



Effect of *Solanum betaceum* Cav. extract on malondialdehyde, testosterone, and Sertoli cells in lead acetate-induced mice

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ABSTRACT

Context: Lead exposure has been scientifically associated with male infertility with an unclear cause. According to studies, lead exposure has a significant association with infertility in both women and men. Lead that is ingested or inhaled and enters the body can increase reactive oxygen species (ROS), which affect sperm quality.

Aims: To evaluate the effects of *Solanum betaceum* extract on malondialdehyde (MDA), testosterone, and Sertoli cells in lead acetate-induced mice.

Methods: 40 male mice weighing 25–30 grams and aged 12–16 weeks were used in this study. In addition, this study is an experimental study with a randomly allocated posttest-only control group design. For 35 days, mice were randomly divided into five groups: K- (the negative control group, distilled water), K+ (the positive control group, lead administration only), P1 (administered with 100 mg/kg BW *S. betaceum* extract), P2 (administered with 200 mg/kg BW *S. betaceum* extract), P3 (administered with 400 mg/kg BW *S. betaceum* extract). On day 36, mice were sacrificed, and the testicular tissues were taken. These tissues were then examined for their MDA levels through an ELISA test and for the number of their Sertoli cells through a histological study. Simultaneously, 3 mL of blood were drawn from the hearts of mice to be analyzed for the testosterone level by an ELISA test.

Results: This study showed that although *S. betaceum* could not significantly affect MDA level ($p > 0.05$), it could significantly affect testosterone level and Sertoli cells.

Conclusion: The administration of *S. betaceum* was able to protect the Sertoli cells and testosterone in mice induced with lead acetate.

Keywords: male infertility; oxidative stress; Sertoli cells; *Solanum betaceum*.

INTRODUCTION

Infertility is a complex and emotionally challenging issue that affects millions of men and women worldwide. It was estimated that male factors contributed to approximately 50% of cases in couples (Sharma et al., 2021). Globally, 48.5 million couples experience infertility. According to UCLA Health's 2020 report, nine of the ten countries with the highest fertility rate were in Africa, followed by Afghanistan. Additionally, male fertility can result from a wide range of factors, including sperm abnormalities, hormone abnormalities, and lead exposure, known to be a potential male reproductive toxin (Agarwal et al., 2021).

There are various ways that lead may enter the body. Once it enters the body, it is absorbed into the bloodstream and inhibits δ -aminolevulinic acid dehydratase (δ -ALAD). It inhibits the activity of the enzyme glucose-6-phosphate dehydrogenase (G6PD) by reducing endogenous antioxidant activity and increasing reactive oxygen species (ROS) (Wahidah et

al., 2020), which can measure malondialdehyde (MDA) levels as oxidative stress biomarkers. High oxidative stress affects pulsatile gonadotropin-releasing hormone (GnRH) in the brain, which disrupts hormone regulation. In this case, increasing oxidative stress may affect the hormone testosterone and Sertoli cell production. Meanwhile, ROS in the testes can also cause blood-testis barrier dysfunction, which has a direct effect on the decrease in epithelial thickness (Jegou and Skinner, 2018).

According to several studies, natural antioxidants have been shown to play a significant role in protecting male reproductive function. Natural antioxidants such as vitamins C and E suggest promising results in an effort to fight against oxidative stress. Moreover, there is one of the inexpensive, easily accessible fruits or vegetables with the highest antioxidant content among other types of tomatoes, which is *Solanum betaceum* Cav. (Terong Belanda or Dutch eggplant), *Solanaceae*, with a CE₅₀ value of 44.25 pg/mL (Noor Atiqah et al., 2014). *S. betaceum* has been reported to have secondary metabolic

components that are quite high in antioxidants, such as flavonoids, tannins, and terpenoids. Flavonoids function to overcome cell membrane damage and are able to bind reactive electrons in them, preventing the formation of oxidative chains (Khaerunnisa, 2018).

The aim of this study was to evaluate the effects of *S. betaceum* extract on MDA, testosterone, and Sertoli cells in lead acetate-induced mice.

METHODOLOGY

Plant material and extraction

Solanum betaceum Cav. fruits were obtained from the Wonosobo Farm, Central Java, Indonesia (GPS coordinates 7.3633°S latitude and 109.9031°E longitude). The sample were identified at the Research Centre for Biology of LIPI, Indonesian Institute of Science, Indonesia. The voucher number of the plant sample was 724/IPH.1.01/If.07/III/2017.

