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Influence of Chitosan from Shrimp Skin to Quality and Quantity of Sperm of Albino Rats after Administration of Lead

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Abstract

Background: Pollution Plumbum (Pb) has become a public health problem in the world, especially in developing countries, such as Asia, Africa and Latin America. For the humans to cause disturbances in the body's organs such as the testes, will indirectly affect the quantity and quality of sperm.

Aim: In the present study, the ability of lead to adversely affect the male reproductive system was investigated and chitosan (CHn) was administered orally to prevent the adverse effects of Pb.

Materials and methods: Thirty Wistar rats, randomised into six groups (n=5), were used for this study. Distilled water administration for 7 weeks (negative control), Pb for 2 weeks + Pb and distilled water for 5 weeks (positive control), Pb for 2 weeks + Pb and chitosan -0.5% for 5 weeks (treatment-1), Pb for 2 weeks + Pb and chitosan-0,75% for 5 weeks (treatment-2), Pb for 2 weeks + Pb and chitosan-1% for 5 weeks (treatment-3). All treatments were for 7 weeks.

Statistical Analysis: Kruskal-Wallis and Mann–Whitney *U*-test were used to analyse the results obtained.

Results: The obtained results showed that Pb caused a significant reduction in the sperm count, sperm motility, normal sperm morphology, and sperm viability, but not a significant increase in ratio of testis/100 g body weight of rat. Chitosan, however, significantly reduced these adverse effects of Pb in cauda epididimis (sperm count, sperm motility, normal sperm morphology, and sperm viability).

Conclusion: These findings lead to the conclusion that chitosan significantly lowered the adverse effects of Pb exposure on the testis as well as Pb-induced oxidative stress.

Keywords: Lead; Quantity and quality of sperm: Chitosan

Introduction

Lead (Pb) as one component of air pollutants have toxic effects on humans and large animals because they interfere with the function of the renal tract, gastrointestinal tract, the nervous system in young, lower fertility, reduce the number of spermatozoa, and increased sperm abnormalities and spontaneous abortions [1]. Plumbum is highly toxic and has a lot of research that says that sulfuric health disorders that can cause blood, liver, kidney, brain, and testes because karsinogenikya effect. It is known that lead can cause oxidative stress by increasing the formation of free radicals and antioxidant systems in the network decrease. Oxidative stress can cause damage to molecules within the cell. Lipid molecules are subjected to oxidative stress will undergo auto - oxidation or better known as lipid peroxides. Proteins that undergo oxidation become dysfunctional and DNA oxidized to mutagens, carcinogens or cause cell death [2].

In men, the effects of lead including lowering the number of spermatozoa and the increasing number of abnormal spermatozoa. Pb toxic effects on the male reproductive function that affect the process of spermatogenesis resulting in decreased quality of semen in the number, morphology, motility and abnormal forms of spermatozoa [3]. There is a lot of research data which showed that Pb alter membrane lipid composition that result in changes in the integrity, permebilitas and function. All this resulted in increased sensitivity to the lipid membrane lipid peroxide [4,5].

Chitosan is a derivative of a natural carbohydrate biopolymer derived from chitin deasetilisasi. Invertebra chitin can be obtained from the sea, insects, fungi and yeast. Type of crustacean containing 20-30% in the eksoskeletonnya [6]. Chitosan is safe for human health because it is non-toxic (non- toxic), can be decomposed naturally (biodegradable) and metal absorber (biosorbents) [7]. According to Nessaa et al. chitosan used in some pharmaceutical industry and biomedical industry (immobilization of enzymes and purification (purification), in the chemical industry (waste treatment) and in the food industry (food preservatives), thickeners (gel) [8]. Chitosan recommended daily dose for treatment is 1-6 grams. From the description above it is necessary to assess whether the shrimp shell chitosan extract a lot of wasted in the environment may improve spermatogenesis and sperm DNA damage prevents white male rats were infertile due to exposure to sulfuric acetate.

Materials and Methods

Thirty (30) adult male Wistar rats (150-250 gm; 11-18 weeks) were used for this study. They were inbred at the Animal House section of the

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Department of Biology, Faculty of Mathematics and Natural Science, University of North Sumatra, Medan - Indonesia. The animals were acclimatized over a period of 2 weeks.

