

## ANTIOXIDANT ACTIVITY OF GINGER (*Zingiber officinale*) OLEORESIN ON THE PROFILE OF SUPEROXIDE DISMUTASE (SOD) IN THE KIDNEY OF RATS UNDER STRESS CONDITIONS

Tutik Wresdiyati <sup>1)</sup>, Made Astawan <sup>2)</sup>, Deddy Muchtadi <sup>2)</sup>, and Yana Nurdiana <sup>2)</sup>

<sup>1)</sup>Department of Anatomy, Physiology, and Pharmacology, Faculty of Veterinary Medicine –IPB

<sup>2)</sup>Department of Food Science and Technology, Faculty of Agricultural Technology –IPB  
Bogor Agricultural University, Jalan Agatis - IPB Darmaga Bogor 16680, INDONESIA.

Diterima 11 Juli 2007/ Disetujui 18 Februari 2008

### ABSTRACT

Stress condition has been reported to decrease intracellular antioxidant-superoxide dismutase (SOD) in the liver and kidney of rats. This study was conducted to evaluate the antioxidant activities of ginger oleoresin on the profile of superoxide dismutase (SOD) in the kidney of rats under stress condition. The stress condition was achieved by five days of fasting together with swimming for 5 min/day. Ginger oleoresin was orally administrated in a dose of 60 mg/KgBW/day for seven days. Drinking water was provided ad libitum to all groups. The treatment of ginger oleoresin significantly decreased malondialdehyde (MDA) levels and increased SOD activity, as well as immunohistochemically, increased the content of copper, zinc-SOD (Cu,Zn-SOD) in the kidney tissues compared to that of untreated group. The antioxidant content in ginger oleoresin such as shogaol, zingeron, and gingerol, etc. were shown to have activities in the kidney tissues of rats under stress condition that is increasing the profile of SOD. Ginger oleoresin treatment in combination both before and after stress gave the best results.

**Key words :** antioxidant, ginger oleoresin, kidney, stress, superoxide dismutase (SOD)

### INTRODUCTION

Wresdiyati and Makita (1995) reported that stressful conditions morphologically altered the kidney of Japanese monkeys, and increased the number of peroxisomes in the organ. It was also reported that stress produced inflammation and decreased the intracellular antioxidant-Cu,Zn-SOD in the liver and kidney of rats (Wresdiyati et al., 2002; Wresdiyati 2003a; Wresdiyati 2003b). These alterations may account for the fasting stress condition, inducing production of reactive oxygen species-free radical and thereby creating the situation known as oxidative stress. Oxidative stress can lead to a variety of biochemical and physiological lesions often resulting in metabolic impairment and cell death. These highly reactive oxygen radicals 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).

Ginger (*Zingiber officinale* Roscoe) has been used in many ways since long time ago. It is known to be effective as an appetite enhancer, an improver of digestive system, and an anti-cold remedy. Oleoresin is a non volatile compound in most of rhizomes. Oleoresin can be obtained by extracted the rhizomes using methanol or ethanol. Rhizomes oleoresin is well known to contain phenolic compounds which play a role as antioxidant. Wresdiyati et al., (2003) and Wresdiyati

(2003a) reported that *in vivo* methanol extract of ginger oleoresin has anti inflammatory effects on the livers and kidneys of rats under stress conditions. Ginger oleoresin has also been reported to have more antioxidative effect than  $\alpha$ -tocopherol *in vitro* (Kikuzaki & Nakatani 1993). However, there are few reports of the antioxidant activities of ginger oleoresin on the profile of intracellular antioxidant-superoxide dismutase (SOD) in kidney tissue, *in vivo*, especially under condition of stress.

The present study was conducted to examine the role of antioxidant activity of ginger extract-oleoresin on the profile of SOD activity in kidney tissue *in vivo* especially under stress condition.

### METHODS

#### Materials and equipments

Ten month old ginger (*Zingiber officinale*) was used for this study. It was obtained from Balitro Cimanggu, Deptan Bogor. A total of 60 male Wistar rats (250  $\pm$  5 g) were used as experimental animals.

Some chemicals used for this study were ethanol, methanol, GF-254, hexane, diethyleter, linoleic acid, ammonium thiocyanate 30%, FeCl<sub>2</sub>.4H<sub>2</sub>O 20mM, HCl 3,5%, Bouin solution, xylol, paraffin, hematoxylin, eosin, monoclonal antibody Cu,Zn-SOD from Sigma (S2147), secondary labelled antibody from Dako (K1491), diaminobenzidine, Na<sub>2</sub>HPO<sub>4</sub>.12H<sub>2</sub>O, NaH<sub>2</sub>PO<sub>4</sub>.H<sub>2</sub>O, NaCl, Trisma HCl, and Trisma Base.

Spectrofotometer, spectrofluorometer Shimadzu RF 540, incubator, oven blower, rotaryvacum-evaporator, centrifuge, tissue embedding console, microtome, humid chamber for immunohistochemical analysis, vortex, and micropipet were used for this study.

**Extraction of ginger oleoresin**

The ginger was cleaned and dried in an oven (40-60°C) for 30-36 hr to obtain dried ginger with 8-11% water content. The dried ginger was ground and then sieved to obtain ginger powder of 30 mesh. A total of 250 g of ginger powder was extracted four times using two kinds of solvents (500 ml), methanol and ethanol. The resulting extract then was sieved under vacuum condition, and distilled using a rotary vacum-evaporator. The resulting oleoresin was a light to dark brown solid materials. The oleoresin was weighed.

**Total phenol of ginger oleoresin analysis**

Total phenol was analysed in both methanol and ethanol extracts of ginger oleoresin by AOAC 1984 method. 0.1 ml oleoresin was added to 75 ml of distilled water, 10 ml Folin-ciocalteu and 10 ml Na<sub>2</sub>CO<sub>3</sub>. The solution was then made up to 100 ml with distilled water, mixed and left at room temperature for 30 minutes. Absorbance of λ760 nm of the solution was then read by a spectrophotometer. The negative control was made by replacing the 0.1 ml oleoresin with 0.1 ml distilled water. The standard of phenol is tannic acid.

**Antioxidant activity analysis of ginger oleoresin**

A 200 ppm ginger oleoresin was added to 2 ml linoleic acid (5C n.m) in 99.8% ethanol, 2 ml phosphate buffer (0.1 M pH 7.0), and 1 ml ionized water. The solution then was incubated at 37°C. Every two days during 14 days, 50 µl of the solution added 2.35 ml ethanol (75%), 50 µl ammonium thiocyanate (30%), and 50 µl FeCl<sub>2</sub>.4H<sub>2</sub>O (20 mM in HCl 3.5%). After 3 minutes, the solution was then observed using spectrofotometer at 500 nm (Chen et al., 1996). Distilled water and alfa tocopherol were also observed for comparison to the ginger oleoresin.

**Identification of ginger oleoresin**

GF-254 plate was heated at 110° C for 4 hours. Ginger oleoresin was put on the start line of the plate, then stored at development media that contain eluent hexane and diethyleter in ratio 3:7. Then let eluent run to the final line. The plate then was taken from the media and shown some separate fractions which have different *Retardation Factor (RF)* from each others. In order to clean and detailed seen of the fractions, reagen *Folin-ciocalteu* was sprayed to the fractions. Rf is ratio distance of oleoresin (start spot to final spot) to the distance of mobile phase.

**Treatment of animals and tissue sampling**

A total of 25 male Wistar rats (250 ± 5 gr BW) were used for this study. The animals were adapted to the situation and conditions of the animal laboratory for 2 weeks, and then divided into 5 treatment groups (Table 1); (1) control group, without treatment of either stress or oleoresin, (2) stress group, treated by fasting and swimming only, without oleoresin, (3) treatment with oleoresin and followed by fasting and swimming, (4) treatment by fasting and swimming followed by treatment with oleoresin, and (5) treatment with oleoresin, followed by the stress regime, and followed again by treatment with oleoresin. Stress condition was created by 5 days of fasting while the animals were given access to only drinking water and making them to swim for 5 min per day. The oleoresin was orally administrated by using a sonde for 7 days. The oleoresin-treated animals were given standard laboratory diet *ad libitum*.

