HAYATI Journal of Biosciences, September 2008, p 105-110 Vol. 15, No. 3
ISSN: 1978-3019

The Effect of Seaweed *Eucheuma cottonii* on Superoxide Dismutase (SOD) Liver of Hypercholesterolemic Rats

TUTIK WRESDIYATI[1]*, ANS BUDI HARTANTA[2], MADE ASTAWAN[2]

[1]Department of Anatomy, Physiology, and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia
[2]Department of Science and Food Technology, Faculty of Agricultural Technology, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia

Received March 10, 2008/Accepted July 31, 2008

Intracellular antioxidant superoxide dismutase (SOD) was reported decreased in the liver and kidney of hypercholesterolemic rats. This study was conducted to observe the effect of seaweed *Eucheuma cottonii* powder on the profile of blood cholesterol and the level of SOD in liver tissues of hypercholesterolemic rats by using immunohistochemical technique. Twenty male Wistar rats were used for this study. Those rats were divided into four groups; (i) negative control group (A), (ii) hypercholesterolemia group treated by 5% seaweed powder (B), (iii) hypercholesterolemia group treated by 10% seaweed powder (C), and (iv) Positive control group or hypercholesterolemia group (D). The experiment was carried out for 35 days. Hypercholesterolemia condition (> 130 mg/dl), except group A, was achieved by feeding the rats with commercial diet containing 1% cholesterol. Drinking water was given ad libitum for 40 days. The results showed that seaweed powder decreased the total cholesterol, low density lipoprotein (LDL), triglyceride, and increased the level of high density lipoprotein (HDL) and SOD status in the liver tissues of hypercholesterolemic rats. The treatment of 10% seaweed powder gave better results than that of 5%. These results suggested that dietary fiber such in the seaweed powder has antioxidant activity.

Key words: superoxide dismutase (SOD), seaweed-*Eucheuma cottonii*, hypercholesterolemia, liver, immunohistochemistry

INTRODUCTION

Wresdiyati *et al*. (2006a,b) reported that hypercholesterolemic conditions altered the liver and kidney tissues of rats, and decreased the level of intracellular antioxidant copper, zinc-superoxide dismutase (Cu,Zn-SOD) in these organs. These alterations may account for the hypercholesterolemic condition, inducing production of reactive oxygen species-free radical. Increased levels of the reactive oxygen species (ROS), reactive nitrogen species (RNS), and other free radicals, create a situation known as oxidative stress. Oxidative stress can be induced by certain factors such as antioxidant deficiency or over production of free radical. The condition can lead to a variety of biochemical and physiological lesions often resulting in metabolic impairment and cell death. These highly reactive oxygens can readily react with various biological macromolecules such as DNA, proteins, lipids, and cause protein destruction. The lesions in turn lead to various diseases and degenerative processes such as, aging and carcinogenesis in human and animals (Halliwell & Gutteridge 1995).

Free-radical scavenger (antioxidant) plays an important role in protection of cells against oxidative stress and maintain a balance between the various toxic oxygen species. The protection can be done by several ways such as prevention, stopping or decreasing of oxidations (Schuler 1990), as well as catalyzing free radicals by intracellular antioxidant enzymes (Mates *et al*. 1999).

The intracellular antioxidant enzymes comprise catalase, glutathione peroxidase, and three isoforms of superoxide dismutase (SOD); copper, zinc (Cu,Zn)-SOD, manganese (Mn)-SOD, and iron (Fe)-SOD (Fridovich 1975). The SOD provides a primary defence against superoxide anion radical generated intracellularly. It was reported that SOD was localized immunohistochemically and immunocytochemically in the human and rat tissues (Marklund 1984; Dobashi *et al*. 1989; Wresdiyati & Makita 1997). SOD was also reported plays important role in physiological processes. Some cases of failed pregnancy in human was caused by the decreasing level of SOD (Sugino *et al*. 2000). Profile of SOD was also reported in pathological condition such as stress, diabetes mellitus and hypercholesterolemia (Wresdiyati *et al.* 2002; Wresdiyati 2003; Wresdiyati *et al*. 2003; Wresdiyati *et al*. 2006a,b), in neoplastic tissues (Keller *et al*. 1991), and in neurons of hypocampus in Alzheimer and Down's syndrome patient (Furuta *et al*. 1995). It was also reported that activity of heart Cu,Zn-SOD decreased after isoproterenol infuse induced oxidative stress in rats (Srivastava *et al*. 2007).

