such as *Myagropsis myagroides*, *Sargassum hensolowianum* and *Sargassum siliquastrum*. Acute elevation in the levels of important marker enzymes such as SGPT and SGOT were normalized through probably their antioxidant properties (Wong *et al*., 1999). Similar hepatoprotective action of number of algae have been reported; *Artimesia*, *Berberis* and *Cyperus* (Gilani and Janbazz, 1995) and *Ulva lactuca* and *Gracilaria edulis* (Sathivel *et al*., 2003). Literature survey of the marine

Corresponding Author: Dr. P. Sampath Kumar, Department of Chemistry and Biosciences, SASTRA University, Srinivasa Ramanujan Centre, Kumbakonam, Tamil Nadu, 612 001, India Tel: +91-9843708269

algae *Gracilaria corticata* revealed that no extensive phytochemical and pharmacological investigations had been carried out.

Keeping the above information in view, the present study was designed to demonstrate the hepatoprotective activity of aqueous extract of *G. corticata* on aflatoxins B_1 induced hepatotoxicity.

MATERIALS AND METHODS

Collection of seaweed: *Gracilaria corticata* were collected from Mandapam station which is located in the Gulf of Mannar region, Rameswaram, India during June to December, 2003. It was authenticated by Dr. N. Kaliaperumal and J.R. Ramalingam, Senior scientists at CMFRI, Mandapam, Ramanathapuram District, Tamilnadu. Algae samples were collected by handpicking. They were washed and freed from extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The samples were then, thoroughly washed with fresh water. They were shade dried, powered, sieved and preserved for further analysis.

Extraction: The powdered material was subjected to Soxhlet extraction using water for 6 h. The solvent was removed *in vacuo* to give an appropriate yield of 12%. The extract was used for the treatment.

Production of AFB_1 and dosage: *Aspergillus parasiticus* NRRL-2000 obtained from Central Clinical Laboratory, Madras Veterinary College, Chennai was used as the source of inoculum for the production of aflatoxin. The fungus was maintained by subculturing it on potato-dextrose-agar slants (Shotwell *et al*., 1966). AFB_1 was produced, characterized and estimated by the method of Romer (1975).

Experimental animal: Male albino Wistar rats weighing about 125 to 150 g (45-60 days old) were maintained under standard experimental conditions (Temperature 27±2°C, relative humidity 60±5% and 12 h light/dark cycle) and had free access to water *ad libitum*. Before the commencement of actual experiment the rats were acclimatized in the above condition for 15 days.

Experimental protocol: Rats of same age and equal weight were selected after acclimatization and divided into 4 groups of 6 animals in each. The animals were fed with the respective experimental feed and water through out the experimental period. All the managemental practices were followed to all animals identically.

Group I : Control group, feed alone for 30 days
Group II : Feed with AFB_1 (1 ppm) for 30 days
Group III : Feed with AFB_1 (1 ppm) + Aqueous extract of *G. corticata* (250 mg kg^{-1}) for 30 days
Group IV : Feed with AFB_1 (1 ppm)+Sylimarin (100 mg kg^{-1}) for 30 days

Morphological parameters: Body weights of individual animals were recorded before and after the experimental period. Six rats from each treatment group were sacrificed after 30 days along with their control group. The animals were fasted overnight, sacrificed by cervical decapitation. Liver was immediately excised after perfusing with physiological saline, the liver was blotted, dried and weighed in the electronic balance.

Biochemical parameters: At the end of the 30 days of experimental duration, the blood samples of each animal were collected in separate tubes, kept at room temperature for 1 h and then centrifuged at 1000 g for 10 min to obtain serum and used for the assay of protein (Lowry *et al*., 1951), Bilirubin (Gupta *et al*., 2007), diagnostic marker enzymes viz., SGOT, SGPT (Sengottuvelu *et al*., 2007), LDH (Ahamed *et al*., 2003) and ALP (Tiez *et al*., 1983).

Statistical analysis: Results are expressed as Mean±SD. One-way ANOVA was carried out and the statistical comparisons among the groups were performed with using a statistical software package program SPSS 7.5.

RESULTS AND DISCUSSION

At the end of the 30 days of the experimental duration, the body weight gain, liver weight, total protein and albumin levels were decreased in group-II rats due to the AFB_1 toxicity. Concentration of serum bilirubin and the activities of serum marker enzymes viz., SGOT, SGPT, LDH and ALP were significantly elevated when compared to the normal (group-I). Co-administration of *G. carticata* extract at a dose of 250 mg kg^{-1} body weight exhibited a significant improvement in body weight gain and liver weight and alters the total protein and albumin to almost normal level (Table 1). Serum levels of bilirubin and marker enzymes were also restored towards normal following the extract treatment (Table 1, 2). The activity of the extract against the AFB_1 induced toxicity was compared with a standard hepatoprotective drug, sylimarin. From the results it is evident that the effect of aqueous extract of *G. carticata* and sylimarin was comparable in all parameters tested.

