ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Effects Glycine max L. Merr on Lipid Peroxidation and Kidney's Histopathology In Lead Intoxication Mice.

Juliana Christyaningsih[1]*, and Rika Yulia[2].

[1]Surabaya Health Polytechnic, Indonesia
[2] Department of Farmacy, Surabaya University, Indonesia.

ABSTRACT

Intoxication of lead (Pb) causes the formation of free radicals that affect the antioxidative defense system, thus speeding up cell damage. Soybean (Glycine max L. Merr) Ijen is one of the varieties of food containing phenolic compounds and flavonoids, which have antioxidant activity. Experimental animals used in this study were the Mus muscullus Balb / c as much as 25 tails, they were divided into 5 groups: placebo, negative control, positive control, test and comparison groups. The negative control, test and comparison group were given Pb at a dose of 25 mg / kg orally for 7 days. After the stages of intoxication, positive control and test groups were given suspensions extract of soybean Ijen varieties at a dose of 1g/ 1 ml for 7 days, the comparison group was given a suspension of vitamin C supplementation at a dose of 64 mg / kg orally for 7 days, and the negative control group were given mucilago CMC Na 0,5% of 1 ml for 7 days. Liver tissue of mice were used to analyze the activity of the catalase enzyme and malondialdehyde (MDA) level. Kidney's tissues were used for histopathological examination. Extract of soybean (Glycine max L.Merr) Ijen varieties decreases the activity of the enzyme catalase and repair damaged Kidneycells of mice that lead intoxication as effective as vitamin C.

Keywords: Glycine max L. Merr, Pb intoxication, MDA, Catase activity, Kidney's histopathology

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Heavy metals cause ecological crisis and when it is accumulated a certain amount in the body causing undesirable biological effects. Lead (Pb) is one of the toxic heavy metals, that is environmental contaminant commonly used in the manufacture of batteries, paints, varnishes and fuel mixture (Mishra et al., 2003; Jayakumar et al., 2008). If lead enters the body, 95% will be bound by erythrocytes and plasma further partly lead will diffuse to soft tissue (bone marrow, nervous system, Kidneys, and liver), hard tissue (bone, nails, hair), stored in the liver, Kidney , brain and skin, and toxic (Ardyanto, 2005). High lead levels will affect the natural antioxidative defense system, by causing increased production of reactive oxygen species (ROS). ROS such as superoxide, H_2O_2, and hydroxyl molecules can cause disturbances in the immune system, nervous system, hematopoietic, and Kidney (Lutz et al., 2011; Jayakumar et al., 2003) as well as associated with disorders of pregnancy (Falcon et al, 2002).

The response of the body in overcoming dependent ROS detoxification mechanism is consisting of an integrated system involving non-enzymatic molecules such as ascorbic acid, α-tocopherol and glutathione and enzymatic activity such as catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), polyphenols oxidase (PPO), peroxidase (POX) and others. The activity of these enzymes can be used as a marker occurrence of lead intoxication. Malondialdehyde (MDA) is dialdehid compound which one of the end products of lipid peroxidation in the body. MDA is a component of cell metabolites produced by free radicals, therefore, a high concentration of MDA which can indicate the presence of oxidation processes in the cell membrane.

Various plants contain active compounds with a variety of defense mechanisms to protect themselves from the harmful effects caused by oxidative stress (Jayakumar et al., 2008). Many of evidence strongly suggests that a synergistic interaction and addictive than natural antioxidant ingredients had a significant protective effect against oxidative damage. Glycine max is the type of crop to the content of polyphenolic compounds that have a variety of biological activity, which is beneficial to health, including preventing oxidative stress (Prakash et al., 2007). Isoflavones as one of polyphenol compounds, a class of phytoestrogens that can participate in redox reactions as free radical scavengers (Albulescu, 2007). Antioxidant ability of a material can be observed by looking at the enzyme activity and the levels of lipid peroxidation products. Some studies indicate that the toxicity of heavy metals associated with lipid peroxidation and reactive oxygen species (ROS).

