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Chitosan as Chelating and Protective Agents from Lead Intoxication in Rat

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lead intoxication; EDTA; chitosan; chelating; protective

Abstract

Increasing accumulation of lead in body causes serious health problems. Chelation is widely used to decrease lead level in body. This research aims the effectivity of chitosan in chelating lead and their protective ability toward liver and kidney for lead acetate-induced rat. Thirty rats were divided into 6 treatment groups. All groups, except control group, were administered by 175 mgkg^{-1} BW lead acetate. Positive control group was treated using EDTA 50 mgkg^{-1} BW. Treatment group 1,2, and 3 were treated using chitosan in dose of 64, 32, and 16 mgkg^{-1} BW dissolved in 2% acetic acid, respectively in 30 days. The effectivity of chitosan was compared to blood lead level. ALT and AST level were measured to determine the protective ability of chitosan. Normal function of kidney was assessed using creatinine level. Results showed that blood lead level from all treatment groups, except negative control group, had no significant difference from control group. EDTA and chitosan ability in chelating lead were proven by low level of AST, ALT, and creatinine in treatment groups. This indicated that there was no significant difference from control group. Chitosan capable of chelating lead and protecting kidney and liver from heavy metal.

How to Cite

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INTRODUCTION

Expanding industries causes increasing number of reported lead pollution incident from working environment. Lead (Pb) pollution from working environment was reported by Marianti et al. (2016). Results showed that 80% of the employees working for more than two years in a brass industry in Pati, Jawa Tengah, had blood lead level above 40 µg/dl. This value passed the threshold limit value for lead assigned by NIOSH (National Institute for Occupational Safety and Health) and CDC (Centre for Disease Control and Prevention), which was 10 µg/dl. Lead is not the raw material of brass industries. However, it is assumed that the raw materials of given industries contain lead. Gunawan, et al. (2013) reported that blood lead level from 41 employees working at a battery melting industry for 1 to 8 years in Kebasen, Adiwerna, Tegal, ranged from 0,6 to 108,3 ppm. Around 51% of total workers in metal melting industry in Ceper, Klaten, has high blood lead level, surpassing quality standards assigned by NIOSH (Ambarwanto, et al., 2015). Lead pollution also occurs in urban areas. This area is mainly affected by industrial waste from its surrounding. Marianti and Prasetya (2013) also reported people living in 56 villages in the district of Tanjung Mas North Semarang were detected of having average blood lead level at 8.304 ppm and peaked at 17.208 ppm. This detection used hair as bio-indicator for lead.

Lead exposure becomes an unavoidable aspect of people working in a heavy metal industry, considering that it is their main occupation. Chronic exposure of lead gives no direct negative effects towards the workers, resulting in late handling of the symptoms. As known to many people, lead exposure gives detrimental effects to physical and mental health. According to Kahn et al. (2009), increasing accumulation of lead in body may cause serious long-term detrimental health effects in multi organ level, including heart, liver, kidney, reproductive organs, brain, and erythrocytes.

High blood lead level will increase oxidative stress that affects tissue stability in many organs. The most susceptible organs exposed to lead are liver and kidney. In hepatocytes, lead will induce peroxidative catalyzation of saturated fat, reduction of N-oxide reductor, and formation of hydroxile radical. Tissue damage will result in increasing alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level in blood. This is a crucial sign of disruption in normal liver function (Khan, 2008). Result of a study in rats shows that ALT activity significantly increases following a Pb only treatment (Omotoso, et al., 2015). High level of oxidative stress also accelerates kidney damage. Kidney damage is marked by several symptoms, which are proteinuria, aminoaciduria, glycosuria, acidosis of kidney tubulars, and cellular gips. Inclusion bodies become prominent in tubular proximal cell in blood lead level of 40-80 µg/dl (Sujatha, et al., 2011).

In order to resolve the lead pollution, sufferers often receive EDTA (Ethylene diamine tetra acetic acid) admission therapy. In many cases of lead toxication, EDTA is administered through intravenous injection. This admission is curative, since people tend to agree of having intravenous injection of EDTA after suffering from the pain. EDTA chelates many types of heavy metals, including lead. However, EDTA admission exerts many side effects. Normal function of kidney must be first examined before admission of a chelating agent.

