ORIGINAL ARTICLE

Improvemnt of lipid profile and antioxidant of hypercholesterolemic albino rats by polysaccharides extracted from the green alga Ulva lactuca Linnaeus

Sherif Hassan a,*, Sanaa Abd El-Twab b,c, Mona Hetta d, Basant Mahmoud e

a Botany department, Faculty of Science, Beni Suef Univ., Egypt
b Zoology department, Faculty of Science, Beni Suef Univ., Egypt
c Department of Biology, Faculty of Science, Hail University, KSA, Egypt
d Pharmacognosy department, Faculty of Pharmacy, Beni Suef Univ., Egypt
e Biochemistry department, Faculty of Science, Beni Suef Univ., Egypt

Received 8 August 2010; revised 5 January 2011; accepted 29 January 2011
Available online 3 February 2011

KEYWORDS
Antioxidants; Hypercholesterolemia; Ulva lactuca; Sulfated polysaccharides; Oxidative stress

Abstract Sulfated polysaccharides from Ulva lactuca were extracted in hot water and precipitated by ethanol then orally gavaged to rats fed on a hypercholesterolemic diet for 21 days to evaluate the antihypercholesterolemic and antioxidant actions. Atorvastatine Ca (Lipitor) was used as a reference drug. The intragastric administration of U. lactuca extract to hypercholesterolemic rats caused significant decrease of serum total lipids, triglycerides, total cholesterol, LDL-cholesterol and vLDL-cholesterol levels. Whereas, HDL-cholesterol concentration was markedly increased by 180%. Aqueous extract showed a significant ameliorative action on elevated atherogenic index, creatine kinase and lactate dehydrogenase activities of hypercholesterolemic group. Furthermore, serum activities of transaminases and alkaline phosphatase were also improved. High fat diet intake caused a highly significantly elevated serum urea, creatinine concentration. These effects were reversed by oral administration of U. lactuca extract. Sulfates polysaccharides extract of U. lactuca ameliorate hepatic enzymatic (catalase, glutathione peroxidase and superoxide dismutase), non-enzymatic (reduced glutathione & total thiol) antioxidant defenses and thiobarbituric acid reactive...
1. Introduction

In recent years, many marine resources have attracted attention in the search for bioactive compounds to develop new drugs and health foods (Huimin et al., 2005). In addition, marine algae are now considered a rich source of antioxidants (Nagai and Yukimoto, 2003). It is known that seaweeds contain numerous bioactive substances that have been shown to lower cholesterol, reduce blood pressure, promote healthy digestion; and antioxidant activity (Raghavendran et al., 2005). Algal polysaccharides have been demonstrated to play an important role as free-radical scavengers in vitro and antioxidants for the prevention of oxidative damage in living organisms (Zhang et al., 2004). Polysaccharide extracted from Ulva pertusa is a group of heteropolysaccharide, mainly composed of rhamnose, xylose, glucose, glucuronic acid, iduronic acid, and sulfate, with smaller amounts of mannoses, arabinose, and galactose (Huimin et al., 2005).

Cholesterol-enriched diet has been reported to adversely affect the health of humans and animal species. The prevalence of dyslipidemia resulting from excess energy intake and physical inactivity is increasing in Egypt. 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 (Sathivel et al., 2008). It has been reported that high levels of fat increase fat-mediated oxidative stress and decrease antioxidative enzyme activity (Slim et al., 1996). In contrast, there are various reports indicating the beneficial effects of antioxidant supplementation in preventing dyslipidemia and cardiovascular disease (Minhajuddin et al., 2005; Gorinstein et al., 2006). Thus, oxidative damage and its consequences may result in many chronic health problems that are attributed to high fat diet.

Many therapeutic agents are available for the management of hypercholesterolemic patients and are employed to promote successful treatment. A number of studies have demonstrated that the use of lipid-lowering drugs can reduce the number of cardiovascular events and mortality from coronary disease (Aronow, 2008). Moreover, a diet restricted in foods high in cholesterol and regular physical exercise should be proposed in the treatment of these patients, contributing significantly to primary health care (De Lorgeril et al., 1999). However, due to certain resistances to dietary restriction and financial limitations to use lipid-lowering drugs, many individuals have turned to alternative treatments to control cholesterol levels. Many of these alternative treatments have been used empirically, lacking scientific studies that would allow for more reliable conclusions (Dickel et al., 2007).