This study used soybean (Glycine max L.Merr) Ijen varieties and as a comparison used vitamin C, an antioxidant compound that has been reported to reduce levels of lead in the blood and liver of male mice (Christyaningsih, 2008), lower levels of lead head of the fetus from the terintoxication lead mice (Christyaningsih, 2010), protect the liver and improve fetal brain histopathology of cerebral cortex (Christyaningsih, 2014).

MATERIALS AND METHODS

Animals:

Male mice (Musmuscullus) strain BALB / C, aged 10 weeks, the average weight of 25-30 g, physically healthy. The use of experimental animals has already got the certificate of conduct worthy of Research Ethics Committee of the Faculty of Veterinary Medicine, Airlangga University. 25 mice were divided into five groups

1. Placebo group (PG), not exposed anyone
2. Negative control group (NCG), on days 1 to 7, exposed to lead acetate at a dose of 25 mg / kg and 0.5% Na CMC mucilago much as 1 mL
3. Positive Control Group (PCG), on days 1 to 7, were given distilled water and on days 8 to 14, given the soy extract dose of 0.23 g / kg in 0.5% Na CMC mucilago much as 1 mL
4. Treatment Group (TG), on days 1 to 7, exposed to lead acetate at a dose of 25 mg / kg and on days 8 to 14, given the soy extract dose of 0.23 g / kg in mucilago CMC Na 0 , 5% in increments of 1 mL.
5. Comparison group (CG), on days 1 to 7, exposed to lead acetate at a dose of 25 mg / kg and on days 8 to 14, were given vitamin C doses of 64 mg / kg

ISSN: 0975-8585

The manufactured condensed extract of Glycine max Ijen varieties :

Soybean seeds were extracted using kinetic maceration method, the first stage using n-hexane. The results of the first stage pulp maceration proceed to the second stage, ie maceration using 90% methanol. The results of the second stage maceration filtrate was concentrated with the aid of a rotary evaporator and electric water bath until thick extract obtained. Extract the contents of flavonoids are then identified qualitatively using Thin Layer Chromatography (TLC). Categorized contains flavonoids if stain blue and green fluorescence appeared on the observation under UV light λ = 365 nm and there are yellow stains after the evaporation of the results of TLC with ammonia vapor. If will be given to the animal through the sonde, soy extract was dissolved in 0.5% Na CMC mucilago.

Measurement of MDA levels in liver:

The liver was transferred into a solution of Phosphate Buffer Solution (PBS) pH 7 to separate the red blood cells and blood clots. Tissue was homogenized in buffer and centrifuged at 3000 rpm for 15 min at 4 °C. Supernatant was separated and analyzed the levels of malondialdehyde using the thiobarbituric acid method Reactive Substances (TBARS) assay. The NWK-MDA01 assay is based on the reaction of MDA with thiobarbituric acid (TBA); MDA-TBA2 forming an adduct that absorbs strongly at 532 nm. Butylated hydroxytoluene (BHT) and EDTA are added to the sample and reaction mixture to minimize oxidation of the lipids that Contribute artifactually during sample processing and the TBA reaction. The temperature of the reaction mixture HAS ALSO been reduced to minimize the decomposition of lipid hydroperoxides, Because much of the MDA is protein bound, mostly as a Schiff base, the pH of the reaction has been optimized to facilitate hydrolysis of the MDA. Additionally, the reaction mixture is subjected to derivative spectrophotometric analysis Resolves that the problem of the variable and nonlinear baseline observed when attempting to measure the A532 absorbance in various biological samples(Northwest, Cat 1-888-449-3091)

Measurement of catalase activity (Biovision, Cat 773-100, Lot 70 473):

The liver was homogenized with the assay buffer in a cold state, further its mix on centrifuge 10,000 g for 15 min at 4 ° C. The activity of catalase enzyme in the supernatant was analyzed with the addition of H_2O_2, stop solution and mix solution developer then read with a spectrophotometer at a wavelength of 570 nm

The Observations of kidney's histopathology:

Kidney was fixed with buffered formalin, embedded in paraffin and stained with Hematoxylin and Eosin. Kidney's histopathology observations based on the percentage of cell damage.

