**ABSTRACT**

Lead is a harmful pollutant from engine exhaust that causes free radicals and has detrimental effects on the testicular tissue. This study aimed to determine the effects of tomato juice on the number of Leydig cells and the diameter of the seminiferous tubules of mice exposed to lead acetate. Twenty-five male mice were divided into five groups. Mice in the control (C- ) group were given placebos. Meanwhile, mice in C+, T1, T2, and T3 groups were exposed to lead acetate at a dose of 100 mg/kg BW/day for 14 days and given tomato juice respectively at 0, 0.16, 0.32, and 0.64 mL/day from day 8 to day 35. On day 36, all mice were sacrificed, and the testes were collected for histological preparation. The result showed that lead exposure in the C+ group decreased (p <0.05) in the number of Leydig cells and the diameter of the seminiferous tubules compared to the mice in group C-. Administration of tomato juice in groups T1, T2, and T3 increased (p <0.05) the number of Leydig cells and the diameter of seminiferous tubule compared to the mice in the C+ and C- groups. However, tomato juice administration to the T3 group decreased the number of Leydig cells and the diameter of the seminiferous tubules (p <0.05) compared to the T2 group. In conclusion, an effective dose of 0.32 mL/day of tomato juice restored Leydig cell number and seminiferous tubules diameter in mice exposed to lead acetate.

**Keywords:** lead acetate, Leydig cell, Lycopersicon esculentum, pollutant, seminiferous tubules, tomato juice

**INTRODUCTION**

Air pollution in big cities is getting worse due to motor vehicle exhaust emissions (Wang et al., 2022). Lead compounds (Plumbum, Pb) are harmful pollutants in engine exhaust when using leaded gasoline (Sassykova et al., 2019). Lead could reduce antioxidant levels and increase the production of free radicals, such as reactive oxygen species (ROS), which results in oxidative stress and lipid peroxidation (Shahid et al., 2014). Increased oxidative stress caused...
damage and decreases the number of Sertoli cells, Leydig cells, and other spermatogenic cells (Asadi et al., 2017). Lipid peroxidation also affects hypothalamus and pituitary gland cells, disrupt the producing of spermatogenesis regulators such as FSH and LH. The decrease in these hormones could interfere with the process of testicular spermatogenesis (Ramswamy and Weinbauer, 2015). The decrease in the process of spermatogenesis caused a decrease in the diameter of the seminiferous tubules and spermatogenic cells and Leydig cells (Gulkesen et al., 2002).

Antioxidants could overcome the toxic effects of oxidative stress on testicular function (Asadi et al., 2017). Tomatoes contained carotenoids, polyphenols, vitamin A, vitamin C, and vitamin E, which could act as antioxidants (Martí et al., 2016). The mechanism of antioxidants against free radicals was to reduce the formation of single oxygen, inhibit the initiation stage and break the propagation stage of the radical chain reaction. Radicals generated from the oxidized antioxidants were stabilized by resonance and become relatively unreactive (Imran et al., 2020). A previous study reported that exposure to lead and administration of tomato paste did not cause significant changes in rat serum testosterone levels (Salawu et al., 2009). This study aimed to evaluate this phenomenon by examining the number of Leydig cell as testosterone producers. In addition, the effect of tomato juice (Lycopersicon esculentum Mill.) on the number of Leydig cells and the diameter of the seminiferous tubules due to exposure to lead acetate has not been studied.

MATERIALS AND METHODS

Tomato juice was made from fresh ripe red tomatoes obtained from Kusuma Agrowisata Batu, Malang regency, East Java, Indonesia. Geographically it is located between 744'55.11" - 826'35.45" South Latitude and 12217'10.90" - 12257'00.00" East Longitude. Tomato juice was made fresh every day before treatment by blending for 2.5 minutes, and the filtrate was separated from the pulp by filtering (Igile, et al., 2016).

Treatment of experimental animals

This study has been approved by the Ethics Commission No 158/HRECC.FODM/IV/2022. Twenty-five male mice (Mus musculus) aged 2-3 months with a body weight of 20-30 grams, were adapted to the conditions of the study site for seven days in an experimental animal laboratory. Mice were fed pellets in the morning and evening, and water was provided ad libitum.

Mice in group C- were given distilled water as a placebo. Whereas mice in groups C+, T1, T2, and T3 were exposed to lead acetate (100 mg/kg BW/day) for 14 days (Yu et al., 2020), and given respectively 0, 0.16, 0.32, and 0.64 mL tomato juice per day, for 28 days from day 8 to day 35 (Handaru et al., 2010). Exposure to lead acetate and administration of tomato juice was carried out orally before feeding using a gastric probe in four hours interval. On day 36, all mice were anesthetized with 0.2 mg/kg BW of ketamine hydrochloride (PT. Dexa Medica) and sacrificed by cervical dislocation. The testes were collected and stored in a plastic pot containing 10% formalin, then histological preparations were made with Hematoxylin and Eosin staining (Machmudia et al., 2021).

