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Antioxidant Effects of Sarang Semut *(Myrmecodia pendans)* on the Apoptosis of Spermatogenic Cells of Rats Exposed to Plumbum.

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ABSTRACT

Active compounds in Sarang Semut are flavonoids, tannins, polyphenols, and tocopherols which serve as primary antioxidants in the body. The existence of these antioxidants enables Sarang Semut to be used to address the free radicals induced by plumbum (Pb). The objective of this study was to analyze antioxidant effects of Sarang Semut on the number of apoptosis spermatogenic cells, malondialdehyde (MDA) levels and superoxide dismutase (SOD) of serum. Compared to the control group, the number of apoptosis of spermatogenic cells and the MDA level of serum of the group exposed to Pb showed a significant increase ($P < 0.05$), and the level of SOD of serum showed a significant decrease ($P < 0.05$). After the provision of the extract and fraction of Sarang Semut, the exposed group showed a significant decrease ($P < 0.05$) in the number of spermatogenic cells and MDA level of serum, and a significant increase ($P < 0.05$) in the level of SOD. Antioxidants of Sarang Semut can decrease the number of apoptosis spermatogenic cells and the MDA level of serum, and increase the level of SOD serum of rats exposed to Pb.

Keywords: *Lead acetate; Sarang Semut; Apoptosis; spermatogenic cells.*

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INTRODUCTION

Plumbum (Pb) is a toxic and dangerous pollutant to human health. Plumbum has toxic effect on the male reproductive system. Studies in experimental animals, especially in rats and rabbits, showed that Pb is toxic to testicular function and tissue. Plumbum can interfere the mitosis of spermatogenic cells and cause a change in the proliferation of sertoli cells. This causes a decrease in the number of sperms in testes and further reduction in the number of epididymis sperms (Corpas et al., 1995). Corpas et al. (2002) mentions that Pb acetate poisoning during spermatogenesis can delay spermiation and immature spermatogenic cells release in the seminiferous tubules of the testes. Plumbum can cause apoptosis of spermatogenic cells in the seminiferous tubules of the testes (Wang et al., 2006; Adhikari et al., 2001). Therefore apoptosis is presumed to be the toxic effect of Pb on spermatogenic cells in the testes.

Plumbum toxic effect on testes is caused by the production of excessive reactive oxygen species (ROS) (Hsu and Guo*,* 2002; Mariola et al., 2004). In addition to the increase in ROS, Pb inhibits the activity of antioxidant enzymes, including glutathione peroxidase, catalase, and superoxide dismutase (Bolin *et al.,* 2006; Patrick, 2006; Ercal et al., 2001), therefore, antioxidant provision may be an alternative therapy.

Myrmecodia pendans known as Sarang Semut is one of Indonesian traditional herbal plants belonging to *Rubiacea,* widely used for the treatment of various types of cancer or tumors, tuberculosis, and rheumatism. Analysis result of antioxidants of crude extract of Sarang Semut show that the extract has antioxidant activity (Subroto and Saputro, 2008). Antioxidant ability of Sarang Semut has also been evidenced by Bustanussalam (2010) that the value of EC_{50} of the fraction of the water of Sarang Semut is 30.66 mg / mL. Antioxidant activity test with 1,1-diphenyl-2-picrilhydrazil (DPPH) method shows the value of EC_{50} of the ethanol extract of Sarang Semut is 3.6830 µg/ mL while the value of EC_{50} of black tea extract is 8.1720 µg/ mL (Utomo et al., 2012).

Sarang Semut is known to contain flavonoids, polyphenols, tannins and tocopherols. Flavonoids, polyphenols, and tocopherols are primary antioxidants (Subroto dan Saputro, 2008). Primary Antioxidant is a compound that can stop the chain reaction of radical formation. Flavonoid is a heavy metals chelating compound in addition to being an antioxidant (Middleton et al, 2000).Tocopherols serve as antioxidant that can neutralize or reduce the negative effects of free radicals from Pb. Data show that antioxidant plays important role in controlling the effects of Plumbum (Khaki and Khaki, 2010).

In addition to flavonoids, polyphenols, and tocopherols, Sarang Semut also contains Zn. The existence of Zn enlarges the ability of Sarang Semut to reduce the toxicity of Pb. This is due to the fact that Zn and Pb compete for binding metallothionin sites in the protein transport in the digestive tract. The competition between Zn and Pb may decrease Pb absorption (Flora et al., 2006). A study shows that zinc supplementation prevents the inhibition of delta

aminolevulinic acid dehydratase (δ -ALAD) and the cellular of SOD increases in the testes of rats exposed to Pb (Batra et al*.,* 1998).

