Arch Pharm Res Vol 27, No 4, 449-453, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

Efficacy of Brown Seaweed Hot Water Extract Against Hcl-ethanol Induced Gastric Mucosal Injury in Rats

Hanumantha Rao Balaji Raghavendran, Arumugam Sathivel, and Thiruvengadam Devaki

Department of Biochemistry and Molecular Biology, University of Madras, Guindy Campus, Chennai 600 025, India

(Received July 20, 2003)

Effect of pre-treatment with hot water extract of marine brown alga *Sargassum polycystum* C.Ag. (100 mg/kg body wt, orally for period of 15 days) on HCl-ethanol (150 mM of HCl-ethanol mixture containing 0.15 N HCl in 70% v/v ethanol given orally) induced gastric mucosal injury in rats was examined with respect to lipid peroxides, antioxidant enzyme status, acid/pepsin and glycoproteins in the gastric mucosa. The levels of lipid peroxides of gastric mucosa and volume, acidity of the gastric juice were increased with decreased levels of antioxidant enzymes and glycoproteins were observed in HCl-ethanol induced rats. The rats pre-treated with seaweed extract prior to HCl-ethanol induction reversed the depleted levels of antioxidant enzymes and reduced the elevated levels of lipid peroxides when compared with HCl-ethanol induced rats. The levels of glycoproteins and alterations in the gastric juice were also maintained at near normal levels in rats pre-treated with seaweed extract. The rats given seaweed extract alone did not show any toxicity, which was confirmed by histopathological studies. These results suggest that the seaweed extract contains some anti-ulcer agents, which may maintain the volume/acidity of gastric juice and improve the gastric mucosa antioxidant defense system against HCl-ethanol induced gastric mucosal injury in rats.

Key words: *Sargassum polycystum*, Gastric injury, Antioxidants, HCl-ethanol, Lipid peroxides, Gastric mucosa

INTRODUCTION

Ulceration refers to the group of disorders of the upper gastrointestinal tract involving principally the most proximal portion of the duodenum and the stomach, which are found to have common involvement of acid/pepsin (Galvin and Szabo, 1992). An imbalance in the levels of acid/pepsin and gastric mucosa turnover results in ulceration. The breakdown of mucosal resistance is considered to be an important factor in ulceration (Mc Guigan, 1994; Piper and Stiel, 1986) HCl-ethanol administration produces ulcerative lesions and lipid peroxidation in the gastric mucosa, which plays an important role in the pathogenesis of the gastric mucosal lesions (Chacin, 1990).

For centuries, seaweeds have been of botanical, industrial and pharmaceutical interest (Abdussalamm, 1990; Chapman and Chapman, 1980). The seaweeds are rich in proteins, vitamins, minerals and sulphated polysaccharides that provide soluble dietary fiber such as alginates and fucoidans (Lahaye and Kaeffer, 1997; Kloareg and Quatrano, 1988; Bobbin dubigon *et al.*, 1997; Jothi Saraswathi *et al.*, 2003). Those are commercially important components of brown seaweed that are used in the pharmaceutical field for treating wounds and gastric ulcers (Nagaoka *et al.*, 2000).

Though many claims have been made in seaweeds for treating various diseases such as gout, cancer and glandular problems (Hoppe and Lerving, 1982), a sporadic status exists regarding anti-ulcer property of seaweeds in animal model. Hence the present study was carried out to assess the protective nature *Sargassum polycystum* C.Ag. against HCl-ethanol induced gastric ulceration in rats.

MATERIALS AND METHODS

Seaweed collection

Sargassum polycystum C.Ag was collected from Gulf of Mannar, Rameswaram, India. Prof. V. Krishnamurthy (Krishnamurthy Institute of Algology, Chennai, India) did

Correspondence to: T. Devaki, M.Sc., M.Phil, Ph.D., Reader, Department of Biochemistry and Molecular Biology, Guindy Campus, Chennai-600 025. India
Tel: 91-2235-1269/ Resi (044) 24412292, Fax: 91-244235-2494
Email: devakit@yahoo.co.uk

the species identification.