Leydig cell count

The number of Leydig cells was counted under a microscope (Olympus BX-53, Shinjuku City, Tokyo, Japan) at 400x magnification in five fields of view of each interstitial tissue. Each field of view was photographed, and the number of Leydig cells was counted and then averaged (Fitri et al., 2019).

Measurement of seminiferous tubules diameter

The diameter (μm) of the seminiferous tubules was measured under a light microscope (Olympus BX-53, Shinjuku City, Tokyo, Japan) at 100x magnification using Image Raster and Optilab (OptiLab Advance V2). The diameter of the seminiferous tubules was measured at the tubular diameter from both edges of the basement membrane. Each sample was measured in three fields of view, three seminiferous tubules, each of which had the

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same or almost the same shape and size, then averaged (Mardatillah et al., 2022).

**Data analysis**

Data were analyzed using one-way Anova followed by Duncan’s Post Hoc test at a 95% confidence level in the Statistical Product and Service Solutions (SPSS) software version 23 for windows.

**RESULTS**

Lead exposure for 14 days in group C+ resulted in a decrease (p <0.05) in the number of Leydig cells and diameter of the seminiferous tubules compared to group C- mice. Administration of tomato juice for 28 days in groups T1, T2, and T3 increased (p <0.05) the number of Leydig cells and the diameter of the seminiferous tubules compared to mice in the C+ and C- groups. However, the administration of tomato juice to the T3 group resulted in a lower number of Leydig cell and seminiferous tubules diameter (p<0.05) than the T2 group.

Microscopic visualization of the number of Leydig cells and the diameter of the seminiferous tubules of mice in each group can be seen in Figure 1 and Figure 2.

**Table 1** Leydig cells count (means ± SD) and the diameter of the seminiferous tubules (µm, means ± SD) of the testes of mice after exposure to lead acetate and administration of tomato juice

<table>
<thead>
<tr>
<th></th>
<th>Leydig cell count</th>
<th>diameter of the seminiferous tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-</td>
<td>6.36 ± 0.43 b</td>
<td>168.70 ± 1.72 b</td>
</tr>
<tr>
<td>C+</td>
<td>4.04 ± 0.41 a</td>
<td>149.74 ± 4.54 a</td>
</tr>
<tr>
<td>T1</td>
<td>7.80 ± 0.58 c</td>
<td>181.18 ± 2.63 c</td>
</tr>
<tr>
<td>T2</td>
<td>14.72 ± 1.0 e</td>
<td>194.55 ± 1.19 e</td>
</tr>
<tr>
<td>T3</td>
<td>10.32 ± 0.99 d</td>
<td>189.22 ± 1.07 d</td>
</tr>
</tbody>
</table>

Different superscripts in the same column showed significant differences (p <0.05); C-: mice were not exposed to lead acetate; C+, T1, T2, T3: mice were exposed to lead acetate (100 mg/kg BW/day) for 14 days, followed by the administration of tomato juice respectively 0, 0.16, 0.32, 0.64 mL/day from day 8 to day 35.

Figure 1 Microscopic view of Leydig cells (blue arrows) of mice after exposure to lead acetate and tomato juice administration (Hematoxylin and Eosin staining, Olympus BX-53, Shinjuku City, Tokyo, Japan at 100x magnification); C-: mice were not exposed to lead acetate; C+, T1, T2, T3:
mice were exposed to lead acetate (100 mg/kg BW/day) for 14 days, followed by the administration of tomato juice respectively 0, 0.16, 0.32, 0.64 mL/day from day 8 to day 35.

**Figure 2** Measurement of the diameter of the seminiferous tubules of mice after exposure to lead acetate and administration of tomato juice (Hematoxylin and Eosin staining, Olympus BX-53, Shinjuku City, Tokyo, Japan at 100x magnification using Image Raster and OptiLab Advance V2) C-: mice were not exposed to lead acetate; C+, T1, T2, T3: mice were exposed to lead acetate (100 mg/kg BW/day) for 14 days, followed by the administration of tomato juice respectively 0, 0.16, 0.32, 0.64 mL/day from day 8 to day 35.

**DISCUSSION**

Exposure to Pb caused decreases in testicular weight, spermatozoa quality, and antioxidant capacity. However, Pb did not cause change in serum testosterone levels. Tomato paste reduced these adverse effects of Pb (Salawu et al., 2009). Tomatoes that were processed into juice or paste had a higher lycopene content than fresh tomatoes (Mendelová et al., 2013).

Lead exposure caused a decrease in the number of Leydig cells compared to normal mice. Lead could cause reproductive toxicity by overproduction of ROS. Excessive amounts of free radicals will cause oxidative stress will cause oxidative stress and cause lipid peroxidation of cell membranes, thereby
damaging cell membranes, including the Leydig cell membranes, resulting in a decrease in the number of Leydig cells. This followed previous studies that exposure to lead with various doses and applications could reduce the number of Leydig cells (Shan et al., 2009; Garu et al., 2011; Hamadouche et al., 2013) and reduce the process of steroidogenesis (Thoreux Manlay et al., 1995). Another impact of decreasing the number of Leydig cell is the disruption of the role of FSH and ICSH. These hormones played an active role in stimulating the Leydig cells to produce testosterone (Zirkin and Papadopoulos, 2018).