Table 1. Animal groups assigned to stress and ginger oleoresin treatments

Group	Treatment		
	Oleoresin*	Stress**	Oleoresin*
C	-	-	-
S	-	+	-
S+O	-	+	+
O+S	+	+	-
O+S+O	+	+	+

C = control, S = stress, S+O = stress+oleoresin, O+S = oleoresin+stress

O+S+O = oleoresin+stress+oleoresin

+ : treated

- : no treatment

\* : 60 mg/KgBW/day for 7 days

\*\* : fasting stress with only drinking distilled water for 5 days and swimming 5 minutes per day

Tissue sampling was carried out at the end of each treatment. Following cervical dislocation kidney tissues were collected from each animal in all groups. Tissues were then divided into three part and subjected to analysis for malondialdehyde (MDA), SOD activity and immunohistochemical analysis of Cu, Zn-SOD.

**MDA analysis**

A total of 50µl of sample or standard (1,1,3,3-tetraethoxypropane) was added to 1 ml thiobarbituric acid (10 mmol TBA in 75 mmol/L phosphate buffer at PH 3.0), then mixed for 5 sec. The solution was incubated for 60 min at 95 °C. Following cooling for 5 min, added 5 ml 1-butanol, then mixed again for 1 min. The solution was then centrifuged at 4000 rpm for 10 minutes, and the supernatant was then observed by using spectrofluorometer at 515 nm (excitation) and 553 nm (emission) (Conti et al., 1991; Raharjo & Sofos 1993).

### SOD activity analysis

The kidney tissue was homogenized in phosphate buffer PH 7.4 (1:0.5) added and centrifuged at 10.000 rpm for 20 min. The lysate was collected and stored at -20°C. A total of 400 µl cold chloroform/etanol 37.5/62.5 (v/v) was added to 150 µl lysate, and mixed for 3 sec. The solution was then centrifuged at 4400 rpm (4°C) for 10 min. The resulted supernatant was collected and stored at 2-8°C. A total of 2.9 ml xanthine and cytochrome c solution was added to the 50 µl supernatant sample, then mixed slowly using vortex. The reaction begin at the time 50 µl xanthine oxidase was added to the solution and mixed again. Observation was carried out using spectrophotometer. For phosphate buffer blanks were used to substitute for the sample. Distilled water was used as control.

### Immunohistochemical analysis for Cu,Zn-SOD

Kidney tissues obtained from the animals of all groups were fixed in Bouin's solution for 24 hr. After fixation, the tissues were then dehydrated in a series of alcohols and cleared in xylol. The tissues were then embedded in paraffin before microtome sectioning (4 µm thickness). Tissue sections were immunohistochemically stained for Cu,Zn-SOD as described previously (Dobashi et al., 1989) with slight modifications. The tissue sections were washed for 15 min with 3 changes of PBS between each step. After deparaffinization and rehydration, the tissue sections were exposed to 3% H<sub>2</sub>O<sub>2</sub> 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 with the primary antibody of Cu,Zn-SOD (Sigma S2147) at 4°C. The tissues were then incubated with enhanced labelled polymer peroxidase (Dako K1491). The reaction product of antigen-antibody was visualized using diaminobenzidine (DAB). The tissue sections were then counterstained with haematoxylin, followed by dehydrated through a series of alcohols, and cleared with xylol. Finally, the sections were mounted on glass slides with entellan. For the control of staining, tissue sections were incubated with PBS instead of Cu,Zn-SOD antibody. The tissue sections of control staining showed a negative reaction with minimal background staining.

The distribution and frequency of positive reaction product on the tissues of control group were compared qualitatively with those of the treatment groups. The Cu,Zn-SOD reaction products were observed in the glomeruli, tubuli proximalis, tubuli distalis of the kidney. 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.

### Data analysis

The amount of oleoresin and total phenol of ginger extract using methanol and ethanol were

statistically analysis using T-test. The MDA level and SOD activities of kidney tissues of all groups of treated animals were statistically analyzed using ANOVA and Duncan test. The immunoreaction products of the Cu,Zn-SOD were observed qualitatively by means of light microscopy.

## RESULTS AND DISCUSSION

### Oleoresin and total phenol of ginger extract

Methanol appeared to extract more oleoresin than ethanol, but the results were not significantly different. However, the phenolic compound in oleoresin following methanol extraction differed from that extracted by ethanol. Methanol extraction yielded total phenols of 647.22 ± 28.66 mg/mL, whereas ethanol extraction resulted in total phenols 522.22 ± 36.33 mg/mL which was significantly different (P<0.05).

Based on the results obtained using methanol and ethanol extraction, oleoresin extracted with methanol was used in the following study on antioxidative activity of ginger oleoresin in animal treatment.

### Antioxidative activity of ginger oleoresin

Antioxidative activity of ginger oleoresin that was compared to control (aquadest) and α-tocopherol were showed in Figure 1. Ginger oleoresin and α-tocopherol showed have antioxidative activities better than control, and antioxidative activity of ginger oleoresin showed the best from others. Antioxidative activity was decided from how can they stop linoleic acid oxidation to resulting peroxide radical. The radical then oxidize Fe<sup>2+</sup> to Fe<sup>3+</sup> and give red colour. Intensity of red colour showed the score of absorbancy. The figure of antioxidative activity of ginger oleoresin showed lower than α-tocopherol (Figure 1).

### Identification of phenolic compound of ginger oleoresin

The identification was done using GF-254 plate. The plate showed several spots of certain active compound such as gingerol at Rf 0.24, zingeron at Rf 0.32, and shogaol at Rf 0.38 (Figure 2). It showed that ginger which used in this study contain active compounds; gingerol, shogaol, and zingeron. In this study, all of active compounds contain in ginger oleoresin (crude extract) were used to treat the experimental animals. We did not use active compound separately. It was reported that antioxidative activity of oleoresin is bigger than that of each phenolic compound in the oleoresin (Kikuzaki and Nakatani, 1993).

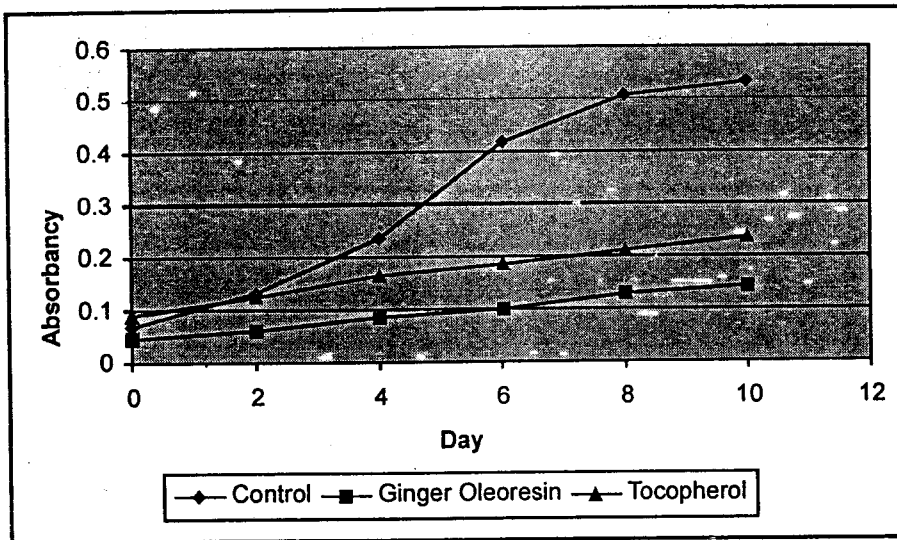


Figure 1. Antioxidative activity of ginger oleoresin,  $\alpha$ -tocopherol and control (aquadest)

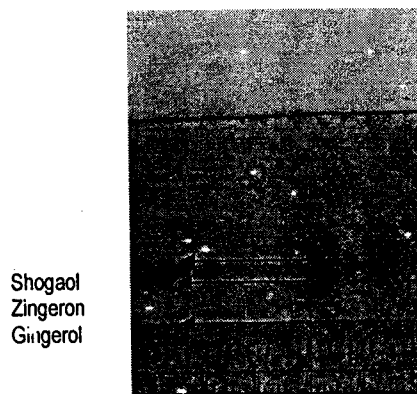


Figure 2. Thin Layer Chromatogram fractionation of phenolic compounds of ginger oleoresin. Ginger oleoresin contain active compounds such as gingerol at (Rf 0.24), zingeron (Rf 0.32), and shogaol (Rf 0.36)

**MDA level of kidney tissues of treated rats**

MDA is one of the final products of lipid peroxidation by free radicals. Thus MDA is used as an indicator of the presence of free radicals and has been widely used for detection of oxidative impairment by free radicals. The high level of MDA indicates a large number of free radicals.