RESULT AND DISCUSION

The Flavonoids in soy can be determined qualitatively by TLC.Results of Identification Flavonoids with TLC.

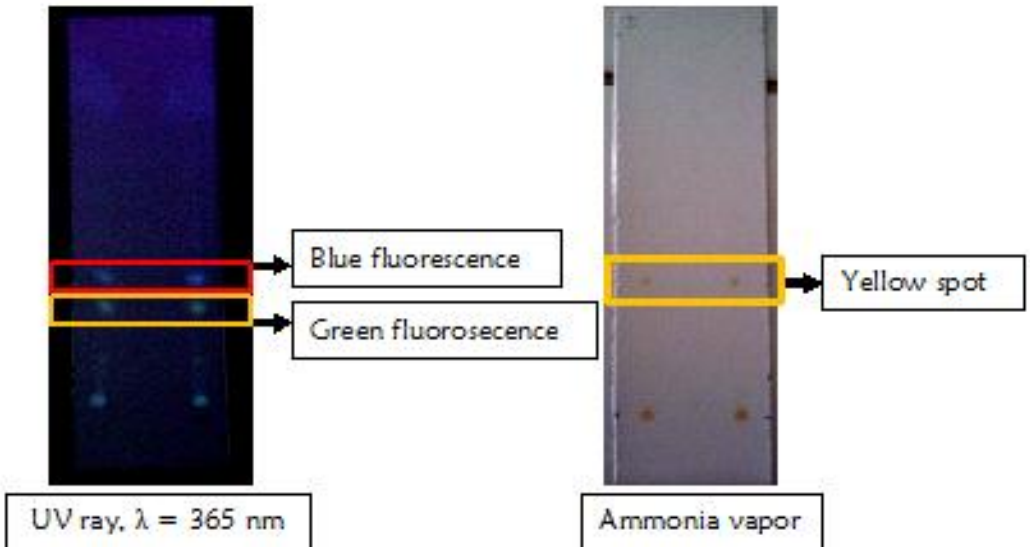
Soy's isoflavones are useful as antioxidants, shown through research on the measurement of MDA, the activity of the enzyme catalase in the liver and Kidney's histopathology in lead intoxication mice. The existence of malondialdehyde in liver showed that the state of lipid peroxidation reactions induced by lead in the experimental group and are shown in the table below.

Table 1: MDA levels (ppm) in liver mice were given extract of soybean Ijen varieties

Mice	PG	NCG	PCG	TG	CG
1.	28,418	58,305	38,013	34,71	18,980
2.	27,272	52,013	23,785	42,575	36,283
3.	23,699	28,418	52,013	23,699	33,137
4.	18,980	41,002	31,931	50,483	29,991
5.	23,785	33,924	41,054	36,493	25,272
Mean	24.431	42.732	37.360	37.592	28.732
SD	3.69	12.39	10.52	9.92	6.80

ISSN: 0975-8585

Figure 1:Identification of Flavonoids in Extract



Data malondialdehyde were analyzed statistically result significant value Levene test was 0.230 and the One-Sample Kolmogorov-Smirnov test was 0.516 so advanced statistical analysis using ANOVA test, and obtained the value 0.033, meaning that there were difference in the results of the study group. Results' LSD Post Hoc Comparison Test were there a significant difference between the placebo group and Negative control group; Placebo and Positive control group; Placebo and treatment groups; Negative control & Comparison group, while the other group was not a significant difference. There were no difference in the levels of MDA in the group of mice with lead intoxication that were given soybean extract compared to the group not given the extract of soybean showed that if the lead is a heavy metal in the body resulting in lipid peroxidation that the situation cannot be corrected with soy extract. Vitamin C was used as a comparison is very effective in reducing lipid peroxidation reactions that MDA levels in the groups did not differ significantly from the placebo group.