More than 70% of Indonesian area is the sea that rich of many kinds of flora and fauna. One of them is seaweed one kind of algae which have four classes: green algae (*Chlorophyceae*), green-blue algae (*Cyanophyceae*), brown algae (*Phaeophyceae*), and red algae (*Rhodopyceae*).

The important compound of seaweed is dietary fiber. Dietary fiber is a complex carbohydrate which undigested by

*Corresponding author. Phone: +62-251-8626064, Fax: +62-251-8629464, E-mail: astawan@indo.net.id

digestive enzymes in intestine. Base on solubility in water, there are two kinds of dietary fiber; soluble and insoluble dietary fiber. Soluble dietary fiber was reported to have role in important physiological procesess, such as to decrease cholesterol and serum glucose level, as well as prevent cardiovascular disease and hypertention. Soluble dietary fiber comprises pectine, beta glucane, gum, mucilase, agar, carageenan, and alginate. Whereas insoluble dietary fiber was reported to have role in prevention of colon cancer, diverticulosis, constipation, and hemorrhoid. Insoluble dietary fiber comprises lygnine, cellulose, and hemicellulose. Related to the function, there are three important compounds in seaweed: agar, carageenan, and alginic acid (Astawan 1998). The present study was conducted to observe the effect of seaweed-*Eucheuma cottonii* powder on the profile of blood cholesterol and intracellular antioxidant copper,zinc-superoxide dismutase (Cu,Zn-SOD) in liver tissues of hypercholesterolemic rats using immunohistochemical technique.

MATERIALS AND METHODS

Seaweed Powder Production and Analysis. Seaweed powder used for this study was *Eucheuma cottonii* and produced as described on Figure 1. Analysis of seaweed powder was carried out to know the content of the dietary fiber (soluble and insoluble dietary fibers) (Asp *et al*. 1983) and proximate analysis was to determine moist, ash, lipid, protein, and carbohydrate by difference (AOAC 1995).

Treatment of Animals and Tissues Preparation. A total of 20 male Wistar rats (250 $\pm$ 5 gr BW) were used for this study. The animals were adapted to the situation and conditions of the animal laboratory for two weeks, and then the blood serum was analyzed its total cholesterol before treatment. The animals were then divided into 4 treatment groups (Table 1); (i) negative control group (A), (ii) hypercholesterolemic group treated by 5% seaweed powder (B), (iii) hypercholesterolemic group treated by 10% seaweed powder (C), and (iv) Positive control group or hypercholesterolemic group (D). The experiment was conducted for 35 days. Hypercholesterolemic condition (>130 mg/dl), except group A, was achieved by feeding the rats with commercial diet containing 1% cholesterol and drinking water *ad libitum* for 40 days.

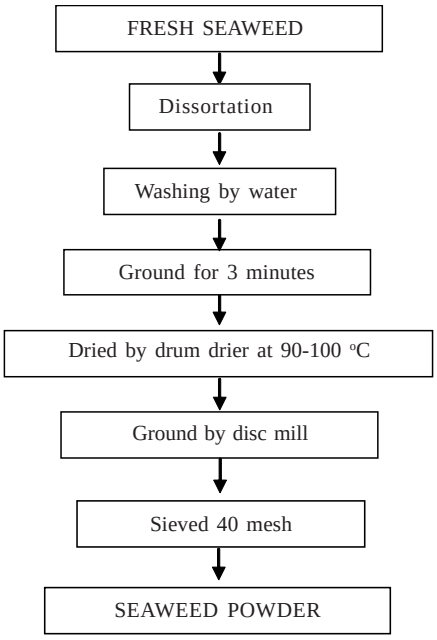

Figure 1. The method of seaweed powder production.

Blood and tissue were sampled at the end of each treatment. Following cervical dislocation liver tissues were collected from each animal in all groups. Blood serum was analyzed for total cholesterol, high denstity lipoprotein (LDL), low density lipoprotein (HDL), and triglyceride. Whereas liver tissues were then processed by paraffin standard method. Specimens were cut into 4 mm-thick sections and subjected to hematoxylin eosin staining and immunohistochemical technique for detection of Cu,Zn-SOD.

Hematoxylin Eosin Staining. The tissue sections were stained with haematoxylin - eosin after deparaffinization and rehydration using standard methods. After staining, they were dehydrated again and followed by clearing with xylol before mounting with entelan.

