Ovozoa 11 No. 3, December 2022
p-ISSN 2302-6464 e-ISSN 2722-967X
Available at https://e-journal.unair.ac.id/OVZ/index

Original article

Tomato (*Lycopersicon esculentum* Mill.) juice restored the number of Leydig cells, and the diameter of the seminiferous tubules of mice (*Mus musculus*) exposed to lead acetate

Revina Ayu Septiani[1*], Iwan Sahrial Hamid[2], Emy Koestanti Sabdoningrum[2], Anwar Ma'ruf[3], Eka Pramyrtha Hestianah[4], Maslichah Mafruchati[4]

[1] Pet and Wild Animals Interest Group Surabaya, [2] Division of Basic Veterinary Science,
[3] Division of Animal Husbandry, [4] Division of Veterinary Anatomy
Faculty of Veterinary Medicine, Universitas Airlangga
[*] Corresponding author, e-mail: revinaayuseptiani@gmail.com

Open access under CC BY – SA license, DOI: 10.20473/ovz.v11i3.2022.123-129
Received February 19 2022, Revised March 6 2022, Accepted March 14 2022
Published online December 2022

How to cite this article: Septiani RA, Hamid IS, Sabdoningrum EK, Ma'ruf A, Hestianah EP, Mafruchati M. 2022. Tomato (*Lycopersicon esculentum* Mill.) juice restored the number of Leydig cells, and the diameter of the seminiferous tubules of mice (*Mus musculus*) exposed to lead acetate. Ovozoa: Journal of Animal Reproduction 11: 123-129.

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*, pollutan, 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 resulted 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 (Ramaswamy 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 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

	Leydig cell count	diameter of the seminiferous tubules
C-	6.36 ± 0.43^{b}	168.70 ± 1.72^{b}
C+	4.04 ± 0.41^{a}	149.74 ± 4.54^{a}
T1	7.80 ± 0.58^{c}	181.18 ± 2.63^{c}
T2	14.72 ± 1.0^{e}	194.55 ± 1.19^{e}
T3	10.32 ± 0.99^{d}	189.22 ± 1.07^{d}

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.

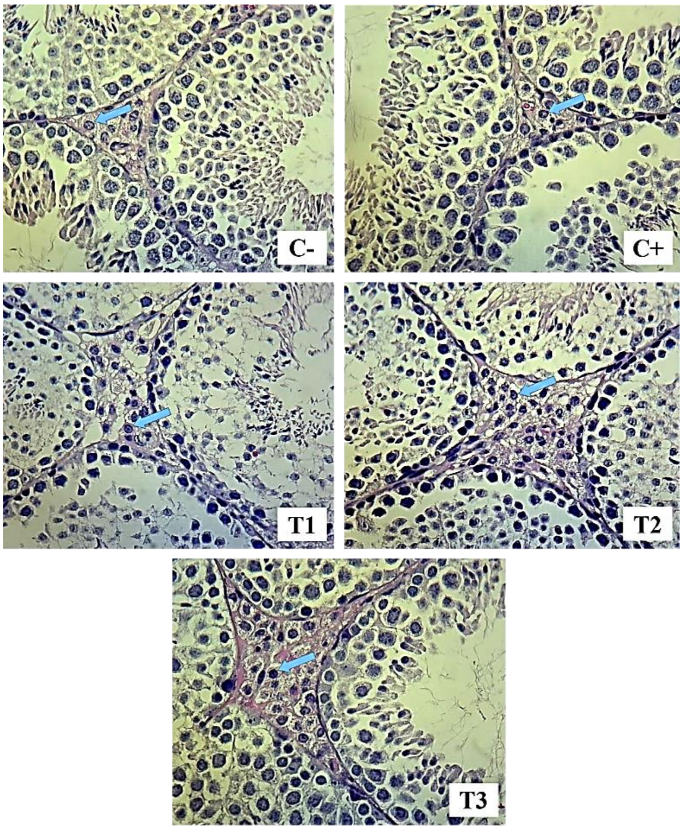

Figure 1 Microscopic view of Leydig cells (blue arrows) of mice after exposure to lead acetate and tomato juice administration (Hematoxylin and Eosin staining, Olympus BX-53, Shinjuku City, Tokyo, Japan at 100x magnification); C-: mice were not exposed to lead acetate; C+, T1, T2, T3: mice were exposed 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.