Preparation of chitosan

White shrimp was obtained from the fisherman in Belawan - Medan. The skin of the head, body and tail, is taken as the material obtained chitin. Then the skin is processed to find chitin through deproteinase process, demineralization, washing and drying until pH neutral and white color. Chitin is then transformed by asetilisasi to obtain chitosan. Discovery process and chitin from shrimp shell chitin into chitosan asetilisasi performed at Integrated Laboratory Faculty of Mathematics and Natural Sciences - USU Medan.

Grouping of animals and treatment

Thirty (30) Wistar rats were housed four per cage in a light-controlled room (12 hr light: 12 hr darkness; lights on at 07.00 hr). Randomised into six groups (n=5), were used for this study. Distilled water administration for 7 weeks (negative control), Pb for 2 weeks + Pb and distilled water for 5 weeks (positive control), Pb for 2 weeks + Pb and chitosan-0.5% for 5 weeks (treatment-1), Pb for 2 weeks + Pb and chitosan-0,75% for 5 weeks (treatment-2), Pb for 2 weeks + Pb and chitosan-1% for 5 weeks (treatment-3), Chitosan-1% for 2 weeks + chitosan-1% for 5 weeks (treatment-4). All treatments were for 7 weeks. Determination of the dose of chitosan based Suharsih (2008) which has been modified.

Animal sacrifice and collection of samples

Twenty-four hours after the last treatment, each animal was sacrificed by cervical dislocation and testis samples were collected via petri dish. Testis and caudal epididymis were excised from each rat.

Collection of data and statistical analysis

Ratio of Testis weight and Body weight: Body weight in rats weighed before was sacrificed. Cauda epididymis and testes were weighed, after that testis was separated with cuda epididymis. The metal accumulations in the testis were determined using AAS. Cauda epididymis in the press with tweezers and sperm removed and placed in a petri dish containing 1 mL of NaCl 0.9%. Two cauda epididimis were taken from each rat for sperm count. Sperm count was determined using the method described by Zaneveld et al. [9]. The data obtained are presented as mean ± SD. The "Control Group (negative) (K1) and positive control (K2)" and the "Test Groups (P1, P2, P3, and P4)" were compared using the Mann-Whitney *U*-test. The significance level was set to a *P*-value <0.05.

Results

The following results were obtained and are presented as mean ± SEM. Level of significance is taken at "*P*-value < 0.05" and/or "*P*-value < 0.01".

Results of Chitosan

Chitosan is derived from shrimp shells and creamy. Before use, chitosan dissolved in 1% acetic acid in accordance with a predetermined dose of treatment. For example, to make chitosan 0.5%, then 0.5 g of chitosan taken that has been provided later in 1% acetic acid added to reach a volume of 100 mL. As well as the manufacture of the other doses (Figure 1).

One kg of crab shell chitosan obtained 50 g or ± 0.5% chitosan obtained. Collecting shells or shells without a throw a first step in exploiting the natural resources are wasted or recycled. If beneficial to the development of anti-toxicity materials, will be better and can reduce useless waste into something useful. For policy makers in public health will be a concern to consider.

Ratio of testis weight (g)/100 g body weight of rats during 7 weeks of treatment

Ratio of Testis Weight (g)/100 g Body Weight of rats was measured at the end of treatment (7 weeks) after administration of Pb (Figure 2). Statistical analysis ANOVA (normal distribution of data and homogeneous variance) showed no significant difference p>0.05 between control and multiple treatment studies with p>0.05 ie, p = 0.076. So, no need to do further tests to distinguish between treatments were carried out (Table 1).

No significant differences Testis/100g Weight Loss Weight ratio

Figure 1: Chitosan derived from shrimp shells. Dry chitosan photos that looked like coarse grains. An arrow is chitosan.