Although some researchers have reported the ability of antioxidants to reduce the negative impacts of Pb, the antioxidant ability of Sarang Semut inhibits the negative impacts of Pb acetate on the apoptosis of spermatogenic cells has not been reported. This study focuses on whether the provision of ethanol extract and ethyl acetate fraction of Sarang Semut orally prevents the apoptosis of spermatogenic cells of rats exposed to Pb.

MATERIALS AND METHODS

Chemicals

Oxiselect^{TM} MDA adduct ELISA kit of Cell Biolabs Inc. was used in the examination of malondialdehyde (MDA) level of serum. *Rat SOD kit* of *medicine*was used for the examination of SOD level*. Tunel-universal apoptosis detection kit of GenScript, Inc.* USA was used for the examination of the apoptosis of spermatogenic cells. Plumbum asetat $(CH_3COO)_2Pb^*3\ H_2O$ (Merck, Germany) was in crystalline form. The ethanol extract and ethyl acetate fraction of Sarang Semut.

Experimental Animal and Research Design

Fifty adult male Wistar strain rats weighing 160-200 g were used as experimental animals in this study. Rats were obtained from The Laboratory of Biochemistry, Faculty of Medicine, University of Airlangga. Experimental animals were placed in a polypropylene cage with a light cycle of 12 hours bright and 12 hours dark, with a temperature of 25° C and humidity ranging from 50% to 65%. Rice bran was used as the base of the cage replaced at any time during cage cleaning. Experimental animals were given food and ad libitum water. Rats feed used was standard feed from PT. Charoen Pokpan Indonesia. All animals were treated in accordance with the principles of laboratory animal treatment. Experimental protocol has been approved by the ethics committee in accordance with the guide of the use, care and treatment of laboratory animals developed by the Research Ethics Committee of the Faculty of Public Health, University of Airlangga Surabaya. The study design used is the separate sample pretest-posttest control group*.*

Grouping and Treatment of Experimental Animal

After the acclimatization for 2 weeks, fifty adult male Wistar strain rats were randomly divided into 2 groups. Group I consisted of 11 rats, group II consisted of 39 rats. For 8 weeks, group I (normal control) was fed standard feed and distilled water. Group II was fed standard feed and Pb acetate at a dose of 1.000 ppm/200 g BW (body weight)/day. After 8 weeks, 4 rats of group I and 4 rats of group II were sacrificed for early examination to examine the number of apoptosis spermatogenic cells, MDA level of serum, and SOD enzyme level of serum. Other rats were continued to the next treatment, in which 7 rats were fed standard feed and distilled

water (group III), while the group which was fed standard feed and Pb acetate at a dose of 1.000 ppm/200g BW/daywas randomly divided into 5 groups, namely group IV which was fed standard feed and Pb acetate at a dose of 1.000 ppm/200g BW/day, group V which was fed standard feed and Pb acetate at a dose of 1.000 ppm/200g BW/day and the extract of Sarang Semut at a dose of 27 mg/200 g BW/today, group VI which was fed standard feed and Pb acetate at a dose of 1.000 ppm/200g BW/day and the extract of Sarang Semut at a dose of 54 mg/200 g BW/day. Group VII was fed standard feed and Pb acetate at a dose of 1.000 ppm/200g BW/day and EtOAc fraction of Sarang Semut with a dose of 4 mg/200g BW/day, group VIII was fed standard feed and Pb acetate at a dose of 1.000 ppm/200g BW/day and EtOAc fraction of Sarang Semut at a dose of 8 mg/200g BW/day, for 8 weeks. Plumbum acetate and the extract and EtOAc fraction of Sarang Semut were given through stomach sonde.

Animal sacrifice and sample collection

Twenty-four hours after the last treatment, all experimental animals were sacrificed by anaesthetizing them with ether, and blood samples were collected intra-cardiac. Blood samples obtained from each rat were put into plain bottles. Serum was obtained with a centrifugation at 3000 rpm for 20 minutes. Blood serum was used for the examination of SOD level through ELISA method (Lequin, 2005) and MDA level through ELISA method (Sevilla et al., 1997). Left testes was cut from each rat and put into a fixative solution of 10% neutral buffer formalin prepared for TUNEL-asay.

TUNEL analysis of apoptosis

Testicular tissues prepared with standard histological method were sliced to a thickness of 5 µm then stained with Tunel-universal apoptosis detection kit. This kit uses TdT enzyme which incorporates labeled nucleotides into a DNA end chain produced by the process of apoptosis. The surgical procedure performed was in accordance with the instruction of the manufacturer. Spermatogenic cells undergoing apoptosis were measured by counting the number of TUNEL cores for each seminiferous tubules section. Selected ten tubules sections approaching round per specimen were assessed and the average number of positive TUNEL cells per section was calculated.

Statistical Analysis

The data obtained are presented as mean ± standard deviation (SD). Data analysis was performed through analysis of variance (ANOVA) and Duncan's test to determine which treatments were different from each other. The level of significance was set to the value of $P < 0.05$.

