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THE EFFECT OF FIG LEAVES (*FICUS CARICA*) ETHANOL EXTRACT ON TOTAL OF SPERMATOGENIC AND LEYDIG CELLS OF MALE MICE (*MES MUSCULUS*) EXPOSED WITH LEAD ACETATE

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ABSTRACT

This study aims to prove the effect of Tin leaves (*Ficus carica*) ethanol extract on the number of spermatogenic cells and the number of Leydig cells of mice exposed with lead acetate. There were 24 male mices as samples and divided into 4 groups. Group C- is a group that was given aquadest for 35 days, group C+ was given lead acetate 50 mg/kg BW for 21 days, groups P1 and P2 were given Tin leaves ethanol extract at a dose of P1 (166.4 mg/kg BW) and P2 (dose 332, 8 mg/kg BW) for 35 days. After one hour both groups were given lead acetate at a dose of 50 mg/kg BW on days 14-35. The result showed a significant difference ($p< 0.05$) on spermatogonia cells from C+ group ($36.20^{a} \pm 2.29$), P1 ($44.97^{b} \pm 2.55$), C- ($48.57^{bc} \pm 4.96$) and P2 ($51.53^{c} \pm 2.94$); on primary spermatocytes C+ group ($34.23^{a} \pm 3.96$), P1 ($47.60^{b} \pm 8.94$), P2 ($55.63^{c} \pm 4.82$), and C- ($69.03^{d} \pm 4.22$); whereas for spermatids C+ group ($44.43^{a} \pm 3.79$), P1 ($60.83^{b} \pm 7.29$), and C- ($77.73^{c} \pm 5.58$); then Leydig cells from C+ group ($23.70^{a} \pm 4.60$), C- ($31.80^{b} \pm 5.29$), and P1 ($28.30^{ab} \pm 3.21$). This study concluded that dose of 332.8 mg/kg BW was an effective dose to protect spermatogenic cells and Leydig cells against lead acetate exposure.

KEY WORDS: Ficus carica ethanol extract, Spermatogenic, Leydig cells, *Mes musculus,* Lead acetate

INTRODUCTION

The development in the industrial field in modern times has been very rapid. The more industries that are built are not followed by an adequate water and waste treatment installation system, consequently more and more industrial waste is discharged into the environment causing environmental pollution, one of the elements contained in environmental pollutants is heavy metals. Environmental pollution that occurs due to heavy metals is a problem that needs attention, because in an environment with high enough heavy metal content it will cause contamination in food, water and air that can cause poisoning, one of the heavy metal pollutants that are most dangerous to human and animal health is lead acetate (Adikwu, 2013).

Lead acetate is a heavy metal that can be obtained in food, drinks and household materials which are polluted through air, water or soil in the environment around us. Lead acetate is toxic and accumulative, causing disruption in the body, one of which is the male reproductive system. In men the effects of lead acetate include lowering the number of spermatozoa, and decreasing libido resulting in infertility (Garu, 2011). Lead exposure below the WHO threshold (400 µg/dl) can reduce spermatozoa concentrations even though it is still clinically within normal limits (I'tisom, 2011). Lead exposure also affects the development of the testes of all age groups and affects cells in the process of spermatogenesis so that it affects the number of spermatogenic cells (Endrinaldi, 2009).

Spermatogenesis itself is a process of formation of spermatozoa that occur in in the seminiferous tubules. Lead acetate can cause a significant decrease in the hormone testosterone, testosterone produced by Leydig cells and is important to use in

the process of spermatogenesis so that there is a significant relationship between testosterone and the number of Leydig cells (Mokhtari, 2011). The hormone testosterone itself is a hormone that plays an important role in maintaining the morphology of spermatogenic cells that are at a developmental stage, so that lead acetate exposure also affects the reduction in the number of Leydig cells (Li, 2018).