Extraction

The seaweed collected was washed in seawater thoroughly to remove epiphytes and then in deionized water to remove the contamination of other algae. Then about 1.5 kg of seaweed fronds extracted twice with 3 liters of boiling water for a period of 1 h. The crude fraction obtained then concentrated, treated with EtOH (80%). The resulting precipitate was filtered and then washed with cold EtOH and dried in a desicator to obtain brownish residue (Yield: 10-12%).

Animals

Male Wistar rats weighing (150-180 g) were fed with standard pelleted diet (M/s. Hindustan Lever Foods, Bangalore, India) and water *ad libitum* and housed under standard conditions. Animals were deprived of food for a period of 24 h before ulcer induction.

The animals were divided into following 4 groups each group with six animals. Group1 was normal control rats, given only standard diet. Group2 rats were ulcer induced (150 mM of HCl-ethanol mixture containing 0.15 N HCl in 70% v/v ethanol given orally). Group3 rats were pre-treated with *S. polycystum* extract (100 mg/kg body wt, orally for period of 15 days prior to HCl-ethanol induction). Group4 rats were given seaweed extract alone (*S.polycystum* 100 mg/kg body wt, orally for period of 15 days.

At the end of the experimental period rats were operated for the collection of gastric juice (Hara *et al.*, 1985). The rats were anesthetized with ether and the abdomen was cut opened through a midline incision below the xiphoid process. The pyloric portion was ligated with silk sutures after which wound was closed and the animals were allowed to retrieve from anesthesia. The animals were sacrificed after 4hrs for collecting gastric juice. The gastric juice was centrifuged and the volume was noted. The stomach portion was inflated using formaline and incised through the greater curvature and examined under a dissection microscope for the number of lesions. Acidity was measured by titrating the gastric juice with 0.1 N NaOH with phenolphthalein as indicator (Takeuchi *et al.*, 1976).

The gastric mucosa was used for estimating protein (Lowry *et al.*, 1951), pepsin (Anson, 1938), hexose, hexosamine (Winzler, 1958), sialic acid (Warren, 1959), lipid peroxides (Ohakawa *et al.*, 1979), glutathione (Ellman, 1959), glutathione peroxidase (Paglia & Valentaine, 1967), glutathione-s-transferase (Habig *et al.*, 1974) superoxide dismutase (Misra & Fridovich, 1972), and catalase (Takahara *et al.*, 1960).

The values were expressed as mean±SD. The statistical difference was analyzed by students *t*-test and *p*-values were expressed.

RESULTS

The Table I shows number of lesions on the gastric mucosa, volume, acidity of gastric juice and pepsin activity of the gastric mucosa in normal control, ulcerated, *S. polycystum* pre-treated rats prior to ulcer induction (Pre-treated ulcer) and *S. polycystum* extract alone. In the group2 rats, the number of lesions was found increased when compared with group3 rats pre-treated with seaweed extract, which showed a significant decrease in the number of lesions. The group2 ulcerated rats showed an elevation in the levels of gastric juice volume, acidity and decreased peptic activity when compared with group3 rats pretreated with seaweed extract, which maintained the levels of gastric juice volume, acidity and peptic activity.

Table II shows the levels of lipid peroxides significantly increasing with decrease in the levels of free radical scavenging enzymes in group2 ulcerated rats whereas in group3 seaweed pretreated rats the levels of antioxidant enzymes were improved with reduced lipid peroxides. The group4 rats pretreated with seaweed extract alone did not show any significant variation in the levels of biochemical parameters thereby indicating the non-toxic nature of seaweed extract, which was confirmed by histopathological studies as shown in (Fig. 1).