The MDA level ( $\mu\text{mol/g}$  protein) of the control group was the lowest,  $947.47 \pm 29.41 \mu\text{mol/g}$ , and statistically different ( $P < 0.05$ ) to the MDA levels of the other groups. The highest MDA level was  $2290.61 \pm 161.99 \mu\text{mol/g}$  which was found in the stress group (Figure 3). This result showed that stress condition could induce the production of free radicals that reactively oxidized lipids. The MDA level in the stress condition group that was treated with ginger oleoresin was significantly lower ( $P < 0.05$ ) compared to that of the

stress group. The MDA level of stress+oleoresin, oleoresin+stress, and oleoresin+stress+oleoresin groups were  $1378.84 \pm 44.93 \mu\text{mol/g}$ ,  $1163.16 \pm 61.23 \mu\text{mol/g}$ , and  $1002.92 \pm 38.32 \mu\text{mol/g}$  respectively. These results showed that ginger oleoresin has antioxidative activity. The antioxidative activity scavenges free radicals resulting from condition of stress. It showed that the treatment of oleoresin before stress (O+S) more effective than oleoresin treatment after stress condition (S+O). However, a combination of both times of treatment, both before and after stress (O+S+O), gave the best result that was not statistically different compared to that of the control group. This means that ginger oleoresin had decreased the number of free radicals to level found in the normal-control group.

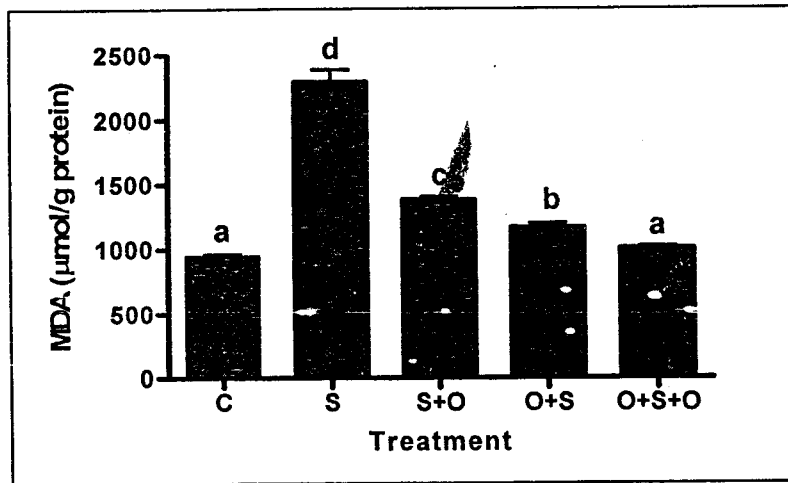


Figure 3. MDA level in the kidney tissues of treated rats. The control (C) group showed the lowest level, while Stress (S) group showed the highest level of MDA. Ginger oleoresin treatment after stress (S+O), before stress (O+S), and combination of both times of treatment (O+S+O) showed decreased MDA level. Different small letters are significantly different (P<0.05).

**SOD activity in the kidney tissues of treated rats**

SOD activity (U/g wet weight) analysis using spectrophotometer showed that the average SOD activity of the stress group was  $1175 \pm 265$  U/g compared to  $4480 \pm 370$  U/g for the control group (P<0.05). The average SOD activity of oleoresin treated groups was higher than that of the stress group, and was  $3195 \pm 185$  U/g in the group which received oleoresin after stress (S+O). This SOD activity was significantly higher (P<0.05) than that of the stress group and not significantly different to that of the control group. This was interpreted to mean that ginger oleoresin treatment

after stress condition (S+O) could increase activity of SOD in the kidney tissue of rats. However, the level remained statistically below the SOD activity in the control group. The average SOD activity of the groups that received oleoresin before stress (O+S) and combination of before and after stress (O+S+O) were  $3745 \pm 385$  U/g and  $3925 \pm 189$  U/g respectively. They were not significantly different to that of the control group. This was interpreted to mean that ginger oleoresin treatment in these latter two groups increased the SOD activity up to the levels under control condition (Figure 4).

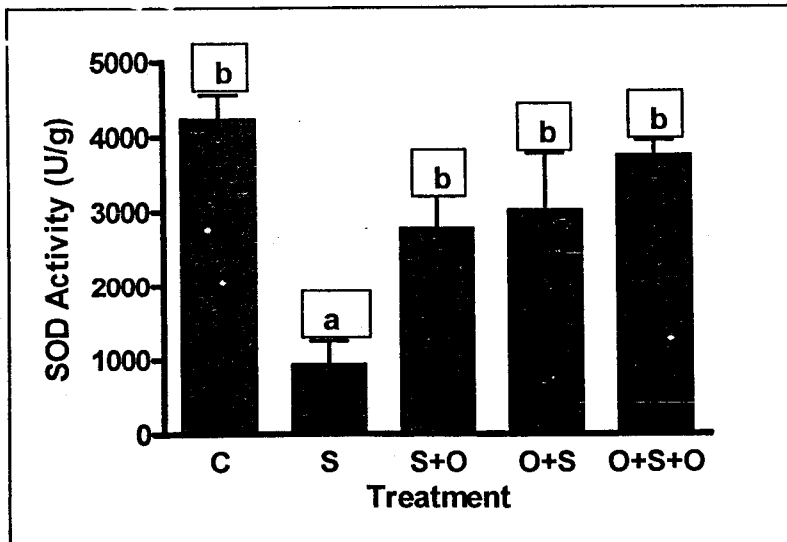


Figure 4. SOD activity in the kidney tissues of treated rats. SOD activity in the control (C) group showed the highest than that of other groups. While the lowest level of SOD activity showed in the stress (S) group. The SOD activity in the ginger oleoresin treated groups; after stress (S+O), before stress (O+S), and combination of both both times of treatment (O+S+O) was higher than that of the stress group and slightly lower than that of the control group. Different small letters are significantly different (P<0.05).

**Immunohistochemically-Cu,Zn-SOD content in the kidneys of treated rats**

The positive reaction products of immunohistochemically localization to the Cu,Zn-SOD showed brown colour in the kidney tissues (Figure 5). The content of the enzyme based on the intensity of brown colour and their distribution in the tissues. The largest amount of Cu,Zn-SOD content was qualitatively found in the control group, which gave the highest intensity of brown colour reaction product and it was distributed in all of part of the tissue. That in the stress group was qualitatively lower compared to that of control group (Table 2 and Figure 5). The oleoresin treated groups showed more Cu,Zn-SOD content compared to that of stress group. The groups that treated ginger oleoresin before stress and combination on both way before and after stress gave higher Cu,Zn-SOD content in the kidney than the group that treated before stress. These results were comparable to the results of SOD activity, where there was an increase of SOD activity in the oleoresin treated groups compared to the stress group.

Table 2. Immunohistochemically-Cu,Zn-SOD content in the kidney tissues of treated rats

Group	Treatment	Content of Cu,Zn-SOD in the kidney
1	C	+++
2	S	+/-
3	S+O	+
4	O+S	++
5	O+S+O	++

C = control, S = stress, S+O = stress+oleoresin,  
 O+S = oleoresin+stress  
 O+S+O = oleoresin+stress+oleoresin

MDA level in the kidney tissues of the stress group was significantly higher compared to that of control group, whereas SOD activity and the Cu,Zn-SOD content in the tissues of the stress group was lower compared to that of control group. These results showed that free radicals were produced under the condition of stress imposed.

It was reported for Japanese monkeys that stress condition resulted in an alteration of the morphological peroxisomes and a remarkable almost three-fold increase in their number in kidney tissues (Wresdiyati & Makita 1995). Increasing the number of peroxisomes may also increase the number of oxidations that take place in the peroxisomes, subsequently more free radicals are produced as a side effect of stress condition. It has also been reported that fasting stress condition increased  $\beta$ -oxidation and cytochrome P-450 oxidase levels (Ishii et al., 1980; Orellana et al., 1992). These conditions result in a remarkable production of free radicals, such as anion superoxide. Anion superoxide can be dismutated by SOD and change to singlet oxygen molecules that able to oxidize unsaturated

fatty acid. The results of this oxidation may alter the cellular membrane as indicated by MDA production.