Exposure to lead acetate can increase the production of Reactive Oxygen Species (ROS) and can resulting in decreased antioxidant reserves which can cause oxidative stress, the increased production of ROS as a result of lead exposure can affect the testes and sperm (Badade, 2011). Efforts to reduce the negative effects of free radicals on reproductive health resulting from exposure to lead acetate can be prevented by administering antioxidants in the body. More natural sources of antioxidants are chosen because they have few side effects (Flora, 2012). Tin leaves can be used as a source of natural antioxidants that can help prevent infection. The content of components such as flavonoids in Tin leaves has the potential as an antioxidant to protect the testes from free radicals and as a high antioxidant (Giorgio, 2000). Based on this description can be the basis of research to determine the effect of ethanol extract Tin leaves (*Ficus carica*) on the number of spermatogenic cells and the number of cells of Leydig testis mice (*Mus musculus*) exposed to lead acetate.

MATERIALS AND METHODS

This study used experimental male mice (*Mus musculus*) aged 2-3 months with a weight range of 20-30 grams with a total of 24 animals. Research materials include tin leaf extract (*Ficus carica*), Lead acetate, ethanol 96% solvent, for maintenance of male mice in the form of drinking water (ad libitum) and experimental animal feed in the form of pellets. 10% formalin is used as a preservative testicle of mice.

Mice (*Mus musculus*) were 24 animals which were divided into 4 treatments each with six animals. Adaptation is done for 7 days so that the animal try not to experience stress. On the 8th day the treatment began with administration of ethanol extract of Tin leaves as preventive for 14 days with two different doses, namely 166.4 mg/kg BW (P1), 332.8 mg/kg BW (P2). Then on the 15th day, red dragon fruit peel extract was given to groups P1 and P2 as preventive and after one hour was given Lead acetate at a dose of 50 mg/kg BW for the K + group was only given Lead acetate at a dose of 50 mg/kg BW and given aquadest for K- group.

This study was divided into four treatments in one treatment with six replications, different doses for P1 and P2. Mice are fed and drink ad libitum. Treatment carried out for 35 days and on the 36th day all mice were sacrificed by means of anesthesia and euthanated with ether for testicular organs.

After 35 days of treatment, all mice were sacrificed first by means of anesthesia and euthanation using ether, then dissected using a surgical instrument to remove the testicular organs by surgery through an incision in the abdominal wall, then the testis was removed.

On testicular sampling, the scrotum is opened then the testicular organs are removed. Furthermore, the testes are washed with physiological solution and then stored in an organ pot containing 10% formalin solution to further make histopathological preparations to see a picture of spermatogenesis which includes the number of spermatogenic cells and the number of Leydig cells.

Testicular histopathological preparations were observed in five fields of view, each field of view consisting of one seminiferous tubule. Histopathological preparations were observed under a microscope at 400x magnification in each seminiferous tubule. Spermatogenic cells consist of spermatogonia, primary spermatocytes, and spermatids. Spermatogenic cells are cells found in several epithelial layers of seminiferous tubules from the outer layer into the lumen, the number of spermatogenic cells is the number of spermatogonium cells, primary spermatocytes and spermatids, spermatogonium cells are the number of cells with a round shape, near the basal membrane, the number of spermatogenic cells is the number of spermatogonium cells, primary spermatocytes and spermatids, spermatogonium cells are the number of cells with a round shape, near the basal membrane, the nucleus is shaped tapering with fine chromatin and a thin core membrane were observed and counted under a microscope. Primary spermatocytes are cells that have the largest size among spermatogenic cells, have a heterochromatic nucleus, are between the basal membrane and tubular lumen, spermatid cells are the number of rounded cells, smaller than spermatocytes, rounded, pale, and bright nuclei observed and counted under a microscope (Sukmaningsih, 2011).

