Effect of Lead Acetate on The Concentration and Sperm Morphology in Mice BALB/C (Mus Musculus)

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EFFECT OF LEAD ACETATE ON THE CONCENTRATION AND SPERM MORPHOLOGY IN MICE BALB/C (MUS MUSCULUS)

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Abstract: Air pollution is mostly dominated by motor vehicle exhaust gas. Lead is one of motor vehicle exhaust gas that is one of toxic heavy metal, one of which is a toxicant for sperm. This study was aimed to demonstrate effect of lead acetate on the concentration and sperm morphology in mice. Mice (Mus musculus) Balb/c divided into 2 groups. K group was the control group, P group was given with lead acetate 0.075 g/Kg BW 1x/day orally for 35 days. Result, all data were normally distributed and homogeneus, then analyzed using One Way Anova test. Result showed significant difference in sperm concentration and normal-shaped sperm morphology. In conclusion, lead acetate can significant reduce sperm concentration and normal-shaped sperm morphology in mice Balb/c (Mus musculus).

Keywords: Lead acetate, sperm concentration, sperm morphology

1. Introduction

Air pollution in Indonesia is increasing because with increasing traffic volume growth, which reached 15% per year (The World Bank Country Study, 1994). The largest air pollution is dominated by motor vehicle exhaust. Lead is one of the results of motor vehicle exhaust (Kusminingrum and Gunawan, 2008) that are toxic to the male reproductive system, especially in spermatozoa (Sokol et al, 2002).

Several studies on the toxicity of lead has been done. Common effects seen in men include: reduced libido, abnormal spermatogenesis (reduced motility and number), chromosomal damage, infertility, abnormal prostatic function and changes in serum testosterone (Flora et al, 2012). Animal studies, administration of lead acetate in mice decreased the number of spermatozoa in the epididymis (dosage 0:25%) and lower sperm concentration at high doses (doses 0.5%) (Wadi and Ahmad, 1999), lower testosterone levels, lower levels of LH and FSH (Hamadouche et al, 2013; Lamia et al 2008), even the administration of lead acetate study in female rats who are breastfeeding decreased testicular weight and volume, as well as a decrease in the diameter of the seminiferous tubules and germinal epithelium in children are breast-fed male rats (Dorostghoal et al, 2011).

Research conducted in humans, men are exposed to the exhaust emissions of motor vehicles obtained an average morphology and sperm concentration is lower if dibandingkankan with men who are not exposed (I'tishom et al, 2008; I'tishom et al, 2011).

This study was aimed to demonstrate effect of lead acetate on the concentration and sperm morphology in mice.

2. Material and Method

Animal: Three months old mice (Mus musculus) Balb/c were used for this study. Mice were housed in controlled rooms temperature and all standard procedures.

Experimental design: Mice (Mus musculus) Balb/c were divided into 2 groups. K group was the control group, P group was given with lead acetate 0.075 g/Kg BW 1x/day orally for 35 days. At the end of the entire study, the mice were sacrificed. Vas deferens were taken for examination of concentration and morphology of the sperm.

Sperm Concentration: Sperm concentration examination steps are 3-5 sperm density estimates in the field of view (magnification 400x), samples sucked with the leukocytes pipette and counted in a Neubauer counting chamber.

Sperm Morphology: The steps of sperm morphology examination are make a smear on a glass object, soak in methanol, safranin solution, wash immersion in phosphate buffer solution, flushing with a phosphate buffer solution, soak in a solution of crystal violet, rinse in distilled water, observe under the microscope with 1000x magnification and oil immersion, calculate sperm in normal-shaped and abnormal-shaped.

Statistical analysis: The results obtained from control and lead-induced animals are expressed as means ± SD. Statistical analysis was use one way Anova test with p value <0.05 was considered significant.

3. Result

Sperm Concentration

Administration of lead acetate reduce mean of sperm concentration. The result of one way Anova test showed significant decrease ($p<0.05$) sperm concentration (table 1).

Table 1 Effect of lead acetate on sperm concentration

Variable	K group (Mean±SD)	P group (Mean±SD)
Sperm Concentration	14.45±4.6	7.77±3.58*

*$p<0.05$

Sperm Morphology

Administration of lead acetate also reduce mean of normal-shaped sperm morphology. The result of one way Anova test showed significant decrease ($p<0.05$) normal-shaped sperm morphology (table 2).

Table 2 Effect of lead acetate on sperm morphology

Variable	K group (Mean±SD)	P group (Mean±SD)
Normal-shaped Sperm Morphology	86.33±3.08	61.67±7.81**

**$p<0.01$

4. Discussion

Induction of lead acetate in this study led to a decrease in sperm concentration and a decrease in normal-shaped sperm morphology in lead acetate-induced group compared with the control group . The same results lead acetate administration to lower the concentration of spermatozoa of mice significantly (Wang et al, 2008). The high concentration of lead in the cement can reduce sperm count significantly (Wu et al, 2012).

These results indicate that the induction of lead acetate also cause a decrease in normal-shaped sperm morphology significantly. Granting 0.5% lead acetate in drinking water for 6 weeks also showed a decrease in the percentage of motile spermatozoa, increase the percentage of abnormal sperm in the epididymis (Wadi and Ahmad, 1999). The same result, namely the provision of lead acetate 100 mg / KgBW increased abnormal sperm shape (Acharya et al, 2003).

Lead is a toxin that is common and persistent environment that can damage cell components and alter the cell's genetic. Lead can increase the production of free radicals and lower the antioxidant reserves in response to damage (Patrick, 2006). Lead in the blood of approximately 95-99% is carried by red blood cells, and binds to hemoglobin. Heaps of lead in the red blood cells and soft tissue allegedly most responsible for the toxic effects to the body (Patocka and Cemy, 2003).

Lead toxicity is able to increase the production of reactive oxygen species (ROS). The mechanism of the toxicity of lead in an increase in ROS through two ways: increase the production of ROS and lower antioxidant reserves. Increased ROS will cause lipid peroxidation, protein peroxidation and DNA oxidation nucleic acids (Ercal et al, 2001). Oxidative stress with the accumulation of ROS, especially $O2^{-}$ and H2O2 thought to contribute to cell damage, apoptosis, and cell death (Handy et al, 2009). Apoptosis that occurs in germ cells in the testes resulting in reduced germ cells resulting in a decrease in the concentration of sperm. As well as the resulting cell damage sperm abnormalities in cell shapes.

Lead can get into the brain resulting in decreased brain weight of mice significantly, increased residual lead in the rat brain, degenerative changes and hypertrophy of the endocrine cells of the pituitary gland, atrophy of the seminiferous tubules, decreased the number of Sertoli cells and Leydig, and a decrease in LH and testosterone in significant (Hamadouche et al, 2013) which resulted in the disruption of the process of spermatogenesis and steroidogenesis in the testes.

5. Conclusion

a. Lead acetate can significant reduce sperm concentration
b. Lead acetate can significant reduce normal-shaped sperm morphology

6. Recommendations

Researcher: Need further research to calculate the Sertoli and Leydig cells, spermatogonia, spermatocytes and spermatids. Measuring the production of LH, FSH, ROS, antioxidants, and MDA as biomarkers of lipid peroxidation.

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