Table III shows significant decrease in the levels of protein, hexosamine, sialic acid, and hexose in group2 ulcerated rats when compared with group1 control rats. The severely depleted levels of glycoproteins were

Table I. Number of lesions, volume, acid output and pepsin activity of the gastric mucosa on HCl-ethanol induced gastric lesion and *S. polycystum* pre-treated experimental rats

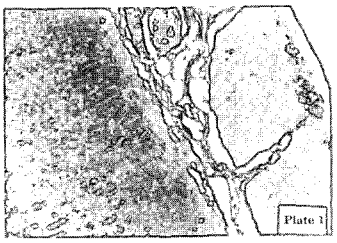
Group	Lesions	Volume (mL/4 h)	Acid output (μEq/4 h)	Pepsin (μmol of tyrosine/4 h)
Group1 (Normal)	–	2.71±0.19	198±20	678±50
Group2 (Ulcer)	14.12±1.2	3.10±0.12**	265±25***	620±42
Group3 (pre-treated ulcer)	6.56±0.61***	2.89±0.2^{NS}	173±14*	661±52^{NS}
Group4 (*S. polycystum alone*)	–	2.75±0.17^{NS}	196±17^{NS}	672±61^{NS}

[s]Values are expressed as the mean ± SD for 6 animals in each group. Comparisons (Group1 vs. Group2, Group3 vs. Group2 and Group4 vs. Group1) *P* values *<0.05, **<0.01, ***<0.001 NS- Non-significant.

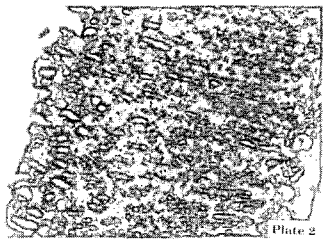
Table II. Levels of lipid peroxides (LPO) and activities of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GSH-Px) and glutathione s-transferase (GST) of gastric mucosa in experimental animals

Group	LPO (nmol/mg Protein)	SOD	CAT	GSH (μmol/mg Protein)	GSH-px (U/mg Protein)	GST (μmol/mg Protein)
Group1 (Normal)	3.97±0.41	4.32±0.20	3.81±0.31	3.92±0.21	222±19.1	4.25±0.56
Group2 (Ulcer)	8.60±0.74^{NS}	2.49±0.13***	1.75±0.08***	1.52±0.11***	169±15.7***	3.19±0.31***
Group3 (Pre-treated ulcer)	4.83±0.37**	3.97±0.32*	3.42±0.27*	3.48±0.24**	198±17.9*	3.72±0.19*
Group4 (*S. polycystum* alone)	3.39±0.32^{NS}	4.43±0.19^{NS}	3.92±0.28^{NS}	3.70±0.28^{NS}	219±20.3^{NS}	4.21±0.19^{NS}

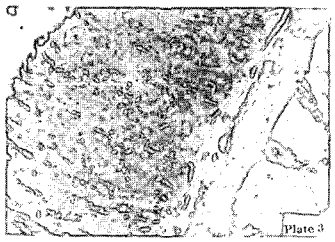
Values are expressed as the mean ± SD for 6 animals in each group. SOD. One unit of SOD activity is the amount of protein requir ed to give 50% inhibition of epinephrine auto oxidation; CAT, μmol of H_2O_2 Consumed/min/mg of protein GSH = nmol g tissue; GSH-px = nmol GSH oxidized/min/mg protein; GST = μmol of 1-chloro-2, 4 dinitrobenzene conjugate formed/min/mg protein; protein, hexose, hexosamine and sialic acid = mg/g.
Comparisons (Group1 vs. Group2, Group3 vs. Group2, and Group4 vs. Group1)
P values *<0.05, **<0.01, ***<0.001 NS-Non-significant.



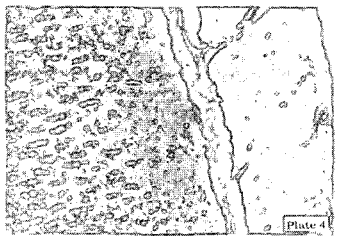
Normal architecture of the control gastric mucosa



Squamous metaplasia of the ulcerated gastric mucosa



Gastric mucosa of pretreated ulcer showing architecture with reduced severity



Normal architeture of gastric mucosa revealed by Sargassum polycystum alone treated rats

Fig. 1.