S. betaceum fruits were dried by fresh drier. Dry powder was extracted by maceration using ethanol solvent for 3 × 24 h three times at room temperature and then separated between filtrate and residue. The ethanol extract was filtered and evaporated with a rotary vacuum evaporator to obtain a viscous extract. The ethanol extract of *S. betaceum* was administered to intervention groups in the form of suspension using 1% CMC with a dose of 100, 200, and 400 mg/kg BW.

Animals and experimental protocol

This study was conducted at the Institute of Tropical Disease Laboratory, Universitas Airlangga, and the Biochemistry Laboratory of the Faculty of Medicine, Universitas Airlangga, within five months (December 2020 to April 2021). All research procedures were approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Airlangga, No. 19/EC/KEPK/FKUA/2020.

This study used a true experimental design with a randomized posttest-only control group design. Furthermore, this study used 40 Balb/C male mice (*Mus musculus*) aged 12–16 weeks and weighing 25–30 g. Animals were administered with food and water *ad libitum*, also handled according to ethical guidelines of the research ethics. These animals were housed under controlled conditions, including an ambient temperature of 22 ± 2°C and a 12-hour light-dark cycle. Mice were acclimated and then randomly divided into five groups: K- (negative control group, administered with distilled water orally using a feeding tube for 35 days); K+

(positive control group, administered with distilled water on days 1-3, and on days 4-35, they were administered with lead acetate solution orally using a feeding tube at a dose of 75 mg/kg BW/day); P1 (administered with *S. betaceum* extract at a dose of 100 mg/kg BW/day on days 1-3, and on days 4-35, one hour after being administered with *S. betaceum* extract at a dose of 100 mg/kg BW/day, they were administered with lead acetate solution at a dose of 75 mg/kg BW/day orally using a feeding tube); P2 (administered with *S. betaceum* extract at a dose of 200 mg/kg BW/day on days 1-3, and on days 4-35, one hour after being administered with *S. betaceum* extract at a dose of 200 mg/kg BW/day, they were administered with lead acetate solution at a dose of 75 mg/kg BW/day orally using a feeding tube); and P3 (administered with *S. betaceum* extract at a dose of 400 mg/kg BW/day on days 1-3, and on days 4-35, one hour after being administered with *S. betaceum* extract at a dose of 400 mg/kg BW/day, they were administered with lead acetate solution at a dose of 75 mg/kg BW/day orally using a feeding tube). On day 36, mice were sacrificed so that the testicular tissues and blood could be collected.

Simultaneously, 75 mg/kg BW of lead acetate was used to disrupt the testicular mice. In addition, the feeding and administration of *Solanum betaceum* extract were done at 7:00–8:00 a.m.

MDA, testosterone, and Sertoli assay

For the MDA assay, tissues were rinsed and homogenized in PBS (pH 7.4) with a glass homogenizer on ice, and MDA levels were measured using the enzyme-linked immunosorbent assay (ELISA) Cat. No. E0625Mo (Biossay Technology Laboratory, Inc., Shanghai, China, 2020). Moreover, for the testosterone assay, blood was drawn from the hearts of the mice. The blood serum was then separated into a new tube, and testosterone levels were measured using the enzyme-linked immunosorbent assay (ELISA) method. In addition, for the Sertoli cell assay, testicular tissues were fixed in neutral buffered formalin 10%, stained with hematoxylin and eosin, and then examined through a light microscope (Olympus BX 41) (Mescher, 2016; Wulandari et al., 2018).

Statistical analysis

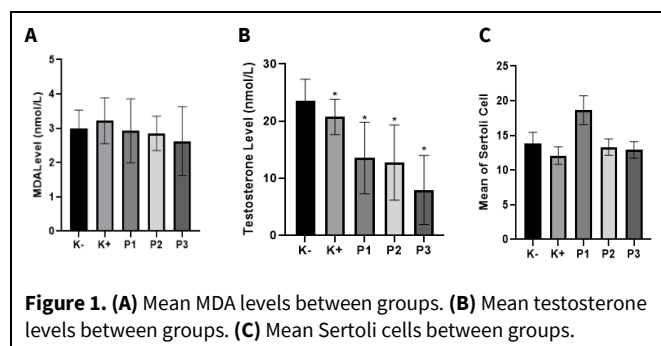
Statistical analysis was performed using social science (SPSS) statistics for Windows, version 21. Moreover, the normality test was conducted using the Shapiro-Wilk test, while the homogeneity test used the Levene test. Furthermore, the statistical analysis used

included a one-way ANOVA, followed by the least significant difference (LSD). The data was presented as mean $\pm$ standard deviation (SD). In addition, the significant level set by the statistical analyses was $p < 0.05$.