Table 2: The enzyme activity of catalase (mU / mL) in liver mice were given extract of soybean Ijen varieties

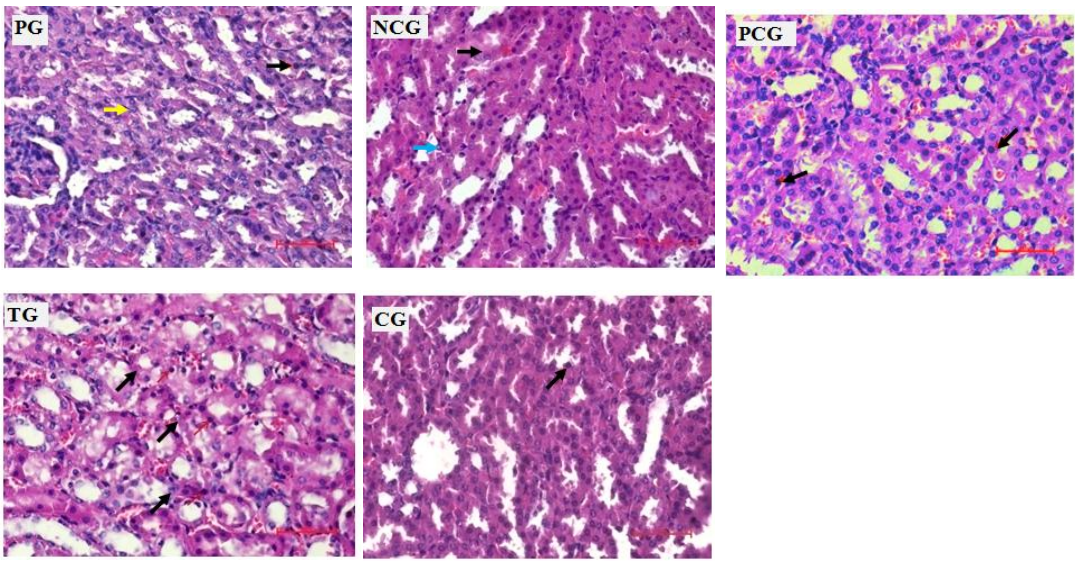
Mice	PG	NCG	PCG	TG	CG
1.	11,15	31,9	30,63	41,99	12,67
2.	13,58	43,63	16,93	48,57	7,64
3.	7,39	75,47	20,24	48,52	13,82
4.	10,97	50,68	23,11	47,19	5,7
5.	17,73	50,85	23,16	52,4	17,2
Mean	12.164	50.506	22.814	47.734	11.406
SD	3.82	15.94	5.06	3.75	4.68

Data activity catalase enzyme were analyzed statistically result Levene test value was 0.189 and the value of One-Sample Kolmogorov-Smirnov test was 0.393 so advanced statistical analysis using ANOVA test, and obtained values of 0.000, meaning that there are differences in the statistical results of the study group. Results LSD Post Hoc Comparison Test was there a significant difference between the placebo group and Negative control group; Placebo and Positive control group; Placebo and treatment groups; Negative control & Comparison group; Negative control and positive control group, positive control and treatment group; positive control and the comparison group; treatment and comparison group while the other group was not a significant difference. There was no significant difference in the activity of the enzyme catalase in the group of mice with lead intoxication that were given the soybean extract compared to the group not given the extract of soybean showed that if the lead is a heavy metal in the body resulting in the formation of hydrogen peroxide that the situation can not be corrected with soy extract. The existence of hydrogen peroxide produced by the body of mice which were given soy extract can be determined by increasing the activity of

catalase in that group compared with placebo. Vitamin C was used as a comparison is very effective in lowering the reaction of hydrogen peroxide so that the catalase activity in the groups did not differ significantly in the placebo group.

Observations of Kidney's Histopathology :

Figure 2: Result of Kidney's Histopathology



Description: Enlargement of the 400x; PG = Placebo Group; NCG = Negative Control Group; PCG = Positive Control Group; TG = Treatment Group; CG = Comparison Group; Black arrows = Cells Damaged; Blue Arrow = Living Cells

Table 3: Percentage of Damage Kidney's Cells

No.	Percentage of Damage Kidney's Cells				
	PG	NCG	PCG	TG	CG
1	8,45	56,16	10,14	28,99	16,22
2	8,96	60,81	10,14	28,77	12,86
3	4,62	47,56	10,53	31,25	12,50
4	10,45	50,00	8,00	29,63	0
5	8,57	49,28	13,70	28,41	0
Mean	8,19	52,64	10,50	29,41	13,86
SD	2,16	5,32	2,05	1,12	2,05