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RESULTS

Apoptosis of spermatogenic cells

Spermatogenic cells observed were spermatogonia, spermatocytes and round spermatids cells. The number of spermatogenic cells undergoing apoptosis of the group exposed to Pb for 8 weeks (group II) and the group exposed to Pb for 16 weeks (group IV) increased significantly ($P<0.05$), compared to their respective control groups. The highest number of apoptotic spermatogenic cells obtained at the group exposed to Pb for 16 weeks (group IV). When combined with the extract and fraction of Sarang Semut, the number of apoptotic spermatogenic cells of groups V, VI, VII and VIII showed a significant decrease ($P <0.05$), compared to the exposed groups (group II and IV) (Table 1).

Table 1: Mean and Standard Deviation of the Number of Spermatogenic cells in Apoptosis of Rats in Treatment Group

Group	Mean ± SD number of spermatogenic cells in apoptosis		
I. Control (8th week)	45.800 [a]	±	3.17385
II. Pb exposure (8thweek)	53,400 [b]	±	4.78400
III. Control (16thweek)	49.933 [b]	±	2.98976
IV. Exposed to Pb (16thweek)	63.300 [c]	±	1.99499
V. Exposed to Pb+ ESS 27 mg/200 g BW	49.666 [ab]	±	4.27910
VI. Exposed to Pb+ ESS 54 mg/200 g BW	51.183 [b]	±	2.45309
VII. Exposed to Pb+FSS 4 mg/200 g BW	52.166 [b]	±	2.96760
VIII. Exposed to Pb + FSS 8 mg/200 g BW	45.900 [a]	±	1.33267

Different superscripts in the same column indicate no significant difference ($p < 0.05$); SD = Deviation Standard, ESS = Extract of Sarang Semut, FSS = Fraction of Sarang Semut

Histological over view of the testes of rats of control group only fed standard feed without Pb acetate showed that fewer cells gave a positive reaction to TUNEL assay staining (marked with red arrows), the arrangement of the seminiferous tubules was dense with intact basal membrane (BM), lumen (L) of tubules contained spermatozoa (Figure 1A). In the group exposed to Pb, the observation in 8th week showed that more cells gave a positive reaction to TUNEL assay staining, the basal membrane was released (Figure 1B). In the group exposed to Pb, observation in 16th week showed that the diameter of the seminiferous tubules shrinked, the arrangement of spermatogenic cells was not in order and fewer, the tubular lumen with few spermatozoa (Figure 1C). The group of rats exposed to Pb and received extract and or fraction of Sarang Semut showed a significant increase in the number of spermatogenic cells. Spermatogenic cells in seminiferous tubules have well developed cytoarchitecture, i.e outermost layer of spermatogonia, primary and secondary spermatocytes middle layers and innermost layer of spermatozoa. The arrangement of the seminiferous tubules was dense with intact basal membrane, and fewer cells gave positive reaction to TUNEL assay staining compared to the group exposed to Pb. (Figure 1D and Figure 1E).