Administration of tomato juice increased the number of Leydig cells compared to mice exposed to lead alone or to normal mice. Tomato (Lycopersicon esculentum Mill.) contained high antioxidants in the form of lycopene, vitamin C, and flavonoids that can neutralize existing free radicals (Ali et al., 2020). Vitamin C and flavonoids function as secondary antioxidants, providing acids in the medium, regenerating the main antioxidant, deactivating metal peroxide contaminants, capturing oxygen, binding singlet oxygen, and converting it into triplet form of oxygen (Munteanu and Apetrei, 2021). Vitamin C and lycopene can capture single oxygen better than beta-carotene and alpha-tocopherol (Ng et al., 2014). Lycopene also had the ability to capture free anions up to ten times compared to beta-carotene and tocopherol (Imran et al., 2020). However, administering tomato juice at 0.64 mL/day resulted in a lower number of Leydig cells than administering a dose of 0.32 mL/day. Administration of antioxidants at higher doses would turn antioxidants into pro-oxidants so that antioxidants no longer function as free radical scavengers (Bast and Haenen, 2013).

Lead exposure caused a decrease in the diameter of the seminiferous tubules compared to normal mice. The decrease in the diameter of the seminiferous tubules was caused by the loss of germ cells, Sertoli cells, and spermatogenic cells (Makhloff et al., 2008). Increased radical formation due to lead induction could reduce endogenous antioxidant reserves causing oxidative stress. Oxidative stress could cause mitochondrial damage that regulated mechanism of apoptosis. Excessive testicular cell apoptosis led to damage and degeneration of the seminiferous tubules. Apoptosis was caused by the release of cytochrome-C proteins (due to ROS attacking the inner and outer mitochondrial membranes). Cytochrome-C was secreted due to an increase in Ca2+. The increase in Ca2+ was caused by the failure of membrane permeability due to the reaction between ROS and membrane lipids and proteins (Asadi et al., 2017). Besides being characterized by the release of cytochrome-C protein, lead induction could increase the expression of caspase-3. Increased expression of caspase-3 gave an indication that testicular cells underwent excessive apoptosis (Xu et al., 2016). The decrease in the diameter of the seminiferous tubules was also caused by the toxic effects of lead on the hypothalamus and pituitary gland. Lead was thought to suppress GnRH secretion produced by the hypothalamus by blocking norepinephrine. A decrease in GnRH secretion resulted in a decrease in LH and FSH secretion (Adikwu et al., 2014). LH played a role in stimulating Leydig cells to produce testosterone, where testosterone was needed to develop spermatogenic cells. FSH also stimulated Sertoli cells to produce androgen-binding protein (ABP) which binds testosterone to stimulate the development of spermatogonia. Decreases in testosterone and FSH affected testicular structures, such as the diameter of the seminiferous tubules and spermatogenic cells (Ghosh et al., 2022).

Administration of tomato juice increased the diameter of the seminiferous tubules compared to mice exposed to lead alone or normal mice. The increase in the process of spermatogenesis could lead to an increase in the diameter of the seminiferous tubules and spermatogenic and Leydig cells. (Guikesen et al., 2002). Apart from containing lycopene, tomatoes also contained flavonoids, vitamin C, and vitamin E, which also function as antioxidants in the body (Imran et al., 2020). Flavonoids had a metabolism that is thought to protect against oxidant-induced cell death through an antioxidant-free mechanisms. When flavonoids enter the stomach, their structural oligomers will split into smaller monomer units (Corcoran et al., 2012). Then in the small intestine, these monomer units would be absorbed in the form of O-methylated glucuronides, O-methylated, and aglycones and enter the portal vein. Flavonoids were further
metabolized and converted into methylated, sulfate, and glucuronide forms. O-methylated will enter the cell and function against apoptosis-induced cell death by hydrogen peroxide. The ability of O-methylated to protect cells was related to their ability to donate hydrogen atoms (Thilakarathna and Rupasinghe, 2013).

Administration of 0.64 mL/day of tomato juice resulted in a smaller diameter of the seminiferous tubules than 0.32 mL/day. Spermatogenesis required a balance of ROS-mediated mitochondrial metabolism and endogenous antioxidants in the spermatozoa environment. Higher doses of antioxidants were accompanied by a lack of ROS for physiological function. Therefore, higher administration of antioxidants (reductants) was followed by a paradox of antioxidants that had detrimental effects on spermatogenic cells (Majzoub et al., 2017; Majzoub and Agarwal, 2018).

CONCLUSION

Administration of tomato juice starting at a dose of 0.32 mL/day for 28 days restored the number of Leydig cells and the diameter of the seminiferous tubules of mice exposed to lead acetate.

REFERENCES


