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Oxidative stress biomarkers in organs of hyperlipidaemic and normal rats fed tropical red seaweed, *Gracilaria changii*

Pei Teng Chan[1] · **Patricia Matanjun**[1] · **Suhaimi Md Yasir**[2] · **Tek Song Tan**[3]

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Abstract The aim of the study was to evaluate the effects of normal and high-cholesterol/fat diet supplemented with 5 and 10 % freeze-dried red seaweed, *Gracilaria changii* powder on rat organs (liver, kidney and heart), lipid peroxidation and antioxidant enzyme activities. The results showed that feeding with atherogenic diet alone caused lipid peroxidation which eventually leads to oxidative stress of the rat's organs. Nevertheless, with 10 % *G. changii* supplementation, it significantly decreased the liver lipid peroxidation by 52.24 %, and the antioxidant enzyme activities were significantly increased by 52.09 to 94.42 %. Similarly, with *G. changii* supplementation, it significantly enhanced the kidney antioxidant enzyme activities. This suggests that *G. changii* suppress oxidative stress and protect the rats' organs. In conclusion, *G. changii* could be a promising functional food ingredient in the management of hyperlipidaemia.

Keywords Lipid peroxidation · Enzymes antioxidants · Hyperlipidaemic · Red seaweed

✉ Patricia Matanjun
patsy@ums.edu.my

[1] Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

[2] Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

[3] Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

Introduction

Hyperlipidaemia is a heterogenous disorder involving multiple aetiologies. It is commonly characterised by an increased flux of free fatty acids (FFAs), raised triglycerides, low density lipoprotein cholesterol (LDL-C) and apolipoprotein B (apoB) levels and reduced plasma high-density lipoprotein cholesterol (HDL-C) concentration as a consequence of metabolic effects, or dietary and lifestyle habits (Micallef and Garg 2009). This condition is known to increase the production of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radical and singlet molecular oxygen through various mechanisms. These ROS are capable of damaging many biological macromolecules such as DNA, RNA, protein and lipids (Fang et al. 2002) and have been implicated in the pathophysiology of heart failure, ischemic heart disease (Prasad et al. 1996), hepatic injury (Scott et al. 2000; Montilla et al. 2006) and chronic renal damage and failure (Baker et al. 1985) in animals and humans. Hence, it is possible that the oxidative stress in hyperlipidaemia may damage organs such as heart, liver and kidney. Despite the fact that there are drugs available clinically for treating hyperlipidaemia, the consumption of functional foods/dietary supplements in lowering/controlling serum cholesterol levels and risk of cardiovascular diseases has gained enormous global acceptance over the years by the general public (Kwok et al. 2010).

Seaweeds are known for their richness in polysaccharides, minerals, omega-3 and 6 polyunsaturated fatty acids (PUFAs), vitamins and also some bioactive substances such as polyphenols and halogenated metabolites that contain anti-hyperlipidaemic (Artiss et al. 2006; Matanjun et al. 2010; Dousip et al. 2014; Chan et al. 2014), antihypertensive (Ren et al. 1994) and antibacterial (e.g., *Staphylococcus*, *Salmonella*) (Vairappan 2003; Rajauria et al. 2012; Fernandes et al. 2014) properties. Hence, the natural antioxidants from

seaweeds can be a very interesting natural source of new compounds with biological activities that could be used for functional ingredients or development of functional foods. They are able to retard the damages from oxidative stress and may be an effective, safe and economical alternative therapy for hyperlipidaemia and organ protection (Matanjun et al. 2009; Cofrades et al. 2010; Holdt and Kraan 2011; O'Sullivan et al. 2011).