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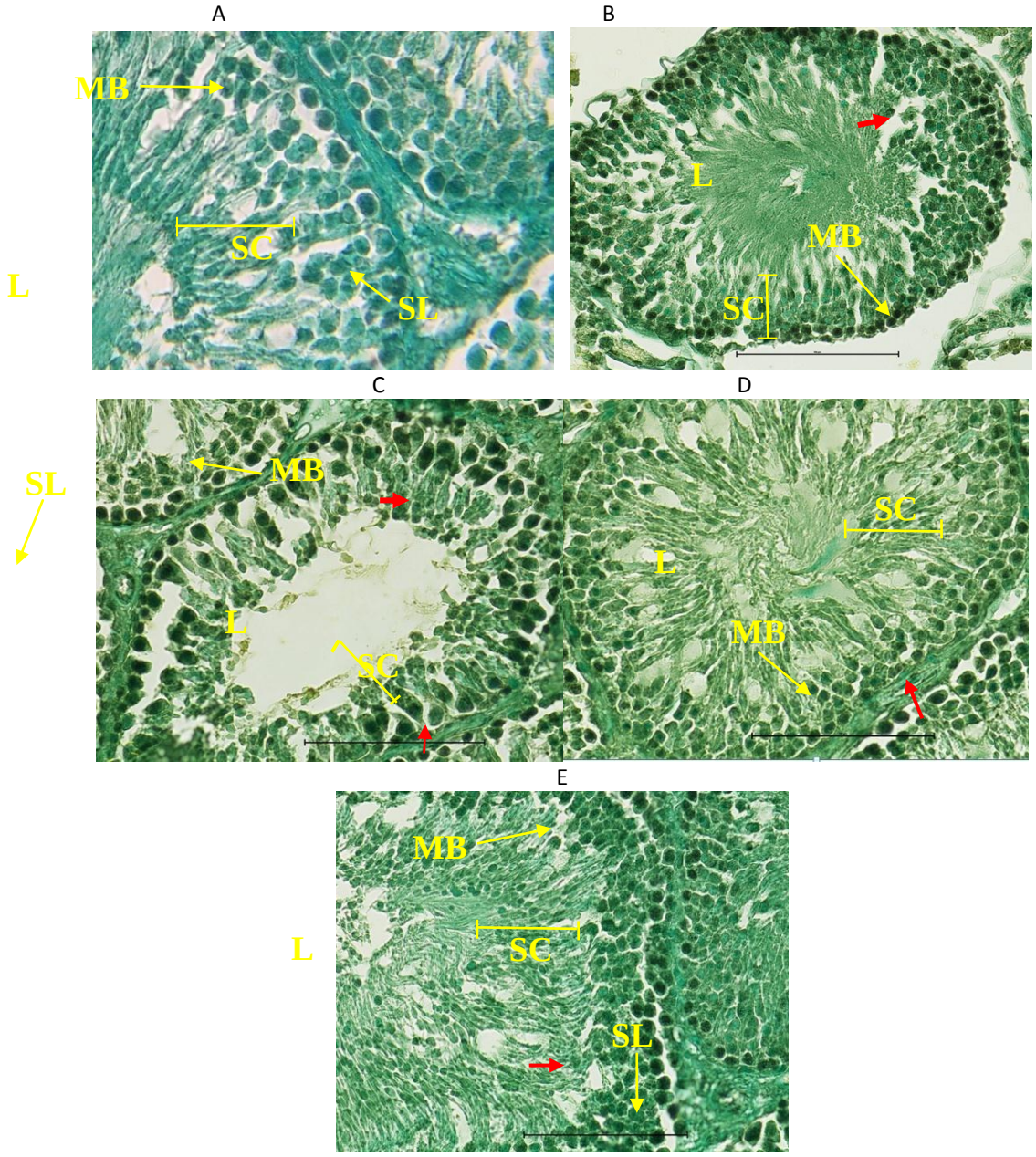

Figure 1: Photomicrograph showing TUNEL-stained cells in the testes of rats treated with 0 ppm Pb (A), 1.000 ppm Pb for 8 weeks (B), 1.000 ppm Pb for 16 weeks (C), 1.000 ppm Pb and Sarang Semut extract (D), 1.000 ppm Pb and Sarang Semut fraction (E). 40 x10 magnification. SL = Leydig cell, SC = spermatogenic cell, L = lumen, MB = Basal membrane.

The Malondialdehyde (MDA) Level of Serum

The MDA level of serum is shown in Table 2. The MDA level of serum of rats of group exposed to Pb for 8 weeks (group II) and of group exposed to Pb for 16 weeks (group IV) showed significant increase ($P < 0.05$) compared to their respective control groups. The highest level of serum MDA of rats was found in the group exposed to Pb for 16 weeks (group IV) and it

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was not different significantly (P > 0.05) from the level of serum MDA of rats of group II. When combined with the extract and fraction of Sarang Semut, the MDA level of serum of rats of group V, VI, VII and VIII showed a significant decrease (P < 0.05), compared to the exposed group (group IV).

Tabel 2: Mean and Standard Deviation of MDA level of serum of Rats in Treatment

Group	Mean ± SD MDA level of Serum of rats (pmol/mL serum)		
I. Control (8th week)	11,475 [a]	±	2,08387
II. Pb exposure (8th week)	31,725 [bc]	±	16,68140
III. Control (16th week)	21,900 [ab]	±	6,16993
IV. Exposed to Pb (16th week)	33,333 [c]	±	13,61935
V. Exposed to Pb+ ESS 27 mg/200 g BW	17,866 [a]	±	6,09973
VI. Exposed to Pb+ ESS 54 mg/200 g BW	17,323 [a]	±	5,74909
VII. Exposed to Pb+FSS 4 mg/200 g BW	18,350 [a]	±	5,54355
VIII. Exposed to Pb + FSS 8 mg/200 g BW	15,450 [a]	±	3,94398

Different superscripts in the same column indicate no significant difference (p < 0.05); SD = Deviation Standard, ESS = Extract of Sarang Semut, FSS = Fraction of Sarang Semut

The Superoxide Dismutase (SOD) Level of Serum

The SOD level of rats of groups II and IV showed a significant decrease (P < 0.05) compared to their respective control groups. Groups V, VI, VII and VIII showed a significant increase (P < 0.05) in the SOD level of serum (Table 3).