The remarkable increase in free radicals production results in more intracellular antioxidant, such as SOD, being needed to catalyze these free radicals. Thus the activities and the cellular content of the antioxidant, SOD, is decreased, as is shown by the SOD activity and the content of Cu,Zn-SOD in the kidney tissues of rats under stress condition. They also relate to the elevated MDA level found under stress condition.

Oral administration of ginger oleoresin decreased MDA level and increased both SOD activity and Cu,Zn-SOD content in the kidney tissues of rats under stress condition. These results showed that antioxidant activities of ginger oleoresin could maintain the profile of SOD in the kidney under stress condition. The ginger used in this study contains some phenolic compounds such as gingerol, zingeron, and shogaol (Figure 2). These phenolic compounds, in ginger extract-oleoresin, have antioxidant activity (Figure 1). The phenolic compounds of ginger oleoresin would appear to neutralized free radicals, such as lipid radicals, to more stable products by transferring their hydrogen molecules to the free radicals. Subsequently, alteration of the cellular membrane is lower and MDA levels are also lower, in this study.

The increased activity of SOD and increased content of Cu,Zn-SOD in the kidney tissues of rats treated with ginger oleoresin under stress condition were both caused by the antioxidant activity of phenolic compounds in the ginger oleoresin. The phenolic compounds neutralized the free radicals, anion superoxide, which would normally be done by SOD. Consequently, intracellular antioxidant-SOD can be saved in both number and activity to neutralize the increase in anion superoxide that results from stress condition. Finally, the SOD activity and Cu,Zn-SOD content in the kidney tissues was higher in the ginger oleoresin treated group compared to that of the stress group without treatment of ginger oleoresin.

Oleoresin treatment, before and after stress condition, had the best effect on kidney tissues of rat by increased SOD activity and Cu,Zn-SOD content, as well as decreased MDA levels. It has been reported that 88 to 95% of phenolic compounds can be absorbed into the body, and that retention in the body is from 62% to 95% (Desminarti 2001). In the current experiments absorption of the phenolic compounds took place in the small intestine. Extending the timespan of oleoresin treatment (before and after stress) in this experiments appeared to have caused more phenolic compounds to be absorbed and retained in the tissues, resulting in a stronger and more positive effect of these compound.

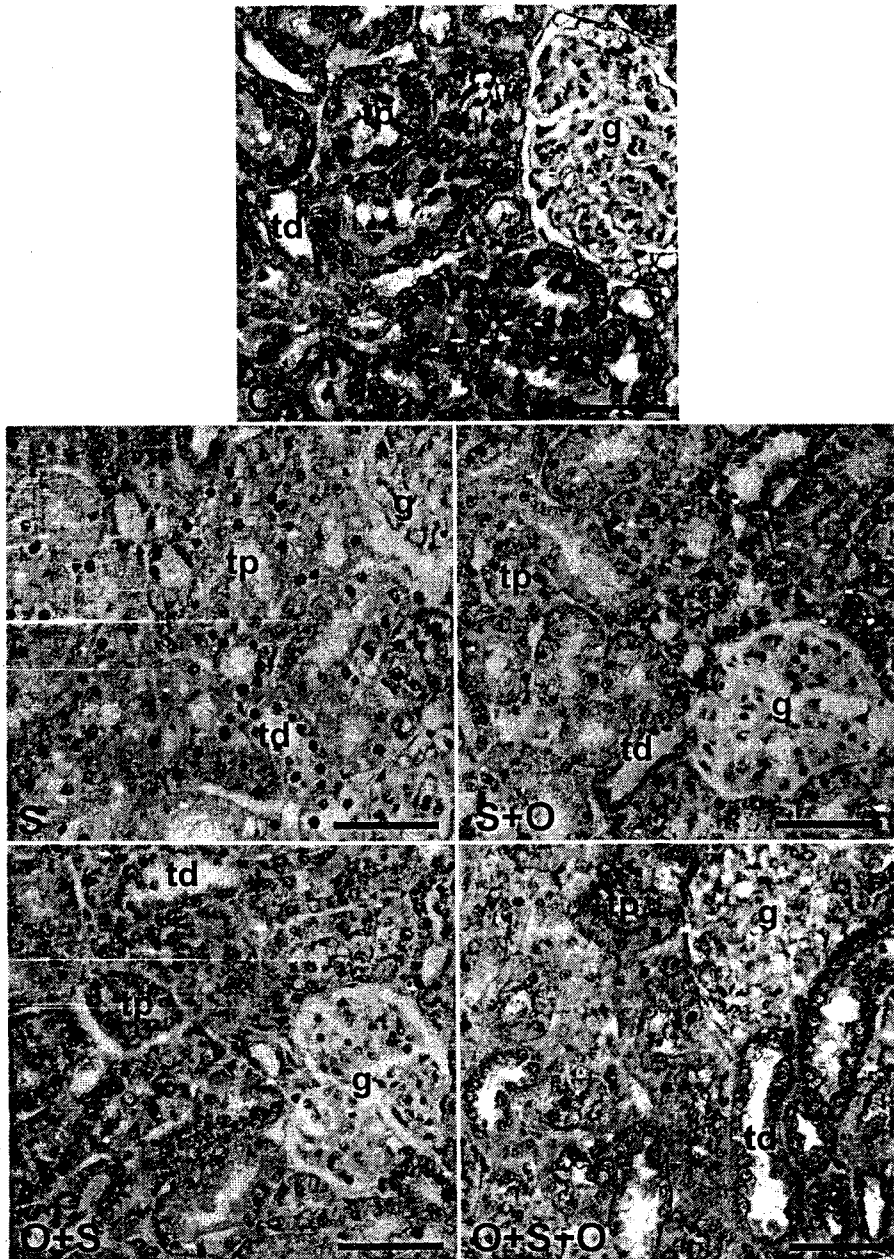


Figure 5. Photomicrograph of immunohistochemical localization of Cu,Zn-SOD in the kidney tissues of treated rats. The content of Cu,Zn-SOD in control (C) group qualitatively showed the highest than that of others group. While the lowest level of Cu,Zn-SOD content showed in the stress (S) group. The Cu,Zn-SOD in the ginger oleoresin treated groups ; after stress (S+O), before stress (O+S), and combination of both times of treatment (O+S+O) qualitatively showed increased compared to that of stress group. g=glomeruli, td=tubuli distalis, tp=tubuli proximalis, — = 50  $\mu$ m (400X magnification).

## CONCLUSION

The study concluded that the antioxidant activity of ginger (*Zingiber officinale*) oleoresin increased SOD activity and the content of Cu,Zn-SOD in the kidney of the rat under stress condition. The antioxidant activity was also shown by the reduced level of MDA in the tissues of rats under stress condition. These results were shown in the groups of rats that were given by ginger oleoresin whether before or after stress condition. However, the group of rats that were given by ginger oleoresin both before and after stress showed the best results.

## ACKNOWLEDGMENT

This study was part of a research project funded by a grant (*Hibah Bersaing X*) from the Ministry of Education of Indonesia No. 103/LII/BPPK-SDM/IV/2002 for Tutik Wresdiyati in 2003.