Table III. Effect of *S. polycystum* extract on HCl-ethanol induced changes in gastric mucosal protein, hexose, hexosamine, and sialic acid in rats

Group	Protein (mg/g)	Hexose (mg/g)	Hexosamine (mg/g)	Sialic acid (mg/g)
Group1 (Normal)	20.1±1.2	14.5±1.32	9.3±0.73	1.50±0.12
Group2 (Ulcer)	12.1±.8***	9.2±0.84***	5.1±0.41***	0.96±0.06***
Group3 (Pre-treated ulcer)	17.9±1.8*	11.4±1.4**	7.8±0.69**	1.53±1.2^{NS}
Group4 (*S. polycystum* alone)	19.4±0.9^{NS}	13.9±1.28^{NS}	8.9±0.71^{NS}	1.72±0.14^{NS}

Values are expressed as the mean ± SD for each 6 animals in each group. Comparisons (Group1 vs. Group2, Group3 vs. Group2 and Group4 vs. Group1) *P* values *<0.05, **<0.01, ***<0.001 NS-Non-significant.

improved in group3 seaweed pre-treated rats when compared with group2 ulcer induced rats.

DISCUSSION

HCl-ethanol induced gastric mucosal lesions may be multifactorial, with static blood flow contributing significantly to the haemorrhagic as well as the necrotic aspects of the tissue injury (Guth *et al.*, 1984). The decrease in the number of lesions may be due the reduction in the levels of gastric secretion (Wormsley, 1974). The alteration in the acidity/volume of the gastric juice is due to the production of HCl, which may increase the permeability of the mucosal membrane (Dayton *et al.*, 1983). The decrease in the

levels of acidity may be due to the anti-ulcer agents in seaweed extract, which may inhibit the acid secretion thereby, offering protection against gastric mucosal lesions. The ulcerated rats showed an alteration in the peptic activity (Juhani, 1982), which was improved in seaweed pre-treated rats showing its efficacy against acid secretion.

The free radical generation in gastric mucosa may exceed the ability of the free radical scavenging enzymes to dismutase the free radical species, which may result in ulceration. The rats pre-treated with seaweed extract showed a significant decrease in the lipid peroxides, as they are important in causing damage to the mucosal membrane (Tappel, 1973). An increase in the levels of free radical scavenging enzymes suggest that the seaweed extract may posses antioxidant property (Anggardiredja *et al.*, 1997), which may improve gastric mucosal defense system in quenching the free radical generated during ulceration. The depletion in the levels of glutathione in gastric mucosa may increase the susceptibility of the mucosal cells to oxygen radical metabolites and acid mediated cell injury in ulcerated rats (Pihan *et al.*, 1987). An improvement in the levels of glutathione in seaweed-pretreated rats protects gastric mucosa from oxidative damage and also regulates the redox state of proteins in the cell surface membrane (Inoue *et al.*, 1987).

The glutathione consumption with oxygen and peroxide radicals may inhibit the glutathione peroxidase, which eliminates hydrogen peroxide and lipid peroxides, leads to the accumulation of oxidants and thereby promote the oxidation of lipids (Yoshikewa *et al.*, 1993). The seaweed pre-treated rats maintained the activity of glutathione peroxidase in order to balance the elevated levels of lipid peroxides by improving the reduced glutathione status. The decreased levels of glutathione will reflect in the levels of glutathione-s-transferase. The ulcerated group showed a decrease in the levels of GST when compared with normal control group; this is because the GST may bind with different lipophilic compounds (i.e. HCl-ethanol may act as a substrate for GSH). Thus GSH and GSH dependent enzymes play a vital role in the gastric ulceration (Morenkova *et al.*, 1987).

According to Davenports theory of H^+ release, histamine stimulates cholinergic nerves, resulting the stimulation of acid and pepsinogen secretion, an elevation in mucosal blood flow and an increase in capillary permeability, resulting a protein loss (Davenport, 1967).

