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Anti-peroxidative and anti-hyperlipidemic nature of *Ulva lactuca* crude polysaccharide on D-Galactosamine induced hepatitis in rats

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ABSTRACT

To find whether pretreatment of *Ulva lactuca* polysaccharide (ULP) extract could be effective against D-Galactosamine (500 mg/kg body weight, i.p.) induced anomaly in rat. Serum total cholesterol (TC), triglycerides (TG), free fatty acid (FFA), phospholipids (PL), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), tissue lipoperoxides (LPO), hepatic protein thiols, non-enzymatic anti-oxidants glutathione (GSH) and vitamins (E and C) were examined using spectrophotometer. The ultra structural changes of liver during D-Galactosamine and protection offered by ULP were examined by electron microscopy. Seaweed histology and chemical composition of polysaccharides in seaweed were examined. Alcian blue staining showed the presence of sulphated polysaccharide with total sugar (65.4%), sulphate (17.4%), and uronic acid (17.2%) content. D-Galactosamine intoxicated rats showed significant ($p < 0.01$) liver damage with acute aberration in serum lipid profile, hepatic protein thiols and tissue non-enzymatic anti-oxidants. Assorted deposits of lipid droplets and abnormal appearance of mitochondria was observed in electron microscopy study. Rats pretreated with ULP (30 mg/kg body weight/day/for 21 days) showed a significant inhibition ($p < 0.05$) against abnormality induced by D-Galactosamine. *U. lactuca* exhibit anti-peroxidative and anti-hyperlipidemic property.

1. Introduction

High level of blood cholesterol is a contributory factor of atherosclerosis and many lipid associated ailments like obesity, heart attacks and stroke and kidney failure. Recent studies have shown that lipid associated disorders are not only attributed to the total serum cholesterol, but also to its distribution among different lipoproteins (Barter and Rye, 1996). The low density lipoproteins (LDLs) are the major carriers of cholesterol towards tissues having atherogenic potential, while the high density lipoproteins (HDLs) carry cholesterol from peripheral tissues to the liver (Kitamura et al., 1994). HDLs thus give protection against many cardiac problems and obesity (Brinton et al., 1990). Although genetic factors recline behind these lipid disorders, in most of the cases it is allied with diets high in saturated fats or trans fats.

Ulvales (Chlorophyta) are very common seaweeds distributed worldwide. Ulvans (from *Ulva lactuca*) constitute a dietary fiber structurally similar to the mammalian glycosaminoglycans but with unexplored biological activities. It contains insoluble polysaccharide and rich in sulphated uronic acid molecules that cannot be degraded by digestive enzymes. *Ulva* (Sea Lettuce) also rich in amino acids, minerals like sodium, zinc, sulphur, iron etc., and essential vitamins like B, B1, C and B9 etc., in addition report shows *U. lactuca* may prevent inflammation, bone decalcification, anemia and useful for children, old and women in pregnancy. Velichko and Shevchenko (1998) have reported that seaweeds as a dietary supplement are found good for prophylaxis of coronary atherosclerosis. Polysaccharides from green alga *Ulva pertusa* have also shown the hyperlipidemic activity (Yu et al., 2003). A study has also showed the anti-hepatotoxic property of green *Ulva reticulata* against acetaminophen induced liver damage (Balaji Raghavendra Rao et al., 2004).

D-Galactosamine hydrochloride (D-GalN) is a known experimental toxin that induces acute hepatitis and other serious health problems that may ultimately lead to death. Absorption of D-GalN affects cell membrane, organelles, and the synthesis of protein and nucleic acid (Keppler et al., 1974). It also decreases the hepatic content of uracil nucleotide resulting in the inhibition of transcription and, consequently, the translation processes (EI-Mofty et al., 1975). This study was attempted to examine whether ULP extract could be effective against D-Galactosamine induced changes in lipid

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profile and non-enzymatic anti-oxidants status during acute liver damage in experimental rats.