Immunohistochemistry. SOD was localized immunohistochemically described previously (Dobashi *et al*. 1989; Wresdiyati 2003) with a slight modification in using of conjugated second antibody and kit of diamino benzidine (DAB). The tissue sections were washed for 15 min with 3 times changes of PBS between each step. After deparaffinization and rehydration, the tissue sections were exposed to 3% H_2O_2 for 10 min to inactivate endogenous peroxidase activity and then to 10% normal goat serum to block nonspecific binding. Following rinsing with PBS, the tissue sections were incubated in primary antibody of copper,zinc-superoxide dismutase (Cu,Zn-SOD, 1:200) (Sigma S2147) at 4 °C for 2 days. The tissues were then incubated with enhanced labelled polymer peroxidase, secodary antibody (Dako K1491). The reaction product of antigen-antibody was visualized using kit of DAB (00-2020) from Zymed Laboratories Inc. The tissue sections were then dehydrated with series of alcohol, and cleared with xylol. Finally, the sections were mounted with entelan. As control of staining, tissue sections were incubated with PBS instead of Cu,Zn-SOD antibody. The tissue sections of control staining showed negative reaction with minimal background staining.

Observation and Data Analysis. The level of total cholesterol, LDL, HDL, and triglyceride were statistically

Table 1. The results of dietary fiber and proximate analysis of seaweed powder

Component	Contents (% dry weight)
Soluble dietary fiber	38.77
Insoluble dietary fiber	43.17
Total dietary fiber	81.94
Water	20.97
Ash	5.11
Protein	5.43
Lipid	1.47
Carbohydrate	87.99

analyzed using ANOVA and Duncan test (Steel & Torrie 1986). Haematoxylin - eosin stained tissue sections were observed under a light microscope. The hepatocytes and interstitial area condition and some histopathological signs of the tissues from the treatment groups were compared to that of the control group.

The immunoreaction products of the SOD were also observed by using a light microscope. The distribution and frequency of positive reaction product on the tissues of control group were compared qualitatively to those of the treatment groups. The qualitatively observation of Cu,Zn-SOD reaction product was done to the nucleus and cytoplasm of hepatocytes. The observation of Cu,Zn-SOD in the tissues was based on the brown colour intensity in the cells and the distribution of the reaction product.

The immunoreaction products of the Cu,Zn-SOD were observed qualitatively by using a light microscopy.

RESULTS

Seaweed Powder Components. The analysis showed that seaweed powder contained a large amount of soluble and insoluble dietary fiber, 81.94%, and carbohydrate, 87.99% of dry weight (Table 1).

Serum Total Cholesterol. Serum total cholesterol in hypercholesterolemic rats that treated with 5% (B) (77.33 mg/dl) and 10% (C) (67.66 mg/dl) seaweed powder was not significantly different ($P > 0.05$) to that of negative control group (A) (78.66 mg/dl) (Figure 2). It means the treatment of seaweed powder decreased serum total cholesterol of hypercholesterolemic rats. Five and ten percent of seaweed powder decreased 1.78 and 13.98%, respectively of serum total cholesterol. While the positive control group, hypercholes-terolemic group (D), showed serum total cholesterol (144.33 mg/dl) significantly higher ($P < 0.05$) than other group.

Low density lipoprotein (LDL) in the negative control group (A), 5% (B), 10% (C) of seaweed powder treatment, and positive control group (D) was 52.7, 47.0, 33.0, and 116.3 mg/dl respectively. The treatment of seaweed powder was significantly ($P < 0.05$) decreased the level of LDL hypercholesterolemic rats. Compared to the hypercholes-terolemic group (D), the level of LDL in 5% (B), and 10% (C) of seaweed powder decreased 59.59 and 71.63%, respectively (Figure 3).

The lowest level of high density lipoprotein (HDL) was showed in the hypercholesterolemic group (D) (13.33 mg/dl). While the highest level of HDL was showed in the hypercholesterolemic rats that treated with 10% seaweed powder (C) (25 mg/dl). It was significantly different ($P < 0.05$) compared to that of hypercholesterolemic group (D). Treatment of 5 and 10% of seaweed powder has no significant effect ($P > 0.05$) on increasing HDL level compared to the negative control group (Figure 4).

Serum triglyceride level of hypercholesterolemic group (D) (74.33 mg/dl) showed the highest and was significantly different ($P < 0.05$) to that of others groups. Treatment of 5 and 10% of seaweed powder significantly decreased ($P < 0.05$) the level of serum tryglyceride of hypercholesterolemic rats (B and C) 31.76 and 36.34%, respectively. The level of serum tryglyceride was not significantly different ($P > 0.05$) to that of negative control group (Figure 5).