Thus, it is essential to develop and utilise effective and natural antioxidants so that they can protect the human body from free radicals and retard the progress of many chronic diseases. Published data indicates that plant polysaccharides in general have antioxidant activities and explored as novel potential antioxidants (Ng et al., 2004; Jiang et al., 2005; Wang and Luo, 2007). The structure and mechanisms of the pharmacological effects of bioactive polysaccharides on diseases have been extensively studied, and more natural polysaccharides with different curative effects have been tested and even applied in therapies. Thus, the present study was designed to evaluate the possible beneficial effect of the extracted sulfated polysaccharide from Ulva lactuca on serum lipid parameters and hepatic oxidative stress parameters in albino rats fed with cholesterol-rich diet.

2. Materials and methods

2.1. Algal collection

U. lactuca Linnaeus was collected from Al-Quser province, Red Sea coast, Egypt (26° 07’ N and 34° 13’ E) in December 2007. The algal material were washed with tap water many times and further washed two times with distilled water to remove epiphytes, salts and sands. They were air dried in shade and ground by a blender to give small size pieces (2 mm) then stored in plastic bags at room temperature in a dry dark place before use.

2.2. Polysaccharides extraction

Dry algal material (100 g) was autoclaved in 3 L of water at 100 °C for 2 h, and the slurry separated and filtered. The filtrate was dialyzed against tap water for 48 h, and then concentrated to about 800 ml under reduced pressure and 95% ethanol (3 L) was added. The mixture was allowed to stand for overnight at room temperature, and the precipitate was collected and washed twice with absolute ethanol (Pengzhan et al., 2003).

2.3. Infra red measurements

Fourier-transformed infrared spectra were recorded from the polysaccharide powder extract (about 1 mg) in KBr (300 mg) pellets on Shimadzu FT-IR 8201 PC spectrophotometer (El-Sayed et al., 2005).

2.4. Polysaccharides hydrolysis and composition analysis

The isolated crude polysaccharides (5 mg) hydrolyzed with 0.5 M H2SO4 at 105 °C for 20 h then neutralized by using BaCO3 and finally centrifuged. The supernatant was concentrated and examined by paper chromatography (PC, descending) using B/A/W (4:1:5 v/v, upper layer) as solvent system. The hydrolysate were also examined by thin layer chromatography (TLC) using the system CHCl3/MeOH/H2O (15:6:2 v/v) (Chen et al., 1997). The spots were visualized by spraying with aniline phthalate reagent. The hydrolysates (20 µl) were also analysed by HPLC HP1050 model equipped with UV detector (set at 192 nm), Hewlett Packard (HPLC laboratory, Agriculture Research center, Cairo) using the following conditions: Column: APS Hypersil column (4.6 × 200mm i.d.), mobile phase: acetonitrile/water (75:25) v/v., flow rate: 2 ml/min.
The sugars were positively identified by matching their retention time data with those of the reference standards, which were also run under identical analytical conditions.

2.5. Animals

White male albino rats *Rattus norvegicus* (Research Institute of Ophthalmology, Giza, Egypt) weighing between 120 and 140 g were used for the evaluation of antioxidant and antihypercholesterolemic actions of sulfated polysaccharides from *U. lactuca*. These animals were housed in stainless steel cages (six in each cage, 36 rat for total represented 6 groups) under ambient temperature of 27 ± 2 °C with 12 h light/dark schedule. They were fed with the standard commercial diet (ATMID Company, Egypt) and provided with tap water for 7 days to be acclimatized with the hold. Then they were weighed and serum triglycerides, total and HDL-cholesterol concentrations were measured.

2.6. Experimental design

Based on data, the normal rats were divided into six groups of six rats each as follows:

Group I served as negative control (given distilled water).