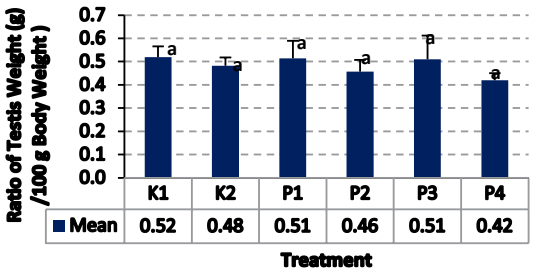

Figure 2: Histogram of the ratio of testis weight/100 g body weight of rats in the same treatment.
Description:K1=control,K2=control+Pb,P1=Pb+chitosan-0.5%, P2=Pb+chitosan-0.75%, P3=Pb+chitosan-1%, P4=chitosan-1%; Lowercase the different treatments were not significantly different (p>0.05) at the 5% level.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.697	5	0.139	2.303	0.076
Within Groups	1.452	24	0.061		
Total	2.149	29			

Table 1: ANOVA results testis/100 weight ratio g body weight of rats during 5 weeks between control and multiple treatment.

among all study treatment (p>0.05). Data does not need to be tested further Dangan Post Hoc Analysis - Bonferroni. Weight ratio testis/100 g Weight was not significantly different (p<0.05) between treatments in the absence of influence of Pb and chitosan significantly to the reduction or testis weight and body weight. Possible influence of Pb and chitosan new look at the level of physiology in the rat testis or body (Figure 2).

Blood lead concentration

Measurement and analysis of blood lead concentrations in rats can be seen in Figure 3. Data of blood lead concentration are not normally distributed and not homogeneous. Data stays that way even after transformation. Data were tested by Kruskal-Wallis and obtained p value<0.05. Thus followed by Mann-Whitney test to determine which groups was difference significantly. Based on this analysis, each group of data specified notation in order to facilitate the reading of data as a whole. Mann-whitney calculation results are written in table 2.

Morphology of rat spermatozoa

Sperm morphology was measured at the end of treatment (5 weeks) after administration of Pb (Figure 4). Result of Kruskal-Wallis statistical analysis (normal distribution of data but not homogeneous) showed significant differences p<0.05 between treatments (Table 3).

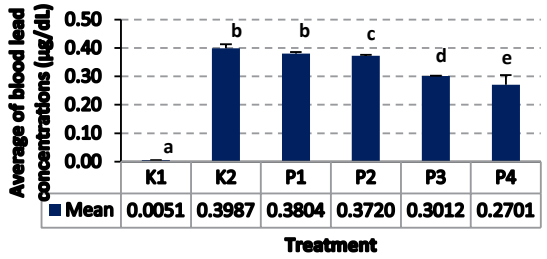

Figure 3: Histogram of average of blood lead concentrations rats after several treatments.
Description: K1=control, K2=control+Pb, P1=Pb+chitosan-0.5%, P2=Pb+chitosan-0.75%, P3=Pb+chitosan-1%, P4=chitosan-1%; Lowercase different the different treatments were significantly different (p<0.05) at the level of 5%.

Groups	Difference of "Sum of Ranks"	Nilai "Asymp. Sig. (2-tailed)"
K1-K2	35.00	0.009
K1-P1	35.00	0.009
K1-P2	35.00	0.009
K1-P3	35.00	0.009
K1-P4	35.00	0.009
K2-P1	17.00	0.076
K2-P2	23.00	0.016
K2-P3	35.00	0.009
K2-P4	35.00	0.009
P1-P2	23.00	0.016
P1-P3	35.00	0.009
P1-P4	35.00	0.009
P2-P3	35.00	0.009
P2-P4	35.00	0.009
P3-P4	35.00	0.009

Table 2: P-value of Mann-Whitney test results for difference "Sum of Ranks" measure blood lead levels between treatment groups.