Histopathology. Histopathological evidence was observed in the liver tissues of all groups stained by haematoxylin and eosin (HE). The hepatocytes were arranged radially to the central vein with the presence of Kupffer cells in their sinusoidal spaces. The liver tissues of hypercholesterolemia group showed histopathological evidences, such as serious

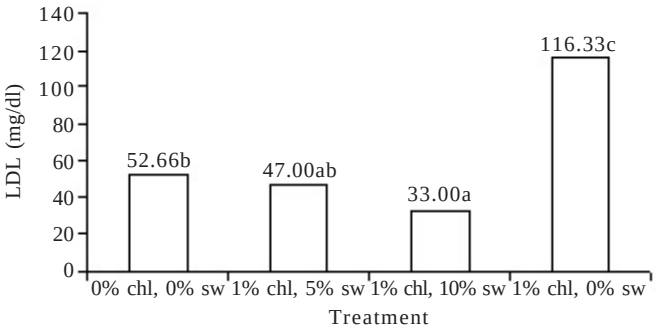

Figure 3. The effect of rat diet containing cholesterol (chl) and seaweed (sw) powder on serum low density lipoprotein (LDL) of rats.

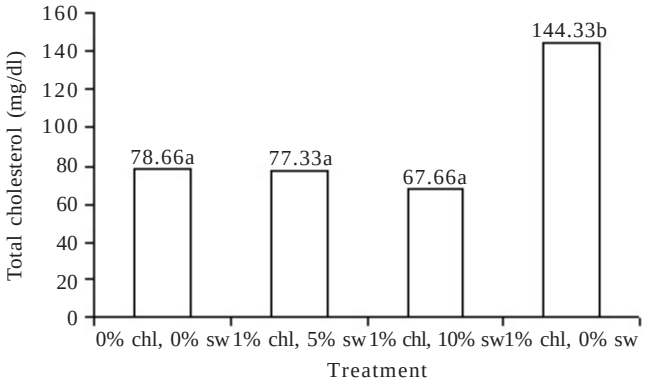

Figure 2. The effect of rat diet containing cholesterol (chl) and seaweed (sw) powder on serum total cholesterol of rats.

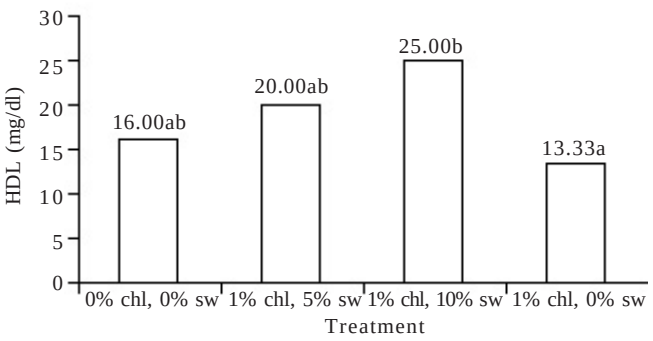

Figure 4. The effect of rat diet containing cholesterol (chl) and seaweed (sw) powder on serum high density lipoprotein (HDL) of rats.

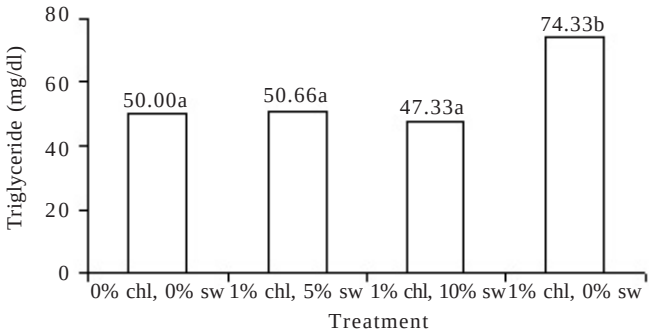

Figure 5. The effects of rat diet containing cholesterol (chl) and seaweed (sw) powder on serum triglyceride of rats.

