



Assessment of preventive effects of *Sargassum polycystum* ethanol extract on hepatic antioxidant defense system in GalN-induced hepatitis in rats

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Abstract

We have examined the protective effect of ethanol extract of *Sargassum polycystum* on hepatic antioxidant status in D-galactosamine-induced hepatitis in rats. Levels of diagnostic marker enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK)] in plasma, lipid peroxides, reduced glutathione and the activities of glutathione dependent antioxidant enzymes [glutathione peroxidase (GPx) and glutathione-S-transferase (GST)] and antiperoxidative enzymes [catalase (CAT) and superoxide dismutase (SOD)] in the liver tissue were determined. Prior oral administration of *S. polycystum* extract [125mg/kg bodyweight/day for 15 days] considerably ($P<0.05$) attenuated the D-galactosamine-induced increases in the levels of diagnostic marker enzymes in plasma of experimental rats. It also demonstrated an antioxidant activity against D-galactosamine-induced hepatitis by inhibiting the stimulation of lipid peroxidation and by preserving the hepatic enzymatic and non-enzymatic antioxidant defense system at near normal. The antihepatotoxic potential of *S. polycystum* might be related to its antioxidant property and membrane stabilizing action.

Keywords: *Sargassum polycystum*, D-galactosamine-induced hepatitis, diagnostic marker enzymes, lipid peroxidation, antioxidant enzymes

INTRODUCTION

Hepatitis is a major public health problem worldwide, responsible for considerable morbidity and mortality from chronic liver disease (Lau and Membreno, 2004). Developing countries like India are also struggling to manage the impact of hepatitis along with the growing burden of obesity, Type II diabetes, hypertension and coronary heart disease (Aggarwal and Ghoshal, 2004). The major abnormalities noticed in hepatitis are lipidemia, peroxidation and loss of plasma membrane integrity. There is an urgent need for the clinical development of safe and non-toxic cytoprotective agents for the adequate management of hepatitis. A better understanding of the processes involved in hepatitis has stimulated the search for new drugs, which could limit the drug-induced hepatic injury.

In recent times, there is a lot of interest in drugs from marine origin with potential benefits. The nutritional value of marine algae has long been recognized in the Orient

than in Western world with limited use as a dietary part. Indian folk medicine claims a lasting cure for viral hepatitis through oral administration of an extract of *Sargassum polycystum* (Brown alga). In traditional medicine, it has also been used to cure eczemia, renal dysfunction, heart ailments, lung diseases, ulcer, scabies and psoriasis, and also to promote the secretion of bile (Raghavendran et al., 2006). It has been reported to possess antilipidemic, antioxidant and membrane stabilizing properties (Raghavendran et al., 2004; Raghavendran et al., 2005). Though the beneficial properties of *S. polycystum* are promising and well studied, the hepatoprotective effects of *S. polycystum* on tissue antioxidant defense system in D-galactosamine induced hepatitis have not yet been explored.

Hepatitis induced by D-galactosamine (GalN) has been reported to show many metabolic and morphologic aberrations in the liver tissue of the experimental animals simi-

lar those observed in human viral hepatitis (Anandan et al., 1998; Kucera et al., 2006). It induces hepatic necrosis by a multiple step mechanism. It mainly results from a depression of uracil nucleotide-dependent biosynthesis of nucleic acids, glycolipids, glycoproteins and glycogen, accompanied by organelle injury, necrosis of hepatocytes, infiltration by inflammatory cells and accumulation of fat (Black et al., 1983; Laconi et al., 1992). Peroxidation of endogenous lipid is a major factor in the cytotoxic action of GalN. A growing body of evidence is emerging which suggests that reactive oxygen-derived free radicals play a crucial role in the pathogenesis of GalN-induced hepatitis (Ohta et al., 2007).

In the present study, an attempt has been made to assess the preventive effects of *S. polycystum* ethanol extract on hepatic antioxidant defense system in GalN-induced hepatitis in rats by virtue of its hypolipidaemic, antiperoxidative and membrane stabilizing properties.

MATERIALS AND METHODS

Chemicals

Epinephrine, tetraethoxy propane and GalN were obtained from M/s. Sigma Chemical Company, St. Louis. MO, USA. All the other chemicals used were of analytical grade.

Plant material

The brown alga, *S. polycystum* collected from Gulf of Mannar, Rameswaram, India was extracted with ethanol in cold for a period of 5 days with occasional shaking. The crude extract was filtered, concentrated and then dried in vacuum. The resulting dried material is used for the experimental purpose.

