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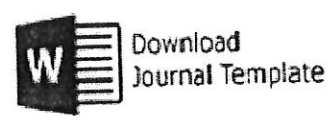
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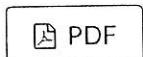
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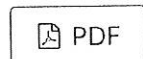


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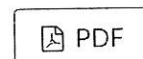
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The Protective Effect of *Solanum betaceum* Extract on Spermatozoa Vitality Exposed to Lead Acetate in Mice

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ABSTRACT

Lead pollution is a serious problem that can cause the number of free radicals to exceed the body's tolerance. This condition can cause damage to cells and body tissues. The toxic effects of lead on the reproductive system result in disruption of the process of spermatogenesis and affect the vitality of spermatozoa. *Solanum betaceum* extract contains high antioxidants which are useful for inhibiting free radicals in the body. The purpose of this study was to analyze the effect of giving various dosages of *Solanum betaceum* extract on the vitality spermatozoa mice exposed to lead acetate. This research was true experimental using randomized post-test only control group design. The total sample was 40 mice which were divided into 5 groups. Lead acetate used a dose of 75 mg/kg BW and various extracts of *Solanum betaceum* namely 100 mg/kg BW, 200 mg/kg BW and 400 mg/kg BW. Acclimatization was carried out for 7 days and continued with maintenance and treatment for 35 days. Statistical tests showed that there was a significant difference with ($p=0.026$). In conclusion, *Solanum betaceum* extract can be used as a protective to increase the vitality of spermatozoa exposed to lead acetate.

Key words: Lead acetate, mice, *Solanum betaceum*, vitality spermatozoa

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BACKGROUND

Epidemiological and experimental studies have been indicated a possible association between lead exposure and the occurrence of damage to the organs of the reproductive system. 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 necrosis of spermatogenic cells are seen. This resulted in impaired spermatozoa vitality (Elgawish and Abdelrazek, 2014). Lead can induce lipid oxidation, especially in unsaturated fatty acid chains. These oxidized lipids undergo a chain reaction to form free radical products. Increasing the number of radicals will result in the decomposition of unsaturated fatty acids into lipids peroxide which is very unstable. Lipid peroxidation will cause structural damage and disruption of spermatozoa metabolism resulting in dead spermatozoa (Cheema *et al.*, 2009).

Lead toxic effects that disrupt the process of spermatogenesis by damaging hormone synthesis and regulation (Kumar, 2018). Studies in rats exposed to lead showed a hyper response to the stimulation of the hormone gonadotropin-releasing hormone (GnRH) (Vigeh *et al.*, 2011). Disruption of GnRH pulsation results in decreased secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Decreased FSH secretion causes the function of Sertoli cells to produce androgen binding protein (ABP). Decreased LH secretion results in decreased production of testosterone hormone in Leydig cells (Oh, 2014).

The addition of antioxidants can inhibit lipid peroxidation reactions (Ayala *et al.*, 2014). *Solanum betaceum* is a fruit that contains quite high natural antioxidants such as anthocyanins, flavonoids, carotenoids, tannins, and saponins (Diep *et al.*, 2020; Khaerunnisa *et al.*, 2019). High antioxidants can reduce lead-induced oxidative stress in experimental animals (Wang *et al.*, 2013). Antioxidants work by inhibiting the formation of reactive oxygen species (ROS), preventing redox reactions that produce new oxidants, and protecting lipophilic antioxidants so that they can strengthen endogenous antioxidants (Kuciel-Lewandowska *et al.*, 2020). *Solanum betaceum* extract is expected to act as an antioxidant by preventing damage to biological membranes due to free radicals and potentially as a sperm protective agent from the influence of lead acetate. Spermatozoa vitality is the percentage of living and healthy spermatozoa in semen.

MATERIALS AND METHODS

Experimental animal criteria

The total samples were 40 mice. The inclusion criteria for experimental animals were male Balb/C, age \pm 12 weeks and initial body weight of 25-30 grams. Criteria for the exclusion of experimental animals were sick and disabled. The criteria for drop out of experimental animals were bodyweight down > 10% and dead. The number of mice used per group was 8 divided with simple random sampling.

All procedures to use animal experiments in this study were approved by the Ethics Committee of Airlangga University, Surabaya, Indonesia, and were performed with strict adherence to the animal research guide and purpose. Mice were chosen as experimental animals because their life cycles are relatively short, easy to handle, and their anatomical and physiological characteristics are well characterized.

