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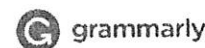
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Ethanollic extract of Dutch eggplants (*Solanum betaceum*) protects spermatozoa motility exposed to lead acetate

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ABSTRACT

Submitted : 2020-05-02
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Lead is a toxic material that can have negative effects on reproductive organs. Lead exposure reduces the activity of endogenous antioxidant enzymes and increases the number of oxidants in the body. High free radicals will affect spermatogenesis and result in decreased motile spermatozoa. Antioxidants are known to protect the motility of spermatozoa, and adequate antioxidants can be found in Dutch eggplants (*Solanum betaceum*). This study aimed to evaluate the effect of *S. betaceum* on spermatozoa motility after exposure to lead acetate. This study is a true experimental design with a randomized post-test-only control group design. Forty male Balb/C mice 12 weeks old were randomly divided into 5 groups: two control groups (C₀, C₁) and three treatment groups (T₁, T₂, T₃). The C₀ received distilled water, and the C₁ received 75 mg/kg BW lead acetate. The T₁, T₂ and T₃ received 100, 200 and 400 mg/kg BW of *S. betaceum*, respectively, an hour before exposed lead acetate. The data were analyzed using one-way ANOVA with a significant level of p < 0.05. A significant increase in the mean total motility of spermatozoa in T₁, T₂, and T₃ was reported. This study indicates that *S. betaceum* have a protective effect on spermatozoa motility when exposed to lead acetate.

ABSTRAK

Timbal adalah bahan beracun yang dapat memberi efek negatif pada organ reproduksi. Paparan timbal mengurangi aktivitas enzim antioksidan endogen dan meningkatkan jumlah oksidan dalam tubuh. Radikal bebas yang tinggi akan mempengaruhi spermatogenesis dan mengakibatkan penurunan spermatozoa motil. Antioksidan telah diketahui dapat memberikan perlindungan pada motilitas spermatozoa dan antioksidan adekuat dapat ditemukan dalam terong Belanda (*Solanum betaceum*). Penelitian ini bertujuan untuk mengetahui pengaruh *S. betaceum* terhadap motilitas spermatozoa yang dipapar timbal asetat. Studi ini adalah eksperimental murni dengan desain *randomized post-test only control group*. Terdapat 40 mencit jantan berumur 12 minggu Balb/C yang secara acak dibagi menjadi 5 kelompok: dua kelompok kontrol (C₀ dan C₁) dan tiga kelompok perlakuan (T₁, T₂, T₃). C₀ menerima aquades dan T₁ menerima 75 mg/kg BB timbal asetat. T₁ menerima 100 mg/kg BB, T₂ menerima 200 mg/kg BB, dan T₃ menerima 400 mg/kg BB *S. betaceum* 1 jam sebelum pemberian asetat timbal. Analisis data menggunakan one way ANOVA dengan nilai (p=0,002). Terdapat peningkatan rerata total motil spermatozoa pada kelompok T₁, T₂, dan T₃. Penelitian ini menunjukkan bahwa *S. betaceum* dapat memberikan efek protektif pada motilitas spermatozoa yang dipapar timbal asetat.

Keywords:
Solanum betaceum;
motility;
spermatozoa;
lead acetate;
mice;

INTRODUCTION

Infertility affects approximately 15% of couples trying to conceive, and a malefactor such as decreased quality of spermatozoa contributes to roughly half of these cases.¹ The incidence of infertility has been increasing in industrial countries from 7%-8% in 1960 to 20%-35% currently. Industrial countries have high environmental pollution. Environmental pollution is one of the factors that contributes to the decline in male fertility.² Environmental pollutant material that is often found daily, especially in industrial and developing countries, is lead (plumbum, Pb).³

Humans are exposed to many toxicants, including a heavy metal lead in the workplace and from the environment. Exposure to lead can result in significant adverse health effects on male reproductive systems.⁴ The most frequent causes of male infertility are associated with spermatozoa motility. Lead can induce a prolonged liquefaction time, thereby reducing spermatozoa motility.⁵ Lead induces lipid oxidation, especially in unsaturated fatty acid chains, which dominate the lipid architecture of the sperm plasma membrane.⁶ 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 lipid peroxide, which is very unstable. Lipid peroxidation is a process that commonly occurs following the production of high levels of reactive oxygen species (ROS).⁷ High oxidants have a strong impact on spermatozoa motility. Spermatozoa are very susceptible to interference in ROS levels. Elevated ROS production can overwhelm the cell's limited antioxidant defenses, leading to dysfunction and loss of fertilizing potential.⁸