Tabel 3: Mean dan Standar Deviasi of Enzyme SOD Level of Serum of Rats in Treatment

Group	Mean ± SD of Enzyme SOD of Serum of rats (U/mL Serum)		
I. Control (8th week)	9,8250 [b]	±	0,48563
II. Pb exposure (8th week)	8,2000 [a]	±	0,35590
III. Control (16th week)	9,5500 [b]	±	0,57533
IV. Exposed to Pb (16th week)	8,4000 [a]	±	0,45607
V. Exposed to Pb+ ESS 27 mg/200 g BW	9,4333 [b]	±	0,53166
VI. Exposed to Pb+ ESS 54 mg/200 g BW	9,7333 [b]	±	0,83347
VII. Exposed to Pb+FSS 4 mg/200 g BW	9,8000 [b]	±	1,14717
VIII. Exposed to Pb + FSS 8 mg/200 g BW	9,8833 [b]	±	0,33116

Different superscripts in the same column indicate no significant difference (p <0.05); SD = Deviation Standard, ESS = Extract of Sarang Semut, FSS = Fraction of Sarang Semut

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DISCUSSION

Apoptosis is an important physiological mechanism to keep cell proliferation balanced with cell death in limiting the number of spermatogenic cells in the seminiferous tubules of the testes. In this case, cell death due to apoptosis plays an important role in limiting the population of male germ cells during spermatogenesis. However, excessive apoptosis can cause damage to the male reproductive function. The decrease in the number of spermatogenic cells in large numbers as a result of toxicant exposure, lack of growth factors, the decrease in the supply of hormones (testosterone, FSH, LH), heat exposure, radiation and chemotherapy are known to be caused by apoptosis. The loss of spermatogenic cells due to apoptosis is the main cause of disruption of spermatogenesis and testicular atrophy. Apoptosis can occur when the testicular environment does not support the ongoing spermatogenesis (Lee et al., 1997).

In this study, spermatogenic cells undergoing apoptosis were identified with TUNEL assay staining. This method relies on the existence of the 3'-OH group generated by DNA fragmentation. Plumbum serves as the inducer of oxidative stress in the blood and soft tissues. There is a correlation between oxidative stress with the apoptosis of spermatogenic cells. Excessive reactive oxygen species (ROS) causes oxidative damage to DNA, it can accelerate the process of apoptosis of spermatogenic cells (Aitken and Roman, 2008). The results of this study show that in the group of rats exposed to Pb, the number of spermatogenic cells undergoing apoptosis are more than that of the rats of the control group not exposed or exposed and received extract or fraction of Sarang Semut. These results are in line with the study conducted by Adhikari et al. (2001) and Wang et al. (2006).

Several researchers have shown that exposure to Pb increases ROS in testes (Mariola et al., 2004). Reactive oxygen species plays an important role in the apoptosis of testicular cells exposed to Pb. There is a positive correlation between the increase in ROS and a higher level of caspase-3 found in the apoptosis of cells in the testis (Wang et al., 2003). Vigeh et al. (2011) describe Pb ability to increase ROS and inhibit antioxidant enzymes and DNA damage. This is in line with Khaki and Khaki (2010) whose research found that there is a significant correlation between ROS with increased DNA damage and the apoptosis of liver cells of Wistar rats exposed to Pb acetate.

Plumbum can cause oxidative stress by increasing the hydroxyl radical in the testes (Ding et al., 2000; Ercal et al., 2001). Hydroxyl radicals react with lipids and membrane proteins resulting in impaired permeability of the membrane system which Pb to the increase in intracellular Ca^{2+}. Increased Ca^{2+} causes mitochondrial depolarization and the release of cytochrome c which in turn caspase 9 and caspase 3 are enabled. Mean while, according to Sakr and Badawy (2011), the increase in intracellular Ca will activate the core nuclease that causes DNA fragmentation.

These results indicate that Sarang Semut is able to reduce the number of apoptotic spermatogenic cells, either in the form of extractor fraction. The approach that can describe it is that Sarang Semut has a component of active materials that can suppress oxidative stress.

Oxidative stress in blood and testicular tissue caused by Pb can be inhibited by compounds contained in Sarang Semut. Identification results show that the extract and fraction of Sarang Semut contain flavonoids, polyphenols, and tocopherols, and this is in line with the study of Subroto Saputro (2008). Flavonoids, polyphenols and tocopherols are primary antioxidants. Primary Antioxidants are compounds that can stop the chain reaction of radical formation. Antioxidants are the main factors of defense against oxidative stress caused by ROS (Agarwal and Prabakaran, 2005).