Free radicals generated on HCl-ethanol induction will ultimately attack the proteins, which may result the depletion of proteins in ulcerated group. Pre-treatment with seaweed extract showed a near normal value of proteins, suggesting a cytoprotective nature against gastric mucosal erosions. The glycoproteins are secreted by exocytosis and apical expulsion in epithelial cell (Dunn and Eisenberg, 1985). The HCl-ethanol mixture may attack the structural organization of glycoprotiens with protruding oligosaccharide chains (Gottschalk, 1972), which may ultimately decrease the levels of glycoproteins in ulcerated rats. An improvement in the levels of glycoproteins in rats pretreated with seaweed extract suggests that it may support the gastric mucosal defense system against HCl-ethanol induced gastric mucosal injury.

Thus the overall protection by seaweed extract against HCl-ethanol induced gastric ulceration in experimental rats suggest that it contains some anti-ulcer agents that may hasten the decomposition of free radicals generated, thereby strengthening the gastric mucosal antioxidant defense system suggesting an antiulcerogenic nature of *Sargassum polycystum*.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. N.Kaliaperumal (CMFRI, Ramnad district, India) for his guidance during the period of collection and Prof. V. Krishnamurthy for his valuable suggestions during the work. We also thank Dr.C.N.Srinivas M.D Pathology [EMS diagnostic and research center. chennai, India] for his help in carrying out histopathological studies.

REFERENCES

Abdussalam, S., Drugs from seaweeds. *Med. Hypo.*, 32, 33-35 (1990).

Anggardiredja, J., Andyani, R., and Muaswanah, H., Antioxidant activity of *Sargassum polycystum* C.Ag. and *laurencia obtusa* from seribu islands. *J. App. phycol.*, 9, 477-479 (1997).

Anson, M. L., The estimation of pepsin, trpsin, papain and cathepsin with hemoglobin. *J. Gen. physiol.*, 22, 79-89 (1938)

Bobbin dubigcon, C., Hoebler, C., Lognone, V., Dagorn-Scaviner, C., Mabeau, S., Barry, J. L., and Lahaye, M., Chemical composition physicochemical properties enzymatic inhibition and fermentative characteristics of dietary fibers from edible seaweeds. *Sci. Aliments*, 17, 619-639 (1997).

Chacin, J., Peptic ulcer a failure of the defence mechanisms of gastro duodenal mucosa. *Gen.*, 44,163-170 (1990).

Chapman, V. J. and Chapman, D. J., Seaweeds and their uses Chapman and Hall London, (1980).

Davenport, H. W., Salicylate damage to the gastric mucosal barrier. *N. Engl. J. Med.*, 276, 1307-1312 (1967)

Dayton, M, T., Kauffman, G. L., and Schlegel, J. F., Gastric bicarbonate appearance with ethanol ingestion Mechanism and significance. *Dig. Dis. Sci.*, 28, 449-455 (1983).

Dunn, D. H. and Eisenberg, M. M., Bokus gastroenterology book 4th edition, Ed. By Haubrich K Roth Schattner Vol 2, p. 897 (1985).

Ellman, G. L., Tissue sulfhydryl groups. *Arch. Biochem. Biophys.*, 82, 70-77 (1959).

Glavin, G. B. and Szabo, S., Experimental gastric mucosal injury laboratory models reveal mechanism of pathogenesis and new therapeutic strategies. *FASEB J.*, 6, 825-831 (1992).

Gottschalk, A., Glycoproteins. Their composition, structure and function, 2nd edition. Elsevier, Amsterdam, (1972).

Guth, P. H., Paulsen, G. E., and Nagata, H., Histological and microcirculatory changes in alcoholinduced gastric lesions in the rat Effect of prostaglandin cytoprotection. *Gastroenterology.*, 87, 1083-1090 (1984).

Habig, W. H., Pabst, M. J., and Jakoby, W. B., Glutathion- s-transferase: the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, 249, 71130-71139 (1974).

Hara, N., Okabe, S., and Takagi K. -A., Effects of gefarnate on acute gastric lesions in rats Folia. *Pharmacology. Jpn.*, 85, 443 (1985).

Hoppe, H. A. and Lerving, T., Marine algae in pharmaceutical science vol 2 (Walter de Gruyter, Berlin), pp 3-48 (1982).

Inoue, M., Satio, Y., Hirato, E., Morino, Y., and Nagase, S., Regulation of redox status of plasma proteins by metabolism and transport of glutathione and related compounds. *J. Protein Chem.*, 6, 207-225 (1987).