EDTA usage tends to be more curative rather than protective. Meanwhile, chronic exposure of lead will defect many organs. Using a protective chelating agent becomes more important due to harmful exposure of lead in many organs. One of the chelating agents that has protective effect is chitosan. Chitosan is a copolymer that consists of (1,4)-d-glucosamine and (1,4)-N-asetil-d-glucosamine. Chitosan is a crystal polysaccharide which is made of chitin deacetilation. Chitin is widely used as nontoxic biodegrading agent. Chitin is different from chitosan since chitin has acetyl group, meanwhile chitosan has NH_2 group. NH_2 group in chitosan will bind to many metal ions. Recent research shows that chitosan effectively absorb those heavy metal ions (Ben-shalom, et al., 2005).

The difference between the structure of chitin and chitosan is on the presence of acetyl in chitin and NH_2 group. NH_2 group in kitosan will interact with metal ions and research showed that Chitosan with efficient to absorb these metal ions (Ben-shalom, et al., 2005).

Several researches have been conducted towards human and animal using orally administered chitosan. Chitosan does not affect food intake, but decrease weight and significantly increases total fat and cholesterol excretion, lessens fat in plasm and liver, also increase lipase lipoprotein activity (Zhang, et al., 2008). chitosan also lowers triglyceride level, cholesterol level, and weight. Chitosan absorbs fat and excrets itthrough faeces, resulting in decreasing fat absorption. Chitosan also increases δ-ALAD enzyme actvity and haemoglobin level of lead acetate-ex-

posed rat (Xu, et al., 2007; Neyrinck, et al., 2009).

Chitosan ability in absorbing blood lead needs further examination, especially its comparative effectivity with EDTA, and also its protective properties for liver and kidney in lead acetate-exposed rat.

METHODS

This research was a true experiment in laboratory scale using Post-Test Control Group research design. This research was conducted in Laboratory of Biology FMIPA UNNES to treat test animals, Unit 1 Integrated Research and Testing Laboratory (LPPT unit 1) Gadjah Mada University, to test blood lead, ALT, AST, and blood serum creatinine level in test animals.

Thirty wistar strain white rats (*Rattus norvegicus* L) that fulfill the inclusion criteria were purposively selected from a population. Criteria for the test animals were male, healthy, ages more than two months old, and weighs more than 180 grams. These rats were divided into six groups. Each group consists of five rats (resulted from Frederer calculation). Group I is a control group, group II is a negative control group treated with 175 mg kg^{-1} BW (Body Weight) lead acetate, group III is a positive control group treated with lead acetate (175 mg kg^{-1}) + EDTA (50 mg kg^{-1}), group IV is the first treatment group (KP 1) treated with lead acetate (175 mg kg^{-1}) + chitosan (64 mg kg^{-1}), group V is the second treatment group (KP 2) treated with lead acetate (175 mg kg^{-1}) + chitosan (32 mg kg^{-1}), group VI is the third treatment group treated with lead acetate (175 mg kg^{-1}) + chitosan (16 mg kg^{-1}). Chitosan were dissolved in 2% acetic acid. Foods and drinks were freely given (*ad libitum*).

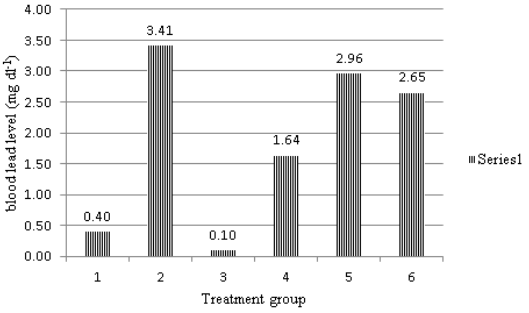
Treatments were given for 30 days. Then, blood was taken using microhematocrite through plexus orbitalis about 3 mL. Obtained whole-blood was then stored in heparin vacutainer tubes. The blood lead level was measured using Atomic Absorbance Spectrophotometry (AAS). The test was conducted at unit 1 LPPT Gadjah Mada University (UGM).

