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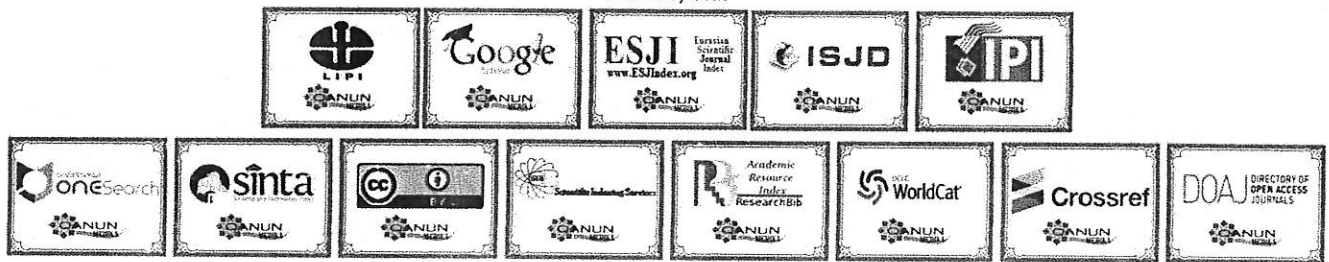
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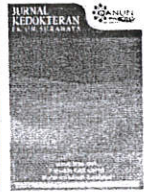
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 Research Article

Solanum betaceum extract give protective effect on spermatozoa morphology of mice exposed to lead acetate

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ABSTRACT

Environmental pollution is one of the factors that contribute to the decline in male fertility. Lead is one of six air pollutants harmful to the reproductive system. One parameter of infertility in men is a decrease in reproductive function observed with increasing abnormalities morphology of spermatozoa. The purpose of this study is to analyze the effect of giving various dosages of *Solanum betaceum* extract on spermatozoa morphology of mice exposed to lead acetate. This study was true experimental using a randomized post-test only control group design. The total sample was 40 male mice Balb/ C taken by simple random sampling technique. Treatment and maintenance of experimental animals for 35 days. Statistical tests with one way Anova showed there were significant differences with p-value 0.005. *Solanum betaceum* extract can be used as a protective agent to improve the normal morphology spermatozoa of mice that exposed to lead acetate.



INTRODUCTION

Environmental pollution is known as one of the factors related to the decline in male fertility (I'tishom, Lubis, Pieters, & Hamdani, 2011). One parameter of infertility in men is a decrease in reproductive function observed with an increase in abnormalities morphology of spermatozoa (Kumar, 2018). Environmental pollutant material that is often found daily, especially in industrial countries and in developing countries is lead (Pb) (Bierkens, Smolders, Holderbeke, & Cornelis, 2011). Indonesia ranks fifth after India, China, Vietnam, and the Philippines as lead polluted countries according to the Political and Economic Risk Consultancy (PERC) (Diana, I'tishom, & Sudjarwo, 2017). Lead acetate given orally in experimental animals can increase levels of Malondialdehyde (MDA) testes and cause changes in the histological features of testicular tissue where interstitial exudation, degeneration, and spermatogenic cell necrosis are seen. This results in impaired spermatozoa quality (Zarghami & Khosrowbeygi, 2005).

Lead can cause fat oxidation in unsaturated fat chains. Lipids that improve oxidation will be repaired chain reactions to make free radical products. Increasing the number of radicals will spend the amount of decomposition of unsaturated fatty acids into lipids peroxide, which is very unstable. Lipid peroxidation will result in damage to the structure of spermatozoa (Acharya, Acharya, & Mishra, 2003). The administration of lead nitrate to male mice for 16 days with concentrations of 120 ppm, 360 ppm, 600 ppm, and 840 ppm, respectively, significantly reduced the process of spermatogenesis in the phases of spermatocytogenesis, meiosis, and spermiogenesis. The toxic effects of lead on the male reproductive system result in testicular atrophy, which affects the morphology of spermatozoa (Suryatini & Rai, 2018).