Jothi saraswathi, S., Babu, B., and Rengaswamy R., Seasonal studies on the alginate and its biochemical composition I: *Sargassum polycystum* (Fucales), phaeophyceae. *Phycological research*, 51(4), 240 (2003).

Juhani, P., Effect of ethanol on peptic activity in the rat stomach. *Digestion*, 23, 97-103 (1982)

Kloareg, B. and Quatrano, R. S., Structure of the cell walls of marine algae and ecophysiological functions of the matrix polysaccharide. *Ocenanogr. Mar. Boil. Annu. Rev.*, 26, 259-315 (1988).

Lahaye, M. and Kaeffer, B., Seaweed dietary fibers structure physicochemical and biological properties relevant to intestinal physiology. *Sci. Aliments.*, 17, 563-584 (1997).

Lowry, O. H., Rosenborough, N. I., Farr, A. I., and Randall, R. J., Protein measurement with folin phenol reagent., *J. Biol. Chem.*, 193, 265-275 (1951).

Mc Guigan, J. E., Peptic ulcer in Harrison's principles of internal medicine, 13th edition ed by Kurt J I Eugene B margin J B Wilson J D and Karper D L Vol 2, 1363 (1994).

Misra, H. P. and Fridovich, I., The role of superoxide anion in the auto- oxidation of epinephrine and a simple assay for superoxidedismutase. *J. Biol. Chem.*, 247, 170-3175 (1972).

Morenkova, S.-A., Tabutsadze, T. U., Fedorova, L. M., and Masenko, N. P., Effect of ulcerogenic agent cysteamine or the content of glutathione and GSH-dependent enzymes in the mucous membrane of the gastro duodenal region in the rat. *Biull. Eksp. Biol. Med.*, 104, 570-572 (1987).

Nagaoka, M., Shibata, H., Kimura-Takagi, I., Hashimoto, S., Aiyama, R., Ueyama, S., and Yokokura, T., Anti-ulcer effects and biological activities of polysaccharide from marine algae. *Biofactors*, 12 (1-4), 267-274 (2000).

Ohakawa, H., Ohishi, N., and Yagi, K., Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.*, 95, 351-358 (1979)

Paglia, D. E. and Valentaine, W. N., Studies on the glutathione and glutathione characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.*, 70, 158-169 (1967).

Pihan, G., Rigello, C., and Szabo, S., Free radicals and lipid peroxidation in ethanol and aspirininduced gastric mucosal injury. *Dig. Dis. Sci.*, 32 1395-1401 (1987)

Piper, D. W. and Stiel, D., Pathogenesis of chronic peptic ulcer, current thinking and clinical implications. *Med. Proc.*, 2, 7-10 (1986).

Takahara, S., Hamiliton, B. H., Nell, J. V., Kobra, T. Y., Ogura, Y., and Nishimura, E. T., Hypocatalasemia A new genetic carrier state. *J. Clin. Invest.*, 29, 610-619 (1960).

Takeuchi, K., Okabe, S., and Takagi, K., A new model of stress ulcers in the rat with pylorus ligation and its pathogenesis. *Am. J. Dig. Dis.*, 21, 782-788 (1976).

Tappel, A. L., Lipid peroxidation damage to cell components. *Fed. Proc.*, 32, 1870-1874 (1973).

Warren, L., The thiobarbituric acid assay of sialic acid. *J. Biol. Chem.*, 234, 1971-1975 (1959).

Winzler, R. J., Determination of serum glycoprotein. *Methods Biochem. Anal* ., 2, 279-311 (1958).

Wormsley, K. G., The pathophysiology of duodenal ulceration. *Gut*, 15, 59-64 (1974).

Yoshikewa, T., Naito, Y., Kishi, A., Tomii, T., Kaneko, T., Linuma, S., Ichikawa, H., Yasuda, M., Takahashi, S., and Kondo, M., Role of active oxygen, lipid peroxidation and antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. *Gut*, 34, 732-737 (1993).