Group II were administered normal rats given *U. lactuca* extract at a dosage of 175 mg/kg body weight dissolved in distilled water, this dose was calculated from the therapeutic dose (250 mg/kg body weight) for mice (Pengzhan et al., 2003). Group III were given the drug Atorvastatine® (purchased from Pfizer-Egypt Company) at a dosage of 10 mg/kg body weight dissolved in distilled water, this dose was calculated from the therapeutic dose (10 mg/day) for human beings (Koter et al., 2002) by using the conversion table of Paget and Barnes (1964). All treatments were given orally and daily for 4 weeks. Normal groups were continued to be provided with the common commercial rat chow.

Hypercholesterolemia was induced to the remaining rat groups by addition of cholesterol powder, bile salt and animallard to the standard diet in percentage of 1%, 0.25% and 4%, respectively (Pengzhan et al., 2003) for about 21 days. Blood samples were withdrawn from lateral tail vein, centrifuged and serum lipid profiles were measured. The considered hypercholesterolemic rats were divided into three groups; hypercholesterolemic group (positive control, group IV) that continued fed with high fat diet throughout the study period (4 weeks). Group V was treated with *U. lactuca* extract (175 mg/kg body weight) and the last one (group VI) was given atorvastatin (10 mg/kg body weight) orally and daily by gastric intubation for 4 weeks.

Body weight gain was calculated from the difference between the initial weight at the beginning and the final weight at the end of the experiment. To reduce the error originating from feeding, all animals were fasted (water was not restricted) for 10 h before measurement.

By the end of the 4th week, animals of different groups were sacrificed under diethyl ether anesthesia. Blood samples were taken, left to coagulate at room temperature and centrifuged at 3000 rpm for 30 min. The clear, non hemolysed, supernatant sera were quickly removed and kept at −20 °C till used for biochemical investigations.

Liver was rapidly excised from each rat; 0.5 g from each liver was homogenized in 5 ml saline solution using Teflon homogenizer. The clear homogenate was used for estimation of non-enzymatic (GSH & T. thiol) and enzymatic (CAT, SOD and GSH-P, ) antioxidant defense system and lipid peroxidation products (TBARS).

2.7. Biochemical examination

Serum total lipids concentration was determined according to the method of Frings et al. (1972) using kits purchased from Diamond Diagnostic (Egypt). Serum triglycerides (TGs) concentration was determined according to the method of Fossati and Prencipe (1982) using reagent kit purchased from Reactivos Spinreact Company (Spain). Serum cholesterol concentration was estimated according to the method of Deeg and Ziegenohrm (1983) and serum HDL-cholesterol concentration was measured according to the method of Burstein et al. (1970) using reagent kit purchased from Spinreact Company (Spain). Serum LDL-cholesterol concentration was determined according to Friedewald et al. (1972) formula:

\[
\text{LDL - Cholesterol} = \frac{\text{Total cholesterol} - \text{HDL - Cholesterol}}{5}
\]

Serum very low density lipoprotein (vLDL) was determined according to Norbert (1995) formula:

\[
v\text{LDL} = \text{Cholesterol conc} - \frac{\text{Triglycerides}}{5}
\]

The Atherogenic index (AI) was calculated by the following formula: (Harnafi et al., 2008)

\[
\text{AI} = (\text{Total cholesterol} - \text{HDL - Cholesterol})/\text{HDL - Cholesterol}
\]

Both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed according to the method of Reitman and Frankel (1957) using reagent kits purchased from Randox Company (UK). Alkaline phosphatase (ALP) activity was determined according to the method described by Belfield and Goldberg (1971) using reagent kits obtained from Bio-Merieux Chemical Company (France). Urea concentration was estimated according to the method of Patton and Crouch (1977) and serum creatinine concentration was measured according to the method of Henry (1974) using reagent kit purchased from Diagnostics Company (Egypt). Serum creatine kinase (CK-NAC) activity was determined according to the method of Young (1975) and lactate dehydrogenase (LDH) activity in serum was determined according to the method of Buhl and Jackson (1978) using reagent kit purchased from Stanbio Laboratories (Texas, USA).