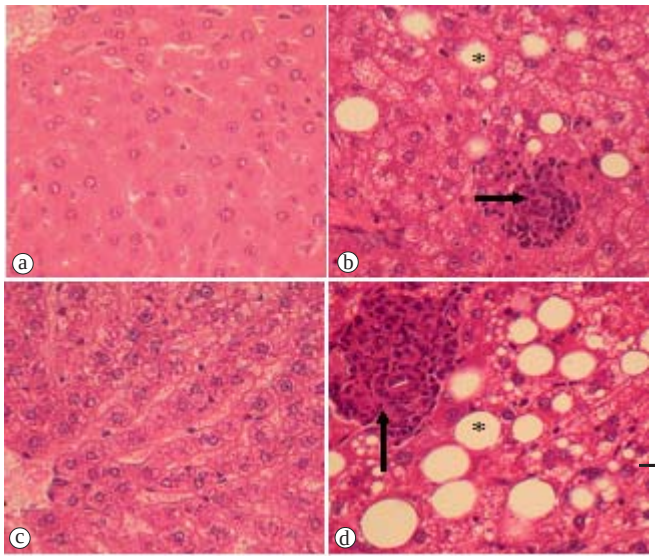

Figure 6. Photomicrograph of liver tissues stained with haematoxylin eosin (HE) showing lipid degeneration (*) and inflammation ( ) in hypercholesterolemic rats. The histopathological evidences showed a recovery by the treatment of seaweed powder (sw), especially in dose of 10% sw. a: 0% cholesterol & 0% sw, b: 1% cholesterol & 5% sw, c: 1% cholesterol & 10% sw, d: 1% cholesterol & 0% sw.

lipid degeneration, remarkable inflammation, as well as degeneration to necrose of some hepatocytes. The treatment of 5 and 10% seaweed powder showed recovery effects to the liver tissues of hypercholesterolemic rats; decreasing the condition of lipid degeneration, inflammation, and degeneration of hepatocytes. Treatment of 10% seaweed powder showed better effects than 5% seaweed powder treatment (Figure 6).

Immunohistochemistry. Cu,Zn-SOD was immunohistochemically localized in the nuclei and cytoplasm of the hepatocytes. The positive reaction product of the enzyme in the liver tissues showed brown colour in the tissues. Qualitatively observation of Cu,Zn-SOD in the tissues of hypercholesterolemic group, positive control group, showed the enzyme content decreased compare to the negative control group (Figure 7). It was shown by the intensity of brown colour reaction product in the positive control group that was lower than that of negative control group. The content of Cu,Zn-SOD in the hypercholesterolemic groups treated with

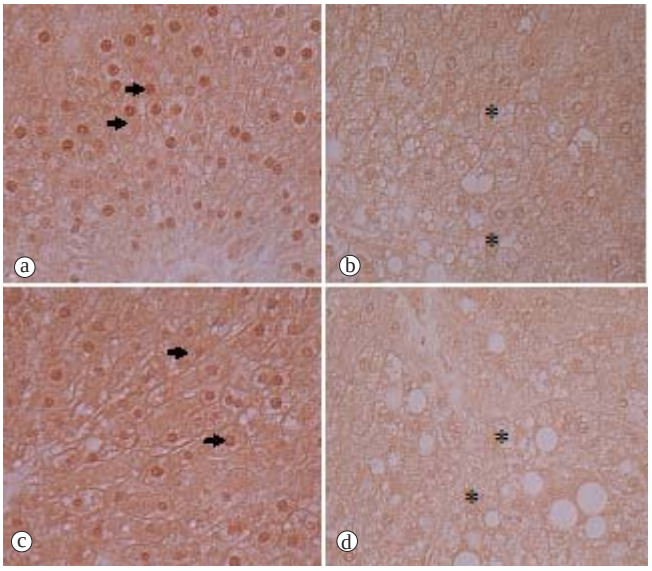

Figure 7. Photomicrograph of antioxidant Cu,Zn-SOD localization using immunohisto-chemical technique in liver tissues of hypercholesterolemic rats treated with seaweed powder. The antioxidant content increased after being treated with seaweed powder (sw), especially in dose of 10% sp. a: 0% cholesterol & 0% sw, b: 1% cholesterol & 5% sw, c: 1% cholesterol & 10% sw, d: 1% cholesterol & 0% sw. *: less imunoreactivity to Cu,Zn-SOD, : high imunoreactivity to Cu,Zn-SOD in both nuclei and cytoplasm of hepatocytes.

seaweed powder increased compared to the positive control group. The treatment of 5% seaweed powder gave slightly increasing on the content of Cu,Zn-SOD, while 10% seaweed powder treatment showed remarkable increased of Cu,Zn-SOD content in the liver tissues of rats compared to that of positive control group (Figure 7).

DISCUSSION

Serum total cholesterol of hypercholesterolemic group (144.33 mg/dl) showed significantly ($P < 0.05$) the highest compared to that of other groups (Figure 2). It showed that feeding with a rat commercial diet containing 1% cholesterol for 40 days increased the level of serum total cholesterol. High concentrations of cholesterol are an indication of a metabolic disorder (sometime inherited) that results in (i) failure to remove lipoproteins from the blood at a sufficient rapid rate, (ii) an overproduction of lipoprotein by the tissues, or (iii) combination of both (i) and (ii). High concentrations of cholesterol may also be caused by (1) lipoprotrein intake that containing cholesterol by receptor – LDL, (2) lipoprotein intake without being mediated by receptor (3) synthesis of cholesterol, and (4) ester cholesterol hydrolysis by ester cholesterol hydrolase (Gurr 1992).