The results show that in the group exposed to Pb without receiving the extract or fraction of Sarang Semut, the number of spermatogenic cells undergoing apoptosis is higher than that of the control group unexposed to Pb and that of the group exposed to Pb and received Sarang Semut. The high number of spermatogenic cells undergoing apoptosis can be due to high MDA level of serum induced by Pb. Possible track to explain this is as follows: Pb induces excessive ROS production. Highly reactive Reactive oxygen species is hydroxyl radical (OH^-) (Xu et al., 2008). Then the reactive radicals attack polyunsaturated fatty acids and cause lipid peroxidation. The final result of lipid peroxidation is MDA. Reactive oxygen species and MDA will attack DNA together or individually. Thus, the DNA damage can occur directly due to ROS or due to ROS derived to MDA. Plumbum-induced DNA damage causes an increase in p53 protein significantly (Xu et al., 2008). The active p53 protein leads to increased expression of Bax protein which is proapoptotic Bax and suppresses the activity of Bcl-2 which is anti-apoptotic (Qiang and Guo, 2001, Xu et al., 2006). Increased Bax triggers an increase in the permeability of the mitochondrial membrane, opens Mitochondria Permeability Transition Pore (MPTP), and allows the mitochondrial to release cytochrome-c into the cytosol through the MPTP (Josephin et al., 2002). Cytochrome-c in the cytoplasm binds to Apaf-1 *(*apoptotic protease activating factor-1*)* and activates caspase-9, followed by a series of caspase cascade, that eventually caspase-3 activates DNA-ase that breaks the DNA chain so that the cells undergo apoptosis (Wang et al*.,* 2006; K haki et al., 2012).

Group of rats exposed to Pb and receiving extract or fraction of Sarang Semut showed lower MDA level than the group exposed to Pb. The approach that can describe it is that the extract and fraction of Sarang Semut contain components of active materials that can prevent lipid peroxidation. Lipid peroxidation can be prevented by the existence of fat-soluble antioxidants such as tocopherol, or antioxidants that can chelate Fe^{2+} or Cu^{2+} as a catalyst for redox reactions (Sadek, 2012). According to Middleton et al*.* (2000) Lipid peroxidation can be prevented at initial stage by radical scavengers and at propagation stage can be prevented by peroxyl radical scavengers. Sarang Semut contains tocopherol. Tocopherol is intracellular antioxidant that protects polyunsaturated fatty acid and cell membranes from oxidative damage. Tocopherol (vitamin E) has been shown to inhibit damage caused by free-radical. Tocopherol is an antioxidant that is very active in preventing lipid peroxidation by seizing lipid peroxyl. Tocopherol will transfer the hydrogen atom (with its single electron). Tocopherol does not only dredgeoxygen radicals from the membrane, but also cuts peroxyl and alkoxyl radicals generated during lipid hydroperoxide conversion that sparks the peroxidation chain reaction. Thus, it prevents the propagation of lipid peroxidation chain reaction (Bansal and Bilaspuri, 2009). Sarang Semut also contains phenols and flavonoids. Flavonoids can chelate iron ions to

form inert complex which can not initiate lipid peroxidation (Sadek, 2012). Flavonoids also serve as free radical scavenger. Flavonoids can reduce the hydroxyl radical (OH •) as the initiator of the chain reaction of lipid peroxidation of the cell membrane in which the end product is MDA, so that flavonoids can prevent the initial of lipid peroxidation chain reaction of cell membranes triggered by the hydroxyl radical (Middleton et al., 2000). The existence of Zn in Sarang Semut is presumed to also be able to reduce MDA level because Zn can inhibit lipid peroxidation by displacing transition metal such as iron and copper from catalytic site.

The results of this study indicate that the group of rats exposed to Pb has lower SOD enzyme level of serum than that of control group unexposed to Pb and of the group exposed to P and received the extract or Sarang Semut. The results of this study are in line with the researches conducted by Salawu et al. (2009), Sudjata et al. (2011), and (Ayinde et al., 2012). Plumbum causes the antioxidant enzyme inhibitory effect by disupting some essential metals required for antioxidant enzyme activity (Sudjata et al., 2011). Plumbum can replace Zn in methaloenzyme. The interaction mentioned has been demonstrated where Pb replaces Zn to form Cu-Pb-SOD (El-Thohomy, 2010). Low level of serum SOD enzyme is one of the causes of the high number of spermatogenic cells undergoing apoptosis in rats exposed to Pb compared to the control group in this study.

The results of this study indicate that Sarang Semut can increase the SOD enzyme level of serum in the form of extract and fraction. The approach that can describe it is that the active materials of Sarang Semut that are able to build a system of protection against oxidative stress damage caused by exposure to Pb. The higher level of SOD enzyme in rats exposed to Pb and received the extract or fraction Sarang Semut compared to rats exposed to Pb may be caused by ameliorative effect of the content of chemical compounds in Sarang Semut, including tocopherols, flavonoids and Zn. A fact has been confirmed by the results of the study by Mollina et al. (2003) in which the provision of α-tocopherol induces an increase in SOD activity in rats undergoing oxidative stress due to induced by ethanol, and the provision of quercetin flavonoids results in higher SOD and GPx activity compared to the ethanol group. The presence of Zn in Sarang Semut enlarge Sarang Semut ability to reduce the toxicity of Pb. Zn contained in Sarang Semut also acts as an antioxidant because Zn becomes the cofactor of free radical scavenger enzyme such as SOD and protects the group of sulfihidril. A study suggests that zinc supplementation prevents the inhibition of δ-ALAD and increased cellular SOD in the testes of rats exposed to Pb (Hsu and Guo, 2002).