Lipid peroxidation reactions can be inhibited by the addition of antioxidants (Chang & Kim, 2018). One of the natural antioxidants that can be used is *Solanum betaceum* extract, which is proven to contain relatively high antioxidants such as anthocyanin, flavonoids, carotenoids, tannins, and saponins (Khaerunnisa, Kusumastuti, Mustika, Aminah, & Suhartati, 2019; Rosadi, Warditiani, & Larasanty, 2018). High antioxidants can reduce lead-induced oxidative stress in experimental animals (Diana et al., 2017). Antioxidant performance by inhibiting the formation of Reactive Oxygen Species (ROS), preventing redox reactions that produce new oxidants, protecting lipophilic antioxidants to strengthen endogenous antioxidants (Hardiningtyas, Purwaningsih, & Handharyani, 2014), and working with testosterone for spermatozoa maturation (Türk et al., 2008). Based on the explanation, *Solanum betaceum* extract is expected to act as an antioxidant by preventing damage to biological membranes due to free radicals and potentially as a spermatozoa protective agent from the influence of lead acetate.

METHODS

This research was conducted after obtaining permission from the committee of Ethics of Medical Faculty, Airlangga University, with letter number 30/EC/KEPK//FKUA/2020. This research is true experimental research using a randomized post-test only control group design to determine the effect of *Solanum betaceum* extract on the spermatozoa morphology of male mice exposed to lead acetate. This research has been conducted in January-February 2020 by involving 40 mice Balb/C.

The research sample was selected by a simple random sampling technique and divided into five groups, namely K0, K1, P1, P2, and P3. K0 received distilled water only. K1 received a lead acetate dose 75 mg/kg BW. P1, P2, and P3 received ethanol extract of *Solanum*



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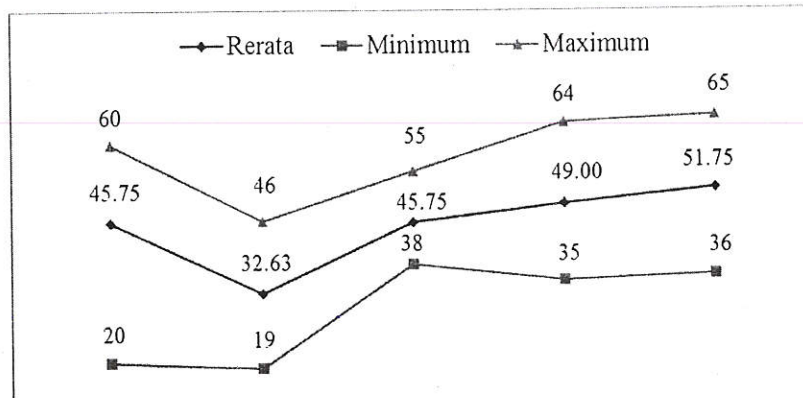
betaceum with three different doses, namely 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW, respectively. The inclusion criteria of this research were male mice Balb/C, age \pm 12 weeks, and initial body weight of 25-30 grams. Research exclusion of experimental animals was sick and died.

After 35 days of treatment, the spermatozoa will be examined to determine its morphology. Spermatozoa suspension was taken using a dissecting kit to remove the epididymis organs. The epididymal fluid was released and suspended with NaCl 0.9% in a microtube. Spermatozoa suspension from cauda epididymis was used for observation. Observation of spermatozoa morphology is done by spermatozoa suspension results dripped on the glass object. Preparations are dried in the air before fixation. The preparation was fixed with methanol for

5 minutes and dried again. Furthermore, the preparation was stained with safranin for 5 minutes and rinsed with phosphate buffer solution, and then stained with violet crystals for 5 minutes. The practices are washed with clean water and dried. The observation is to see the spermatozoa deformity and its percentage, using a 1000x magnification microscope.

The normality and homogeneity of the data are tested first. Data was said to be normal and homogeneous if the p-value >0.05 . The normality test used Shapiro-Wilk because of the data <50 . After the data is declared normal and homogeneous, it is continued with Anova test. P-value <0.05 is the significance value of the variables analyzed with one way Anova. After an Anova test shows the significant result, then continue with the Post hoc test to know which group is different from the others.

RESULTS



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