To protect cells against oxidative damage by oxidants produced during the oxygen metabolism, an antioxidant system has presumably evolved in aerobic organism. Oxidative stress markers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) had been frequently used in studies to assess redox status in response to exercise, nutrition or exposure to xenobiotic. There are several studies on the protective effect of different seaweeds species supplementation on organs of rats and mice (Bocanegra et al. 2003, 2006, 2008; Dhanapal et al. 2009; Motshakeri et al. 2014), but to our knowledge, no studies have been done on the protective effect of the tropical red seaweed, *Gracilaria changii*. Moreover, the previous studies reported the hypolipidaemic properties (Chan et al. 2014) and also high-antioxidant activities (Chan et al. 2015) of *G. changii*, this showed the potential of this seaweed to reduce oxidative stress and to improve the endogenous enzyme antioxidant activities. Thus, the objectives of the present study are aimed to investigate the effects of *G. changii* supplementation on the organs (liver, kidney and heart), lipid peroxidation and antioxidant enzyme activities (SOD, CAT and GSH-Px) of normal and high-cholesterol/fat-diet-induced hyperlipidaemic male Sprague–Dawley rats.

Methods and materials

Gracilaria changii was collected from the mangrove area of Santubong, Sarawak, Malaysia. It was then brought back to the laboratory in Universiti Malaysia Sabah packed in a cool condition. The seaweed was then cleaned with distilled water to remove epiphytes, sand and debris and immediately placed in a freezer (−40 °C) and freeze-dried for 24 h. The dried sample was then ground to powder using a Waring blender to pass through a 0.85 mm (pore size) screen and then stored in a sealed bag at −40 °C. A voucher specimen (FSMP 01) of the seaweed was preserved in the Biochemistry Laboratory, Faculty of Food Science and Nutrition, Universiti Malaysia Sabah.

Animals and diets

Seventy male Sprague–Dawley laboratory rats (4 to 5 months) were purchased and housed in cages and maintained in a room temperature of 22±2 °C with a 12-h light, 12-h dark cycle. All the rats were provided with a commercial diet and tap water ad libitum for acclimatisation period of 1 week. They were handled according to the guidelines approved by the university's animal ethics committee. After acclimatisation, the rats were then weighed and randomly divided into two groups, to be fed with either a high-cholesterol/fat diet (n=40) or normal diet (n=30) for 4 weeks. The high-cholesterol and high-fat diet comprised of 2 % of non-oxidised cholesterol, 1 % cholic acid and 20 % corn oil. Cholic acid was added to improve cholesterol absorption by the rats' intestine. All diets were prepared from C.P.Myanah (Thailand) which provides the macro and micronutrients required by adult rats. The compositions and chemical constituents of the experimental diets are shown in Table 1 (Chan et al. 2014).

After 4-week induction period, starting week 0 (baseline), the rats were weighed and divided into seven experimental groups of ten animals each: (1) high-cholesterol/fat diet (HF), (2) high cholesterol/fat + 5 % *G. changii* powder (HF+5 %), (3) high cholesterol/fat + 10 % *G. changii* powder (HF+10 %), (4) high cholesterol/fat + 2.05 mg kg^{-1} body weight Atorvastatin (positive control) (HF + Ator) (equivalent to human dosage, 20 mg) (Reagan-Shaw et al. 2008), (5) normal diet (NF), (6) normal diet + 5 % *G. changii* powder (NF+5 %), and (7) normal diet + 10 % *G. changii* powder (NF+10 %). The rats were fed with different diets (as mentioned above) for 8 weeks before they were sacrificed. During the experimental period, the rats were given free access to food and water. The feed was replenished daily.

At the end of the experimental period, the rats were euthanised by exsanguination under diethyl ether and their organs (liver, heart and kidney) were removed, rinsed with physiological saline, blot dried and homogenised for enzymatic antioxidants analysis and lipid peroxidation.

Preparation of organs post mitochondrial supernatant

The organs post mitochondrial supernatant (PMS) was prepared according to Shah and Iqbal (2010). The organs (liver, kidney, heart) (1 g) were homogenised with ice cold 10 mL potassium phosphate buffer (50 mM containing 1.17 % NaCl) using Polytron homogeniser. The homogenates were centrifuged at 875×g for 15 min at 4 °C, and then the supernatant was transferred to a new centrifuge tube and then centrifuged again at 10,000×g for 30 min at 4 °C to obtain PMS. The PMS was decanted to a new centrifuge tube and kept at −40 °C for further antioxidant enzyme assays and lipid peroxidation assay. The protein content of organ PMS was determined by bicinchoninic acid kit (BCA) (Sigma, USA) using bovine serum albumin as standard.