From the data in Table 3 when analyzed using SPSS 12, showed statistical significance at Lavene = 0.000 and Kolmogorov Smirnov = 0.134 so advanced statistical analysis by Kruskal Wallis test and showed a significance of 0.001, which means that there is a significant difference in the experimental group. To different test each group, we analyze the Mann-Whitney test and showed no significant difference between groups in the PG and NCG; PG and TG; NCG and PCG; NCG and TG; NCG and CG; PCG and TG; TG and CG. There were differences in the percentage of Kidney tissue damage in the group of mice that were given the lead terintoksikasi soybean extract compared to the group not given the extract of soy. Data shown that if the lead is a heavy metal in the body resulting in Kidney tissue damage, which can be fixed with soy extract, although not as good in the group given vitamin C. Kidney tissue damage in the group given only reached 52.64% Pb. Vitamin C effectively prevents damage to the Kidney cells of mice with lead intoxication than the placebo group.

Nurahman's Research (2013) obtain results that tempe was made from black soybeans if consumed by humans can boost the immune system through enhanced T cell proliferation against hydrogen peroxide,

increasing the proliferation of B cells, the activity of SOD, catalase and glutahione peroxide, thus it can be concluded that consumption of black soybean tempeh can boost imune system, particularly the cellular immune system. These results are clearly different from the present study, since the use of soybean Ijen varieties can not dampen the reaction of H_2O_2 formation. The possibility that the difference is Nurahman using fermented soybeans and induction material using Salmonella typhimurium whereas in this study using the original soy extracts and induction materials using lead.

Similar research conducted by Arifyanto (2012) who used mice as experimental animals produce soybean extract that soy can lower the rat liver cell damage and in increasing doses of soy tempeh extract followed by an increase in the protective effect against rat liver cell damage induced by paracetamol. These results are similar to this study that soy extract is able to reduce tissue damage in mice, mean soy contains ingredients that can reduce tissue damage.

Results of Hamida (2013) conducted by showed that 1000 mg / kg of nutrient enriched soybean tempe (NESTE) can significantly reduce the activity of the enzyme Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), cholesterol, triglicerida (TG), malondialdehyde (MDA) and nitric oxide (NO). On the other hand, the extract also increased the activity of the enzyme superoxide dismutase (SOD) and ferric Reducing Antioxidant Potential (FRAP). On histological examination results at 1000 mg / kg treatment group NESTE showed that the extract is able to recover damages hepatocytes structure so that it can be concluded that NESTE produced through the fermentation process can improve the hepatoprotective and antioxidant effects in vivo. Different results with this study, because the levels of MDA were not affected by administration of soy extract on mice group terintoksikasi lead though to the effects of the network gives the same result that is able to restore damaged tissue structure.

The Goddess' Research (2006) using CCl_4 to create animal models of liver damage caused by free radicals. Xenobiotic metabolism involving cytochrome P450, activate CCl_4 to be more reactive and be as free radicals, which causes liver damage in experimental animals. Lecithinin soy was administered orally in male rats that had been induced with CCl_4, has a hepatoprotective effect, the parameters examined is the enzyme activity of liver function tests (SGOT, SGPT, γ-glutamyltranspeptidase) and liver histopathology. The results showed that administration of CCl_4 1 ml / kg, single dose, intraperitoneally affect enzyme activity Liver Function Test (LFT) in serum was significantly ($P < 0.05$) and make the rat liver cell damage is very significant histopathological examination ($P < 0.01$) compared with controls. Results of linear regression analysis showed that the administration of a dose of 90 mg lecithin, 180 mg, and 360 mg / kg in rats induced CCl_4 LFT resulted in a decreased enzyme activity and liver cell damage siring with increasing doses of lecithin. In addition to the content of isoflavones in soy extract obtained lecithin is also thought to have played a role in repairing tissue histopathology.