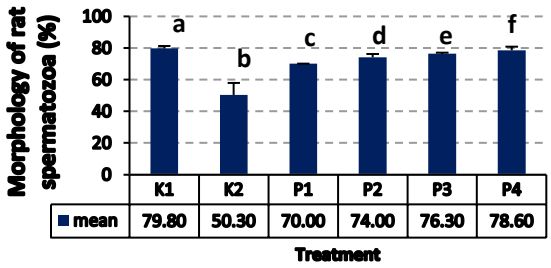

Figure 4: Histogram average normal morphology of rat spermatozoa after multiple treatments.
Description: K1=control, K2=control+Pb, P1=Pb+ chitosan-0.5%, P2=Pb+chitosan-0.75%, P3=Pb+ chitosan-1%, P4=chitosan-1%; Lowercase different the different treatments were significantly different (p<0.05) at the level of 5%.

Groups	Difference "Sum of Ranks"	Value "Asymp. Sig. (2-tailed)"
K1-K2	25	0.009
K1-P1	25	0.005
K1-P2	25	0.007
K1-P3	25	0.009
K1-P4	6	0.525
K2-P1	25	0.005
K2-P2	25	0.007
K2-P3	25	0.009
K2-P4	25	0.008
P1-P2	20	0.014
P1-P3	25	0.005
P1-P4	25	0.005
P2-P3	25	0.009
P2-P4	25	0.009
P3-P4	15	0.112

Table 3: P-value of Mann-Whitney test results for difference "Sum of Ranks" measure blood lead levels between treatment groups.

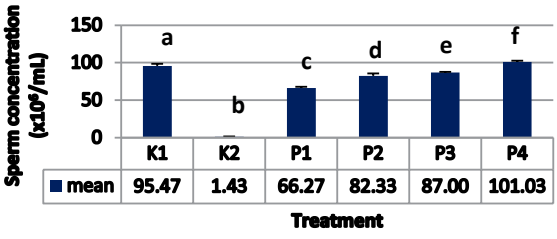

Figure 5: Histogram of average of sperm concentration rats after several treatments.
Description: K1=control, K2=control +Pb, P1=Pb+chitosan-0.5%, P2=Pb+chitosan-0.75%, P3=Pb+chitosan-1%, P4=chitosan-1%; Lowercase different the different treatments was significantly different (p<0.05) at the 5% level.

Number of rat spermatozoa

Measurement and analysis of the number of rat spermatozoa can be seen in figure 5 data on the number of spermatozoa with normal distribution rats but not homogeneous. Once transformed data remains normal distribution but not homogeneous. Then the data was tested with the Kruskal-Wallis and obtained p value <0.05. Thus followed by Mann-Whitney test to determine which groups were significantly

different. Based on this analysis, each group of data specified notation in order to facilitate the reading of data as a whole. Mann-whitney calculation results are written in table 3. The observation and analysis of the number of spermatozoa seen rats decreased when given Pb and increased with addition of chitosan. Compared with the controls and the addition of chitosan, Pb significant negative effect ($p < 0.05$) on the number of rat spermatozoa.

Discussion

Weight ratio testis/100 g body weight had no significant differences ($p > 0.05$) between control and other treatments. It shows the change of quantity and quality of sperm does not directly affect testis weight and body weight of rats. Given the possibility of Pb contents have not been able to influence the overall factors related to body weight and testicular determinant. The testes are non spermatogenic tissue such as smooth muscle tissue and the tunica albugenia. According Isradji (2011), Pb-acetate had no significant effect ($p > 0.05$) on the weight and volume of the testes.

Blood Pb levels seen on providing the highest Pb (K2) were not significantly different from P1 (Pb+chitosan-0.5%). If distinguished from control (K1), proved that the administration of Pb can cause an increase in blood Pb rats ($p < 0.05$). This allows the disruption of other functions in the body of rats fed Pb. In this study relate to the structure and function of the male reproductive or spermatogenesis. Ardyanto (2005), Plumbum enter the body primarily through the respiratory system and the digestive tract, while absorsbsi through the skin is very small so it can be ignored [11]. Diabsorsbsi of lead transported by the blood to the organs of the body, and as much as 95% Pb in the blood bound by erythrocytes. Excretion of Pb in several ways, the most important is through the kidneys and gastrointestinal tract, Pb excretion via urine as 75-80%, by 15% and other feces through bile, sweat, hair and nails.

