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Profile Germinal Cells of Rat (*Rattus norvegicus*) at the Chitosan Administration after Exposure Plumbum (Pb)

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Abstract: Reproductive health is often compromised because of the environmental pollution by heavy metals such as of lead, cadmium, mercury,etc. Testis is a main organ of repruduction that can be observed when the accumulation of lead in the body. Chitosan is a substance that is being developed to prevent disturbance of heavy metals on reproductive health.The study used a completely randomized design (CRD) 30 male rats (150-200 g; 8-12 weeks), which consists of six treatment groups and 5 replications. The groupsconsist of control (C1), control + Pb (C2), Pb + 0.5% chitosan (T1), Pb + 0.75% chitosan (T2), Pb + 1% chitosan (T3), chitosan 1% (T4). Every group was observed several parameters such as number of measuring germ cells from spermatogonia to spermatids. The results showed a significant difference ($p<0.05$) between treatment groups on all test parameters. Spermatogonial mitoses efficiency coefficient, meiotic index, general spermatogenesis efficiency, the cellular losses occurrence during the meiotic prophase, calculate of proportions such as spermatogonia A/Sertoli cells, primary spermatocytes (I) in pre-leptoteneleptotene/Sertoli cells, spermatocytes I in pachytene/Sertoli cells, rounded spermatids/Sertoli cells, and the total germinative cells /Sertoli cells.
Keywords: Plumbum,germ cell, chitosan.

Introduction

Germ cell is the result of several stages of the process of spermatogenesis in rat[1,2].This germ cells to form multiple layers of germ cells or spermatogensis stage in the seminiferous tubules[3,4].The stages of the seminiferous tubule epithelium beginning of spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids[5].Sperm are formed will ripen themselves in exactly in the cauda epididymis epididymis[6,7,8].

Pollution caused by the presence of lead (Pb) in nature has been shown to cause health problems of male rats. One occurred in reproductive health disorders such as the process of spermatogenesis in the testis[9,8,10]. Several other research showed that chitosan could become an obstacle to the adverse effects of lead (Pb).[11]Chitosan, which can be formed by deacetylation of chitin, is the most important derivative of chitin. Chitosan in partially converted crab shell waste is a powerful chelating agent and interacts very efficiently with transition metal ions[12,13].For most of the chelating adsorbent, the functional groups with the donor atoms are normally attached to the metal ions, thus leading to a donor–acceptor interaction between chitosan and the metal ions[14].

Shrimp shell is a material that is often wasted and can be processed into useful materials through a chemical process into chitosan. This study aims to look at the function of chitosan in inhibiting the antifertility effect of Pb on cells spermatogenic.

Experimental

This study uses a white male rats (*Rattus norvegicus*) wistar adult weight of 200-250 g, aged 10-12 weeks as many as 48 animals. The rats obtained from the Center for Disease Investigation (BPPV) field then maintained and adapted in the laboratory of Biological Departmen Faculty of Mathematics and Natural Sciences - USU Medan. The provision of food and beverage adlibitum derived from Charoen Pokphand Indonesia Tbk (CP551).

The measuring of the ratcage is 40 cm x 20 cm x 15 cm. Manufacture of chitosan derived from shrimp shells in Belawan conducted at the Laboratory of Integrated Faculty of Mathematics and Natural Sciences University of Sumatra Utara-Medan. Source of Pb from Pb-acetate dissolved in glacial acetic acid. Pb-acetate was given 0.5 ml (10 mg Pb/kg of body weight of rats) orally 4 hours before dicekokkan chitosan for 7 weeks. After the treatment is done made preparations testicular thickness of 5 lm. The process of networking by creating blocks of paraffin after fixed with Bouin and through standard processes and colored with Haemotoksilin eosin (HE)[15].

The study used a completely randomized design (CRD) 30 male rats (150-200 g; 8-12 weeks), which consists of six treatment groups and 5 replications according Feferer (1963) formula; $(t-1)(n-1)\geq 15$. The groupsconsist of control (C1), control + Pb (C2), Pb + 0.5% chitosan (T1), Pb + 0.75% chitosan (T2), Pb + 1% chitosan (T3), chitosan 1% (T4).Each treatmengroups was done 7 weeks.