The hypercholesterolemic groups that treated with seaweed powder both 5 and 10% showed serum total cholesterol decreased significantly ($P < 0.05$) compared to the positive control group. The decreasing concentration of cholesterol reached to the level of negative control group (Figure 2). Dietary fiber of seaweed (Table 1) in intestine may bind cholesterol directly, bind bile salts, and inhibit the

circulation of enterohepatic bile salts. The mechanism increase the rate of lipid, cholesterol and bile salts excretion via feces. Subsequently, the level of cholesterol decreased in this study, as reported by Anderson (1994) that dietary fiber decreased the concentration of cholesterol.

The level of LDL in the hypercholesterolemic groups treated with seaweed powder both 5 and 10% also decreased significantly ($P < 0.05$) compared to that in hypercholes terolemic group. LDL transported cholesterol from liver to the pheripheral tissues. It contains 60-70% of total cholesterol (Jeppesen *et al*. 1998), so there is a relationship beetwen LDL and total cholesterol in the same direction. If total cholesterol decreased then the level of LDL also decreased. Dietary fiber treatment decreased concentration of LDL because there is an inhibition of cholesterol absorbtion in the intestine, subsequently increased the excretion rate of bile salts and cholesterol that bind to the dietary fiber. Because the bile salts are produced from cholesterol, so the rate of cholesterol metabolism increase and subsequently the total cholesterol and LDL decreased. Ara *et al*. (2002) also reported that ethanol extracts of five seaweed species *Solieria robusta, Lyengaria stellata, Colpomenia sinuosa, Spatoglossum asperum,* and *Caulerpa racemosa* significantly decreased the serum total cholesterol, triglyceride, and LDL levels in normal, triton-induced and died-induced hyperlipidaemic rats.

High density lipoprotein (HDL) transports cholesterol from pheripheral tissues to liver, an opposite function of LDL. Dietary fiber of seaweed stimulates the bile salts excretion via feces, so that the reabsortion and transportation of cholesterol to the liver decreased. If LDL concentration decreased then more HDL is needed to obtain cholesterol for bile salts synthesis. This condition stimulates synthesis HDL in the liver then concentration of HDL increased in blood serum, as shown in the hypercholesterolemic group treated with seaweed powder, especially in dose of 10%. The increase of HDL level is very important in decreasing the risk of atherosclerosis. One point increasing of HDL level can decrease 2-3% of the risk to coronary heart disease. The increasing of HDL level was reported prevent myocardial infarctions (MIS) (Johnston 2000).

Triglyceride level of hypercholesterolemic groups showed significantly ($P < 0.05$) the highest compared to the other groups. Serum triglyceride level of hypercholesterolemic groups treated with seaweed powder both 5 and 10% significantly decreased to the the level of negative control group. It is because of cholesterol, LDL, and tryglyceride absorbtion in the same shape namely micell and chilomicrone. The decreasing of triglyceride level have similar patern to the decreasing level of cholesterol and LDL. In the intestine, the micell and chilomicrone can be destroyed by dietary fiber.

Lipid degeneration in the liver tissues stained haematoxylin-eosin of hypercholesterolemic group showed a high level of lipid concentration in the tissues that fail to be transported and metabolized properly as usual as normal condition. It was reported that hypercholesterolemic condition increased lipid peroxidation which give side effect to overproduction of free radicals. To get stable, free radicals take some electrons from biomolecular components that build cell structures such as lipid, protein, and carbohydrate than free radicals caused impairment of cell membrane by change of fluidity, cross linking, structure, and function of cell membrane. These conditions can lead to the cell degeneration and finally necrose or cell dead (Halliwell & Gutteridge 1995). Necrose cells stimulate inflammation condition (Forest *et al*. 1994) as showed in the hypercholesterolemic group.

The synthesis of bile salts takes place in the liver as a main gate to eliminate cholesterol. The synthesis was initiated by 7 α-hydroxylation reaction. 7 α-hydroxylase catalizes the reaction requiring oxygen, NADPH, and cytochrome P-450 oxidase. The hypercholesterolemic condition increases bile salt synthesis then more oxygen and NADPH are needed, as well as increasing activity of cytochrome P-450 oxidase. Cytochrome P-450 oxidase also played a role in the reticulum endoplasmic metabolism resulting in free radicals, anion superoxide (Dhaunsi *et al*. 1992). The increasing activity of cytochrome P-450 produces more free radicals. It was also reported that hyperlipidemic condition increased xanthine oxidoreductase (XO) producing free radicals (Scheuer *et al*. 2000). Therefore, we need more antioxidant to scavenge the free radicals remarkably increasing in hypercholesterolemic condition. Consequently, the antioxidant especially Cu,Zn-SOD decreased in the liver tissues as shown in the hypercholesterolemic group in this study (Figure 7).