At th 31st day, blood was retaken for 2 mL to make serum for enzyme ALT, AST and creatinine level measurement. ALT and AST level were treated using photometric method. Creatinine level was measured using deproteinase method. All measurements were conducted unit 1 LPPT UGM.

Data was analyzed using one way ANOVA. When the result showed significant difference, the test was continued with Duncans Multiple Range Test (DMRT). Statistic analysis used SPSS software version 17.

RESULT AND DISCUSSION

Results of average blood lead level among treatment groups is shown below at Figure 1.



Information: 1) Control group; 2) Negative control group (Lead-acetate 175 mg kg^{-1}); 3) Positive control group (Lead-acetate 175 mg kg^{-1}+ NaEDTA 50 mg kg^{-1}); 4) Treatment group 1 (Lead-acetate 175 mg kg^{-1}+ Chitosan 64 mg kg^{-1}); 5) Treatment group 2 (Lead-acetate 175 mg kg^{-1}+ Chitosan 32 mg kg^{-1}); 6) Treatment group 3 (Lead-acetate 175 mg kg^{-1}+ Chitosan 16 mg kg^{-1})

Figure 1. Comparison of blood lead level from lead-acetate-exposed rat treated with EDTA and chitosan.

Results of normality and homogeneity analysis from research data showed that blood lead level data was normally distributed and homogen. Because of that, further statistical analysis using one way ANOVA is required. One way ANOVA results showed that there was a significant difference among treatment groups, proven by F value of 4.862 at significance level of < 0.05. Significant difference on variance analysis leads to further test using Duncan Multiple Range Test (DMRT). Results of DMRT is shown below at Table 1.

Table 1. Duncan Multiple Range Test Result of Blood Lead Level from Lead-acetate Exposed Rat

Treatment	Average
Control group	0.09600^{a}
Positive control group (EDTA)	0.09600^{a}
Treatment group 3 (chitosan 64 mg)	0.81480^{a}
Treatment group 4 (chitosan 32 mg)	1.35960^{a}
Treatment group 5 (chitosan 16 mg)	1.84760^{a}
Negative control group	4.17600^{b}

* Different letters show significant difference at the level of $p<0.05$

DMRT analysis results showed that, except

negative control group, all treated group not significant difference with control group. Chitosan-treated groups did not differ significantly with both control group and EDTA-treated group but not significant difference with negative control group. This means that chitosan posses the property of chelating lead-acetate.

Research showed that negative control has the highest exposure of lead compared to another groups. Ibrahim, et al. (2012). It is assumed due to the absence of chelating agents that reduce lead contamintaion inside the body of the rats. Lead elimination fully depends on the ability of body to detoxify the given compound. It is not quite effective to reduce the lead contamination in blood. Lead itself is excreted through urine around 75-80% from its total amount, 15% through faeces, and the rest of it through bile, sweat, nail, and hair (Palar, 2008). Lead excretion through digestive tracts is mainly affected by passive and active tracts of saliva gland, pancreas, many glands on intestinal wall, epithelial cell regeneration, and bile excretion. Elimination of anorganic lead passes through kidney (major) and breast milk (minor), whereas organic lead is eliminated through urine, faeces (major), and sweat (Kosnet, 2012). Since all the rats used in this research are male, main pathway of lead elimination from the body goes through the kidney.

As widely known, lead in blood is bound to erythrocyte for around 25 days. It settles inside the smooth tissue for 40 days and 25 years inside the bone. Settling lead can be transmitted through the blood once blood lead level decreases. It causes most cases of lead toxication. Slow excretion of lead causes lead to easily settles inside the body tissue and health disturbance.

Different results are shown by groups treated with chelating agents, which are chitosan and EDTA. Measured blood lead level in these groups shows no significant difference from control group, means that the blood lead level of both groups are almost equal. Measured blood lead level from positive control group, which is treated using EDTA, is equal to control group. Whereas chitosan-treated group has higher blood lead level compared to positive control and control group. However, statistical analysis does not give any significant difference. This means that chitosan has equal ability to EDTA to chelate blood lead. According to average measured blood lead level, chitosan at dose of 64 $mgkg^{-1}$ exerts the best result to lower the blood lead level compared to the chitosan on dose of 32 $mgkg^{-1}$ and 16 $mgkg^{-1}$