Animals

Wistar strain male albino rats, weighing 120 – 150 g were selected for the study. The animals were housed individually in polypropylene cages under hygienic conditions and maintained at normal room temperature ($28 \pm 2^\circ\text{C}$, humidity 60 - 70%, 12 h light/ dark cycle). The animals were allowed food and water *ad libitum*. The experiment was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of experiments on Animals (CPCSEA), New Delhi, India.

Induction of hepatitis

The hepatitis was induced in experimental rats by injecting GalN [500 mg (dissolved in physiological saline)/kg body weight/day], i.p. for 2 days (Anandan et al., 1998).

Experimental protocol

Seven days after acclimatization, the animals were divided into four groups of 6 rats each. Group I served as control. Group II were normal animals orally administered with *S. polycystum* extract (125

mg [dissolved in distilled water]/kg body weight/day), by intragastric intubation, for 15 days. Group III animals were intraperitoneally (i.p.) injected with GalN [500 mg (dissolved in physiological saline)/kg body weight/day], i.p. for 2 days] for the induction of hepatitis. Group IV animals were orally pretreated with *S. polycystum* extract (125 mg/kg body weight/day for 15 days, and then i.p. injected with GalN [500 mg (dissolved in physiological saline)/kg body weight / day], i.p. for 2 days]. Group I and Group II animals were injected with physiological saline alone for 2 days.

At the end of the experimental period, that is, 24 h after last injection of GalN, the experimental animals were killed, blood was collected using sodium citrate as anticoagulant, and the plasma separated was used for the determination of diagnostic marker enzymes. The liver tissue was excised immediately and washed with chilled isotonic saline. The liver tissue homogenates prepared in ice cold 0.1M Tris- HCl buffer, pH 7.2 were used for the determination of lipid peroxides (LPO), reduced glutathione (GSH) and antioxidant enzymes.

Biochemical assays

The activities of alanine aminotransferase [EC 2.6.1.2] (ALT) and aspartate aminotransferase [EC 2.6.1.1] (AST) in plasma were determined spectrophotometrically by the method of Mohur and Cook (1957). The lactate dehydrogenase [EC 1.1.1.27] (LDH) activity in plasma was assayed according to the method of King (1965). Tissue lipid peroxidation level in the presence of promoters such as ascorbic acid, ferrous sulphate (FeSO_4) and *tert*-butyl hydroperoxide (*t*-BH) was determined as TBA-reactive substances by the method described by Ohkawa et al. (1979). GSH was determined by the method of Ellman (1959). Glutathione peroxidase [EC 1.11.1.9] (GPx) activity was measured by the method of Paglia and Valentine (1967). Glutathione-S-transferase [EC 2.5.1.18] (GST) activity was determined by the method of Habig et al. (1974). Catalase [EC 1.11.1.6] (CAT) activity was assayed according to the method of Takahara et al. (1960). Superoxide dismutase [EC 1.15.1.1] (SOD) activity was determined according to the method of Misra and Fridovich (1974).

Statistical analysis

All data were analyzed using ANOVA with the aid of SPSS 10.0 for Windows. Data obtained were expressed as mean \pm SD. Multiple comparisons of the means were separated using the Duncan Multiple Range Test at 5% probability.

RESULTS AND DISCUSSION

Indigenous herbs and plants have received recent attention for the treatment of hepatitis. As a consequence of an increasing demand for the biodiversity in screening programs seeking therapeutic drugs from natural products, there is now a greater interest in the marine organism especially marine algae (Faulkner, 1993). Seaweeds are low in fats, but contain bioactive compounds like sulphated polysaccharides, which are potential natural antioxidants not common in land plants (Lahaye and Kaffer, 1997). Previous reports (Matsukawa, 1997; Yan et al., 1998) have shown that *Sargassum* Sp. are found to have the highest free radical scavenging property. The focus of the current study was to evaluate the effects of

Table 1. Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in plasma of normal and experimental groups of rats.

Groups	Group I	Group II	Group III	Group IV
ALT	84.5 ± 7.26 ^a	86.9 ± 7.05 ^a	329 ± 28.6 ^b	118 ± 9.41 ^c
AST	95.2 ± 7.11 ^a	88.7 ± 7.48 ^a	376 ± 32.3 ^b	135 ± 9.94 ^c
LDH	164 ± 11.2 ^a	152 ± 12.4 ^a	344 ± 27.3 ^b	187 ± 14.1 ^c

Results are mean ± SD for 6 animals. Values expressed: ALT, AST, and LDH, mol pyruvate liberated h⁻¹l⁻¹. Values that have a different superscript letter (a, b, c) differ significantly with each other ($P < 0.05$; Duncan's multiple range test).