RESULTS AND DISCUSSION

Effect of *S. betaceum* extract administration on decreasing MDA levels in mice exposed to lead acetate

Based on the results of this study and the one-way ANOVA statistical tests, it was observed that there were no significant differences in all experimental groups ($p = 0.625$). However, it can be seen from the descriptive data in Fig. 1A above that the average value of MDA levels in the group of mice exposed to lead was higher (3.222 nm/L) than the control group (2.993 nm/L).



Lead can have an effect on the emergence of oxidative stress by causing ROS-free radicals to form. According to Kovacik et al. (2016), MDA is a reactive compound that serves as a marker for oxidative stress and lipid peroxidation in the biological system as well as indirectly indicating the appearance of cellular damage caused by free radicals. The presence of free radicals in the form of carcinogenic compounds, H_2O_2 , O_2^- , OH^- , and decreased endogenous antioxidant activity cause oxidative stress, which triggers the production of ROS (Lopes et al., 2016). Moreover, the MDA levels decreased in the treatment group administered with lead and *S. betaceum* extract at different doses, with the lowest MDA levels in the treatment group administered with a maximum dose of *S. betaceum* extract (2.623 nm/L). Based on the study by Trilaksani (2003), MDA levels could be reduced by administering antioxidants. Furthermore, Khaerunnisa (2018) obtained the results of secondary metabolic screening analysis using various methods in the ethanol extract of *S. betaceum*, including flavonoids, tannins, and terpenoids. Flavonoids can increase the activity of antioxidant enzymes in the body,

such as glutathione (GSH) (Speisky et al., 2022). The ability of the GSH enzyme to convert H_2O_2 molecules and lipid peroxide into H_2O makes it a free radical quencher (Xu et al., 2019). The administration of antioxidants before lead exposure aimed to protect enzymes from oxidative hazards caused by lead exposure (Rendón-Ramírez et al., 2014). In addition, the results of the aforementioned studies support this study's results. Flavonoid compounds in *S. betaceum* extract could act as antioxidants, reducing MDA levels in the testicles of mice exposed to lead acetate. However, further research is still required to investigate the significance of the statistical test when *S. betaceum* is administered in increased doses to lead-exposed mice.

The effect of *S. betaceum* extract administration on increasing testosterone levels in mice exposed to lead acetate

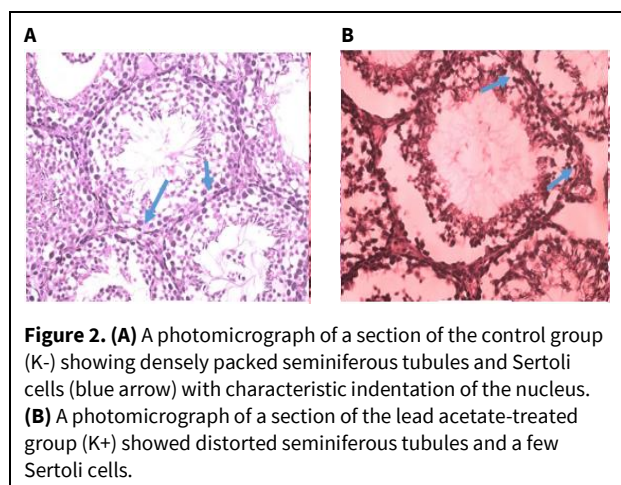
Fig. 1B from this study shows that testosterone levels significantly decreased in the negative control group (K-) compared to the positive control group (K+). This indicates that lead acetate decreased the levels of the hormone testosterone. Moreover, the Mann-Whitney test and t-test for the testosterone levels in each group showed that in all treatment groups, *S. betaceum* extract at doses of 100, 200, and 400 mg/kg BW showed a significant decrease in the control group ($p < 0.05$). The testosterone levels decreased more significantly with increasing *S. betaceum* extract doses administered. It was due to the toxic nature of lead acetate exposure. Lead in the blood caused inhibition of an enzyme involved in heme biosynthesis, namely the δ -ALAD enzyme, which caused an increase of aminolevulinic acid, and the result was an increase in the production of ROS. The increase in ROS was due to decreased endogenous antioxidant reserves, causing oxidative stress (Diana, 2017).