## REFERENCES

- AOAC. 1984. Official Methodes of Analysis of the Assosiation of Official Agricultural Chemist. Washington : AOAC Inc.
- Chen, H.M.K., Muramoto, K., and Yamauchi, F. 1996. Structural Analysis of Antioxidative Peptides from Soybean  $\beta$ -Conglicinin. *Agria Food Chem* 43:574-578.
- Conti, M., Moramd, P.C., Levillain, P., and Lemonnier, A. 1991. Improve Flurometric Determination of Malonaldehyde. *J Clin Chem* 37/7 : 1273-1275.
- Desminarti, S. 2001. Study on Dietary Fibers and Natural Antioxidant of some Plants and Their Absortion and Retention in the Rats [Thesis]. Bogor : Faculty of Agricultural Technology, Bogor Agricultural University.
- Dobashi, K., Asayama, K., Kato, K., Kobayashi, M., and Kawaoui, A. 1989. Immuhistochemical localization and quantitative analysis of superoxide dismutase in rat tissue. *Acta Histochem Cytochem* 22:351-365.
- Halliwell, B., and Guttridge, J.M.C. 1995. *Free radicals in biology and medicine*. 3<sup>rd</sup> Ed. Oxford : Clarendon Press. Pp.301
- Ishii, H., Horie, S., and Suga T. 1980. Physiological role of peroxisomal  $\beta$ -oxidation in the liver of fasted rats. *J Biochem* 87:1855-1858
- Kikuzaki, H., and Nakatani, N. 1993. Antioxidant effect of some ginger constituents. *J Food Sci* 58:1407-1410.
- Orellana, M., Fuentes, O., Rosenbluth, H., Lara, M., and Valdes, F. 1992. Modulation of rats liver peroxisomal and microsomal fatty acids oxidation by starvation. *FEBS* 310:193-196.
- Raharjo, S., and Sofos, J.N. 1993. Methodology for Measuring Malonaldehyde as a Product of Lipid Peroxidation in Muscle Tissues : a Review. *Meat Sci* 35:145-169.
- Wresdiyati, T., and Makita, T. 1995. Remarkable increase of peroxisomes in the renal tubule cells of Japanese monkeys under fasting stress. *Pathophysiol* 2:177-182.
- Wresdiyati, T., Mamba, K., Adnyane, I.K.M., and Aisyah, U.S. 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.
- Wresdiyati, T. 2003a. The utilization of ginger (*Zingiber officinale*) oleoresin as anti inflammation on the liver of rats under stress condition. *J Vet* 4:154-163.
- Wresdiyati, T. 2003b. Immunohistochemical study of oxygen-free radical scavenger superoxide dismutase (Cu,Zn-SOD) in the liver of rats under stress condition. *Biotra* 8:107-112
- Wresdiyati, T., Astawan, M., and Adnyane, I.K.M. 2003. Anti inflammation activity of ginger (*Zingiber officinale*) extract on the kidney of rats under stress condition. *J Tech and Food Industry*. 14:113-120



## PENGHAMBATAN OKSIDASI LDL DAN AKUMULASI KOLESTEROL PADA MAKROFAG OLEH EKSTRAK TEMULAWAK (*Curcuma xanthorrhiza* Roxb)

[The Inhibition of Low Density Lipoprotein Oxidation and Cholesterol Accumulation on the Macrophage by Temulawak Extract]

Aisyah Tri Septiana<sup>1)</sup>, Hidayah Dwiyanti<sup>1)</sup>, Deddy Muchtadi<sup>2)</sup>, dan Fransiska Zakaria<sup>2)</sup>

<sup>1)</sup> Jurusan Teknologi Pertanian, Fakultas Pertanian, UNSOED Purwokerto

<sup>2)</sup> Program Studi Ilmu Pangan, Fakultas Teknologi Pertanian, IPB Bogor

Diterima 15 Desember 2006 / Disetujui 10 Mei 2007

### ABSTRACT

Coronary heart disease is caused among others by atherosclerosis, which is the result of oxidized low density lipoprotein (LDL) and cholesterol accumulation on the macrophage. This were reported to be inhibited by temulawak (*Curcuma xanthorrhiza* Roxb). The objective of this study was to find out the types and concentrations of temulawak extract which could inhibit LDL oxidation, and to find out the effect of temulawak extract on the accumulation of cholesterol on macrophage. Temulawak was extracted by water, ethanol, acetone and dichlorometane. Inhibition of LDL oxidation was analyzed by measuring the level of malonaldehyde content of the oxidized LDL-CuSO<sub>4</sub> which were given water extract, ethanol extract, acetone extract and dichlorometane extract. of temulawak at concentrations of 43 µg, 430 µg, and 4300 µg per ml of LDL. The percentage of malonaldehyde reduction due to addition of water, ethanol, acetone and dichlorometane extract were 44.27; 47.68; 51.83 and 61.2 respectively. The inhibition of LDL oxidation by temulawak extract depends on the concentrations. The percentage of malonaldehyde reduction due to addition of temulawak extract of 43 µg, 430 µg, and 4300 µg per ml of LDL were 43.63; 56.72; and 53.89. Concentrations of temulawak extract resulting in the highest inhibition of LDL oxidation was 430 µg/ml LDL. Temulawak extract tends to inhibit cholesterol accumulation on the macrophage. There is a correlation between the inhibition of cholesterol accumulation on the macrophage and the inhibition of LDL oxidation by temulawak extract.

**Key words :** Low density lipoprotein, macrophage, cholesterol, temulawak (*Curcuma xanthorrhiza* Roxb)

### PENDAHULUAN

Saat ini, penyakit jantung koroner (PJK) merupakan penyebab utama kematian penduduk dunia termasuk penduduk Indonesia berusia di atas 40 tahun. Pada tahun 1995, PJK menyebabkan kematian sekitar 15 juta jiwa atau sekitar 30 persen dari total penyakit penyebab kematian, pada tahun 2020 diperkirakan meningkat mencapai 40 persen (WHO, 2001).

Penyakit Jantung Koroner (PJK) antara lain disebabkan oleh aterosklerosis, yaitu penyakit degeneratif pada arteri besar dan menengah yang ditandai dengan penimbunan lipid dan fibrosis. *Low density lipoprotein* (LDL) atau lipoprotein densitas rendah yang teroksidasi merupakan faktor penting dalam pembentukan aterosklerosis. LDL yang telah teroksidasi dapat dikenali oleh reseptor *scavenger* makrofag tetapi tidak dikenali oleh reseptor LDL. Pengambilan LDL yang termodifikasikan oleh makrofag melalui reseptor *scavenger* dapat mengakibatkan akumulasi kolesterol yang selanjutnya tersimpan di dalam bentuk titik-titik lemak, sehingga makrofag berubah menjadi sel-sel menyerupai sel busa (Brown dan Goldstein, 1983).

Antioksidan memberikan perlindungan kepada LDL dari proses oksidasi (Septiana, 2001).

Salah satu jenis tanaman obat yang berpotensi sebagai antioksidan alami adalah temulawak (*Curcuma xanthorrhiza* Roxb) yang termasuk di dalam famili *Zingiberaceae*. Rimpang temulawak mengandung kurkuminoid. Komponen utama kurkuminoid temulawak yaitu kurkumin dan desmetoksikurkumin (Purseglowe et al., 1981). Ekstrak etanol-air dari kunyit yang mengandung 10 % kurkumin dapat menghambat oksidasi LDL atau lipoprotein densitas rendah (Ramirez-Tortosa et al., 1998), sehingga diduga temulawak yang mengandung kurkumin dapat menghambat oksidasi LDL sebagai tahap awal dari aterosklerosis.

Kadar dan aktivitas kurkumin dan antioksidan lain yang terekstraksi oleh berbagai pelarut berbagai polaritas, seperti air, etanol, aseton, dan diklorometan mungkin berbeda sehingga penghambatan oksidasi LDL dan akumulasi kolesterol yang dihasilkan berbeda-beda pula. Selain itu, hasil penelitian Septiana (2001) menunjukkan bahwa ekstrak air jahe yang sangat polar lebih mampu menghambat akumulasi kolesterol dari pada ekstrak diklorometan jahe meskipun aktivitas antioksidan ekstrak air lebih kecil dari ekstrak diklorometan jahe. Untuk melihat pengaruh aktivitas ekstrak temulawak terhadap penghambatan akumulasi kolesterol pada makrofag maka berbagai ekstrak

temulawak disuplementasikan pada LDL karena secara *in vivo* di dalam tubuh, temulawak yang dikonsumsi dapat tersuplementasi di dalam LDL.

Selain pelarut, konsentrasi antioksidan diduga juga berpengaruh terhadap aktivitas antioksidan dari ekstrak temulawak tersebut. Esterbauer et al., (1991) melaporkan bahwa suplementasi  $\alpha$ -tokoferol sebesar 1000 nmol/ml plasma (BM 430,7) (setara 430  $\mu$ g/plasma) terbukti paling mampu menghambat terjadinya oksidasi dibandingkan dengan konsentrasi 125, 250, maupun 500 nmol/ml plasma.