Observations were made with a microscope at a magnification of 400x or preparations testis germ cells in the seminiferous tubules of the testes of rats. Tubules used is a round or nearly round. Preparations with HE staining was used as the principal observations on histological testicular tissue.

After treatment, the rats anesthetized and their testes in preparation for making a histological preparations. The parameters to be analyzed, among others: spermatogonial mitoses efficiency coefficient (proportion betweenthe number of primary spermatocytes in preleptotene/leptotene and the number of spermatogonia A), meiotic index (proportion between the number of rounded spermatids and the number of pachytene primary spermatocytes), general spermatogenesis efficiency (proportion between the rounded spermatids and the number of type A spermatogonia), the cellular losses occurrence during the meiotic prophase (proportion between the primary spermatocytes number in pre-leptotene/leptotene and the pachytene primary spermatocytes number). And then, calculate of proportions: spermatogonia A/Sertoli cells;primary spermatocytes (I) in pre-leptoteneleptotene/Sertoli cells; spermatocytes I in pachytene/Sertoli cells; rounded spermatids/Sertoli cells; and the total germinative cells /Sertoli cells. All the proportions were calculatedusing the cellular counts of the stage 1 of thecycle.

For this parameter spermatogenic cell counts performed on stage VII of the seminiferous epithelium cycle and data computation result is corrected by a correction factor Abercrombie[16].To obtain the exact number of germ cells, correction using Abercrombie correction factor by the following formula:

$$P = A\frac{M}{L+M}$$

Description: P = the average number of cores per slice; A rough calculation = Total core per slice; M = thickness incisions (5 micron); L = Mean diameter of the core.

Results and Discussion

Results of the study can be seen in Figures 1 and 2 below.Based on Figure 1 can be said that Hp can interfere with the formation process of the formation of the stages of spermatogenesis. The number of germ cells from spermatogonia, primary spermatocytes preleptoten / leptoten and spermatid is very low when compared to controls without Pb. Ilyas[13,10] found that Pb may cause the occurrence of germ cell death via apoptosis event.