Lipid peroxidation

The organ PMS thiobarbituric acid reactive substances (TBARS) were measured according to the method of

Table 1 The composition and chemical constituents of experimental diet (%)

Components	Experimental group					
	NF	NF+5 %	NF+10 %	HF	HF+5 %	HF+10 %
Standard diet	100	95	90	77	72	67
Cholesterol	–	–	–	2	2	2
Cholic acid	–	–	–	1	1	1
Corn oil	–	–	–	20	20	20
G. changii powder	–	5	10	–	5	10
Total (%)	100	100	100	100	100	100
Moisture	6.73 ± 0.18^{a}	6.36 ± 0.12^{a}	6.64 ± 0.29^{a}	5.29 ± 0.10^{b}	5.24 ± 0.04^{b}	5.48 ± 0.35^{b}
Ash	5.75 ± 0.18^{d}	7.50 ± 0.22^{b}	8.30 ± 0.30^{a}	4.83 ± 0.04^{f}	$6.26\pm0.28^{c, d}$	6.74 ± 0.27^{c}
Protein	18.26 ± 0.16^{a}	18.28 ± 0.03^{a}	18.51 ± 0.42^{a}	14.37 ± 0.07^{b}	14.38 ± 0.05^{b}	13.85 ± 0.05^{b}
Fat	3.96 ± 0.87^{c}	2.12 ± 0.33^{c}	2.03 ± 0.10^{c}	23.10 ± 0.87^{a}	22.10 ± 0.52^{ab}	20.45 ± 0.27^{b}
Soluble dietary fibre	0.79 ± 0.13^{c}	2.83 ± 0.14^{b}	5.06 ± 0.21^{a}	0.82 ± 0.13^{c}	2.57 ± 0.04^{b}	5.12 ± 0.13^{a}
Insoluble dietary fibre	1.72 ± 0.23^{d}	$2.32\pm0.29^{b, c}$	3.47 ± 0.30^{a}	0.90 ± 0.07^{e}	$1.89\pm0.04^{c, d}$	2.88 ± 0.06^{b}
Total dietary fibre	2.51 ± 0.19^{d}	5.15 ± 0.24^{b}	8.53 ± 0.39^{a}	1.72 ± 0.20^{e}	4.46 ± 0.08^{c}	7.99 ± 0.11^{a}
Energy (kcal)	365.34 ± 0.65^{d}	354.99 ± 1.03^{e}	350.99 ± 2.34^{e}	475.02 ± 4.36^{a}	464.52 ± 2.18^{b}	453.53 ± 1.67^{c}

Data are mean±SEM

NF normal feed, *NF+5%* normal feed + 5 % *G. changii*, *NF+10%* normal feed + 10 % *G. changii*, *HF* high-cholesterol/fat feed, *HF+5%* high-cholesterol/fat feed + 5 % *G. changii*, *HF+10%* high-cholesterol/fat feed + 10 % *G. changii*

[a–f] Different letters within the same row indicate significant difference at $p<0.05$

Sivoňová et al. (2007) with slight modification. The proteins of the diluted organ PMS (0.5 mL) were removed by adding 1 mL of 14 % trichloroacetic acid (TCA), and the mixture was centrifuged at 875×*g* for 10 min at 4 °C. Then the supernatant (0.5 mL) was mixed with 20 μL butylated hydroxytoluene (BHT) (0.02 %) to prevent further oxidation. Subsequently, 1.0 mL thiobarbituric acid (TBA) (0.6 %) was added to the mixture and incubated at 90 °C for 30 min. After cooling, the absorbance was measured at 535 nm. The concentration of MDA was calculated using molar extinction coefficient of MDA (1.56×10^{5} M^{-1} cm^{-1}). The results are expressed as nmol MDA mg^{-1} protein.