2. Materials and methods

2.1. Sample collection and extraction

U. lactuca (Sea Lettuce) was collected from Mandapam region (Gulf of Mannar). Prof. V. Krishnamurthy (Krishnamurthy Institute of Algology, Chennai, India) did the authentication of species. The collected sample was washed with seawater and deionized to remove extraneous matter such as epiphytes and contamination from other algae. Then sample was again washed with fresh water and air shade dried. Sulphated polysaccharide extraction procedure by Ray and Lahaye (1995) was followed with some modifications. Briefly, 500 g ($n = 5$) of air shade dried algae were roughly cut and autoclaved in 5 l of water at 100 °C for 3 h. The slurry was separated by gauze and filtered. The filtrate was dialyzed against tap water for 48 h, then concentrated to about 1000 ml under reduced pressure and then 95% ethanol (5 l) was added. The mixture was allowed to stand for overnight at room temperature. The precipitate was collected and washed twice with absolute ethanol, then dried at 50 °C. The crude polysaccharide extract was stored at 4 ° C and used for animal experiments. Total carbohydrate contents were estimated with the phenol–sulphuric method using rhamnose as standard (Dubois et al., 1956). Uronic acids were measured by colorimeter using glucuronic acid as standard (Thibault, 1979). Sulphate contents were determined from about 10 mg of various samples after 2 mol l^{-1} HCl hydrolysis (2 h at 100 °C) (Kawai et al., 1969).

2.2. Histochemical analysis of U. lactuca

The sectional and macerated seaweed material was dehydrated by passing through ascending and descending alcoholic (100–50%) series and kept in water. The section was then stained with diluted 5% aluminium sulphate solution of toluidine blue (TB) at pH 4.4. The sectional seaweed showed deep purple color indicating the presence of sulphated polysaccharide. Alcian blue (5% 8GX of 100 ml acid water at pH 5.0) a specific stain for sulphated polysaccharide was also used for the further confirmation. The section stained with alcian blue turned purple that indicated the presence of sulphated polysaccharide.

2.3. Animals

Adult male Albino rats of Wistar strain weighing about 120–130 g were used in this study. They were maintained in clean, sterile, polypropylene cages and fed with commercial pelleted rat chow (M/s. Hindustan Lever Ltd., Bangalore, India), water ad libitum and kept in a well ventilated room with 12 h light/dark cycles throughout the experimental period. This study was conducted as per the guidelines of the animal ethical committee of our institution.

2.4. Experimental protocol

Group I rats, received the normal diet and served as control, Group II rats were given single intraperitoneal injection of D-Galactosamine (500 mg/kg body weight). Group III rats were given *U. lactuca* extract for 21 days (30 mg/kg body weight, p.o). Group IV comprised rats were given *U. lactuca* extract for 21 days prior to the induction of D-Galactosamine experimental hepatitis.

At the end of experimental period, rats were anesthetized with sodium pentobarbitone (25 mg/kg body weight intraperitoneally) and sacrificed by cervical decapitation. The liver tissue was excised immediately and washed using ice-cold saline. The blood samples taken from the experimental animals without any anticoagulant were centrifuged at 3000g for 10 min to obtain clear serum. Serum levels of total cholesterol (Parekh and Jung, 1970), triglycerides (Folch et al., 1970), free fatty acid (Hron and Menahan, 1981), phospholipids, HDL, and LDL fractions were separated from serum according to the dual precipitation method (Friedewald et al., 1972). The esterified cholesterol was calculated from the difference between the total cholesterol and free cholesterol levels. VLDL determined from serum triglyceride level. The levels of glutathione (Moron et al., 1979), tissue lipid peroxides, (Ohkawa et al., 1979), Vitamin E, (Desai, 1984) and ascorbic acid (Omaye et al., 1979) were estimated.