This research also proves that chitosan posses the potential to become a new chelating agent for chronic lead exposure. Such property is supported by the chemical structure of chitosan which has NH_2 group on its chain. This group will bind to metal ions. Amina group of chitosan has an active side that forms a complex with stable metal ions. Chitosan effectively absorbs those metal ions. Admission of 1% and 2% of chitosan by deacetylation degree of 64%, 65%, and 75% on lead-acetate exposed mice increases ALAD enzyme activity and Hb level (Suharsih 2008). The increase itself differs significantly from the control group. Escalation of ALAD enzyme acitivity and Hb level in blood can happen due to low blood lead level. This indicates that chitosan lowers blood lead level (Ben-shalom, et al., 2005)

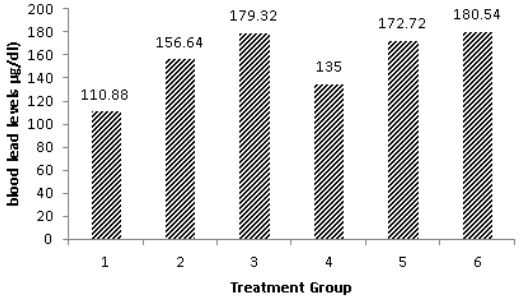
Chitosan is proven to have lower ability to chelate heavy metal ions compared to EDTA (although statistically is not significantly different). EDTA has more chain to bind heavy metal compared to chitosan. EDTA usually binds metal cation through two amin groups and four carboxylate groups. At the end, there are six chains for EDTA to bind heavy metal. Meanwhile chitosan only has three amina groups that can bind to heavy metal. However, chitosan may exert equal ability to bind heavy metal as EDTA (Ngah & Fathinathan, 2010).

Another advantage of using chitosan as chelating agent is that chitosan is not toxic compared to EDTA. Lowest dose of EDTA that still exerts toxic effects towards animal is 750 $mgkg^{-1}day^{-1}$. The most common side effect from EDTA chelation therapy is burning feeling on where the medication is injected. Other side effects include gastrointestinal problems, such as nausea and vomiting, as well as headache. In some cases, patients receiving EDTA chelation therapy may also experience sudden drop in blood sugar (hypoglycemia) or in blood pressure (hypotension). Another side effect of EDTA therapy is kidney failure. This is because EDTA binds minerals and causes the kidneys to filter them out. However, in the process, it can overwork the kidney, leading to a condition known as acute tubular necrosis.

EDTA admission for human must be administered through intravenal injection to cure lead toxication. This will be difficult since most chronic lead toxication sufferers do not feel certain symptoms. Most people will reject infuse treatment before getting the symptoms. This will lead people to not seek for help. It is easier to administer chitosan through per oral pathway to those people who are prone to lead exposure, for example workers in brass industry.

AST Level

Comparison of AST level in blood serum between treatment groups is shown below at Figure 2.



Information: 1) Control group; 2) Negative control group (Lead-acetate 175 mg kg^{-1}); 3) Positive control group (Lead-acetate 175 mg kg^{-1}+ NaEDTA 50 mg kg^{-1}); 4) Treatment group 1 (Lead-acetate 175 mg kg^{-1}+ Chitosan 64 mg kg^{-1}); 5) Treatment group 2 (Lead-acetate 175 mg kg^{-1}+ Chitosan 32 mg kg^{-1}); 6) Treatment group 3 (Lead-acetate 175 mg kg^{-1}+ Chitosan 16 mg kg^{-1})

Figure 2. Comparison of AST level in blood serum from lead-acetate exposed rat between each treatment group.

Results of normality and homogeneity analysis showed that obtained research data of AST level in blood serum was classified as normal and homogen. Further parametric analysis using one way ANOVA method was then chosen.

One way ANOVA results showed that there was a significant difference between each treatment group, proven by F value of 5,084 at significance level of 0.003. This leads to another analysis using Duncan Multiple Range Test (DMRT). Results of given further analysis is shown below at Table 2.