Tujuan penelitian ini adalah untuk membandingkan penghambatan oksidasi LDL dengan perlakuan suplementasi LDL menggunakan berbagai ekstrak temulawak hasil ekstraksi pelarut air, etanol, aseton, dan diklorometan pada konsentrasi yang berbeda. Penelitian juga bertujuan untuk mengetahui pengaruh polaritas ekstrak temulawak terhadap akumulasi kolesterol makrofag.

## METODOLOGI

### Bahan dan alat

Bahan baku yang digunakan untuk penelitian adalah rimpang temulawak (*Curcuma xanthorrhiza*), yang berumur 10 bulan dari Perkebunan BALITRO Cimanggu, Bogor. Pelarut yang digunakan untuk ekstraksi adalah etanol, diklorometan dan aseton (Merck) dan air.

Isolasi LDL manusia membutuhkan darah lelaki yang sehat, EDTA, NaCl, KBr dan  $\text{NaHCO}_3$ . Analisis LDL membutuhkan NaCl,  $\text{NaHCO}_3$ , EDTA, TCA,  $\text{CuSO}_4$  masing-masing dari Merck, thiobarbituric acid/TBA dan tetra etoksi propana (Sigma) serta air bebas ion (UGM). Isolasi makrofag peritoneal menggunakan mencit Balb-C jantan, tioglikolat, tripan blue, RPMI-1640, serum janin sapi/fetal bovine serum (FBS), N-2-hidroksimetil-piperazine-N-2-etan-sulfonic acid (HEPES), L-glutamin, penisillin-streptomisin, fungizon, dan fosfat bufer salin (PBS). Analisis kadar kolesterol makrofag membutuhkan tetrametil amonium hidroksida-isopropanol (TMH-I), tetrakloroetilen, dan metilbutirat serta bahan-bahan lain untuk analisis protein sel dan kadar malonaldehid (MDA) dari LDL.

Peralatan yang digunakan adalah: blender, parut, pengering beku/*freeze dryer*, timbangan analitik, shaker, evaporator vakum berputar (*rotary vacuum evaporator*) dengan penangas air dan pompa vakum, pendingin dan pembeku, vortex, spektrofotometer UV-vis, mikropipet, penangas air, ultrasentrifus, HPLC (*high performance liquid chromatography*), sentrifus, inkubator  $\text{CO}_2$  dan peralatan gelas.

### Ekstraksi rimpang temulawak

Mula-mula rimpang temulawak diparut, sebagian diekstraksi menggunakan air. Ekstraksi menggunakan air dilakukan dengan cara menambahkan

1 bagian rimpang yang sudah hancur dengan 5 bagian air, diperas, disaring berturut-turut menggunakan kain saring, kertas saring, dan whatman nomor 42. Temulawak parut dan ekstrak air basah dikeringkan menggunakan pengering beku sehingga diperoleh hancuran temulawak kering dan ekstrak air. Hancuran temulawak kering disaring menggunakan pengayak 40 mesh sehingga dihasilkan bubuk temulawak. Bubuk temulawak diekstraksi menggunakan pelarut etanol, aseton, dan diklorometana menggunakan metode Septiana et al., (2002). Sebanyak 100 gram bubuk temulawak di ekstraksi 3 kali menggunakan pelarut tersebut (masing masing 500 ml) pada suhu kamar. Bubuk temulawak serta ekstrak air, etanol, aseton, dan ekstrak diklorometan diuji kadar antioksidannya dengan menganalisis kadar total fenol (Andarwulan dan Shetty, 1999)

### Isolasi LDL

Pada prinsipnya pemisahan LDL dilakukan setelah  $\beta$  very low density lipoprotein (VLDL) yang mempunyai densitas (d) lebih kecil dari 1,006 g/ml dipisahkan menggunakan larutan pemisah densitas yaitu 0,9 % NaCl dan 0,01 % EDTA (b/v) dan ultrasentrifugasi. Kemudian fraksi yang telah dikurangi  $\beta$  VLDLnya diatur densitasnya sampai 1,080 g/ml, dan memisahkan fraksi yang densitasnya (d) lebih besar dari 1,063 g/ml dengan larutan pemisah densitas dan ultrasentrifugasi (Sulistiyani dan St. Clair, 1997).

### Uji penghambatan oksidasi LDL

Suplementasi antioksidan dari ekstrak temulawak pada LDL dilakukan dengan melarutkan antioksidan sebanyak 43, 430, dan 4300  $\mu$ g/ml LDL dalam 10  $\mu$ l pelarut dan kemudian dilakukan inkubasi antioksidan tersebut dengan LDL selama 3 jam, dan selanjutnya dilakukan oksidasi. Oksidasi LDL dilakukan dengan menginkubasi LDL yang telah disuplementasikan dengan antioksidan menggunakan 5  $\mu$ M  $\text{CuSO}_4$  pada larutan 0,9 % NaCl - 1 mM  $\text{NaHCO}_3$  pH 7,4 suhu 37°C selama 90 menit. Reaksi dihentikan dengan penambahan EDTA (konsentrasi akhir 0,1 %) seperti yang dilakukan Suzukawa et al., (1994). Lipid LDL yang teroksidasi diukur dengan menganalisis kadar malonaldehid (Kikuzaki dan Nakatani, 1993). Selain itu dilakukan analisis kadar protein LDL menggunakan metode Lowry (Sulistiyani dan St. Clair, 1997).

### Rancangan percobaan

Penelitian dilaksanakan secara eksperimental menggunakan rancangan dasar Rancangan Acak Lengkap (RAL) pola faktorial 4 x 3 (Sudjana, 1989). Percobaan diulang 3 kali. Faktor yang dicoba meliputi jenis ekstrak (ekstrak air, etanol, aseton, dan ekstrak diklorometan temulawak) dan konsentrasi ekstrak (43, 430, dan 4300  $\mu$ g/ml LDL). Analisis yang dilakukan

adalah kadar malonaldehid dari LDL yang disuplementasikan berbagai ekstrak temulawak. Sebagai data pendukung dilakukan analisis kadar fenol di dalam ekstrak dan kadar protein LDL.

Percobaan akumulasi kolesterol pada makrofag menggunakan Rancangan Acak Lengkap (RAL) dengan faktor perlakuan jenis ekstrak temulawak (ekstrak air, etanol, aseton, dan ekstrak diklorometan temulawak), kontrol (+), kontrol (-) dan  $\alpha$ -tokoferol. Konsentrasi ekstrak temulawak yang disuplementasikan berdasarkan konsentrasi terbaik pada analisis kadar malonaldehid LDL. Percobaan akumulasi kolesterol diulang sebanyak 3 kali sehingga diperoleh 21 unit percobaan.

## HASIL DAN PEMBAHASAN

### Kadar malonaldehid LDL

Malonaldehid (MDA) merupakan salah satu produk hasil peroksidasi asam lemak tidak jenuh. Perbedaan nilai MDA terkait dengan reaksi oksidasi yang terjadi. Kadar MDA berbanding terbalik dengan aktivitas antioksidan. Kadar MDA yang tinggi menunjukkan aktivitas antioksidan yang rendah begitu juga sebaliknya. Rerata nilai MDA LDL yang disuplementasikan dengan berbagai ekstrak temulawak dapat dilihat pada Tabel 1.

Tabel 1. Aktivitas antioksidan ekstrak temulawak berdasarkan pelarutnya

Sampel	Rerata kadar MDA (nmol MDA/ mg prot)	Pengurangan kadar MDA dari kontrol (+) (%)
Kontrol (-)	64,17	-
Kontrol (+)	90,46	-
Ekstrak air	50,41	44,27
Ekstrak etanol	47,33	47,68
Ekstrak aseton	43,57	51,83
Ekstrak diklorometan	35,10	61,20
$\alpha$ -tokoferol	51,65	42,90

Keterangan : Kontrol (-) : LDL tanpa ekstrak temulawak dan tanpa prooksidan  
Kontrol (+) : LDL tanpa ekstrak temulawak, diberi prooksidan  $\text{CuSO}_4$   
Semua sampel dioksidasikan pada  $37^\circ\text{C}$  selama 90 menit

Nilai MDA keempat ekstrak temulawak ternyata lebih rendah dari kontrol (+) yaitu LDL yang tidak diberi ekstrak temulawak tetapi diberi prooksidan  $\text{CuSO}_4$  dan lebih rendah dari pada  $\alpha$ -tokoferol. Hal ini menunjukkan keempatnya berpotensi sebagai sumber antioksidan alami. Ekstrak temulawak yang mengandung senyawa kurkuminoid yaitu kurkumin dan desmetoksikurkumin diduga mampu menghambat peroksidasi lipid tak jenuh ganda yang ada pada LDL. Suroyo (2001) menyatakan

bahwa kurkumin berpotensi menghambat peroksidasi lipid.