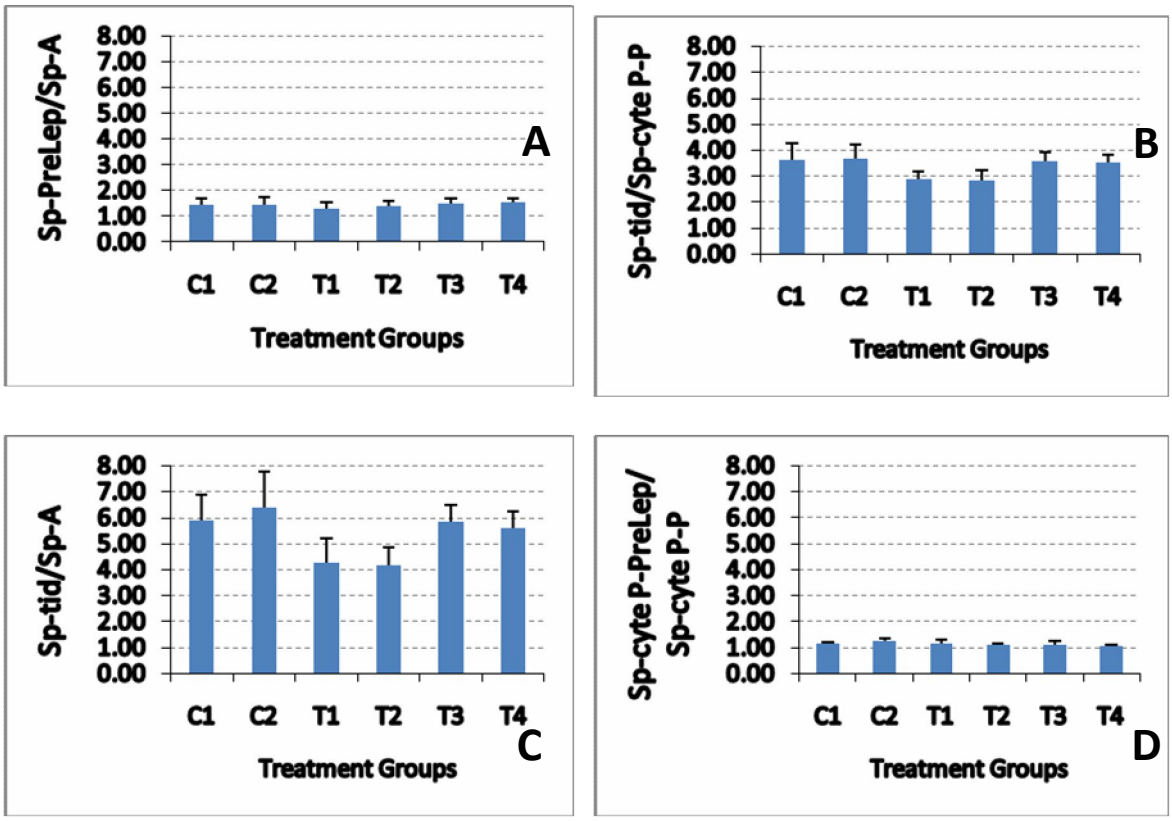

Figure 1. Gambaran beberapa perbandingan perkembangan spermatogenesis tikus.

(A)Spermatogonial mitoses efficiency coefficient (proportion between the number of primary spermatocytes in preleptotene/leptotene and the number of spermatogonia A(Sp-cyte P-PreLep/Sp-A), (B) meiotic index (proportion between the number of rounded spermatids and the number of pachytene primary spermatocytes (Sp-tid/Sp-cyte P-P), (C) general spermatogenesis efficiency (proportion between the rounded spermatids and the number of type A spermatogonia (Sp-tid/Sp-A), (D) the cellular losses occurrence during the meiotic prophase (proportion between the primary spermatocytes number in pre-leptotene/leptotene and the pachytene primary spermatocytes number (Sp-cyte P-PreLep/Sp-cyte P-P). Note; Control (C1),control+Pb (C2), Pb+0.5% chitosan (T1), Pb+0.75% chitosan (T2), Pb+1% chitosan (T3), and chitosan 1% (T4).

Pb poisoning has been recognized as a major public health risks, particularly in developing countries. Although a variety of work and public health measures have been taken to control lead exposure, lead poisoning cases are still being reported. Pb exposure produces a variety of deleterious effects on the hematopoietic, renal, reproductive and central nervous system, primarily through increased oxidative stress. These changes play an important role in the manifestation of the disease. Modulation of cellular thiols for protection against reactive oxygen species (ROS) has been used as a therapeutic strategy against lead poisoning. N-acetylcysteine, α-lipoic acid, vitamin E, quercetin and some herbal extracts showed prophylaxis against damage of Pb in in vitro or in vivo. Nanoencapsulation new therapeutic strategies that are used to treat poisoning manifestations result from induction Pb[9,10].

Provision of chitosan could prevent the influence of Pb in the body. In Figure 1 we can see that the administration of chitosan 1% (T3) has a very good effectiveness can prevent disturbance of Pb in the body. According to research of Hadi[11]that chitosan can prevent disturbance of Pb in the body.According Nadapdap[12], that chitosan has the ability to bind Pb before entering into a blood vessel.Pb can become free radicals that cause increased ROS in cells that trigger cell death via caspase cascade activity. Such as statements of Ilyas[2] and Nandi[17],that the death of germ cells may be through the activity of caspase 3 which is executor of apoptosis. Caspase 3 may lead to a fragmentation of the DNA so that the cells continue to die through apoptosis.