REFERENCES

[1] Adhikari N., Sinha N., Narayan R., and Soxena D. 2001. Lead induced cell death in testes of young rats. *Journal of Applied Toxicology*, 21: 275–277.

[2] Agarwal A., and Prabakaran S.A. 2005. Oxidative stress and antioxidants in male infertility: a difficult balance. *Iranian Journal of Reproductive Medicine,* 3: 1-8.

[3] Aitken J.R., and Roman D.S. 2008. Antioxidant systems and oxidative stress in the testes. *Oxidative Medicine and Cellular Longevity*, 1:15-24.

[4] Ayinde O.C., Ogunnowo S., and Ogedegbe R.A. 2012. Influence of vitamin C and vitamin E on testicular zinc content and testicular toxicity in lead xposed albino rats. *BioMed Central Pharmacology and Toxicology,* 13: 1-8.

[5] Bansal A.K., and Bilaspuri G.S. 2009. Antioxidant effect of vitamin E on motility, viability and lipid peroxidation of cattle spermatozoa under oxidative stress. *Animal Science Papers and Reports*, 27: 5-14.

[6] Batra N., Nehru B., and Bansal M.P. 1998. The effect of zinc supplementation on the effects of lead on the rat testis. *Reproductive Toxicology*, 12: 535-540.

[7] Bolin C.M., Basha R., Cox D., Zawia N.H., Maloney B., Lahiri D.K., Fardozo-Pelaez. 2006. Exposure to lead and the developmental origin of oxidative DNA damage in the aging brain. *Federation of American Societies Experimental Biology Journal*, 20: 788–790.

[8] Bustanussalam, 2010. Penentuan struktur molekul dari fraksi air tumbuhan sarang semut (*Myrmecodia pendans*) yang mempunyai aktivitas sitotoksik dan antioksidan. Bogor: Institut Pertanian Bogor

[9] Corpas I., Gaspar I., Martinez S., Codesal J., and Candelas S. 1995. Testicular alteration in rats due to gestational and early lactational administration of lead. *Reproductive Toxicology,* 9: 307- 313.

[10] Corpas I., Castillo M., Marquina D., and Benito M.J. 2002. Lead intoxication in gestational and lactation periods alters the development of male reproductive organs. *Ecotoxicology and Environmental Safety,* 53: 259-266.

[11] Ding Y., Gonick H.C., and Vaziri N.D. 2000. Lead promotion hydroxyl radical generation and lipid peroxidation in culture aortic endothelial cels. *American Journal of Hypertension,* 13: 552-555.

[12] El-Tohamy M.M., and El-Nattat W.S. 2010. Effect of antioxidant on lead-induced oxidative damage and reproductive dysfunction in male rabbits. *Journal American Science,* 6: 613–622.

[13] Emrah C., Dhsan H., Suleyman A., Selda T., Ozgur B., and Hakim C. 2007. The effects of sulfur-containing compounds on total antioxidant levels of liver, kidney and brain in lead-exposed rats. *Turkiye Klinikleri Journal Medical Sciences,* 27: 823-8.

[14] Ercal N., Orhan G.H., and Burns N.A. 2001 Toxic metals and oxidative stress part I: Mechanisms involved in metal induced oxidative damage. *Current Topics Medicinal Chemistry,* 1*:* 529-539*.*

[15] Flora J.S, Govinder F., and Geetu S. 2006. Environmental occurrence, health effects and management of lead poisoning. In: *Lead Chemistry, Analytical Aspects, Environmental Impact and Health Effects* (Edited by: Cassas J.S. and Sordo J), Oxford: Elsevier, pp 158-220.

[16] Hsu P.C., and Guo Y.L. 2002. Antioxidant nutrients and lead toxicity. *Toxicology,* 180:33-44.

[17] Josephin S., Napolitano M., and Singh K., 2002. Mitochondria as target for detection and treatment of cancer. Cambridge: University Press, pp 1-11.

[18] Khaki A.A., and Khaki A. 2010. Antioxidant effect of ginger to prevents lead-induced liver tissue apoptosis in rat. *Journal of Medicinal Plants Research* 4: 1492-1495.

[19] Khaki A., Imani S.A.M., and Golzar F.S. 2012. Effect of rosmanic acid on male sex hormone (testosterone-FSH-LH) and testis apoptosis after exposure to electromagnetic field (EMF) in rats. *African Journal of Pharmacy and Pharmacology,* 6: 248-252.