Adequate antioxidants are needed to increase male fertility.⁹ One fruit

that contains high antioxidants is *Solanum betaceum* or Dutch eggplants. *Solanum betaceum* is a subtropical non-climacteric fruit that produces fruit throughout the year.¹⁰ *Solanum betaceum* fruit is very rich in nutrients and chemical compounds needed by the body.¹¹ Several previous studies indicate that lead exposure can be inhibited by administering exogenous antioxidants.¹² Antioxidants can bind to free radical compounds.¹³

Solanum betaceum is a natural antioxidant that has been shown to contain quite high antioxidants such as anthocyanin, flavonoids, and tannins.¹⁴ Flavonoid compounds are secondary metabolite compounds that act as antioxidants because they are beneficial in preventing cell damage due to oxidative stress.¹⁵ Flavonoids that function as antioxidants are flavonoids that have hydroxyl groups (-OH) because they can donate protons (H atoms) to free radicals so that free radicals become stable.¹⁶ Anthocyanin is a natural source of antioxidants that can be used to minimize oxidation reactions and ward off free radicals.¹⁷ Ellagic acid in tannins reacts with free radicals because of its ability to bind metal ions, which are powerful antioxidants against lipid peroxide.¹⁸ *Solanum betaceum* extract acts as an antioxidant that can protect the biological membrane of sperm from damage due to free radicals and has the potential to protect the motility of spermatozoa from the influence of lead.

MATERIALS AND METHODS

Study design and site

This is true-experimental research with a randomized post-test only control group design. Forty male mice were obtained from Surabaya Veterinaria Center. Maintenance of mice and treatment were located at the Laboratory Animals Experiment,

Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya. The observation of the quality of the spermatozoa was located at the Medical Biology Laboratory, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

Animal criteria, standard diet, and bedding

Forty clinically healthy male Balb/C mice, 12 weeks old, with an initial body weight of 25-30 g were used in this study. Acclimatization was performed before use for 7 days to adjust to the new environment. All mice were found to be in a clinically acceptable condition and were assigned to the study. Animals that are sick and die will be excluded from the study.

Animals were housed in five cages with a size of 500 x 300 x 150 mm for 35 days based on the spermatogenesis cycle. The cages were well placed so that all experimental animals obtained good environmental conditions, such as light, temperature, and humidity, that were homogeneous and constant. There were five groups, namely, C₀, C₁, T₁, T₂, and T₃. C₀ was receiving distilled water. C₁ was receiving lead acetate. T₁, T₂, and T₃ were given lead acetate after receiving *S. betaceum*. All treatments were given orally. Each group (8 mice) received the same standard diet *ad libitum*. The sawdust used for the base of the cage was changed depending on needs or at least every 3 days to keep the cage clean and for animal health.

Lead acetate preparation

Lead acetate used a dose of 75 mg/kg BW, dissolved in distilled water and saved at room temperature. Lead acetate solution was given as much as 0.1 mL.

***Solanum betaceum* extract preparation**

The extracted material used in this study was the ethanolic extract of *S. betaceum*. *Solanum betaceum* fruit was dried by a fresh dryer. Dry powder was extracted by maceration 3 x 24 h 3 times at room temperature. The ethanol extract of *S. betaceum* was dissolved in distilled water before being given to mice orally. The extract was given in 3 different doses, namely, T₁ 100 mg/kg BW, T₂ 200 mg/kg BW, and P₃ 400 mg/kg BW. Different doses were administered in the Medical Biochemistry Laboratory, Faculty of Medicine, Universitas Airlangga. *Solanum betaceum* solution was given as much as 0.1 mL.

The instrument and measurement of spermatozoa motility

Mice were sacrificed by giving chloroform solution and then surgically performed. After sacrificing the animals, the spermatozoa was examined to determine their motility. The procedure for checking spermatozoa motility was carried out in each group. Spermatozoa suspension was obtained from the epididymis using a dissecting kit. The cauda epididymis was separated by cutting the proximal portion of the epididymal corpus and the distal portion of the vas deferens. Subsequently, the cauda epididymis was put into a petri dish containing 1 mL of NaCl 0.9%, and the proximal portion of the cauda was cut with scissors and then pressed gently until the secretion of epididymal fluid was released and suspended in NaCl 0.9%. Spermatozoa suspension from the cauda epididymis was used for observation approximately 30 min after collection.