The treatment of seaweed powder in the hypercholes terolemic groups decreased the level of serum total cholesterol, LDL, and triglyceride. It was caused by increasing the cholesterol and bile salts excretion via feces, followed by the decrease of the activities supporting in bile salt synthesis from cholesterol. Free radical production, as the side effects of these reactions, also decreases. Subsequently, seaweed powder treatment prevents the processes of hepatocyte degeneration in the liver tissues including inflammation and necrose. This condition can also save the utility of intracellular antioxidant especially Cu,Zn-SOD to neutralize the increase in anion superoxide on hypercholesterolemic condition. These results also showed that seaweed *E. cottonii* powder has an antioxidant activity, which could maintain the profile of SOD in the liver tissues under hypercholesterolemic condition. The antioxidant content in seaweed would appear to neutralized free radicals, such as lipid radicals, to more stable products. Subsequently, (i) the alteration of the cellular membrane is lower and the hepatocyte degeneration including inflammation and necrose are also lower, (ii) intracellular antioxidant-Cu,Zn-SOD can be saved in to neutralize the increase of anion superoxide resulting from hypercholes-terolemic condition. Finally, the Cu,Zn-SOD content in the liver tissues was higher in the 10% seaweed powder treated group compared to that of the hypercholesterolemic group without treatment of seaweed powder. The 5% seaweed powder may not enough contain of antioxidant compound that can be used to neutralized the free radicals produced under hypercholesterolemic condition.

It was reported that red seaweed *Callophyllis japonica* exhibits antioxidant properties, *in vitro* (Kang *et al.* 2005). Chen *et al.* (2006) also reported that agaro-oligosaccharides derived from red seaweed can exert their *in vitro* and *in vivo*

hepatoprotective effect through scavenging oxidative damage induce by reactive oxygen species (ROS). Brown seaweed-Tasco was also reported enhance antioxidant status and immune function in heat-induced oxidative stress in lambs (Saker *et al*. 2004).

The study concluded that the dietary fiber of seaweed decreased serum total cholesterol, LDL, and triglyceride, meanwhile increasing the HDL level of hypercholesterolemic rats. The treatment of seaweed powder increased immunohistochemically the content of Cu,Zn-SOD in the liver tissues of the rat under hypercholecterolemic condition. The treatments was also prevent the hepatocytes degeneration including inflammation and necrose. The dose of 10% seaweed powder gave better results than that of 5%. These results suggested to the hypercholesterolemic patients to consume frequently of dietary fibers.

ACKNOWLEDGEMENT

This study was part of a research project funded by a grant (Basic Research) from the Ministry of Education of Indonesia No.026/SPPP/PP-PM/DP3M/IV/2005 to Tutik Wresdiyati in 2005.

REFERENCES

Anderson H. 1994. Effects of cabohydrates on the excretion of bile acids, cholesterol, and fat from the small bowel. *Am J Clin Nutr* 59:785 (suppl).

[AOAC] Association of Official Analytical Chemistry. 1995. Method of Analysis. Washington: Association of Official Analytical Chemistry.

Ara J, Sultana V, Qasim R, Ahmad VU. 2002. Hypolipidaemic activity of seaweed from Karachi coast. *Phytother Res* 16:479-483.

Asp NG, Johansson CG, Hallmer H, Siljestrom M. 1983. Rapid enzymatic assay of insoluble and soluble dietary fiber. *J Agric Food Chem* 31:476-482.

Astawan M. 1998. Penggunaan serat makanan untuk pencegahan berbagai penyakit. *J Ilmu Tek Pangan* 3:41-51.

Chen H, Yan X, Zhu P, Lin J. 2006. Antioxidant activity and hepatoprotective potential of agaro-oligosaccharides *in vitro* and *in vivo*. *Nutr J* 2:5-31.

Dhaunsi GS *et al*. 1992. Demonstration of Cu,Zn-superoxide dismutase in rat liver peroxisomes. *J Biol Chem* 267:6870-6873.