Antioxidant enzyme activities

Superoxide dismutase (SOD) The SOD activity of organ PMS was determined according to Marklund and Marklund (1974) with slight modification. Organ PMS (0.1 mL) was topped up with Tris–HCl (50 mM, pH 8.2) to 1.80 mL. The reaction was initiated with the addition of 0.2 mL pyrogallol (0.2 mM). For the control, the organ PMS samples were substituted with an equal amount of Tris–HCl (50 mM, pH 8.2). The changes of absorbance at 420 nm were recorded for 5 min at 30-s intervals, after an initial lag time of 30 s. One unit SOD activity is defined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50 % per min. The SOD activity of organ PMS was expressed in U mg^{-1} protein.

Catalase (CAT) The CAT activity was determined according to Aebi (1984). Diluted organ PMS (0.1 mL) was added with potassium phosphate buffer to 2 mL. Then 1 mL of 30 mM hydrogen peroxide (H_2O_2) was added to initiate the reaction. Catalase activity was determined by measuring the rate of decomposition of H_2O_2 at 240 nm for 2 min at 30-s intervals. A molar extinction coefficient of 43.6 M^{-1} cm^{-1} was used to determine the CAT activity. One unit of CAT activity was defined as the μmol of H_2O_2 degraded min^{-1}. The CAT activity of organ PMS was expressed as U mg^{-1} protein.

Glutathione peroxidase (GSH-Px) The GSH-Px activity was measured according to the method of Cejková et al. (2004) and Khan (2009) with slight modifications. The GSH-Px in the sample catalysed the oxidation of reduced glutathione (GSH) to glutathione disulfide (GSSG) by H_2O_2. The reaction mixture consisted of a phosphate buffer (50 mM, pH7.2), EDTA (1 mM), sodium azide (1 mM), glutathione reductase (1 U mL^{-1}), GSH (1 mM) and NADPH (0.2 mM). Diluted organ PMS (0.05 mL) was added to 0.45 mL reaction mixture and then topped up to 2 mL with K-phosphate buffer (50 mM, pH 7.0), then 0.01 mL H_2O_2 (0.25 mM) was added to initiate the reaction. The GSH-Px activity was determined by measuring the rate of disappearance of NADPH at 340 nm for 3 min at room temperature, after an initial lag time of 15 s. The rate of reaction of two blanks were also measured in order to obtain the net GSH-Px activity: (blank 1) reagent mixture (0.45 mL), in addition of 0.01 mL H_2O_2 and the mixture

was topped up to 2 mL with potassium phosphate buffer (50 mM, pH 7) and (blank 2) sample (0.05 mL) was added with 0.45 mL reaction mixture and topped up to 2 mL with K-phosphate buffer (50 mM, pH 7). The net GSH-Px activity was calculated by subtracting the rate of reaction of blank 1 and blank 2 from the rate of reaction of organ PMS. The GSH-Px activity was expressed as nmol NADPH oxidised min^{-1} mg^{-1} protein using a molar extinction coefficient of 6.22×10^3 M^{-1} cm^{-1}. One unit of GSH-Px was defined as nmol NADPH oxidised min^{-1}. The GSH-Px activity of organ PMS was expressed as U mg^{-1} protein.

Statistical analysis

All data are expressed as mean±standard error of mean (SEM). Data were analysed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using SPSS statistical software version 17.0 for Windows. A significant difference was considered at level of $p<0.05$.

Results and discussion

Liver

The liver lipid peroxidation and antioxidant enzyme activities are presented in Table 2. The liver MDA level of the HF group (2.01±0.14 nmol mg^{-1} protein) was significantly higher than NF group (1.08±0.16 nmol mg^{-1} protein). This suggested that the liver MDA of HF group was due to the oxidative stress imposed on this organ by a cholesterol-rich diet. The hepatic MDA concentration of HF+5 %, HF+10 % and HF+Ator groups was significantly different compared to the HF being reduced by 26.37, 52.24 and 35.82 %, respectively, compared to the HF group. This suggests that addition of *G. changii* powder to the high-cholesterol/fat diet significantly reduced hepatic lipid peroxidation. On the other hand, the hepatic MDA concentrations of the NF, NF+5 % and NF+10 % groups showed no significant difference among them.