The levels of hepatic reduced glutathione (GSH) and total thiol (T. thiol) were determined by the methods of Beutler et al. (1963) and Koster et al. (1986), respectively. The activity of hepatic superoxide dismutase (SOD) was measured according to the method of Marklund and Marklund (1974). Liver catalase (CAT) was determined according to the technique of Cohen et al. (1970) and the results were expressed in terms of the first-order reaction rate constant (K). Glutathione peroxidase (GSH-P, ) was estimated according to the methods of Pinto and Bartley (1969). Thiobarbituric acid reactive substances (TBARS) indicative of lipid peroxidation was determined by the thiobarbituric acid reaction in the liver tissue according to the method of Preuss et al. (1998).
2.8. Chemicals

All chemicals and reagents used in the present study were of the highest purity available, and were purchased from Sigma (St Louis, MO, USA) and Merck (Darmstadt, Germany) Companies.

3. Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA, PC-STAT program, 1995) followed by least significant difference (LSD) analysis to compare various groups with each other. Results were expressed as mean ± standard deviation and values of \( P > 0.05 \) were considered statistically, non-significantly different, while those of \( P \leq 0.05 \) and \( P \leq 0.01 \) were statistically significantly and highly significantly different, respectively.

4. Results and discussion

The current study was designed to examine the cholesterol lowering, antioxidative activity and possible mechanism(s) of green alga \( U.\ lactuca \).

4.1. Infra red measurements

IR (cm\(^{-1}\)) spectrum of \( U.\ lactuca \) polysaccharide extract revealed presence of absorption bands at 1633.3, 1263.2 and 1051.3 cm\(^{-1}\) that indicated the presence of C–O of uronic acids, the stretching vibration of S–O of sulfate and the vibration of C–O–C bridge of glucosides, respectively. The signal at 789.8 cm\(^{-1}\) might demonstrate to the binding vibration of C–O–S of sulfate in equatorial position. The previous gained data dealt with the presence of sulfate functional groups in our extract (Pengzhan et al., 2003) (Fig. 1).

4.2. Polysaccharides composition

The monosugars content of \( U.\ lactuca \) obtained after hydrolysis and subjected to HPLC analysis, proved the presence of rhamnose, galactose, glucose, arabinose, xylose, mannose glucuronic acid and galacturonic acid at various retention times (Table 1).

4.3. Biochemical examination

4.3.1. Serum lipid profile

The present results elucidated high significant increase in serum total lipids, total cholesterol, triglyceride, LDL-cholesterol and vLDL-cholesterol concentrations in hypercholesterolemic control rats and highly significant decreased HDL-cholesterol levels as compared to normal rats (Table 2). These results run in parallel with those of other investigators (Jang et al., 2008). The hypercholesterolemic effect may be ascribed to the increased dietary cholesterol intake (Zulet et al., 1999) and subsequently increased rate of intestinal cholesterol absorption (Mathé, 1995). Lard fat is rich in saturated fatty acids, known to increase serum and LDL-cholesterol, and in monounsaturated fatty acids, which can increase serum triacylglycerols (Higashi et al., 1997; Lichtenstein et al., 1998). In addition, increased serum concentration of triglycerides may be attributed to decreased clearance of triglycerides secondary to decreased activity of lipoprotein lipase (LPL) (Nofer et al., 2002). The high level of LDL-cholesterol found in hypercholesterolemic rats may be attributed to a down regulation in LDL receptors by cholesterol and saturated fatty acids included in the diet (Mustad et al., 1997). Reduction in HDL following cholesterol feeding may be due to contributed acceleration of apoA-I clearance from the plasma based on cholesterol-enriched diets (Sorci-Thomas et al., 1989).

Treatment of hypercholesterolemic rats with \( U.\ lactuca \) polysaccharide extract induced marked significant decrease of serum total lipids (–61%), total cholesterol (–49.6%), triglycerides (–66%) and LDL-cholesterol (–93%) concentrations as compared to the hypercholesterolemic rats. Rats treated with algal extract showed improved high significant level of HDL-cholesterol (+180%, as compared to hypercholesterolemic rats), which may be due to the ability of the extract to hasten the decomposition of free radical species generated during cholesterol administration (Raghavendran et al., 2005; Godard et al., 2009). Where, HDL-C is a free radical scavenger and prevents peroxidation of beta lipoproteins (Chander and Kapoor, 1990). The polysaccharides from \( U.\ lactuca \).
lactuca showed high anti-hyperlipidemic activity in rats but the mechanism by which extract regulate TC, TG and LDL-C is unknown.