Solanum betaceum extract

The extracted material used in this study was the ethanol extract of *Solanum betaceum*. *Solanum betaceum* was dried by a fresh dryer. Dry powder was extracted by

maceration for 3 x 24 hours 3 times at room temperature. Ethanol extract of *Solanum betaceum* was dissolved with distilled water before giving to mice orally. The extract will be given in 3 different doses, namely P₁ as much as 100 mg/kg BW, P₂ as much as 200 mg/kg BW and P₃ as much as 400 mg/kg BW.

Lead acetate

Lead acetate used a dose of 75 mg / kg BW. Lead is dissolved with distilled water and saved at room temperature. Lead acetate is given orally 1 hour after administration of *Solanumbetaceum* extract in P₁, P₂, and P₃ while K₁ is just given lead acetate.

Standard diet and bedding

The standard diet for mice was \pm 3-4 g pellets/day/tail and drinking water in the bottle \pm 3 ml/day/tail. The sawdust used for the base of the cage is changed depending on needs or minimal every 3 days to keep the cage clean and for animal health. There were 5 cages with size 500 x 300 x 150 mm (length x width x height). Each cage consists of 8 mice.

Spermatozoa vitality measurement

After 35 days of treatment, the spermatozoa will be examined to determine its vitality. The procedure for checking spermatozoa vitality was carried out in each group. Spermatozoa suspension was taken using a dissecting kit to remove the epididymis organs. Subsequently, the cauda epididymis was put into a petri dish containing 1 ml of NaCl 0.9%, the proximal portion of the cauda was cut with scissors and then pressed gently until the secretion of epididymal fluid was released and suspended with NaCl 0.9%.

The technique of calculating the percentage of the life of spermatozoa or vitality was done to use eosin coloring. It works by dripping one drop of fresh cement on the tip of the object-glass using a micropipette. Eosin solution was dropped one drop near fresh semen then both were mixed. The mixture was covered with another object glass at the end which forms an angle of 45 °C and was pulled towards the other end. Let stand 30 seconds then observed at 400 x magnification. Spermatozoa die painted red while the living does not absorb the color. Count live and dead spermatozoa until they reach 200 spermatozoa, then calculated to get the percentage.

Statistical analysis

Statistical analysis was performed by one-way ANOVA. Data analysis was used by SPSS. Data were presented as means and standard errors. $P < 0.05$ were considered significant.

RESULTS

The means taken in Table 1 is the percentage of live spermatozoa. The highest mean was 54.63% in the K₀ group which given distilled water and the lowest mean was 36.38% in the K₁ group which given lead acetate. The mean vitality of spermatozoa which were given extracts in groups P₁, P₂ and P₃ increased with increasing doses namely 42.13%, 42.75% and 46.88%, respectively. The result of staining eosin can be seen in the Figure.

To see the spermatozoa vitality data distributed normally, a normality test was conducted with Shapiro-Wilk because the data amounted to < 50 data. Based on the normality test in Table 2 showed that the distribution of spermatozoa vitality data in each group was normal with ($P < 0.09$), then the analysis is continued with a homogeneity test. Based on Table 2 homogeneity test showed the distribution of spermatozoa vitality data in each group was not homogeneous with ($p = 0.001$). After the data is declared normal but did

not have a homogeneous variant then proceed with the ANOVABrown-Forsythe test showed that there was a significant difference in the spermatozoa vitality with a ($p=0.026$).

DISCUSSION

Relationship between Lead Exposure and Spermatozoa Vitality

Lead poisoning can occur because metal compounds enter the body and pass through a multi-toxic environment (Wani *et al.*, 2015). The lead will accumulate in the body and cause health problems because it can have toxic effects on various body systems including endocrine and the reproductive system (O'Grady and Perron, 2011). Lead exposure given to the K_1 group resulted in many dead spermatozoa which were indicated by a significant reduction in the mean number of live spermatozoa compared to the K_0 group. The difference between K_1 and K_0 group was quite, 18.25%. Lead exposure in this study resulted in a decrease in spermatozoa vitality due to the oxidative damage caused. This is following the same research which states that lead toxic effects can produce ROS, reduce endogenous antioxidants and cause a state of oxidative stress (Sitaresmi *et al.*, 2017).