The average number of Leydig cells in the seminiferous tubules, Leydig cells are found in the interstitial portion of the seminiferous tubules, some cells attach close to capillaries. Leydig cells are surrounded by fibroblasts, macrophages and binding tissue. Leydig cells have a single nucleus that is round, with two nucleuses that are eccentric and cytoplasmic (Colon, 2007). Leydig cells were observed in five fields of view, each field of view consisting of one seminiferous tubule. Histopathological preparations were observed under a microscope at 400x magnification in each seminiferous tubule.

Data on the number of spermatogenic cells and Leydig testis cells of mice (*Mus musculus*) were analyzed statistically using the One-Way ANOVA (Analysis of Variance) method to determine differences between treatments. If there is a significant difference ($p < 0.05$) followed by Duncan's Multiple Range test. The level of significance used was ($p < 0.05$).

RESULTS

Number of Spermatogenic cells and Leydig cells

The measurement of Spermatogenic cell's number and Leydig cell's number were carried out on the 36th day of the study conducted in the treatment groups C-, C+, T1, and T2 which can be seen in Table 1.

The results of this study indicate that the smallest number of spermatogenic cells was obtained in the group of mice exposed to Lead acetate 50 mg/kgBW without giving Tin leaves ethanol extract therapy. These data prove that lead acetate causes toxicity to spermatogenic cells by reducing the number of spermatogenic cells in the testes of mice. This is in line with research which proves that there is a barrier to spermatogenesis in mice exposed to Lead acetate 50 mg/kg BW for 28 days by mouth (Diana, 2017).

Figure 1 showed that there is a diversity numbers of spermatogonia, primary spermatocyte, spermatid, and Leydig cells which are influenced by the variety of doses. The higher doses given, the higher number in spermatogenia cells, primary spermatocyte, spermatid, and Leydig cells.

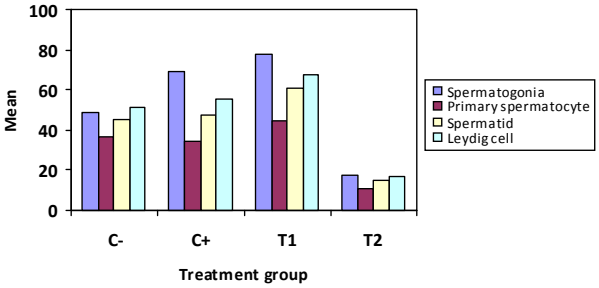

Fig. 1. Diagram of spermatogenic cells (spermatogonia, primary spermatocyte, spermatid) and Leydig cells number in male mice (*Mus musculus*) given Tin ethanol extract which then exposed to lead acetate

DISCUSSION

Exposure to lead acetate results in increased mitochondrial activity, organelles responsible for oxygen metabolism and energy production for the body, resulting in an excessive increase in ROS levels in testicular tissue that can trigger oxidative stress (Praharjaya, 2017). The testis is the site of the process of spermatogenesis which is very vulnerable to oxidation and free radicals because it has a small amount of superoxide dismutase. These free radicals will cause interference with spermatogenesis and spermatogenic cell membranes. Spermatogenic cell membranes contain large amounts of monounsaturated fatty acids (Sukmaningsih, 2011). The results of this study indicate that administration of ethanol extract of Tin leaves can maintain the number of spermatogenic cells due to toxicity of Lead acetate which induces oxidative stress can be inhibited by compounds contained in Tin leaf extract.

Table 1. Mean and standard deviation of Spermatogenic cells (spermatogonia, primary spermatocyte, and spermatid) and Leydig cells on mice exposed to lead acetate given Tin ethanol extract in various doses.