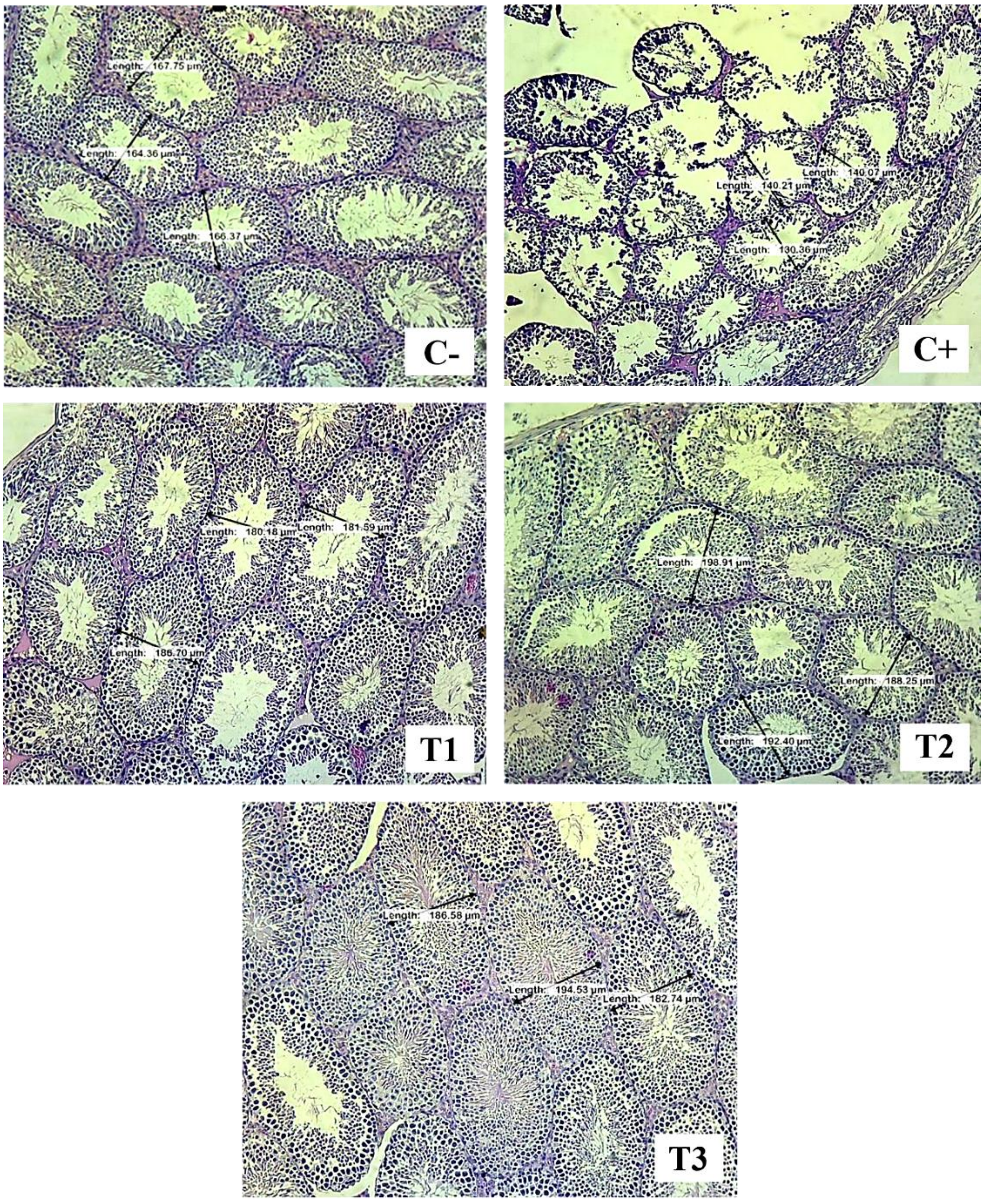

Figure 2 Measurement of the diameter of the seminiferous tubules of mice after exposure to lead acetate and administration of tomato juice (Hematoxylin and Eosin staining, Olympus BX-53, Shinjuku City, Tokyo, Japan at 100x magnification using Image Raster and OptiLab Advance V2) C-: mice were not exposed 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 esclentum* 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 (Makhlouf *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. (Gulkesen *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

Adikwu E, Deo O, Geoffrey O-Bp, Enimeya Da. 2014. Lead Organ and Tissue Toxicity: Roles of Mitigating Agents (Part2). Br J Pharmacol. 5: 1-15.

Ali MY, Sina AA, Khandker SS, Neesa L, Tanvir EM, Kabir A, Khalil MI, Gan SH. 2020. Nutritional Composition and Bioactive Compounds in Tomatoes and Their Impact on Human Health and Disease: A Review. Foods 10: 45.

Asadi N, Bahmani M, Kheradmand A, Rafieian-Kopaei M. 2017. The Impact of Oxidative Stress on Testicular Function and the Role of Antioxidants in Improving it: A Review. J Clin Diagn Res. 11: IE01-IE05.

Bast A, Haenen GR. 2013. Ten misconceptions about antioxidants. Trends Pharmacol Sci. 34: 430-6.

Corcoran MP, McKay DL, Blumberg JB. 2012. Flavonoid basics: chemistry, sources, mechanisms of action, and safety. J Nutr Gerontol Geriatr. 31: 176-89.