The oxidative stress in the organs was assessed by measuring MDA and the activity of antioxidant enzymes such as SOD, CAT and GSH-Px. The liver SOD, CAT and GSH-Px activity of the hyperlipidaemic rats were significantly lower than normal rats (Table 2). The SOD activities of the HF and NF group were 0.77±0.07 and 1.48±0.08 U mg^{-1} protein, respectively. The feeding of high-cholesterol/fat diet significantly decreased the liver SOD activity by 47.97 % as compared to the NF group. The liver SOD activities of NF+5 % and NF+10 % were not significantly different from the NF group. On the other hand, the liver SOD activity of HF+5 % was 0.97±0.06 U mg^{-1} protein (an increase of 20.62 %), while for HF+10 %, it showed a significant increase of 57.15 % with a SOD activity of 1.21±0.13 U mg^{-1} protein as compared to the rats fed with cholesterol diet alone. No significant difference was observed in the SOD activity of the HF + Ator group compared to the HF group.

The CAT activities of NF, NF+5 % and NF+10 % groups were not significantly different (Table 2). As for the hyperlipidaemic groups, the liver CAT activities of HF and HF + Ator were significantly the lowest among the groups with values of 238.36±21.33 and 246.79±16.16 U mg^{-1} protein, respectively. Meanwhile, for the hyperlipidaemic treatment groups (HF+5 % and HF+10 %), the CAT levels were 413.08± 52.67 and 459.41±41.79 U mg^{-1} protein, respectively. These results indicate that the supplementation of 5 and 10 % *G. changii* powder to the high-cholesterol/fat diet significantly increased the liver CAT activity by 73.30 and 92.73 % compared to the HF group. In fact, the CAT levels of HF+5 % and HF+10 % were similar to the NF group. This indicates that *G. changii* powder is able to ameliorate oxidative stress in the liver and also bring the levels of these endogenous antioxidant enzymes to a level comparable to normal rats.

Table 2 The liver lipid peroxidation and antioxidant enzyme activities of the experimental groups

Experimental groups	MDA (nmol mg^{-1} protein)	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	GSH-Px (U mg^{-1} protein)
NF	$1.08\pm0.16^{b, c}$	$1.48\pm0.08^{a, b}$	$376.55\pm24.83^{a, b}$	81.71 ± 6.89^{a}
NF+5 %	0.74 ± 0.07^{c}	$1.48\pm0.10^{a, b}$	347.97 ± 17.44^{b}	78.45 ± 6.88^{a}
NF+10 %	0.73 ± 0.06^{c}	1.65 ± 0.24^{a}	$373.92\pm31.62^{a, b}$	84.68 ± 5.99^{a}
HF	2.01 ± 0.14^{a}	0.77 ± 0.07^{d}	238.36 ± 21.33^{c}	40.24 ± 1.03^{c}
HF+5 %	1.48 ± 0.26^{b}	$0.97\pm0.06^{c, d}$	$413.08\pm52.67^{a, b}$	56.20 ± 4.99^{b}
HF+10 %	$0.96\pm0.23^{b, c}$	$1.21\pm0.13^{b, c}$	459.41 ± 41.79^{a}	61.20 ± 6.20^{b}
HF + Ator	$1.29\pm0.26^{b, c}$	0.72 ± 0.14^{d}	246.79 ± 16.16^{c}	39.23 ± 3.94^{c}

Data are mean±SEM, $n=10$ rats per group

[a–d] Different letters within the same column indicate significant difference at $p<0.05$

NF normal feed, *NF+5%* normal feed + 5 % *G. changii*, *NF+10%* normal feed + 10 %*G. changii*, *HF* high-cholesterol/fat feed, *HF+5%* high-cholesterol/fat feed + 5 % *G. changii*, *HF+10%* high-cholesterol/fat feed + 10 % *G. changii*, *HF + Ator* high-cholesterol/fat feed + Atorvastatin

Similar to SOD and CAT, the GSH-Px activities of HF (40.24±1.03 U mg^{-1} protein) and HF + Ator (39.23±3.94 U mg^{-1} protein) group were the lowest among the experimental groups. The GSH-Px activity of NF group was 81.71 ±6.89 U mg^{-1} protein, while for the normal treatment groups (NF+5 % and NF+10 %) the GSH-Px levels were 78.45±6.88 and 84.68±5.99 U mg^{-1} protein, respectively (no significant difference was observed). On the other hand, the GSH-Px levels of the HF+5 % and HF+10 % groups were significantly higher in the HF+10 % with the activity increased by 52.09 % as compared to the HF group, whereas for HF+5 %, the increment was 39.66 %.