Table 2. Level of lipid peroxidation (LPO) in the presence of promoters (2mm) ascorbic acid, ferrous sulphate (FeSO₄) and *tert*-butyl hydroperoxide (*t*-BH) in the liver tissue of normal and experimental groups of rats

Groups	Group I	Group II	Group III	Group IV
Basal	0.82 ± 0.06 ^{a,b}	0.76 ± 0.05 ^a	2.15 ± 0.18 ^b	0.91 ± 0.07 ^c
Ascorbic acid	2.96 ± 0.22 ^a	2.82 ± 0.15 ^a	4.93 ± 0.25 ^b	3.04 ± 0.17 ^c
FeSO ₄	4.15 ± 0.28 ^a	3.94 ± 0.25 ^a	7.18 ± 0.61 ^b	4.31 ± 0.29 ^c
<i>t</i> -BH	5.84 ± 0.37 ^{a,c}	5.61 ± 0.34 ^a	9.54 ± 0.72 ^b	6.27 ± 0.46 ^c

Results are mean ± SD for 6 animals. Values expressed: LPO, nmol MDA released mg⁻¹ protein. Values that have a different superscript letter (a, b, c) differ significantly with each other ($P < 0.05$; Duncan's multiple range test).

of ethanol extract of *S. polycystum* for its antioxidant and membrane-stabilizing properties during GalN-induced hepatitis in rats.

Significant ($P < 0.05$) increase was observed in the levels of diagnostic marker enzymes (ALT, AST and LDH) in plasma of Group III GalN-injected rats as compared to that of Group I control rats (Table 1). This is an indicative of the cellular leakages and loss of functional integrity of cell membrane in liver. The present observation is in agreement with earlier reported studies (Anandan and Devaki, 1999; Kume et al., 2006), which have shown that the amount of diagnostic marker enzymes present in plasma is directly proportional to the number of necrotic cells present in the liver tissue. Oral pre-treatment with *S. polycystum* significantly ($P < 0.05$) attenuated the GalN-induced elevation in the levels of these diagnostic marker enzymes in plasma of Group IV animals as compared to Group III GalN-injected rats, demonstrating the cytoprotective activity of *S. polycystum*. These findings indicated that the ethanol extract of *S. polycystum* preserved the structural integrity of the hepatocellular membrane and liver cell architecture damage caused by GalN. Previous studies have shown that natural antioxidant molecules impart stabilization to cell membranes in relation to the degree of their free radical scavenging ability (Sabeena Farvin et al., 2004; Ganesan et al., 2007). Hence, it is possible that likewise *S. polycystum* may also prolong the viability of liver cell membranes from GalN-induced necrotic damage by its membrane stabilizing action.

Lipid peroxidation *in vivo* has been identified as one of the basic deteriorative reaction in cellular mechanisms of the D-galactosamine-induced hepatitis. In the present study, there was a significant ($P < 0.05$) rise observed in the level of lipid peroxides in the liver tissue of GalN-administered rats as compared to Group I control, suggesting an enhanced oxidative stress in GalN-induced hepatitis condition (Table 2). This present observation concurs with earlier reported studies (Anandan et al., 1998; Vimal and Devaki, 2004), which showed that enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. Our results also suggested that GalN-administered rats might be less resistant and more susceptible to lipid peroxidation in the presence of promoters like ascorbate, FeSO₄ and *t*-BH (Table 2). The rats administered with *S. polycystum* extract showed significant ($P < 0.05$) decrease in the level of lipid peroxidation in the liver tissue as compared to Group III GalN-treated rats, indicating the antioxidant property of *S. polycystum* against GalN-induced lipid peroxidation.

Glutathione is one of the abundant tripeptide non-enzymatic biological antioxidants present in the liver (Anderson, 1998). It acts as a substrate for H₂O₂ removing enzyme glutathione peroxidase and for dehydroascorbate reductase (Ahmed and Khater, 2001). It also plays a critical role in cellular function, which includes the maintenance of membrane protein, the removal of free oxygen radicals such as peroxy radical, superoxide radical, alkoxy radical, translocation of amino acids across cell mem-

Table 3. Level of reduced glutathione (GSH) and the activities of glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) in the liver tissue of normal and experimental groups of rats.