CONCLUSIONS AND SUGGESTIONS

The results of this study concluded extract of soybean (Glycine max) Ijen varieties is not effective in reducing the levels of MDA and the activity of the enzyme catalase in the liver of mice with lead intoxication compared with vitamin C. The extract of soybean (Glycine max) Ijenvarieties and vitamin C are equally effective in repairing damaged Kidney cells of mice with lead intoxication.

REFERENCES

[1] Albulescu,M.andM.Popovici,2007.Isoflavones-biochemistry,pharmacology and therapeuticuse.*Reveu RoumainedeChimie*, 52(6):537-550.

[2] Ardyanto, Denny, 2005, Deteksi Pencemaran Timah Hitam (Pb) Dalam Darah Masyarakat yang Terpajan Timbal (Plumbum), *Jurnal Kesehatan Lingkungan*, 2(1), hal. 67-76.

[3] Christyaningsih J., 2008, Timbal dalam hepar dan ginjal pada kasus intoksikasi timbal dengan suplementasi vitamin C, *Pelita, Jan 1:23-28*

[4] Christyaningsih J., 2010, Pengaruh Asam askorbat terhadap kadar timbal fetus dan aktivitas enzim cytochrome P450 1A1 (CYP1A1) pada induk mencit terintoksikasi timbal,Berkala Penelitian Hayati, vol 16 no 1:27-31

[5] Christyaningsih J., 2014, Effect of Vitamin C on xenobiotic metabolism and histopathology of fetal brain of lead exposed mice, *Research Journal of Pharmaceutical, Biological and Chemistry Sciences*, 5(4):855-864

[6] Dewi, Lestari, 2006,*The Protective Effects of Lecithin on The Hepatotoxicity of Carbontetrachlorida Induction in Male Rats (Rattus norvegicus)*, Thesis, Unair

[7] ElandaRahmat Arifyanto, S. Bambang Widjokongko, Ipop Sjarifah, 2012, Efek Hepatoprotektor Ekstrak Tempe Kedelai Pada Mencit (Mus Musculus) Yang Diinduksi Parasetamol, *Nexus Kedokteran Translasional,*vol 1 no 2

[8] Falcon, M, Vinas, P, and Luna, A, 2002, Placental lead and outcome of pregnancy, *Environmental Toxicology and Pharmacology* (7), p.123-127

[9] Hamidah Mohd Yusof, Norlaily Mohd Ali, Swee Keong Yeap, Wan Yong Ho, Boon Kee Beh, Soo Peng Koh, Kamariah Long, Suraini Abdul Aziz, andNoorjahan Banu Alitheen, 2013, Hepatoprotective Effect of Fermented Soybean (Nutrient Enriched Soybean Tempe) against Alcohol-Induced Liver Damage in Mice, *Evidence-Based Complementary and Alternative Medicine*, Volume 2013

[10] Jayakumar K., Vijayarengan P., Changxing Z., Gomathinayagam M., Jaleel CA., 2008. Soil applied cobalt alters the nodulation, leg-haemoglobin content and antioxidant status of *Glycine max* (L.) Merr. *Biointerfaces* 67 : 272–275

[11] Lutz M, Henríquez C, E scobar M (2011). Chemical composition and antioxidant properties of mature and baby artichokes (Cynara scolymus L.), raw and cooked. *Journal of Food Composition and Analysis,*24(1):49-54.

[12] Mishra KP, Singh VK, Rani R, et al. 2003. Effect of lead exposure on the immune response of some occupationally exposed individuals. *Toxicology* 188:251-259

[13] Nurahman, Mary Astuti, Suparmo dan Marsetyawan HNE Soesatyo, 2013, The role of black soybean tempe increasing antioxidant enzym activity and human lymphocyte proliferation in vivo, *International Journal of Current Microbiology and Applied Sciences*,(2)9:316-327

[14] Prakash,D.,Upadhyay,G.,Singh,B.N.,& Singh,H. B.(2007).Antioxidant and free radical-scavenging activities of seeds and agri- wastes of some varieties of soybean (Glycinemax).FoodChemistry,104, 783–790