High levels of oxidative stress in the brain can disrupt the function of the hypothalamus so that the pulsatile GnRH is also disrupted. Disruption of pulsatile GnRH causes the secretion of follicle-stimulating hormone, and luteinizing hormone are also disrupted. At the testicular level, disruption of hormone regulation causes a decrease in the production of the hormone testosterone (Barkur and Bairy, 2015; Suryatini et al., 2018). Moreover, according to Ul Haq et al. (2016), anthocyanin compounds are flavonoid derivative compounds whose content is highest in *S. betaceum*, and their antioxidant activity is higher than vitamins C and E. This may be correlated with an increase in the toxicity of the flavonoid content in the *S. betaceum* itself. High concentrations of flavonoids contained in extracts have

been found to be cytotoxic by inducing severe oxidative stress. In addition, prooxidants cause oxidative stress, which can exacerbate cell damage because it can cause mitochondrial dysfunction, resulting in cell necrosis (Abrori et al., 2019). In fact, there are so many factors that have an effect on testosterone levels, and these factors may function as a feedback mechanism to increase or decrease testosterone levels (Riachy et al., 2020). Therefore, further research related to this is crucial.

The effect of *S. betaceum* extract administration on increasing Sertoli cells in mice exposed to lead acetate

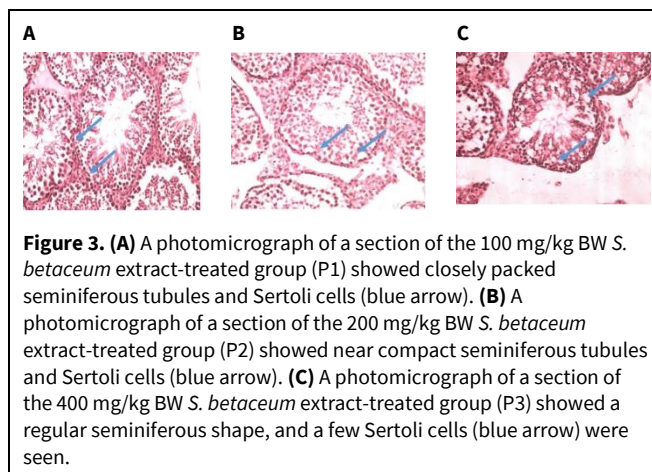
This study used the ANOVA test to compare the means of different groups and continued with the LSD posthoc test to find an optimum dose of *S. betaceum* extract ($p < 0.05$). The highest mean value (18.65 ± 2.08) was observed in P1, in which the treatment was administered *S. betaceum* extract at a dose of 100 mg/kg BW/day (Fig. 1C).



This study revealed that lead acetate exposure could significantly reduce the number of Sertoli cells in Balb/C mice. This can be seen in Fig. 1C, which compares the positive control groups that had the lowest mean number of Sertoli cells compared to the entire group. Moreover, the results of this study were supported by other studies showing that lead acetate exposure could reduce the number of Sertoli cells (Shubina and Dudenkova, 2016). Furthermore, recently has been reported that chronic exposure to lead resulted in structural changes in the intracellular junction of epithelial cells in the seminiferous tubules (Ramos-Treviño et al., 2018). The longer the lead exposure, the more structural changes there are in the seminiferous tubules, including Sertoli cells. This occurs because Sertoli cells are often the main target of toxic compounds,

such as lead, that can pass the blood-testis barrier (Gao et al., 2015). Additionally, Sertoli cells play an essential role in maintaining the integrity of the germinal epithelium (Wong and Khan, 2022) (Figs. 2 and 3).

Lead exposure results in an increase in ROS, which is the cause of testicular toxicity (Sudjarwo et al., 2017). Increased ROS results in adhesion and instability of cell membrane structure through lipid oxidation (Banjarnahor and Artanti, 2015; Sudjarwo et al., 2017). Moreover, increased ROS production makes the body's defense mechanism of endogenous antioxidants insufficient and requires exogenous antioxidants (Asadi et al., 2017). Furthermore, Sertoli cells have an essential regulatory role in testicular differentiation and fetal masculinization, anti-Mullerian hormone secretion, induce fetal Leydig cell development, and even play a role in the development of adult Leydig cells (Richburg et al., 2018; Wong and Khan, 2022). Additionally, research conducted by Rebourcet et al. (2017) showed that Sertoli cells have an essential role in maintaining Leydig cells. Sertoli cell ablation in adult mice results in a decrease in the number of Leydig cells. The aforementioned research supports the results of this study. It was discovered that in the treatment group exposed to lead acetate, the number of Sertoli cells decreased significantly.



CONCLUSION

Solanum betaceum is a natural compound that protects the Sertoli cells of mice induced with lead acetate, but it could not significantly change the MDA level or decrease the testosterone level.

CONFLICT OF INTEREST

The authors declare no conflicts of interest with the data contained in the document.

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