Dobashi K, Asayama K, Kato K, Kobayashi M, Kawaoi A. 1989. Immuhistochemical localization and quantitative analysis of superoxide dismutase in rat tissue. *Acta Histochem Cytochem* 22:351-365.

Forrest VJ, Kang YH, Mc Clain DE, Robinson DH, Ramakrishnan N. 1994. Oxidative stress apoptosis prevented by trolox. *Free Rad Biol Med* 16:675-683.

Fridovich I. 1975. Superoxide dismutases. *Ann Rev Biochem* 44:147-159.

Furuta A *et al*. 1995. Localization of superoxide dismutase in Alzheimer's disease and Down's syndrome neocortex and hippocampus. *Am J Pathol* 146:357-367.

Gurr MI. 1992. *Role of Fats in Food and Nutrition*. 2nd ed. London: Elsevier Applied Science. p 156-159.

Halliwell B, Guttridge JMC. 1995. *Free Radicals in Biology and Medicine*. 3rd Ed. Oxford: Clarendon Pr. p 301.

Jeppesen J, Hein HD, Suadicani, Gyntelberg F. 1998. Tryglyceride concentration and ischaemic heart diseases in the copenhagen male study. *Circulation* 97:1029-1036.

Johnston JD. 2000. Cholesterol synthesis and metabolism. *Clinic chall moder hyperchol* 97:1029-1036.

Kang KA *et al*. 2005. Antioxidant activity of ethanol extract of *Callophyllis japonica*. *Phytother Res* 19:506-510.

Keller GA, Warner TG, Steimer KS, Hallewell RA. 1991. Cu,Zn superoxide dismutase is a peroxisomal enzyme in human fibroblasts and hepatoma cells. *Proc Natl Acad Sci USA* 88:7381-7385.

Marklund SL. 1984. Extracellular superoxide dsimutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species. *Biochem J* 222:649-655.

Mates JM, Gomez CP, Castro IN. 1999. Antioxidant enzymes and human diseases. *Clin Biochem* 32:595-603.

Saker KE, Fike JH, Veit H, Ward DL. 2004. Brown seaweed- (Tasco) treated conserved forage enhances antioxidant status and immune function in heat-stressed wether lambs. *J Anim Physiol Nutr (Berl)* 88:122-130.

Srivastava S *et al*. 2007. Downregulation of CuZn-superoxide dismutase contributes to β-adrenergic receptor-mediated oxidative stress in the heart. *Cardiovas Res* 74:445-455.

Scheuer H *et al*. 2000. Oxidant stress in hyperlipidemia-induced renal damage. *Am J Physiol Renal Physiol* 278:F63-74.

Schuler P. 1990. Natural antioxidants exploitedcommercially. In: Hudson BJF (ed). *Food Antioxidants*. London: Elsevier Applied Science. p 99-170.

Steel RGD, Torrie JH. 1986. *Principles and Procedures of Statistics, A Biometrical Approach*. Singapore: McGraw-Hill Book Company.

Sugino N *et al*. 2000. Decreased superoxide dismutase expression and increased concentrations of lipid peroxide and prostaglandin F2a in the decidua of failed pregnancy. *Mol Human Reprod* 6:642-647.

Wresdiyati T. 2003. Immunohistochemical study of oxygen-free radical scavenger superoxide dismutase (Cu,Zn-SOD) in the liver of rats under stress condition. *Biota* 8:107-112.

Wresdiyati T, Astawan M, Hastanti LY. 2006a. Profil imunohistokimia antioksidan superoksida dismutase (SOD) pada jaringan hati tikus di bawah kondisi hiperkolesterolemia. *Hayati* 13:85-89.

Wresdiyati T, Astawan M, Nurwati VD. 2006b. Kondisi hiperkolesterolemia turunkan level antioksidan superoksida dismutase (SOD) pada jaringan ginjal tikus: suatu kajian imunohistokimia. *J Sain Vet* 24:168-176.

Wresdiyati T, Lelana RPA, Adnyane IKM, Noor K. 2003. Immunohistochemical study of superoxide dismutase (SOD) in the liver of diabetic experiment *Macaca fascicularis*. *Hayati* 10:61-65.

Wresdiyati T, Makita T. 1997. Immunocytochemical localization of Cu, Zn-SOD (Cooper, zinc-superoxide dismutase) in the renal tubules and glomerulus of rat kidney. *Mol Biol Cell* 8:342.

Wresdiyati T, Mamba K, Adnyane IKM, Aisyah US. 2002. The effect of stress condition on the intracellular antioxidant copper,zinc-superoxide dismutase in the rat kidney: an immunohistochemical study. *Hayati* 9:85-88.