Membranes function to protect organs in cells and as filters on intracellular and extracellular surfaces (Walters *et al.*, 2020). Dead spermatozoa showed that the plasma membrane is damaged due to lipid peroxidation so that the metabolism of the spermatozoa is disturbed and the permeability of the membrane is very high (Christova *et al.*, 2004). This causes the eosin dye to pass through the membrane and cause the spermatozoa to die marked with dark pink spermatozoa. The high level of dead spermatozoa occurred in the K_1 group which was only given lead acetate. Giving lead as a non-essential metal can increase ROS compounds. ROS compounds or free radicals are atoms or groups whose outer orbitals have unpaired electrons. These materials are magnetic, reactive, and oxidants that can cause cell damage or death (Samarghandian *et al.*, 2013).

Relationship between *Solanum betaceum* and Spermatozoa Vitality exposed to lead acetate

The negative effects of free radicals can be overcome by administering exogenous antioxidants (Sarangarajan *et al.*, 2017). Exogenous antioxidants can be obtained by consuming foods that contain high antioxidants such as *Solanum betaceum*. *Solanum betaceum* is thought to play an excellent role as an antioxidant against free radicals especially testicular organs exposed to lead. The testes have developed a sophisticated arrangement of antioxidant systems consisting of enzymatic and nonenzymatic constituents. Antioxidant defense systems are major importance of the testicular mechanism protecting itself from free radical damage (Aitken and Roman, 2008).

Antioxidants from *Solanum betaceum* extract can provide protective effects to increase the vitality of mice spermatozoa in groups P1, P2, and P3. This is following the function of antioxidants which can delay, prevent, or eliminate oxidative damage from a highly reactive target molecule such as free radicals (Lobo *et al.*, 2010). High antioxidants can reduce lead-induced oxidative stress in animal experiments (Diana *et al.*, 2017). Administration of antioxidants resulting in an increased percentage of spermatozoa life. The group given extract showed that the spermatozoa plasma membrane can be maintained characterized by the dye eosin not being able to penetrate the spermatozoa head so that the spermatozoa head did not absorb dyes or was white (Talwar and Hayatnagarkar, 2015).

CONCLUSION

This study showed that the administration of *Solanum betaceum* extract had a significant effect on spermatozoa vitality with a ($p=0.026$). The highest increase in the mean percentage of live spermatozoa is shown by giving the highest dose of 400 mg/kg BW. This situation can be caused by the antioxidants contained in the extract of *Solanum betaceum* can provide a protective effect to prevent lipid peroxidation and protect the integrity of the spermatozoa membrane.

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Table 1 Means and standard error of spermatozoa vitality

Group	Mean	Standard error
K0	54.63	2.994
K1	36.38	6.400
P1	42.13	2.567
P2	42.75	3.400
P3	46.88	2.142

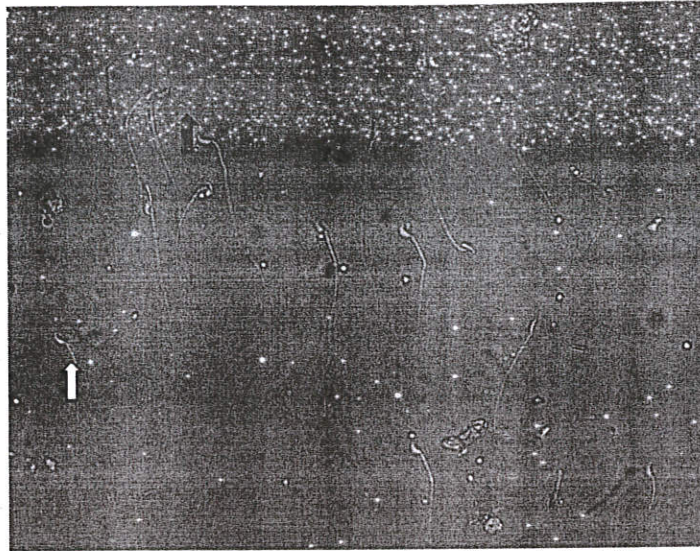


Figure Sperm vitality appearance of the sample, live (↷) and die (↴)

Table 2 Statistical test results on the vitality of spermatozoa

Group	Normality test	Homogeneity test	ANOVA test
	Sig.	Sig.	Sig.
K0	0.825		
K1	0.097		
P1	0.283	0.001	0.026
P2	0.921		
P3	0.593		

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