4.3.2. Atherogenic index, creatine kinase and lactate dehydrogenase

Table 3 revealed that, the hypercholesterolemic control rats exhibited a profound increase in atherogenic index (AI) as compared to normal ones. This atherogenicity thought to be due to the atherogenic lipoprotein subclasses commonly associated with hyperlipidemia (Hodis, 1999). The result indicated that, treatment using U. lactuca extract and atorvastatin exhibited significant decrease of AI (94% and 92.4%, respectively, as compared to hypercholesterolemic control rats) that might be ascribed to their plasma lipid-lowering activity. Cytosolic enzymes, lactate dehydrogenase (LDH) and creatine kinase,
served as the diagnostic markers of myocardial tissue damage. They leak out from the damaged tissues to the blood stream when the cell membrane becomes permeable or rupture (Gürgün et al., 2008). Therefore, elevated enzymes activity in serum of hypercholesterolemic control rats reflects the alterations in plasma membrane integrity and/or permeability. The declined enzymes activity secondary to drug and the alga treatments might be ascribed to their ability to maintain membrane integrity thereby restricting the leakage of these enzymes (Rajadurai and Stanley Mainzen Prince, 2006; Zhou et al., 2008).

4.3.3. Liver enzymes activity and kidney functions
In this study, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were significantly high in high-cholesterol fed diet than in normal rats, these results agreed with those of Sudhahar et al. (2007). High fat diet intake caused a highly significantly elevated serum urea and creatinine concentrations of hypercholesterolemic control rats as compared to normal rats (Table 4). Enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis is possible, an acceptable postulate to interpret the elevated levels of urea, creatinine, due to their antioxidant activity, emphasised with the previous observation where algal polysaccharide supplementation could circumvent the hypercholesterolemic condition (Table 5). Qi et al. (2006) showed the strong anti-oxidant activity (in vitro) of high sulfate content in polysaccharide fraction from the alga Porphyra haitaisan.

Apart from enzymatic antioxidants, non-enzymatic antioxidants, hepatic reduced glutathione (GSH) and total thiol (T. thiol) play a vital role in protecting cells from oxidative damage (Table 5). GSH and T. thiol in hypercholesterolemic rats decreased significantly (−23% and −59%, respectively) when compared with the normal ones. These reductions may be due to the increased utilization of these anti-oxidants for quenching enormous free radicals produced during hypercholesterolemic condition (Table 5). Qi et al. (2006) showed the strong anti-oxidant activity (in vitro) of high sulfate content in polysaccharide fraction from U. pertusa. Thus, the increased GSH and T. thiol content in both hypercholesterolemic groups may be attributed to the ability of atorvastatin and U. lactuca polysaccharides to improve the defensive nature of liver against free radicals (Qi et al., 2006).

Lipid peroxidation, measured as thiorbituric acid reactive species (TBARS), is a free-radical mediated propagation of oxidative insult to polysaturated fatty acids involving several types of free radicals (Korkina and Afanas’ev, 1997). The hypercholesterolemic rats of the present study exhibited high significant elevation of hepatic TBARS concentration as compared to normal control group (Table 5). An increase of TBARS levels, in animals fed with a high cholesterol diet has been previously reported (Shukla et al., 2004; Visavadiya and Narasimhacharya, 2007). There was a positive correlation between plasma total cholesterol and triacylglycerol concentrations and free radicals generation (Chen and Li, 2007). An abnormal rise in lipid peroxidation was reduced with atorvastatin and the polysaccharide administration, due to their antioxidant activity, emphasised through in vitro experiments (Qi et al., 2006). This is in line with the previous observation where algal polysaccharide supplementation could circumvent the in vitro oxidation of linoleic acid (Tannin et al., 2005). Sulfated polysaccharides supplementation significantly prevented the oxidative damage to macromolecules by minimizing the levels of lipid peroxidation (Josephine et al., 2008).