Hasil penelitian memperlihatkan bahwa suplementasi ekstrak temulawak pada LDL mengurangi konsentrasi MDA dari LDL yang teroksidasi. Berdasarkan uji terhadap MDA, minuman anggur merah dan jus anggur merah (Miyagi et al., 1997), ekstrak jahe (Septiana, 2001), serta ekstrak etanol-air (hidroalkoholik) dari kunyit yang mengandung 10 % kurkumin (Ramirez-Tortosa et al., 1998) dapat menghambat oksidasi LDL manusia. Seperti halnya pada kunyit, kurkumin merupakan komponen fenolik utama pada temulawak (data tidak ditampilkan).

Penghambatan pembentukan MDA dari LDL teroksidasi (penghambatan oksidasi LDL) oleh ekstrak temulawak sejalan dengan kadar total fenol ekstrak temulawak yang dikandungnya. Hasil penelitian Septiana et al., (2004) menunjukkan bahwa pada umumnya ekstrak zingiberaceae yang mempunyai aktivitas antioksidan tinggi mempunyai kadar total fenol yang tinggi pula. Menurut Gordon (1990) senyawa fenolik berfungsi sebagai antioksidan karena kemampuannya uaiam memberikan atom hidrogen secara cepat kepada radikal lipid, sedangkan radikal bebas yang berasal dari antioksidan senyawa fenol ini lebih stabil daripada radikal bebasnya.

Selain total fenol, aktivitas antioksidan diduga dipengaruhi pula oleh polaritas pelarut yang digunakan untuk mengekstrak. Aktivitas antioksidan ekstrak diklorometan yang lebih tinggi, diduga karena ekstrak diklorometan lebih mudah kontak dengan LDL sehingga lebih melindungi LDL dari oksidasi dibandingkan dengan ekstrak temulawak hasil ekstraksi pelarut yang lain. Substrat LDL yang digunakan bersifat kurang polar, sama dengan diklorometan yang bersifat kurang polar juga sehingga ekstrak diklorometan lebih mampu melindungi LDL dari oksidasi.

Rerata kadar MDA perlakuan konsentrasi ekstrak temulawak 43, 430 dan 4300  $\mu\text{g/ml}$  LDL berturut-turut adalah 50,99; 39,61 dan 41,71 nmol/mg prot (Tabel 2). Pada konsentrasi yang sama (4300  $\mu\text{g/ml}$  LDL), ekstrak temulawak lebih mampu menghambat pembentukan malonaldehid daripada  $\alpha$ -tokoferol (41,71 vs 51,65 nmol/ml LDL). Hasil penelitian menunjukkan bahwa komponen fenolik pada minuman anggur merah lebih mampu menghambat LDL teroksidasi  $\text{CuSO}_4$  dibandingkan dengan  $\alpha$ -tokoferol (Frankel et al., 1993). Penghambatan terhadap malonaldehid dari ekstrak temulawak (Septiana et al., 2004) maupun dari ekstrak kunyit (Jitoe et al., 1992) yang mengandung senyawa kurkuminoid pada asam linoleat, lebih tinggi dari pada  $\alpha$ -tokoferol.

Tabel 2. Aktivitas antioksidan ekstrak temulawak berdasarkan konsentrasinya

Sampel	Rerata kadar MDA (nmol MDA/ mg prot)	Pengurangan kadar MDA dari kontrol (%)
Kontrol (-)	64,17	-
Kontrol (+)	90,46	-
43 µg/ml LDL	50,99	43,63
430 µg/ml LDL	39,61	56,21
4300 µg/ml LDL	41,71	53,89

Keterangan : Kontrol (-) : LDL tanpa ekstrak temulawak dan tanpa prooksidan  
 Kontrol (+) : LDL tanpa ekstrak temulawak, diberi prooksidan CuSO<sub>4</sub>  
 Semua sampel dioksidasi pada 37°C selama 90 menit

Penggunaan tingkat konsentrasi ekstrak temulawak berpengaruh nyata terhadap pembentukan malonaldehid. Konsentrasi sebesar 430 µg/ml LDL paling efektif dalam menghambat pembentukan malonaldehid dari pada konsentrasi 43 µg/ml LDL dan 4300 µg/ml LDL. Gordon (1991) mengemukakan bahwa keefektifan fenol sebagai antioksidan memiliki rentang tertentu sehingga dapat terjadi pada konsentrasi yang rendah fenol menjadi kurang efektif berperan sebagai antioksidan dan pada konsentrasi yang tinggi fenol dapat bersifat sebagai prooksidan. Berdasarkan hasil penelitian ini, konsentrasi ekstrak temulawak yang terbaik dipilih untuk uji penghambatan akumulasi kolesterol dalam makrofag yaitu konsentrasi 430 µg/ml LDL.

#### Akumulasi kolesterol pada makrofag

Dalam uji ini, sel makrofag diinkubasi dengan LDL yang telah disuplementasikan dengan ekstrak temulawak selama 24 jam. Selama inkubasi sel, LDL ditelan oleh sel makrofag dengan mekanisme endositosis melalui reseptor *scavenger* sehingga terjadi akumulasi kolesterol di dalam sel.

Pada uji penghambatan kolesterol di dalam makrofag digunakan kontrol (+) dan kontrol (-) sebagai pembandingan dan sebagai antioksidan standar digunakan  $\alpha$ -tokoferol. Kontrol (+) adalah sel makrofag yang diinkubasikan dengan LDL yang telah diberi 5 µM CuSO<sub>4</sub> sebagai oksidator tanpa adanya suplementasi ekstrak temulawak. Sedangkan kontrol (-) terdiri dari sel makrofag saja. Kedua kontrol tersebut digunakan untuk melihat ada tidaknya aktivitas antioksidan ekstrak temulawak pada LDL dibandingkan dengan sampel dan

kontrolnya. Nilai rerata akumulasi kolesterol (kolesterol ester) dapat dilihat pada Tabel 3.

Data persentase penghambatan yang diperoleh menunjukkan bahwa suplementasi ekstrak temulawak pada LDL menurunkan persen penghambatan akumulasi kolesterol ester dibandingkan dengan kontrol (+). Hal ini diduga karena aktivitas antioksidan pada LDL cenderung berpengaruh terhadap akumulasi kolesterol ester pada makrofag. Semakin berkurangnya LDL yang teroksidasikan, pengambilan LDL tersebut oleh reseptor pemangsa makrofag akan semakin berkurang sehingga cenderung menghambat akumulasi kolesterol pada makrofag dan pembentukan sel busa seperti yang terjadi pada ekstrak aseton, etanol dan ekstrak air temulawak maupun  $\alpha$  tokoferol. Menurut Brown dan Goldstein (1983), sel busa berasal dari makrofag yang mengambil LDL yang telah teroksidasikan melalui reseptor pemangsa makrofag tanpa adanya pengaturan arus balik sehingga kolesterol ester akan terakumulasi.

Ekstrak diklorometan mempunyai persentase penghambatan akumulasi kolesterol ester terendah dibandingkan dengan ekstrak temulawak lainnya. Hasil ini mungkin dipengaruhi oleh hidrofobisitas reseptor permukaan makrofag. Kemungkinan reseptor makrofag bersifat hidrofobik, pengurangan hidrofobisitas reseptor makrofag mungkin lebih menghambat pengikatan partikel ke reseptor makrofag (Weinberg, 1999). Ekstrak diklorometana kemungkinan tidak mengurangi hidrofobisitas reseptor makrofag, sehingga kurang menghambat pengambilan LDL teroksidasi ke reseptor makrofag.