After administration of chitosan, especially in group P3 (Pb+chitosan-1%) seen a decrease in blood lead levels of rats significantly ($p < 0.05$). The possibility of this is due to the inhibition of Pb by chitosan which will fit into the system of blood vessels in the small intestine. After administration of Pb through the stomach (pencekokan), Pb continues to flow into the small intestine. Intestinal villi to have many anatomical structures contain many blood vessels will absorb Pb contained around it. Contained chitosan will bind Pb around the intestines before entering the small intestine. So that the provision of treatment Pb (K2) will look a lot more content in the blood Pb compared with control rats ($p < 0,05$). Such statements and Vanugopel Lusanckey, that as a chelating chitosan can bind transition metals by binding metal complexes Pb and form bonds that make it are polar (hydrophilic), and excreted through the urine. Plumbum also issued in the form of a salt of uric acid, hipuric acid, and creatinine. Plumbum that goes through the digestive tract and is not absorbed in the gastrointestinal tract is excreted with feces.

Plumbum seen decreased significantly when administration of chitosan-1%, but remained significantly different from control (addition of water only). This may be due to contamination of drinking water or food. Such statements Hariono (2005), that the Pb in drinking water can come from contamination of pipes, solder, and water faucets [12]. The content of Pb in water at 15 mg/L is considered still safe to eat. Pb in foods derived from contamination of canned food and leaded solder.

Giving Pb can decrease the number of morphologically normal spermatozoa to nearly 50%. This may be due to toxic effects caused by Pb. Sharma and Garu (2011), reported his research shows that exposure to lead, especially affect the testicles, then the effect to suppress the hypothalamic-pituitary-axis testes, resulting in testicular histology, sperm morphology and relationships of various cells in the testis [13]. Mating males were exposed to lead and not be exposed females showed reduced fertility in males exposed. While the provision of chitosan 0.5, 0.75, and 1% showed significant differences in increasing sperm morphology of rats ($p < 0.05$) after being pressured by Pb. Giving chitosan-1% for 5 weeks caused the rat sperm morphology than the control and other treatments.

In the research work of Acharya et al. using 30 strains of Swiss mice, 6 mice as controls were injected intraperitoneally with distilled water, 24 mice were given a single dose of Pb-acetate (200 mg/kg bw) intraperitoneally, every week 6 rats treated with Pb-acetate in the testes shut down and taken for examination [14]. From the first week to the fourth week, there is evidence of a decrease in testicular weight with an increased incidence of sperm abnormalities (abnormal sperm morphology) and a decrease in the number of spermatozoa per week constantly.

Plumbum significantly suppress sperm count, normal morphology of spermatozoa. This is consistent with the hypothesis that plumbun can negatively affect the quantity (amount) and normal morphology of spermatozoa. Probably caused by heavy metals as Pb is toxic to the body those are organism. The nature of the cause of ROS (Reactive Oxygene Species) causes oxidative stress. Many free radicals can disrupt the cell structure from the cell membrane to the cell nucleus.

The effect of the decrease in the quantity of Pb and quality of sperm can also through its effect on the hypothalamic-pituitary-axis of the testis (Figure 3). Cause disruption to the hypothalamic LHRH or FSHRH reduced production or decreased. Thereby suppressing effect on work and causes the pituitary LH and FSH production can not be met. LH causes decreased activity of Leydig cell testosterone intratesticular so reduced that resulted in disruption of spermatogenesis. The low FSH may lead to reduced ABP (Androgen Binding Protein) so not a lot that can bind to testosterone in the seminiferous tubules of the testes. This causes disruption to the growth and development of germ cells. Abnormally formed sperm (abnormal) and can also reduce sperm can live (reduced sperm viability). Finally the number of sperm produced in the testes guard will be reduced in number. Sharma and Umesh, states that the reproductive effects of Pb is very complex and appears to involve multiple pathways, not all fully understood [14]. Reproductive dysfunction due to Pb showed distinct morphological changes, lower sperm quality and sperm morphology change. Investigation of the effects of chronic Pb acetate on the development of the reproductive system of Swiss albino rats showed exposure to Pb suppress the hypothalamic-pituitary-testes, thus changing the testicular histology, sperm morphology and relationships of germ cells in the testis.

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