[20] Kiran K.B., Erika B., Rashidi M., Prabhakara R.Y., Sharada R., and Rajanna B. 2008. Lead-induced increase in antioxidant enzymes and lipid peroxidation products in developing rat brain. *Biometals,* 21:9-16.

[21] Lee J., John H.R., Sarah C.Y., and Boekelheide K. 1997. The fas system Is a key regulator of germ cell apoptosis in the testis. *Endocrinology,* 138: 2081-2088.

[22] Lequin RM, 2005. Enzyme Immunoassay (EIA)/Enzyme-Linked Immunosorbent Assay (ELISA). *Clinical Chemistry,* 51: 2415-2418.

[23] Mariola M., Michalska T., and Wiszniewska B. 2004. Detection of lead-induced oxidative stress in the rat epididymis by chemiluminescence. *Chemosphere,* 57: 1553-1562.

[24] Middleton E., Kandaswami C., and Theoharides T.C. 2000. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacological Review,* 52: 673-751.

[25] Molina M.F., Reus I.S, Iglesias I., and Benedi J. 2003. Quercetin, a flavonoid antioxidant, prevents and protects against ethanol-induced oxidative stress in mouse Liver. *Biological and Pharmaceutical Bulletin,* 26: 1398—1402.

[26] Patrick L.N.D. 2006. Lead toxicity part II: the role of free radical damage and the use of antioksidan in the pathology and treatment of lead toxicity. *Alternative Medicine review,* 11: 114-127.

[27] Sadek K.M. 2012. Barley phenolic compounds impedes oxidative stress in lead acetate intoxicated rabbits. *Oxidants Antioxidants Medical Science*, 1: 141-146.

[28] Sakr S.A. and Badawy G.M. 2011. Effect of ginger (*Zingiber officinale* R.) on metiram-inhibited spermatogenesis and induced apoptosis in albino mice. *Journal of Applied Pharmaceutical Science,* 1: 131-136.

[29] Salawu O., Olusola A., Olutunde P.F., Uthman A.Y, Ablodun A., and Anthony A. 2009. Tomato (*Lycopersicon esculentum*) prevents lead-induced testicular toxicity. *Journal Human Reproductive Science*, 2: 30-34.

[30] Sevilla C.L., Mahle N.H., Eliezer N., Uzieblo A., O'Hara S.M., Nokubo M, Miller R., Rouzer C.A., and Marnett L.J. 1997. Development of monoclonal antibodies to the malondialdehyde-deoxyguanosine adduct, pyrimidopurinone. *Chemical Research Toxicology,* 10: 172-180.

[31] Subroto M.A., dan Saputro H. 2008. Gempur Penyakit dengan Sarang Semut. Jakarta: Penebar Swadaya, pp11-32.

[32] Sujatha K., Srilatha C.H., Anjanejului Y., and Amaravathi P., 2011. Lead acetate induced nephrotoxicity in wistar albino rats apathological, imunohistochemical and ultrastruktural studies. *International Journal of Pharmacy and Biological Sciences,* 2: 459-469.

[33] Utomo A.B., Suprijono A., Risdianto A. 2010. Uji aktivitas antioksidan kombinasi ekstrak sarang semut (*Myrmecodia pendans*) & ekstrak teh hitam (*Camellia sinensis O.K.var.assamic*a (mast.)) dengan metode DPPH (1,1-difenil-2-pikrilhidrazil). *Jurnal StiFar*, 14: 42-52.

[34] Vigeh M., Smith D.R., and Chi-Hsu P. 2011. How does lead induce male infertility. *Iranian Journal of Reproductive Medicine*, 9: 1-8.

[35] Wang X, Sharma R, Sikka S, Thomas A, Falcone T, Agarwal A. 2003. Oxidative stress is associated with increased apoptosis leading to spermatozoa DNA damage in patients with male factor infertility fertility and sterility. Fertil Steril 80:531–5.

[36] Wang C., Zhang Y., Liang J., Shan G., Wang Y., and Shi Q. 2006. Impacts of ascorbic acid and thiamine supplementation at different concentrations on lead toxicity in testis. *Clinica Cimica Acta,* 370: 82-88.

[37] Xu J., Lian L.J., Wu C., Wang X., Fu W.Y., and Xu L. 2008. Lead induces oxidative stress, DNA damage and alteration of p53, Bax and Bcl-2 expressions in mice. *Journal Food and Chemical Toxicology,* 46: 1488-1494.

[38] Ye X.B., Fu H, Zhu J.L, Ni W.M, Lu Y.W, Kuang X.Y, Yang S.L, Shu B.X. 1999. A study on oxidative stress in lead-exposed workers. *Journal Toxicology Environment Health* A, 57: 161–172.