Evaluation of hepatic antioxidant capacities of Spirogyra neglecta (Hassall) Kützing in rats

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ABSTRACT
Free radicals are one of the causes of chronic and degenerative diseases. Antioxidants can protect the progression of free radical mediated disorders. The aim of this study was to evaluate the antioxidant activity of Spirogyra neglecta (Hassall) Kützing in rats. The rats were divided into 5 groups. Group 1 served as control. Groups 2 and 3 were administered hot water extract of S. neglecta at 50 and 200 mg/kg bw, respectively, while groups 4 and 5 were fed 1% and 4% S. neglecta mixed diet, resp., for 13 weeks. Antioxidant enzymes were evaluated in livers of the rats. The activities of catalase and glutathione reductase were significantly increased in the group fed 50 mg/kg of the extract, compared with the control group. Glutathione peroxidase activity was also significantly higher in the group fed 50 and 200 mg/kg of the extract. The study suggests that S. neglecta may enhance antioxidant systems in the rat liver.

KEY WORDS: antioxidant; green algae; Spirogyra neglecta (Hassall) Kützing

Introduction
Reactive oxygen species include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide and various lipid peroxides (Lee & Min, 2004). They are generated in all living organisms. These free radicals can react with various biomolecules in cells, leading to cell damage and death. This phenomenon appears to be a major contributor in chronic and degenerative diseases such as cancer, diabetes, arthritis, and atherosclerosis (Sapakal et al., 2008). Antioxidants can either trap or destroy free radicals and other reactive oxygen species, thus preventing oxidative stress-related diseases (Ak & Gülçin, 2008). The antioxidant system includes endogenous enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase, as well as non-enzymatic antioxidants such as glutathione, ascorbic acid and tocopherol (Sapakal et al., 2008). Consumption of fruit and vegetables rich in natural antioxidants is associated with the prevention of degenerative diseases (Ferrari, 2004; Veerapur et al., 2009).

Algae are a great source of natural compounds that are widely known and consumed in Asian countries. Numerous studies have investigated compounds found in algae for their antibiotic, antiviral, antioxidant, anti-inflammatory and cytotoxic activities (Lordan et al., 2011). Spirulina is a blue-green microalga from the Cyanobacterium gender and contains a phycocyanin with pharmaceutical and antioxidant properties (Estrada et al., 2001). Brown algal polyphenols function as antioxidants, antibacterial and anti-fungal compounds (Kuda et al., 2007; Shibata et al., 2006). Seaweeds are known to contain reactive antioxidant molecules, such as ascorbate, glutathione, carotenoids, catechins and polysaccharides (Yuan et al., 2005). Polysaccharides in various algae have been demonstrated to act as free radical scavengers in vivo and in vitro (Jiao et al., 2011). However, most previous studies have focused on seaweed, blue-green, red and brown algae. There is little information concerning freshwater green algae and their pharmacological and medical application.

Spirogyra spp. are filamentous freshwater green algae. These algae are consumed as food in northern Thailand. Spirogyra neglecta (Hassall) Kützing contains high amounts of protein, carbohydrate, fat, sulfate and dietary fiber (Phinyo et al., 2012). S. neglecta is beneficial to the...
Materials and methods

Chemicals
β-dihydronicotinamide adenine phosphosphanucleotide (β-NADPH) was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Cupric chloride (CuCl₂) was purchased from BDH (BDH Chemicals Ltd, Poole, England). All other chemicals used were purchased from Sigma-Aldrich Co. (St. Louis, Mo, USA).

Materials
Spirogyra spp. was collected from the cultivation pool of Na Kuha village, Saun Kuen Sub district, Muang District, Phrae Province, Thailand, during October and November 2010. The fresh algae were identified and authenticated according to the morphology both of vegetative cells and sex cells and their habitat by the method of John et al. (2002). The algae were extracted with distilled water at 100°C for 2 h. The hot water extract was filtered with Whatman filter paper no. 1 and lyophilized with a freeze dryer.

Chemical composition analysis
The alga S. neglecta was analyzed for total carbohydrate, sulfate, chlorophyll and total phenolic compounds. Carbohydrate was determined by the phenol-H₂SO₄ method using glucose as the standard (Dubois et al., 1956). Sulfate was measured by the method of Craigie and Wen (1984) using K₂SO₄ as standard. Total phenolic compounds were determined by the Folin-Ciocalteu method using gallic acid as standard (Emmons et al., 1999). Chlorophyll was determined according to the method described by Proctor (1981).

Animals
Male Wistar rats weighing between 120–150 g were purchased from the National Laboratory Animal Center, Salaya, Nakorn Pathom, Thailand. They were housed in the animal care center of the Faculty of Medicine, Chiang Mai University, under controlled environmental conditions of 24°C and 12 h light-dark cycle. The experimental protocol was approved by the Animal Ethics Committee of the Faculty of Medicine, Chiang Mai University.