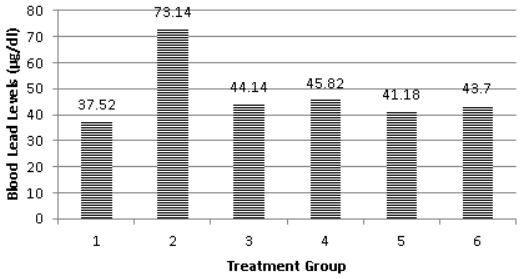
Table 2. Duncan Multiple Range Test Result of AST Level in Blood Serum from Lead-acetate Exposed Rat

Treatment	Average*
Control group	110.880[a]
Treatment group 3	135.000[a]
Negative control group	156.640[bc]
Treatment group 2	172.720[bc]
Positive Control Group	179.320[c]
Treatment group 1	180.540[c]

* Different letters show significant difference at the level of $p<0.05$

ALT Level

Results of ALT level measurement in blood serum from each teratment group is shown below at Figure 3.



Information: 1) Control group; 2) Negative control group (Lead-acetate 175 mg kg^{-1}); 3) Positive control group (Lead-acetate 175 mg kg^{-1}+ NaEDTA 50 mg kg^{-1}); 4) Treatment group 1 (Lead-acetate 175 mg kg^{-1}+ Chitosan 64 mg kg^{-1}); 5) Treatment group 2 (Lead-acetate 175 mg kg^{-1}+ Chitosan 32 mg kg^{-1}); 6) Treatment group 3 (Lead-acetate 175 mg kg^{-1}+ Chitosan 16 mg kg^{-1})

Figure 3. Comparison of ALT level in blood serum from lead-acetate exposed rat between each treatment group.

Result of normality and homogeneity analysis showed that ALT level in blood serum of lead-acetate exposed rat was classified as normal and homogen. One way ANOVA was then chosen for parametric analysis.

One Way ANOVA results in a significant difference between each treatment group for F value of 8.691 at significance level of < 0,05. Duncan Multiple Range Test (DMRT) was applied for further analysis. The result is shown below at Table 3.

Table 3. Duncan Multiple Range Test Result of ALT Level in Blood Serum from Lead-acetate Exposed Rat

Treatment	Average
Control group	37.520[a]
Treatment group 2	41.180[a]
Treatment group 3	43.750[a]
Positive Control group	44.140[a]
Treatment group 1	45.820[a]
Negative control group	73.140[b]

* Different letters show significant difference at the level of $p<0.05$

According to the further test, it is known that all treatment groups do not significantly differ from control group, except negative control group. This means that chelating agents, both EDTA and chitosan, have the ability to protect normal function of liver from negative effects exerted by lead exposure. Where as negative control group, which were exposed by lead-acetate without any chelating agent, has high higher ALT level compared to any other group. High ALT level signs on disruption of liver function.

Statistical analysis results shows that AST level in blood serum of lead-acetate exposed rats is higher compared to control group. Similar condition is also found in ALT level. This indicates that there is a liver malfunction in rats exposed by lead-acetate. Ibrahim et al. (2012) also reported that stimulation in ALT and AST level were gradually paralleled with increasing Pb^{2+} ingested doses. The present result of the liver function parameters (ALT dan AST) shows the damage in liver cells of Pb^{2+} intoxicated animal. These observations reported that lead had hepatotoxic effect. The present result shows that effect of lead acetate on transaminase activity is independent. The high plasma ALT and AST activities are accompanied by high liver microsomal membrane fluidity, free radical generation, and alteration in liver tissue histogram.

Statistical analysis of AST level shows that all treatment groups gives different result from control groups. Only a group treated with chitosan in dose of 64 $mgkg^{-1}$ that gives no significantly different result from control group. Negative control group is not significantly different from a treatment group treated with chitosan in dose of 32 $mgkg^{-1}$ positive control group, and a treatment group with chitosan in dose of 16 $mgkg^{-1}$.