Groups	Group I	Group II	Group III	Group IV
GSH	5.18 ± 0.32 ^{a,b}	5.42 ± 0.37 ^b	2.64 ± 0.18 ^c	4.93 ± 0.22 ^a
GPx	2.45 ± 0.19 ^{a,b}	2.52 ± 0.17 ^b	1.27 ± 0.08 ^c	2.29 ± 0.14 ^a
GST	1256 ± 114 ^{a,b}	1218 ± 105 ^a	673 ± 58.4 ^b	1102 ± 98.5 ^c
CAT	9.24 ± 0.72 ^{a,c}	9.43 ± 0.68 ^a	4.47 ± 0.28 ^b	8.73 ± 0.51 ^c
SOD	4.11 ± 0.24 ^a	4.26 ± 0.19 ^a	1.95 ± 0.12 ^b	3.67 ± 0.21 ^c

Results are mean ± SD for 6 animals. Values expressed: GSH, nmol g⁻¹ wet tissue; GPx, nmol GSH oxidized min⁻¹ mg⁻¹ protein; GST, mol 1-chloro-2,4-dinitrobenzene conjugate formed min⁻¹ mg⁻¹ protein; CAT, nmol H₂O₂ decomposed min⁻¹ mg⁻¹ protein; SOD, one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation. Values that have a different superscript letter (a, b, c) differ significantly with each other ($P < 0.05$; Duncan's multiple range test).

branes, the detoxification of foreign compounds and biotransformation of drugs (Comporti et al., 1991; Muriel et al., 1992). In the present study, significant ($P < 0.05$) decline observed in the level of GSH in Group III rats compared to Group I normal control animals (Table 3), which is in accordance with earlier investigations (Anandan et al., 1999; Adaramoye and Adeyemi, 2006), indicated that the tissue antioxidant status was being operated at diminished level in GalN-induced hepatitis. Reduction noticed in the level of GSH in GalN-induced hepatitis was either due to increased degradation or decreased synthesis of glutathione. Prior oral administration of *S. polycystum* Group IV rats resulted in the elevation of GSH level, which protects the hepatocellular membranes from the oxidative damaging action of lipid peroxides.

Significant ($P < 0.05$) decline was noticed in the activities of GSH-dependent antioxidant enzymes, GPx and GST, in the liver tissue of Group III rats compared to normal controls (Table 3), reflecting an increased oxidative stress in GalN-induced hepatitis. This is in accordance with earlier reports (Vimal and Devaki, 2004; Ravikumar et al., 2005). GPx offers protection to the cellular and subcellular membranes from the peroxidative damage by eliminating hydrogen peroxide and lipid peroxide. GST binds to many different lipophilic drugs; so it would be expected to bind GalN and acts as an enzyme for GSH conjugation reactions (Anandan et al., 1998). Inhibition of these enzymes may leads to the accumulation of these oxidants and makes liver cell membranes more susceptible to oxidative damage. GSH and GSH-dependent enzyme systems may be directly related to the pathogenic mechanisms of GalN-induced hepatitis. In the present study, the administration of *S. polycystum* maintained the activities of these enzymes at near normal as compared to Group III GalN-administered animals. It probably did so by counteracting the free radicals produced by D-galactosamine.

Activities of antiperoxidative enzymes (SOD and CAT) were also significantly ($P < 0.05$) decreased in the liver tissue of GalN-injected rats as compared to controls (Table 3). This concurs with earlier reported studies (Anandan et al., 1998; Vimal and Devaki, 2004). Reduction in the acti-

vities of the antiperoxidative enzymes in GalN-induced hepatitis might be due to the increased generation of reactive oxygen radicals such as superoxide and hydrogen peroxide, which in turn leads to the inactivation of these enzyme activities. The animals orally administered with *S. polycystum* in the present study showed a significant ($P < 0.05$) reduction in the level of lipid peroxidation along with a marked rise in the activities of superoxide dismutase and catalase, thus indicating the antioxidant nature of *S. polycystum* in experimentally induced oxidative stress condition. The antioxidant effect is probably due to the presence of free radical scavenging sulphated compounds in *S. polycystum*. The unpaired electron present in the hydroxyl radical (OH[•]) generated during D-galactosamine-induced hepatitis might have been trapped for dismutation by the free radical scavenging sulphated compounds present in *S. polycystum*.

In conclusion, the results of the present study indicate that the prior administration of *S. polycystum* at 125 mg/kg body weight/day for 15 days prevents the GalN-induced hepatitis in rats. In the present study, the prior treatment with *S. polycystum* significantly ($P < 0.05$) prevented all these GalN-induced alterations in the activities antioxidant enzymes and maintained the rats at a near normal status. The normal rats receiving *S. polycystum* alone did not show any significant change when compared with normal rats, indicating that it does not *per se* have any adverse effects. The overall hepatoprotective effect of *S. polycystum* is probably related to a counteraction of free radicals by its antioxidant property, or to a strengthening of hepatocellular membrane by its membrane stabilizing action, or to its ability to maintain near to normal status the activities of free radical enzymes and the level of GSH, which protect hepatocellular membrane against oxidative damage by decreasing lipid peroxidation.

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