Experimental protocol
All rats were randomly divided into 5 groups of 6 animals each with treatment groups as follows: Group 1 served as control, receiving distilled water and basal diet, Groups 2 and 3 received 50 and 200 mg/kg body weight of S. neglecta extract via intragavage for 13 weeks, respectively, Groups 4 and 5 were fed with S. neglecta mixed diets at 1% and 4% for 13 weeks, respectively. All animals were sacrificed at the end of the 13th week by diethyl ether. Liver samples were removed for biochemical analysis. Blood samples were collected to determine liver function enzymes.

Liver function tests
Alanine aminotransferase and aspartate aminotransferase enzymes in serum were measured spectrophotometrically using commercial Olympus kits (Olympus Corp., Tokyo, Japan).

Determination of oxidative stress and antioxidant status in rat livers
Lipid peroxidation
The lipid peroxidation levels in livers were measured in terms of thiobarbituric acid reactive substance (TBARS) according the method of Fujiwara et al. (2003). The reaction mixture consisted of 10% liver homogenate, 50% trichloroacetic acid and 0.67% thiobarbituric acid aqueous solution. The reaction mixture was heated in a boiling water bath for 10 minutes. The tubes were placed on ice to stop the reaction, n-butanol was added, then mixed and centrifuged at 3,000 rpm for 20 minutes. The supernatant was measured for its absorbance at 532 nm. TBARS generation was calculated based on a standard curve of MDA.

Liver cytosol preparation
Frozen livers were homogenized with ice-cold buffer containing 1.5% KCl and 0.25 mM PMSF using a Polytron homogenizer. The homogenates were centrifuged at 10,000 g for 20 minutes at 4°C. The supernatants were further centrifuged at 100,000 g for 60 minutes at 4°C. The cytosols were stored at –80°C until analysis. The protein content in rat liver cytosols was assayed via the Lowry method.

Glutathione
Glutathione was measured according to the method of Akerboom and Sies (1981) based on the reaction with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). The amount of glutathione was expressed as nmol/mg protein.

Superoxide dismutase (SOD)
This assay is based on the inhibition of nitroblue tetrazolium (NBT) reduction by superoxide radicals produced by the xanthine/xanthine oxidase system (Sun et al., 1988). The SOD activity was expressed as U/mg protein.

Catalase
Catalase activity was determined by measuring the decomposition of hydrogen peroxide according to the method of Aebi (1984). Catalase activity was expressed as nmol of hydrogen peroxide reduced/min/mg protein.

Glutathione peroxidase (GPx)
GPx activity was determined by NADPH oxidation in a coupled reaction system containing l-butyldihydroperoxide.
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(t-BHA) and oxidized glutathione (Nagalakshmi & Prasad, 2001). GPx activity was expressed as μmol of β-NADPH oxidized/min/mg protein.

Glutathione reductase (GR)

GR activity was determined according to the method of Carlberg and Mannervik (1983) by measuring the amount of NADPH consumed during the conversion of oxidized glutathione (GSSG) to reduced glutathione at 340 nm. GR activity was expressed as nmol of β-NADPH oxidized/min/mg protein.

Statistical analysis

The experimental results are expressed as mean of 6 animals in each group±SD. The statistical significance of differences between groups was analyzed by one way analysis of variance (ANOVA) with LSD for post hoc tests.

Results

The yield of hot water extract of *S. neglecta* was approximately 25%. Total carbohydrate and sulfate in *S. neglecta* were 33.94±1.46% and 1.08±0.01%, respectively. Chlorophyl content in dried *S. neglecta* and hot water extract from *S. neglecta* were 15.44 and 9.91 mg/g and total phenolic compounds were 72.98±14.34 and 151.02±15.39 mg GAE/g, respectively. Table 1 shows body weights and liver weights in *S. neglecta* treated and control groups. The food and water intake in all *S. neglecta* treated groups were not significantly different when compared with the control group. AST and ALT activities were not significantly changed in treatment groups as compared with the control group. No significant differences were observed in glutathione and TBARS levels in either treated group as compared with the control group (Table 2). Antioxidant enzymes were evaluated in livers of the rats. The activities of catalase and GR in the liver were significantly (*p<0.05*) increased only in the 50 mg/kg *S. neglecta* treated group (Figures 1 and 2). GPx activity was significantly (*p<0.05*) increased in livers of rats fed with 50 and 200 mg/kg of *S. neglecta* as compared with controls (Figure 3). SOD was not significantly changed in the treatment groups as compared with the control group (Figure 4).