Observation of the motility of

spermatozoa was performed by dripping approximately 10-15 μ L into the glass object and then covered with a cover glass. Spermatozoa motility is observed under a microscope with a magnification of 200-400x. Evaluate at least 200 spermatozoa with a calculating machine and assess the movements of spermatozoa that occur by assessing 3 categories and calculating their percentage. Spermatozoa motility assessment criteria are spermatozoa moving forward (progressive), spermatozoa moving but not forward, only spinning, or moving in place (non progressive), and spermatozoa are silent or not moving (immotile).

Statistical analysis

The results are given as the means \pm standard deviation (SD). Data were analyzed using one-way ANOVA and post hoc tests with a significance level

of pvalue <0.05 . The data were normally distributed.

Ethical consideration

This study was approved by the Research Ethics Committee of Faculty of Medicine, Universitas Airlangga, Surabaya, with registration number: 30/EC/KEPK//FKUA/2020.

RESULTS

Spermatozoa motility assessment criteria, namely progressive, nonprogressive, and immotile are shown in FIGURE 1. The highest mean number of immovable or immotile spermatozoa in the C_1 group was given lead acetate. The lowest mean of progressive and nonprogressive motile spermatozoa was in the C_1 group that was given lead acetate.

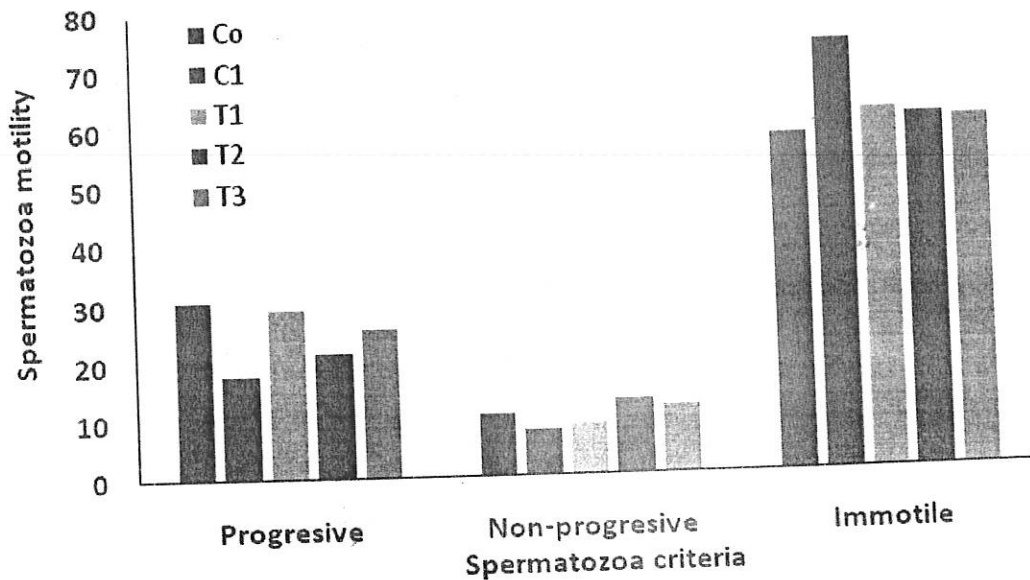


FIGURE 1. Average spermatozoa motility of 5 treatment groups (n=40).

Statistical analysis results of each group can be seen in TABLE 1. Each group consisted of 8 male mice. The average measurement results were

obtained by combining progressive and nonprogressive motility as total motile. The mean of the total motile in each group was different. The highest mean in the

C₀ group was 45.13%, and the lowest in the C₁ group was 25.88%. The mean value of total motile spermatozoa in groups T₁, T₂, and T₃ increased with increasing doses. To see the spermatozoa motility data normally distributed, a normality test with Shapiro-Wilk was performed because the data amounted to <50 data points. The spermatozoa motility data distribution of each group was normal with a p value >0.05, and then the analysis was continued with a homogeneity test. The homogeneity test showed that the distribution of spermatozoa motility data

in each group was homogeneous with a p value of 0.256 and then continued with the ANOVA difference test. ANOVA showed that there was a significant difference in spermatozoa motility with a p value of 0.002.

The post hoc LSD test showed that there were significant differences in total motile spermatozoa (p <0.05) as shown in TABLE 2. Superscript letters show significant differences, namely, C₀ and C₁ (0.000), C₁ with T₁ (0.004), T₁ with T₂ (0.003), and C₁ with T₃ (0.001).