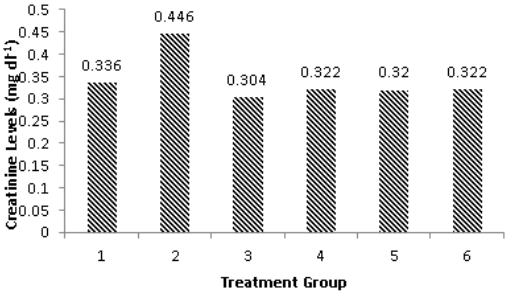
Per oral lead exposure will cause lead absorption in jejunum tract. Absorbed lead will move upward into vena porta hepatica to enter liver. In liver, hepatocyte will inhibite enzymes, interference with essential cations, and change membrane and receptor structure. Lead accumulation induces hepatotoxicity through the depletion of glutathione and protein, resulting in enhanced production of reactive oxygen species (ROS) such as peroxide ion, hydroxyl radical and H_2O_2. These ROS increase peroxidation lipids and cell membrane damage. According to Prasanthi et al. (2010) Pb-induced decrease in free radical scavenging enzymes is mostly attributed to the high affinity of Pb for sulfhydryl groups or metal co-factor in these enzymes and molecules. Pb also binds to the –SH groups of Catalase (CAT), Superoxide dismutase (SOD), Xantine Oxidation (XO). This leads another inhibition of the following antioxidant enzymes activities. The inhibitory effects of Pb on various enzymes will result in impaired antioxidant defenses by cells and render cells more vulnerable to oxidative attacks.

Damages in hepatocytes can be detected from the presence of aminotransferase aspartate enzyme (AST), or widely known as glutamic oxaloacetate transaminase (GOT) and alanine aminotransferase (ALT) or commonly known as glutamic pyruvate transaminase (GPT). These transaminase enzymes are included into nonfunctional plasm enzymes that is not held accountable for physiological function inside the blood. Normally, these enzymes cannot be found in blood. Transaminase existence inside the blood at high level signs tissue damage (Hidayat, et al., 2013). These two enzymes will be excreted for hepatocytes once hepatocytes are damaged (necrosis). Tissue damage will increase the level of these two enzymes in blood serum (Gajawat, et al., 2006; Wibowo, et al., 2006). Increasing level of ALT and AST in blood serum of lead-acetate exposed rats is also reported by Khan (2008).

Groups treated with EDTA and chitosan shows lower ALT and AST level. This means that both chelating agents are able to protect normal function of liver. Both chelating agents inhibit lead infiltration to blood vessel through per oral administration. EDTA and chitosan will bind dissolved lead to amina and carboxylate groups. However, there will still be accumulated lead inside the liver due to chronic exposure. The physiological disruption is not severe compared to rats in negative control group. This condition is proven by average blood AST and ALT level from negative control group which is higher than other groups.

Creatinine Level

Result of creatinine measurement from each treatment groups is shown below at Figure 4.



Information: 1) Control group; 2) Negative control group (Lead-acetate 175 mg kg^{-1}); 3) Positive control group (Lead-acetate 175 mg kg^{-1}+ NaEDTA 50 mg kg^{-1}); 4) Treatment group 1 (Lead-acetate 175 mg kg^{-1}+ Chitosan 64 mg kg^{-1}); 5) Treatment group 2 (Lead-acetate 175 mg kg^{-1}+ Chitosan 32 mg kg^{-1}); 6) Treatment group 3 (Lead-acetate 175 mg kg^{-1}+ Chitosan 16 mg kg^{-1})

Figure 4. Comparison of Creatinine level in blood serum from lead-acetate exposed rat between each treatment group.

Results of normality and homogeneity analysis showed that creatinine level data is normally distributed and homogen. This allowed

further analysis of one way ANAVA. One way ANOVA showed a significant difference between each treatment group, proven by F value of 2.773 at significance level of < 0.05. Duncan Multiple Range Test was chosen for another analysis and the result is shown at Table 4.

Table 4. Duncan Multiple Range Test Result of Creatinine Level in Blood Serum from Lead-acetate Exposed Rat

Treatment	Average
Positive control group	0.3040[a]
Treatment group 3	0.3200[a]
Treatment group 1	0.3220[a]
Treatment group	0.3220[a]
Control group	0.3360[a]
Negative control group	0.4460[b]

* Different letters show significant difference at the level of $p<0.05$

According to the result of further test, it is known that all treatment groups is not significantly different from control group (normal rats), except the negative control group. This shows that both EDTA and chitosan protect normal function of kidney from lead-acetate effect. Negative control group shows higher creatinine level compared to other group, since it is only exposed to lead-acetate without any chelating agent. Increasing creatinine level becomes a sign for kidney malfunction.