Table 1. General appearance and liver weight of *S. neglecta* treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Food intake (g/rat/day)</th>
<th>Water intake (ml/rat/day)</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>% body weight change absolutes</th>
<th>Liver weight (g)</th>
<th>Relative liver weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21±3</td>
<td>32±12</td>
<td>101±4</td>
<td>428±10</td>
<td>324.4±18.4</td>
<td>13.3±2.7</td>
<td>3.0±0.4</td>
</tr>
<tr>
<td><em>S. neglecta</em> 50 mg/kg</td>
<td>21±4</td>
<td>39±9</td>
<td>100±3</td>
<td>441±42</td>
<td>340.7±37.4</td>
<td>13.4±1.7</td>
<td>2.9±0.3</td>
</tr>
<tr>
<td><em>S. neglecta</em> 200 mg/kg</td>
<td>23±4</td>
<td>37±11</td>
<td>100±4</td>
<td>461±43</td>
<td>360.8±37.6</td>
<td>13.5±1.5</td>
<td>2.9±0.5</td>
</tr>
<tr>
<td>1% <em>S. neglecta</em> mixed diet</td>
<td>23±3</td>
<td>33±8</td>
<td>102±4</td>
<td>467±49</td>
<td>358.4±38.2</td>
<td>12.7±1.3</td>
<td>2.9±0.3</td>
</tr>
<tr>
<td>4% <em>S. neglecta</em> mixed diet</td>
<td>22±3</td>
<td>31±8</td>
<td>103±4</td>
<td>435±17</td>
<td>324.9±21.7</td>
<td>13.1±1.6</td>
<td>3.1±0.4</td>
</tr>
</tbody>
</table>

Values are means ± SD, n=6

Table 2. Effects of *S. neglecta* on the level of serum AST and ALT activities and hepatic TBARS and glutathione in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/l)</th>
<th>ALT (U/l)</th>
<th>TBARS (nmole/mg protein)</th>
<th>GSH (nmole/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76±7</td>
<td>32±8</td>
<td>0.14±0.06</td>
<td>5.99±2.83</td>
</tr>
<tr>
<td><em>S. neglecta</em> 50 mg/kg</td>
<td>99±22</td>
<td>30±4</td>
<td>0.15±0.04</td>
<td>7.29±1.57</td>
</tr>
<tr>
<td><em>S. neglecta</em> 200 mg/kg</td>
<td>83±12</td>
<td>31±6</td>
<td>0.16±0.05</td>
<td>8.06±1.37</td>
</tr>
<tr>
<td>1% <em>S. neglecta</em> mixed diet</td>
<td>81±5</td>
<td>33±3</td>
<td>0.16±0.05</td>
<td>6.19±1.43</td>
</tr>
<tr>
<td>4% <em>S. neglecta</em> mixed diet</td>
<td>83±8</td>
<td>33±6</td>
<td>0.16±0.05</td>
<td>6.38±1.51</td>
</tr>
</tbody>
</table>

Values are means ± SD, n=6

Antioxidant enzymes play a crucial role in cellular defense oxidative stress. Catalase is an enzyme which converts hydrogen peroxide to water and molecular oxygen, thus preventing the formation of extremely dangerous hydroxyl radicals from hydrogen peroxide. It has long been known that hydroxyl radicals can destroy biomolecules leading to pathological alterations (Limon-Pacheco & Gonsebatt, 2009). Thus induction of catalase activity by S. neglecta treatment may result in a decrease in deleterious effects due to hydrogen peroxide. GPx plays an important role in protecting cells from free radicals generated by peroxide decomposition (Limon-Pacheco & Gonsebatt, 2009). GR is a ubiquitous NADPH-dependent enzyme protecting against oxidative damage within the cell by maintenance of appropriate levels of intracellular glutathione (Sapakal et al., 2008). The enhancement of these antioxidant enzyme activities in livers of rats treated with hot water extract of S. neglecta indicated that S. neglecta may protect against cellular and tissue damage due to hydrogen peroxides, peroxides and hydroxyl radicals.

The antioxidant potentials of algae in human health have been discussed elsewhere (Cornish & Garbary, 2010). The major groups of antioxidant compounds in macroalgae are phenolic compounds, polyphenols, sulfated polysaccharides, carotenoids and vitamins. Phenolic compounds and polyphenols can chelate metal ions, prevent radical generation and indirectly modulate the activities and alter the expression levels of significant proteins, such as antioxidant and detoxifying enzymes (Ferguson, 2001; Ferguson et al., 2004). Many studies have shown that the sulfated polysaccharides in algae possess various pharmacological activities including antioxidant activities (Jiao et al., 2011). Zhang et al. (2003) reported that polysaccharide fractions from the alga Porphyra haitanesis (Rhodephyta) could increase antioxidant enzymes in aging mice. Song et al. (2010) demonstrated the inhibitory effect of polysaccharides extracted from the green alga Bryopsis plumose on superoxide radical and DPPH. In this study, we found that the total amount of phenolic compounds, total carbohydrates and sulfate of hot water extract were greater than those of raw materials. This may be one reason why the administration of the hot water extract of S. neglecta enhanced antioxidant systems in rat liver. Vitamin E and β-carotene, fat-soluble vitamins, and vitamin C, a heat unstable vitamin, are well-known antioxidants in algae. These antioxidant compounds might be excluded from this study due to the hot water preparation of S. neglecta extract. Furthermore, chlorophyl, green pigments found in algae and higher plants, exhibited various biological properties including anticancer and antioxidant activities (Ferruzzi & Blakeslee, 2007). The present study showed that the content of chlorophyl in dried S. neglecta was higher than in the hot water extract. This indicated that chlorophyl might not be the important antioxidant substances in S. neglecta. Further studies are needed to identify and determine polysaccharides and the other polar compounds of S. neglecta and their antioxidant activities.
Acknowledgements
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Conflict of interest statement
Authors declare no conflict of interest.

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