Consumption of high cholesterol/fat causes oxidative stress to the rat organs, especially the liver because liver is the main organ that is involved in lipid metabolism and thus prone to potential oxidative damage in condition of hyperlipidaemia (Lecumberri et al. 2007). The oxidative stress results from an imbalance between the production of free radicals and the effectiveness of the antioxidant defence system (Zhu et al. 2008). This was evident with the significant increase in liver MDA concentration and decreased in SOD, CAT and GSH-Px activity of the HF group as compared to the NF group. A reduction in the activity of these enzymes is associated with the accumulation of highly reactive free radicals, leading to harmful effects such as loss of integrity and function of cell membranes (Raja et al. 2012). This was in agreement with a previous study showing the supplementation of high-cholesterol/fat diet alone significantly decreased the antioxidant activity of hepatic enzymes in rats (Vázquez-Castilla et al. 2013). Nevertheless, with the supplementation of 10 % of *G. changii* powder to high-cholesterol/fat diet, there was a significant reduction of the liver lipid peroxidation resulting in a recovery of the activity of the antioxidant enzymes to values similar to those of normal groups. The increased activities of SOD, CAT and GSH-Px in *G. changii*-treated groups can be attributed to the antioxidant potential of *G. changii* against injury caused by free radicals. *G. changii* contains considerable amounts of polyphenols and flavonoids. The polyphenol content ranged from 0.64 to 1.41 mg PGE g^{-1} freeze-dried sample, while flavonoids ranged from 1.72 to 6.89 mg RE g^{-1} freeze-dried sample (Chan et al. 2015). Previous findings had suggested that samples rich in polyphenols and flavonoids were able to increase the levels of antioxidant enzymes in antherogenic diet rats by suppressing lipid peroxidation through activation of the antioxidant enzymes (Visavadiya and Narasimhacharya 2007; Kang et al. 2012; Raja et al. 2012; Vázquez-Castilla et al. 2013). Additionally, a previous study showed that diet supplemented with *G. changii* exhibited hypolipidaemic properties (Chan et al. 2014). Nutrients such as omega-3 PUFAs, α-tocopherol, vitamin C and selenium in *G. changii* also contribute to the reduction of lipid peroxidation and increased antioxidant enzymes in the rat organs. Avramovic et al. (2012) showed daily supplementation of omega-3 PUFAs (30 mg EPA+45 mg DHA) significantly reduced lipid peroxidation and increased the SOD activity of rat brain tissues. Similarly, rats treated with selenium (0.5 ppm) significantly increased the hepatic, muscle and erythrocytes GSH-Px activity of the exercise rats (Brady et al. 1979).

Kidney

The kidney MDA level and the antioxidant enzymes activitie are shown in Table 3. The kidney MDA level of the HF group had no significant difference from the NF group. The lipid peroxidation of kidney for HF, HF+5 %, HF+10 % and HF + Ator groups showed no significant difference.

The kidney SOD activity of the HF group (1.37±0.27 U mg^{-1} protein) was significantly lower than in the NF group (2.04±0.16 U mg^{-1} protein) by 32.84 %. The SOD activity of the normal rats fed with diet containing 5 and 10 % *G. changii* powder showed no significant difference to the NF group. Meanwhile, the SOD activities for HF+5 % and HF+10 % were 2.01±0.15 and 2.72±0.22 U mg^{-1} protein, respectively, indicating that the diet supplemented with 10 % seaweed powder significantly increased kidney SOD activity by 98.54 %, comparable to the rats fed with normal diet. The SOD activity of the HF + Ator group (1.34±0.27 U mg^{-1} protein) was not significantly different from the HF group.