TABLE 1. The difference in spermatozoa motility between the intervention and the control groups

Groups	Mean± SD	Normality	Homogeneity	p
C ₀	45.13±5.489	0.631		
C ₁	25.88± 10.092	0.732		
T ₁	37.25± 5.574	0.347	0.256	0.002
T ₂	37.88± 8.526	0.073		
T ₃	38.75± 6.319	0.499		

C₀: normal mice; C₁: exposed to lead acetate only; T₁: 100 mg/kg BW + exposed to lead acetate; T₂: exposed to lead acetate + 200 mg/kg BW; T₃: exposed to lead acetate + 200 mg/kg; SD: standard deviation

TABLE 2. Post hoc LSD test for total motility of spermatozoa

Groups	C ₀	C ₁	T ₁	T ₂
C ₁	0.000a	-	-	-
T ₁	0.238	0.004b	-	-
T ₂	0.265	0.003c	0.947	-
T ₃	0.374	0.001d	0.766	0.817

DISCUSSION

In this study, the C₁ group that was only given lead acetate, showed the lowest total motile rate. These results showed that lead toxicity can reduce total motile spermatozoa. Lead-induced oxidative stress causes major damage to sperm quality by disrupting the antioxidant and reactive oxygen

species (ROS) balance, thus resulting in abnormalities of spermatogenesis.¹⁹ Increased ROS products cause oxidative stress (OS) due to an imbalance of antioxidants and oxidants.²⁰ Although ROS are necessary for the normal physiological function of sperm, excessive oxidative stress can cause increased susceptibility to DNA damage, potentially leading to infertility and reducing sperm

motility.²¹ Oxidative stress has been identified as an area of great attention because ROS and their metabolites can attack enzymatic systems. Spermatozoa have minimal endogenous antioxidant defenses and are very dependent on exogenous enzyme sources during spermatogenesis.²²

Spermatozoa are rich in mitochondria to supply much energy that is required for motility.²³ The movement of spermatozoa requires some ATP, which is used to move the flagellate apparatus.²⁴ ROS-induced damage to mitochondrial DNA leads to decreased ATP and energy availability, impeding sperm motility. The presence of dysfunctional spermatozoa in the semen significantly elevates the production of ROS, which in turn affects its mitochondrial function and sperm motility.²⁵ Disturbances in mitochondrial respiration function can result in decreased motility and fertility, which can be seen in the C₁ group. The cytology of animals that lead poisoning describes the occurrence of asthenozoospermia or a condition when sperm cannot move swiftly.²⁶

The balance between ROS and antioxidants is necessary to maintain physiological oxidative mechanisms and to minimize the risk of cellular injury.²⁷ If ROS accumulate, endogenous antioxidant defenses will not be sufficient.²⁸ Exogenous antioxidants are needed to inhibit cell damage that occurs. *Solanum betaceum* fruit contains high antioxidants that the body needs.²⁹ The mean value indicates that there was a significant difference between the groups given lead (C₁) and the group's given extracts of *S. betaceum* and lead acetate (T₁, T₂, and T₃). The antioxidant content in the *S. betaceum* extract can function as a protective agent to prevent cell damage caused by free radicals, with the mean total motile values of the T₁, T₂, and T₃ groups increasing by 37.63, 37.88, and 38.75%, respectively,

compared to the C₁ group. Spermatozoa motility significantly increases with increasing dose.³⁰ Administration of antioxidants can increase the body's metabolism and accelerate the formation of ATP so that spermatozoa motility will increase.³¹ This happens because in the cell plasma membrane, there are many macromolecules in the form of proteins, lipoproteins, and glycoproteins that function as enzymes and receptors. Spermatozoa require several enzymes and receptors for their movement.³²

The results of this study showed that the administration of ethanolic *S. betaceum* extracts significantly affected the total motility of spermatozoa. The highest percentage increase in the mean total motility is shown by giving the highest dose of 400 mg/kg body weight. The higher the dose of antioxidant administration, the more antioxidant content obtained will increase the motility of mouse spermatozoa.³³ Other studies have shown an improvement in mitochondrial function as a producer of ATP after exogenous antioxidants are given. ATP will be better synthesized as an energy source to drive flagella if given adequate antioxidants.³⁴

CONCLUSION

The findings of this study shows that there is a significant difference in motility between the intervention and the control groups. *Solanum betaceum* extract has a protective effect by increasing the total motility of spermatozoa. The highest increase in the mean percentage of total motile spermatozoa was shown by giving the highest dose of 400 mg/kg BW in the T₃ group.

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