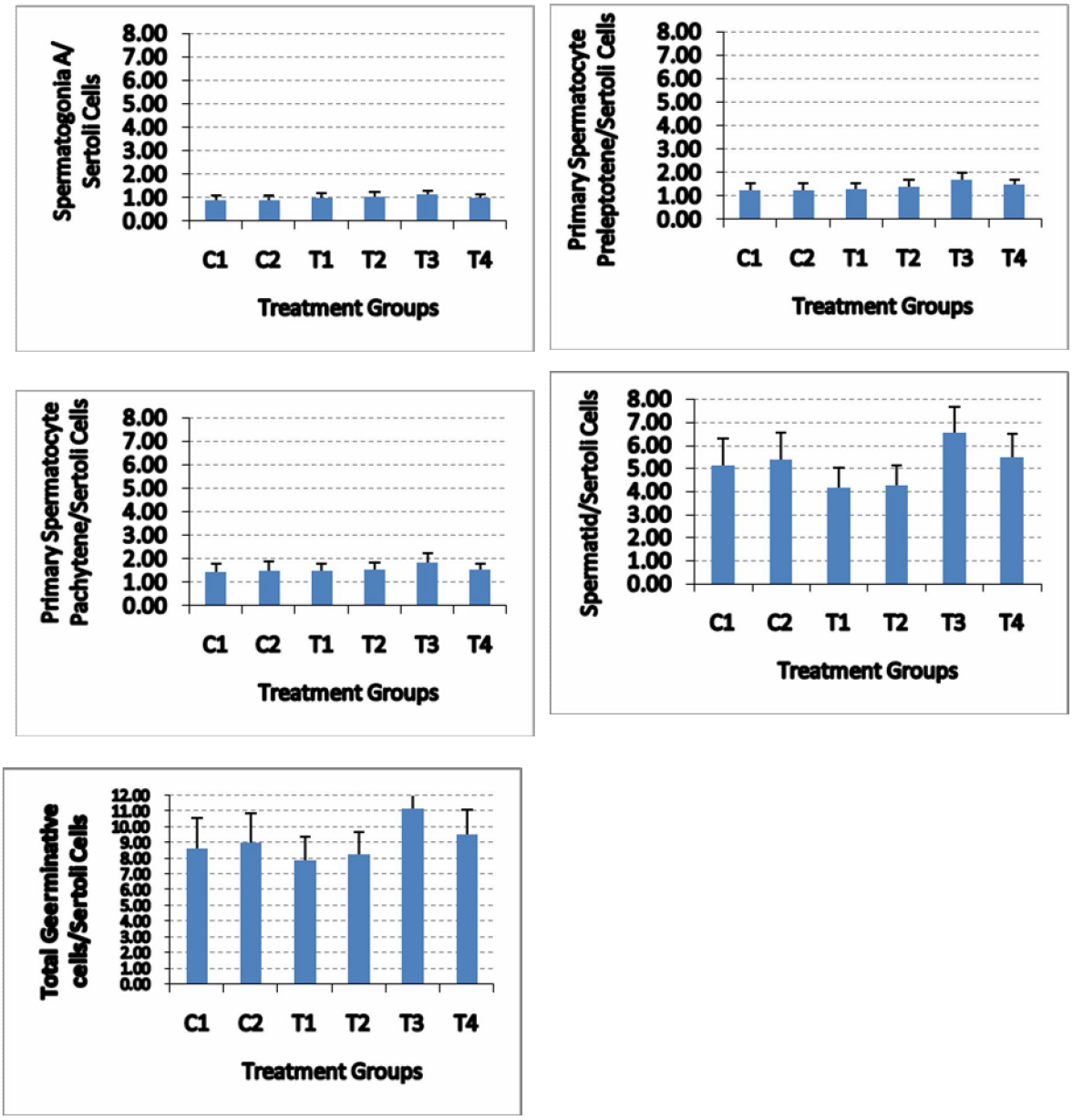

Figure 2. Proporsi beberapa parameter sel germinal dengan sel sertoli. Note; Control (C1),control+Pb (C2), Pb+0.5% chitosan (T1), Pb+0.75% chitosan (T2), Pb+1% chitosan (T3), and chitosan 1% (T4).

In Figure 2 it can be seen that the proportion of the number of germ cells by Sertoli cells is very strong. This proves that the Sertoli cells is essential dala stages of germ cell development from spermatogonia, spermatocytes and spermatids. Appropriate research Griswold[18] and Walker[19]thatsignaling pathways that are regulated by FSH and testosterone as well as the resulting metabolicand gene expression changes that occur as related to Sertoli cell proliferation, differentiation and the support ofspermatogenesis.Sertoli cells are the somatic cells of the testis that are essential for testis formation and spermatogenesis. Sertoli cells facilitate the progression of germ cells to spermatozoa via direct contact and by controlling the environment milieu within the seminiferous tubules. The regulation of spermatogenesis by FSH and testosterone occurs by the action of these hormones on the Sertoli cells. While the action of testosterone is necessary for spermatogenesis, the action of FSH minimally serves to promote spermatogenic output by increasing the number of Sertoli cells**.**

Conclusions

It was concluded that chitosan can reduce the negative influence of Pb-acetate against measuring germcellsnumber each seminiferous tubules.

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