Tabel 3. Persentase penghambatan akumulasi kolesterol ester

Sampel	Rerata akumulasi kolesterol ester (µg / µg prot)	Pengurangan akumulasi kolesterol ester dari kontrol (+) (%)
K (+)	45,03	-
K (-)	25,56	-
Ekstrak air	37,08	17,65
Ekstrak etanol	24,27	46,11
Ekstrak aseton	20,26	55
Ekstrak diklorometan	41,33	8,22
$\alpha$ -tokoferol	38,70	14,06

Berdasarkan hasil penelitian yang telah dipaparkan diatas, dapat diketahui bahwa suplementasi ekstrak temulawak pada LDL mampu melindungi LDL dari proses oksidasi dan cenderung menurunkan persen penghambatan akumulasi kolesterol ester pada makrofag. Dengan demikian, ekstrak temulawak kemungkinan dapat dijadikan sumber antioksidan alami penghambat aterosklerosis.

Banyak penelitian telah dilakukan yang menunjukkan bahwa antioksidan alami memberikan perlindungan terhadap LDL dari proses oksidasi dan menghambat akumulasi kolesterol pada makrofag. Hasil penelitian De Whalley et al., (1990) menunjukkan flavonoid menghambat oksidasi LDL dan menghambat sitotoksitas LDL teroksidasi oleh makrofag. Suplementasi vitamin E ( $\alpha$ -tokoferol) pada LDL (Hirano et al., 1997) dan plasma sebelum isolasi LDL (Esterbauer et al., 1991) mampu menghambat oksidasi LDL. Hasil penelitian Suzukawa et al., (1994) menunjukkan bahwa suplementasi vitamin E pada plasma sebelum isolasi LDL, mampu menghambat akumulasi kolesterol ester dan mencegah stimulasi pembentukan kolesterol ester pada makrofag.

### KESIMPULAN

Suplementasi berbagai jenis dan konsentrasi ekstrak temulawak pada LDL mempengaruhi penghambatan oksidasi LDL.

Ekstrak diklorometan temulawak paling mampu menghambat oksidasi pada LDL (61,20 %) dibandingkan dengan ekstrak aseton, ekstrak etanol dan ekstrak air yang menghambat oksidasi LDL berturut-turut sebesar 51,83 %, 47,68 % dan 44,27 %.

Penggunaan konsentrasi 430  $\mu$ g/ml LDL paling efektif dalam menghambat oksidasi LDL (56,21 %) dibandingkan konsentrasi 43 dan 4300  $\mu$ g/ml LDL yang menghambat oksidasi LDL berturut-turut sebesar 43,63 % dan 53,89 %.

Ekstrak temulawak cenderung dapat menurunkan akumulasi kolesterol pada makrofag.

Terdapat hubungan di antara penghambatan akumulasi kolesterol pada makrofag dengan penghambatan oksidasi LDL oleh ekstrak temulawak

### SARAN

Perlu penelitian lebih lanjut tentang penghambatan oksidasi LDL dan penghambatan akumulasi kolesterol oleh ekstrak temulawak secara *in vivo*.

### DAFTAR PUSTAKA

- Andarwulan, N and K. Shetty. 1999. Phenolic Content in Differentiated Tissue Culture of Transformed and Agrobacterium-Transformed Roots of Anise (*Pimpinella anisum* L.). *J Agric Food Chem*, 47 : 1776-1780.
- Brown, M. S. and J. L. Goldstein. 1983. Lipoprotein Metabolism in Macrophage. *Annu Rev Biochem.* 52:223-261.
- De Whalley, C. V., S. M. Rankin, J. R. S. Hoult, W. Jessup and D. S Leake. 1990. Flavonoid inhibits the Oxidative Modification of Low Density Lipoproteins. *Biochem Pharmacol* 39: 1743-1749.
- Esterbauer, H., M. D. Rotheneder., G. Striegl., and G. Waeg. 1991. Role of Vitamin E in Preventing the Oxidation of Low Density Lipoprotein. *J. Clin Nutr* 53: 314S-321S.
- Frankel, E. N., J. Kanner, J. B. German, E. Parks and J. E Kinsella. 1993. Inhibition of Oxidation of Human Low Density Lipoprotein by Phenolic Substance in Red Wine. *Lancet.* 341: 454-457.
- Gordon, 1990. The Mechanism of Antioxidant Action *in vitro*. In: B. J. F. Hudson (Ed.), *Food Antioxidants*. Elsevier Applied Science. London and New York. Pp. 1-15.
- Hirano, R., K. Kondo., T. Imawoto., O., Igarasih and H. Itakura. 1997. Effects of Antioxidant on the Oxidative Susceptibility of Low Density Lipoprotein. *J. Nutr Sci Vitaminol* 13: 435-444.
- Jitoe, A., T. Masuda, I.G.P. Tengah, D.N. Suprpta, I.W. Gara, and N. Nakatani. 1992. Antioxidant Activity of Tropical Ginger Extracts and Analysis of The Contained Curcuminoids. *J Agric Food Chem* 40 : 1337-1340
- Kikuzaki, H. and N. Nakatani. 1993. Antioxidant Effect of Some Ginger Constituents. *J of Food Sci* 58 (6) : 1407-1410.
- Miyagi, Y., K. Miwa and H. Inoue. 1997. Inhibition of Human Low Density Lipoprotein Oxidation by Flavonoids in Red Wine and Grape juice. *Am. J. Cardiology.* 80: 1627-1631.
- Purseglove, J. W., E. G. Brown, C. L. Green and S. R. J. Robbins. 1981. *Spices Vol II*. Longman Inc, New York.
- Ramirez-Tortosa, M.C, C.M. Aguilera, M.A Carrion Gutierrez, A Ramirez Bosca and A. Gill. 1998. Curcumin Ethanol-aqueous Extract Inhibits *In Vitro* Human Low Density Lipoprotein Lipoxydation. Dalam : *Functional Foods : The Consumer, The Products and The Evidence*. 111-115. M. J. Sadler and M. Saltmarsh (Eds). The Royal Society of Chemistry. Cambridge.
- Septiana, A.T. 2001. Aktivitas Ekstrak Jahe (*Zingiber officinale* Roscoe) dalam Pencegahan Oksidasi

- Lipoprotein Densitas Rendah (LDL) dan Akumulasi Kolesterol pada Makrofag. *Disertasi Program Pasca Sarjana IPB, Bogor.*
- Septiana A.T., D. Muchtadi, dan F.R .Zakaria. 2002.** Aktivitas antioksidan ekstrak diklorometana dan air jahe (*Zingiber officinale* Roscoe) pada asam linoleat. *Jurnal Teknol dan Industri Pangan XIII (2) : 105-110.*
- Septiana, A.T., H. Dwiyantri, D. Muchtadi, dan F. Zakaria. 2004.** Kajian Antioksidan Zingiberaceae sebagai Penghambat Oksidasi Lipoprotein Densitas Rendah (LDL) dan Akumulasi Kolesterol pada Makrofag. *Laporan Penelitian Hibah Pekerti. Fakultas Pertanian UNSOED.*
- Sudjana. 1989.** *Desain dan Analisis Eksperimen.* Tarsito. Bandung. 412 hal.
- Sulistiyani, and St. Clair. 1997.** Effect of Estradiol on Metabolism of Acetylated Low Density Lipoprotein by Macrophage in Culture. *Atheroscler. Thromb Vasc. Biol.* 17: 1691-1700.
- Suroyo, M. F. 2001.** Pengaruh Penambahan Kurkumin dan Waktu Reoksigensi Terhadap Jumlah Radikal Bebas Pada Proses Reperfusion Jantung Marmut Terisolasi. *Skripsi. FTP IPB. Bogor.*
- Suzukawa, M., M. Abbey, P. Clifton, and P.J. Nestel. 1994.** Effect of Supplementing with Vitamin E on the Uptake of Low Density Lipoprotein and The Stimulation of Cholesteryl Ester Formation in Macrophage. *Atherosclerosis* 110 : 77-86.
- Weinberg, J.B. 1999.** Mononuclear phagocytes. Dalam : *Wintrrobe's Clinical Hematology.* Vol 1. Lee, G.R, J. Lukens, J.P. Greer, J. Foester, G.M. Rodgers (eds). Tenth edition. Lippincott Williams and Wilkins. Baltimore.
- WHO. 2001.** *Heart Disease.* <http://www.who.int/>. Diakses 5 Maret 2005.