### Table 5: Effects of U. lactuca extract and atorvastatin supplementation on hepatic oxidative stress parameters of normal and hypercholesterolemic rats.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>CAT (Kx10⁻²)</th>
<th>GSH-Px (U/g)</th>
<th>SOD (U/g)</th>
<th>GSH (nmol/100mg tissue)</th>
<th>T. Thiol (nmol/100mg tissue)</th>
<th>TBARS (nmol/100mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Gr. I)</td>
<td>53.7 ± 4.2a</td>
<td>1.26 ± 0.22a</td>
<td>5.07 ± 0.30b</td>
<td>0.64 ± 0.03a</td>
<td>12.05 ± 2.25a</td>
<td>0.53 ± 0.03d</td>
</tr>
<tr>
<td>U. lactuca (Gr. II)</td>
<td>43.4 ± 2.2b</td>
<td>0.74 ± 0.02bc</td>
<td>3.43 ± 0.36d</td>
<td>0.48 ± 0.01b</td>
<td>7.05 ± 0.22b</td>
<td>0.56 ± 0.03d</td>
</tr>
<tr>
<td>Atorvastatine (Gr. III)</td>
<td>37.1 ± 2.9f</td>
<td>0.63 ± 0.02e</td>
<td>3.10 ± 0.29g</td>
<td>0.49 ± 0.01ed</td>
<td>7.53 ± 0.25f</td>
<td>0.74 ± 0.01e</td>
</tr>
<tr>
<td>Hypercholesterolemic (Gr. IV)</td>
<td>19.2 ± 2.0d</td>
<td>0.51 ± 0.05d</td>
<td>2.72 ± 0.33f</td>
<td>0.49 ± 0.01bcd</td>
<td>4.93 ± 0.53d</td>
<td>0.75 ± 0.04f</td>
</tr>
<tr>
<td>U. lactuca (Gr. V)</td>
<td>34.0 ± 2.0c</td>
<td>0.63 ± 0.14c</td>
<td>5.72 ± 0.28e</td>
<td>0.50 ± 0.01bc</td>
<td>9.80 ± 0.06b</td>
<td>0.59 ± 0.04c</td>
</tr>
<tr>
<td>Atorvastatine (Gr. VI)</td>
<td>45.0 ± 2.4h</td>
<td>0.76 ± 0.02b</td>
<td>4.08 ± 0.25f</td>
<td>0.51 ± 0.01b</td>
<td>7.12 ± 0.13h</td>
<td>0.63 ± 0.01b</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>3.21</td>
<td>0.11</td>
<td>0.36</td>
<td>0.02</td>
<td>1.13</td>
<td>0.03</td>
</tr>
<tr>
<td>LSD 1%</td>
<td>4.33</td>
<td>0.15</td>
<td>0.49</td>
<td>0.02</td>
<td>1.52</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Gr, Group; CAT, Liver catalase; K, first-order reaction rate constant; GSH-Px, Glutathione peroxidase; SOD, superoxide dismutase; GSH, hepatic reduced glutathione; T. Thiol, total thiol; TBARS, Thiobarbituric acid reactive substances; LSD, least significance difference; Data are expressed as mean ± SD (n = 6). Means which are not significantly different have the same symbol; significance level at P ≤ 0.05.
Improvement of lipid profile and antioxidant of hypercholesterolemic albino rats

5. Conclusions

In conclusion, the supplementation of sulfated polysaccharide extract of *U. lactuca* to hypercholesterolemic rats is effective in decreasing the oxidative stress and hypercholesterolemia development, by increasing the activities of antioxidant enzymes (CAT, GSH-Px, and SOD), non-enzymes (GSH & T. thiol) and limiting lipid peroxidation process. However, further clinical studies are necessary to assess the benefits and safety of sulfated polysaccharide extracts before using for human beings and approval by Food and Drug Administration (FDA) to be used as drugs.

References