The kidney CAT activity of normal control and treatment groups ranged from 190.51±11.50 to 197.58±12.90 U mg^{-1} protein, with no significant difference among the groups. As for the HF group, the CAT activity was significantly lower than NF group by 20.73 %. The CAT activities of the HF+5 % and HF+10 % groups were 172.60±5.60 and 192.97±13.71 U mg^{-1} protein, respectively. The high-cholesterol/fat diet supplemented with 10 % *G. changii* significantly enhanced the CAT activity in the kidney by 23.21 % as compared to HF group, with the recovery of the enzyme activity similar to the NF group.

There were no significant differences in the GSH-Px activity of the NF, NF+5 % and NF+10 % groups, although the supplementation of 5 and 10 % *G. changii* to the normal diet increased the GSH-Px activity by 3.10 and 12.70 %, respectively. For the HF group, the GSH-Px activity in the kidney was 89.78±3.88 U mg^{-1} protein, and the activity showed no significant difference with HF+5 % (84.76±2.97 U mg^{-1} protein) and HF + Ator (80.49±5.70 U mg^{-1} protein). Meanwhile, the HF+10 % group showed a significantly higher kidney GSH-Px activity of 109.95±8.85 U mg^{-1} protein, an increment of 22.47 % compared to the HF group.

The decrease in antioxidant enzyme activities in organs has been associated with oxidative stress (Montilla et al. 2006; Bouderbala et al. 2008; Green et al. 2012). In the current study, although feeding a high-cholesterol/fat diet alone did

Table 3 Kidney lipid peroxidation and antioxidants enzymes activities of the experimental groups

Experimental groups	MDA (nmol mg^{-1} protein)	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	GSH-Px (U mg^{-1} protein)
NF	0.89±0.14[a]	2.04±0.16[b]	197.58±12.90[a]	85.25±2.67[b]
NF+5 %	0.93±0.14[a]	2.10±0.15[a, b]	191.57±9.48[a]	87.89±4.68[b]
NF+10 %	0.85±0.09[a]	2.22±0.27[a, b]	190.51±11.50[a]	96.08±6.51[a, b]
HF	0.97±0.10[a]	1.37±0.27[c]	156.61±6.31[b]	89.78±3.88[b]
HF+5 %	1.00±0.19[a]	2.01±0.15[b]	172.60±5.60[a, b]	84.76±2.97[b]
HF+10 %	1.20±0.12[a]	2.72±0.22[a]	192.97±13.71[a]	109.95±8.85[a]
HF + Ator	0.83±0.13[a]	1.34±0.27[c]	154.94±7.45[b]	80.49±5.70[b]

Date are mean±SEM, $n=10$ rats per group

[a–b] Different letters within same column indicate significant difference at $p<0.05$

NF normal feed, *NF+5%* normal feed + 5 % *G. changii*, *NF+10%* normal feed + 10 % *G. changii*, *HF* high-cholesterol/fat feed, *HF+5%* high-cholesterol/fat feed + 5 % *G. changii*, *HF+10%* high-cholesterol/fat feed + 10 % *G. changii*, *HF + Ator* high-cholesterol/fat feed + Atorvastatin

not show a significant difference in rat kidney MDA levels, it significantly reduced the antioxidant enzyme activity as compared to normal rats. This was similar to the HF+5 % and HF + Ator groups. In contrast, supplementation of 10 % *G. changii* to high-cholesterol/fat diet significantly increased the enzymes antioxidant activity to a value comparable to the NF group. Anila and Vijayalakshmi (2003) reported that rats fed with high-cholesterol/fat diet supplemented with flavonoids significantly improve the in vivo antioxidant enzyme activities in the kidney by suppressing lipid peroxidation and enhancing the antioxidant enzyme activities. Montilla et al. (2006) also reported that the antioxidant enzyme activities (SOD, CAT, GSH-Px) in the kidney were significantly increased with supplementation of red wine to high-cholesterol diet, showing protective effect of red wine. This may indicate that addition of 10 % of *G. changii* to the high-cholesterol/fat diet resulted in the resumption/stabilisation of the antioxidant/oxidant level in the kidney of hyperlipidaemic rats to equilibrium.