Treatment Groups	Spermatogonia	Primary spermatocyte	Spermatid	Leydig cells
C-	$48.57^{bc} \pm 4.96$	$69.03^{d} \pm 4.22$	$77.73^{c} \pm 5.58$	$17.80^{b} \pm 3.24$
C+	$36.20^{a} \pm 2.29$	$34.23^{a} \pm 3.96$	$44.43^{a} \pm 3.79$	$10.66^{a} \pm 1.59$
T1	$44.97^{b} \pm 2.55$	$47.60^{b} \pm 8.94$	$60.83^{b} \pm 7.29$	$14.93^{b} \pm 3.29$
T2	$51.53^{c} \pm 2.94$	$55.63^{c} \pm 4.82$	$67.63^{b} \pm 7.49$	$16.86^{b} \pm 0.99$

Note: Different superscripts $^{a, b, bc, c, d}$ in the same column show significant differences ($p < 0.05$).

Tin leaves contain natural antioxidant compounds, namely flavonoids. Flavonoids have an antioxidant function that can bind to free radicals. If free radicals bind to antioxidants, the free radicals become unstable, free radicals are unstable means to reduce interference with cell membranes, so that the cells can avoid damage. Tin leaf extract with a dose of 166.4 mg/kg BW has not been able to maintain the total number of spermatogenic cells of male mice exposed to lead acetate totally, this is presumably due to high oxidative stress and the amount of antioxidants that have not been able to stabilize the ROS caused by Lead acetate exposure. In this research it was found that a dose of 332.8 mg/kg BW resulted in a higher number of spermatogenic cells compared to a dose of 166.4 mg/kg BW.

The results of this study prove that the ethanol extract of Tin leaves (*Ficus carica*) can maintain the number of Leydig cells of mice (*Mus musculus*) that are exposed to Lead acetate, as a protective agent of Tin leaves ethanol extract (*Ficus carica*) is able to maintain the number of Leydig cells at a dose of 166.4 mg/kg BW, but the results given by this dose have not been maximized. Based on the results of this study the optimum dose of Tin leaf ethanol extract is 332.8 mg/kg BW because at that dose the highest number of Leydig cells was obtained compared to other treatment groups other than the control.

Administration of lead acetate at a dose of 50 mg/kg BW orally for 35 days has been shown to reduce the number of Leydig cells in male mice (*Mus musculus*) exposed to Lead acetate. The decline in the number of Leydig cells is caused by metabolic disorders in Leydig cells due to lipid peroxidation and failure of ATP formation. Failure to form ATP will stimulate anaerobic glycolysis by mitochondria and cause lactic acid buildup so that it manifests in a decrease in intracellular pH. A decrease in intracellular pH causes the compaction of cell nucleus chromatin, if it continues it will disrupt RNA synthesis and result in cell necrosis (Praharjaya, 2017).

The number of Leydig cells was obtained in the treatment group which was given ethanol extract Tin leaves with a Tin dose of 332.8 mg/kg BW. The results of this study indicate that the ethanol extract of Tin leaves can maintain the number of Leydig cells. Leydig cells are responsible for testosterone secretion, and the amount is positively correlated with testosterone concentration. Lead acetate related to various structures and chemical changes, at low concentrations can cause apoptosis, whereas at high doses can cause necrosis. Flavonoids are polyphenol compounds that play a role in antioxidant activity in Tin leaves (Sumardika, 2012). Tin leaf extract flavonoids include exogenous antioxidants whose mechanism of action is secondary by cutting chain oxidation reactions and free radicals or by capturing free radicals (Sayuti, 2015).

Tin leaf extract with a dose of 166.4 mg / kg BW was able to maintain the number of Leydig cells in male mice exposed to lead acetate. However, this dose still cannot maintain the total number of Leydig cells, this is presumably due to the high oxidative stress and the amount of antioxidants that have not been able to stabilize the ROS caused by exposure to Lead acetate. In this study it was found that a dose of 332.8 mg/kg BW resulted in a higher number of Leydig cells compared to a dose of 166.4 mg/kg BW.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that the administration of ethanol extract of Tin leaves (*Ficus carica*) at a dose of 332.8 mg/kg BW can maintain the number of spermatogenic cells and the number of mice leydig cells (*Mus musculus*) exposed to Lead acetate.

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