Kidney is an important organ in toxic compund excretion process, including lead. Lead is excreted through urine aroung 75-80% from its total amount, 15% through faeces, and the rest of it through bile, sweat, nail, and hair (Palar, 2008). Chelating agents can affect metal toxicity by mobilizing the toxic metal mainly into urine (Flora & Pachauri, 2010). This condition leads to high workload for kidney to excrete toxin from the body.

The effect of lead on renal function can be attributed into the alterations in the antioxidant defensive system, resulting in kidney injury. Lead accumulation inside the cells causes severe damage to mitochondria. Many cells are unable to perform normal functions and this condition causes oxidative stress (Sujatha, et al., 2011). Lead induces oxidative damage by reducing anti defense mechanism at cellular level and it increases apoptotic bodies in proximal convoluted tubules of kidneys. Lactate dehydrogenase (LDH) activity intoxicates rats with lead acetate (Ibrahim, et al*.,* 2012).

Further analysis result shows that almost all treatment groups is not significantly different from control group (normal rats), except the negative control group. This indicates that both EDTA and chitosan has the abitility of protecting kidney from lead exposure. Creatinine level in negative control group shows higher number compared to other groups treated with chelating agents. This also indicates a kidney malfunction. According to statistical analysis result, chitosan is able to protect kidney as good as EDTA, although average measured creatinine level from chitosan-treated group is higher than EDTA-treated group.

CONCLUSION

Chitosan capable of chelating lead and protecting kidney and liver from lead intoxication. Researchers suggest more organ examinations for further research. Another method aside from AAS to measure blood lead level may also be considered since AAS method requires relatively large amount of blood to validate the result.

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REFERENCES

Ambarwanto, S. T., Nurjazuli, & Raharjo, M. (2015). The relationship between Blood Lead Level (BLL) with Hypertention on Gen Metal Casting Industry Workers In Klaten Ceper. *Jurnal Kesehatan Lingkungan Indonesia,* 14(2), 35-40.

Ben-shalom, N., Kudabaeva, N., & Borisover, M. (2005). Copper-binding efficacy of water-soluble chitosans: characterization by aqueous binding isotherms. *Chemosphere*, 59(9), 1309-1315.

Flora, S. J. S. & Pachauri, V. (2010). *Review* Chelation in Metal Intoxication. *International Journal of Environmental Research and Public Health. 7*(7), 2745-2788.

Gajawat, S., Sancheti, G., & Goyal, P. K. (2006). Protection Against Lead Induced Hepatic Lesion in Swiss Albino Mice by absorbis Acid. *Pharmologi online,* 1,140-149.

Gunawan, L., Setiani, O., & Suhartono, S. (2013). Hubungan Kadar Timah Hitam dalam Darah dengan Jumlah Lekosit, Trombosit, dan Aktifitas Superoxide Dismutase (SOD) pada Pekerja Timah Hitam di Kabupaten Tegal. *Jurnal Kesehatan Lingkungan Indonesia*, 12(2), 106-110.

Haouas, Z., Sallem, A., Zidi, I., Hichri, H., Mzali, I., & Mehdi, M. (2014). Hepatotoxic effects of lead acetate in rats: histopathological and cy-

totoxic studies. *Journal of Cytology & Histology*, 5(5), 1.

Hidayat, A., Christijanti, W., & Marianti, A. (2013). Pengaruh Vitamin E terhadap Kadar ALT dan AST Tikus Putih Galur Wistra yang Dipapar Timbal. *Unnes Journal of Life Science,* 2(1), 16-21.

Ibrahim, N. M., Eweis, E. A., El-Beltagi, H. S., & Abdel-Mobdy, Y. E. (2012). Effect of lead acetate toxicity on experimental male albino rat. *Asian Pacific Journal of Tropical Biomedicine*, 2(1), 41-46.

Khan, M. S. H., Mostofa, M., Jahan, M. S., Sayed, M. A., & Hossain, M. A. (2008). Effect Of Garlic and Vitamin B-Complex in Lead Acetate Induced Toxicities in mice. *Bangladesh Society for Veterinary Medicine,* 6(2), 203-210.