Heart

There were no significant differences between the different diet groups in the MDA levels (Table 4). Similarly, there were no statistically significant differences in the heart SOD activity between the different groups (Table 4)

The heart CAT activity for the NF group was 56.83± 10.19 U mg^{-1} protein. And supplementation of 5 and 10 % *G. changii* powder to the normal diet had no significant effect on the CAT activity compared to the NF group. Similar results were also observed in the hyperlipidaemic group.

The GSH-Px activity for the groups fed the diets enriched with 5 and 10 % *G. changii* powder was decreased by 17.01 and 18.29 %, compared to the NF group (Table 4). The GSH-Px activity of the HF group (41.91±2.37 U mg^{-1} protein) was significantly lower by 32.79 % compared to the NF group. As for the HF+5 % and HF+10 % group, the GSH-Px activities were significantly higher by 21.36 and 37.44 % than the HF group, while the

Table 4 Heart lipid peroxidation and antioxidant enzyme activities of the experimental groups

Experimental groups	MDA (nmol mg^{-1} protein)	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	GSH-Px (U mg^{-1} protein)
NF	2.06±0.37[a]	1.01±0.12[a]	56.83±10.19[a, b]	62.36±2.23[a]
NF+5 %	1.56±0.28[a]	1.07±0.12[a]	54.91±10.16[a, b]	51.75±2.62b[c]
NF+10 %	1.64±0.15[a]	1.15±0.07[a]	59.45±7.95[a]	50.95±2.26[b, c]
HF	2.13±0.27[a]	0.78±0.10[a]	33.67±3.69[b]	41.91±2.37[d]
HF+5 %	2.00±0.18[a]	0.83±0.15[a]	52.11±3.31[a, b]	50.86±3.39[b, c]
HF+10 %	2.08±0.28[a]	1.03±0.12[a]	55.38±8.49[a, b]	57.60±3.05[a, b]
HF + Ator	1.73±0.12[a]	0.79±0.13[a]	34.56±1.78[b]	43.91±4.04[c, d]

Date are mean±SEM, $n=10$ rats per group

[a–d] Different letters within the same column indicate significant difference at $p<0.05$

NF normal feed, *NF+5%* normal feed + 5 % *G. changii*, *NF+10%* normal feed + 10 % *G. changii*, *HF* high-cholesterol/fat feed, *HF+5%* high-cholesterol/fat feed + 5 % *G. changii*, *HF+10%* high-cholesterol/fat feed + 10 % *G. changii*, *HF + Ator* high-cholesterol/fat feed + Atorvastatin

Atorvastatin-treated group showed no significant difference compared to the HF group.

The current findings showed that the liver and kidney tissues were more susceptible to oxidative stress as compared to the heart tissues, as a high cholesterol/diet alone significantly decreased the antioxidant enzyme activities as compared to the normal diet. On the other hand, heart tissues showed no significant differences among all the experimental groups. The variable effects of the experimental diets and the type of organs on the activity of the studied enzymes indicate that the responses of these enzymes involved in antioxidant defence vary greatly depending on several factors such as oxygen consumption, susceptibility to oxidant, antioxidant enzyme levels, the origin of the oxidative stress and the type or source of dietary antioxidants (Lecumberri et al. 2007; Liu et al. 2012). Tissues and organs have different rates of metabolic activity and oxygen consumption possibly due to differences in mitochondrial biogenesis and the occurrence of oxidant-induced degeneration (Liu et al. 2012). The level of antioxidant enzymes also varies in different organs, for example, GSH and cysteine are lower in the brain than the liver, kidney or muscle (Limón-Pacheco and Gonsebatt 2009).

In conclusion, the supplementation of *G. changii* powder to the high-cholesterol/fat diet was able to decrease lipid peroxidation and increase antioxidant enzyme activities in organs, thus, decreasing the oxidative stress caused by the lipogenic diet. This seaweed could provide a protective effect to the rat's organs and can be suggested for potential applications as a functional foods ingredient for human consumption.

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