2.5. Transmission electron microscopy of liver tissue

A portion of the liver tissue was instantaneously immersed in 25 g/l of glutaraldehyde solution, buffered with 0.1 mol/l sodium cacodylate (pH 7.4). The specimen was then placed in the buffer fixative medium, followed by washing with sodium cacodylate and fixation in 20 g/l osmium tetroxide buffered with 0.1 mol/l sodium cacodylate. After dehydration in a graded series of alcohol and propylene oxide, the tissues were transferred to the propylene oxide: ethanol mixture (1:1) and embedded in resin. The specimens were mounted on epoxy resin blocks and left in the oven at 65 °C for 72 h. Thin sections were cut with an ultra microtome, stained with uranyl acetate and lead citrate, and then examined under an EM-9A electron microscope.

2.6. Statistics

Results are presented as mean ± S.D. The significance of difference among the groups were assessed using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparison test, SPSS software version 10. Significance was set at level $p < 0.05$, $p < 0.01$.

3. Results and discussion

As shown in Fig. 1, significantly elevated levels of serum total cholesterol, free cholesterol and depleted ester cholesterol ($p < 0.01$) were evident in rat intoxicated with D-Galactosamine (Group II) as compared to normal control (Group I) animals. A study by Cartwright et al. (1982) has reported that administration of D-Galactosamine in rats caused a significant decrease in the levels of cholesterol esters and an increase in the free cholesterol levels. However the *U. lactuca* (Group IV) extract prevented the severe increase in the levels of total cholesterol and free cholesterol ($p < 0.05$) as compared to rats intoxicated with D-Galactosamine alone. However, the prevention was not significant when compared with control rats fed with normal diet.

As shown in Fig. 2, significantly elevated levels of ($p < 0.01$) serum triglycerides, free fatty acids, and depleted phospholipids were obvious. The rats that received *U. lactuca* extract as pretreatment significantly inhibited these ($p < 0.05$) severe modulations as compared to alteration induced by D-Galactosamine alone. D-Galactosamine is known to cause fatty changes in the liver, disorganization of nucleoli, disappearance of glycogen granules and distorted shape changes in mitochondria. A number of agents that produce liver injury also cause the accumulation of abnormal amounts of fat, predominantly triglycerides, in the parenchymal cells. In general, triglyceride accumulation might be from an imbalance between the rate of synthesis and the rate of release of triglycerides by the parenchymal cells into the systemic circulation. Cartwright et al., have reported the increased accumulation of triglycerides during D-Galactosamine induced hepatitis in rats. Our study observations suggest that the ULP may have the ability to prevent the acute increase in the levels of free fatty acids, triglyceride, and total cholesterol in both serum and tissue (Dianzani, 1978). The *U. lactuca* is reported to contain some sulphur compounds (D-cysteinolic acid), which are capable of reducing the excessive accumulation of intracellular triglyceride (approx. 40%). As shown in Fig. 3, animals intoxicated with D-Galactosamine (Group II) showed a considerable reduction ($p < 0.01$) in the levels of HDL and VLDL with concomitant elevation of LDL when compared with control. The animals pretreated with seaweed extract (Group IV) showed a substantial improvement ($p < 0.05$) in the levels of HDL and VLDL with a parallel inhibitory action on the elevation of LDL level as compared to animals intoxicated with D-Galactosamine (Group II). A study has shown that Ulvan, an active polysaccharide of green weeds limits hyperlipidemia in rats and mice. The different effects of Ulvans on lipid metabolism might due to the changes of physical and physiological properties, such as viscosity and molecular weight, induced by degradation. The mechanism by which extract significantly improve the levels of serum HDL-cholesterol and TG remains unknown. But the elevations of total and LDL-cholesterol were considered to be related with biosynthesis and uptake of cholesterol. Wong et al. (1999) have reported that some seaweed, such as *Ecklonia cava*, *Colpomenia sinuosa*, *Sargassum hemiphyllum*, did not reduce but rather elevated serum cholesterol levels due to the increase of endogenous synthesis of cholesterol in the liver (Hirayama et al., 2004). The