Khan, D. A., Qayyum, S., Saleem, S., & Khan, F. A. (2009). Evaluation of lead body burden in occupational workers by lead mobilization test. JPMA. *The Journal of the Pakistan Medical Association*, 59(6), 350-354

Kosnett, M. J. (2012). Heavy Metals Intoxication and Chelators (Chapter 57). *Basic and Clinical Pharmacology*. 12th edition. Editor Katzung, B,G. Masters, SB., Trevor, AJ.. Mc Graw-Hill Companies Inc.

Marianti, A., Anies, A., & Abdurachim, H. R. S. (2016). Causality Pattern Among The Blood Lead, Monoamine Oxidase A, and Serotonin Levels In Brass Home Industry Workers Chronically Exposed By Lead. *Songklanakarin Journal . Science. Technology*, 38(2), 147-153.

Marianti, A. & Prasetya, A. T. (2013). Rambut sebagai Bioindikator Pencemaran Timbal pada Penduduk di Kecamatan Semarang Utara. *Biosaintifika: Journal of Biology & Biology Education*. 5(1), 10-15.

Neyrinck, A. M., Bindels, L. B., De Backer, F., Pachikian, B. D., Cani, P. D., & Delzenne, N. M. (2009). Dietary supplementation with chitosan derived from mushrooms changes adipocytokine profile in diet-induced obese mice, a phenomenon linked to its lipid-lowering action. *International Immunopharmacology,* 9(6), 767-773.

Ngah, W. S. W., & Fatinathan, S. (2010). Pb(II) biosorption using chitosan and chitosan derivatives beads: Equilibrium, ion exchange and mechanism studies. *Journal of Environmental Sciences,* 22(3), 338-346.

Omotoso, B. R., Abiodun, A. A., Ijomone, O. M., & Adewole, S. O. (2015). Lead-Induced Damage on Hepatocytes and Hepatic Reticular Fibres in Rats; Protective Role of Aqueous Extract of Moringa oleifera Leaves (Lam). *Journal of Biosciences and Medicines*, 3(5), 27-35.

Palar, H. (2008). *Pencemaran dan Toksikologi Logam Berat*. Jakarta: Penerbit PT Rineka Cipta.

Prasanthi, R. J., Devi, C. B., Basha, D. C., Reddy, N. S., & Reddy, G. R. (2010). Calcium and zinc supplementation protects lead (Pb)-induced perturbations in antioxidant enzymes and lipid peroxidation in developing mouse brain. *International Journal of Developmental Neuroscience*, 28(2), 161-167.

Suharsih. (2008). Pengaruh Derajat Deasetilasi Kitosan terhadap kadar Plumbum (Pb) Darah dan Aktivitas Enzim Delta Aminolevulinic Acid Dehydratase (δ-ALAD) Mencit Albino (Mus musculus L). *Tesis.* Medan: Sekolah Pasca Sarjana Universitas Sumatera Utara.

Sujatha, K., Srilatha, C. H., Anjaneyulu, Y., & Amaravathi, P. (2011). Lead acetate induced nephrotoxicity in wistar albino rats, pathological, immunohistochemical and ultra structural studies. *International Journal of Pharma and Bio Sciences*, 2(2), B459-B469.

Wibowo, A. W. L. Maslachah & Bijanti, R. (2008). Pengaruh pemberian Perasan Buah Mengkudu (*Morinda citrifolia*) Terhadap Kadar AST dan ALT Tikus Putih (Rattus norvegicus) Diet tinggi Lemak. *Jurnal Veterineria Medika Universitas Airlangga,* 1, 1-5

Xu, G. F., Huang, X. D., Qiu, L. L., Wu, J. B., & Hu, Y. Q. (2007). Mechanism study of chitosan on lipid metabolism in hyperlipidemic rats. *Asia Pacific journal of clinical nutrition,* 16(S1), 313-317.

Zhang, X., Yang, L., Li, Y., Li, H., Wang, W., & Ye, B. (2012). Impacts of lead/zinc mining and smelting on the environment and human health in China. *Environmental monitoring and assessment*, 184(4), 2261-2273.