Table 1: Effect of aqueous extract of *G. corticata* on body weight gain, liver weight, total protein, albumin and total bilirubin

Treatment groups	Parameters: Body weight gain (g)	Liver weight (g)	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Total bilirubin (mg dL^{-1})
I	24.36±1.89	3.06±0.24	7.71±0.56	4.79±0.37	0.41±0.045
II	13.83±1.02*	2.05±0.18*	6.02±0.61*	3.28±0.26*	1.35± 0.16*
III	20.51±1.46	3.13±0.26	7.02±0.52	4.11±0.29	0.57± 0.02
IV	22.93±1.56	3.50±0.19	7.43±0.61	4.60±0.38	0.54±0.028

Values are expressed as Mean±SD (For six animals in each group), *Values are statistically significant at $p<0.05$ when compared with control (group I)

Table 2: Effect of aqueous extract of *G. corticata* on serum marker enzymes

Treatment groups	Parameters (IU L^{-1}): SGOT	SGPT	LDH	ALP
I	106.05±9.560	41.53±3.590	103.66±7.890	55.55±4.55
II	224.08±19.65*	129.60±11.21*	206.00±16.58*	160.20±13.24*
III	131.51±8.650	51.01±3.110	132.16±11.65	70.67±5.37
IV	131.80±11.25	40.04±2.980	113.66±9.140	62.99±4.21

Values are expressed as Mean±SD (For six animals in each group). *Values are statistically significant at $p<0.05$ when compared with control (group I)

The present study demonstrates, the hepatoprotective effect of *G. corticata* on AFB_1 induced liver injury in rats. The liver weight decreased in aflatoxin induced hepatotoxic conditions may largely be due to protein degradation during tumour growth. Protein metabolic perturbations in host, although causing tissue waste may themselves favour the growth of tumour itself. The tumour growth elicited marked loss of body weight in growing asitic hepatoma bearing rats (Tessitore *et al*., 1987). This may be due to decreased food intake and/or absorption, which contribute to muscle waste in tumour Lachexia (Pain *et al*., 1984). In this study, rats treated with AFB_1, body weight gain and liver weight were significantly reduced and increased after treatment with aqueous extract and sylimarin.

The results in the present study indicate that 250 mg kg^{-1} of aqueous extract of *G. corticata* was able to reduce all the elevated biochemical parameters due to the hepatotoxin intoxication. The reduced levels of total protein and albumin due to aflalotoxin induced hepatotoxicity. Hypoalbuminemia is most frequent in advanced chronic liver diseases. Hence decline in total protein content can be deemed as an useful index of severity of cellular dysfunction in chronic liver disease (Balamurugan and Muthusamy, 2008). The reduction is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of P_{450} leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver (Pattanayak and Priyashree, 2008). In rats administered with aqueous extract of *G. corticata* and sylimarin along with the AFB_1, the levels of protein and albumin were resumed to near normal levels. The total protein level was raised suggesting the stabilization of endoplasmic reticulum leading to protein synthesis.

Determining the activities of serum marker enzymes like SGOT, SGPT, LDH and ALP can make assessment of liver function. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released in to the blood stream and their determination in the serum is a useful quantitative marker of the extent and type of hepatocellular damage (Balamurugan and Muthusamy, 2008). The rise in SGOT activity is almost always due to hepatocellular damage and is usually accompanied by raise in SGPT (Ravikumar *et al*., 2005). In the present study, the data in Table 2 reveals that, the animals of toxic control group recorded high level of serum marker enzymes viz., SGOT, SGPT, LDH and ALP indicating severe hepatic damage due to toxic effect of AFB_1, the serum marker enzyme levels in the standard drug treated rats showed almost normal values indicating the protective effect of silymarin. The animals treated with aqueous extract of *G. corticata* at the dose of 250 mg kg^{-1} recorded significant reduction in the serum marker enzyme levels which is in comparison to the values of silymarin treated group indicating the effect of the algae in stabilizing the plasmamembrane and in protecting the hepatocytes from the toxic effect of aflatoxin. Alkaline phosphatase (ALP) is the prototype of these enzyme that reflects the pathological alteration in biliary flow (Bhakta *et al*., 1999). Determination of serum bilirubin represents as an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary diseases and severe disturbance of hepatocellular function (Martin and Friedman, 1992). Stabilization in the levels of serum bilirubin in the *G. corticata* treated groups as compared to aflatoxin alone fed group clearly indicates the improvement in the functional status of the liver.

The present investigation indicates the aqueous extract of *G. corticata* had therapeutic and preventive efficiencies in AFB_1 induced hepatotoxicity in rats. Hence, it is possible that the mechanism of hepatoprotuction by *G. corticata* may be due to its antioxidant action. Further studies are recommended to elucidate the antioxidant activity and mechanism of the hepatoprotective action of this algae.

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