Fitri PE, Wurlina W, Chusniati S, Suwanti LT, Plumeriastuti H, Mufasirin M. 2019. Effect of honey in spermatogenesis staging and leydig cells in mice (Mus musculus) infected by Toxoplasma gondii. Ovozoa: J Anim Reprod. 8: 10-6.

Garu U, Sharma R, and Barber I. 2011. Effect of Lead Toxicity on Developing Testis of Mice. Int J Pharmaceu Sci Res. 2: 2403-7.

Ghosh A, Tripathy A, Ghosh D. 2022. Impact of Endocrine Disrupting Chemicals (on Reproductive Health of Human. Proc Zool Soc. 75: 16-30.

Gulkesen KH, Erdogru T, Sargin CF, Karpuzoglu G. 2002. Expression of Extracellular Matrix Proteins and Vimentin in Testes of Azoospermic Man: an Immunohistochemical and Morphometric Study. Asian J Androl. 4: 55-60.

Hamadouche NA, Sadi N, Aoues A. 2013. Lead Toxicity and the Hypothalamic- Pituitary-Testicularaxis. Not Sci Biol. 5: 1-16.

Handaru M, MN Sri, Indah SNK. 2010. Tomatoes Juice Reduce Bronchus Epithelial Cell in Rat with Sub Chronic Exposed to Cigarette Smoke. Jurnal Kedokteran Brawijaya. 26: 32-36.

Igile GO, Ekpe OO, Essien NM, Bassey SC, Agiang MA. 2016. Quality Characteristics of Tomato Juice Produced and Preserved with and without its Seed. Donnish J Food Sci Technol. 2: 1-9.

Imran M, Ghorat F, Ul-Haq I, Ur-Rehman H, Aslam F, Heydari M, Shariati MA, Okuskhanova E, Yessimbekov Z, Thiruvengadam M, Hashempur MH, Rebezov M. 2020. Lycopene as a Natural Antioxidant Used to Prevent Human Health Disorders. Antioxidants 9: 706.

Machmudia A, Eliyani H, Widjiati W, Wurlina W. 2021. Effect of α-tocopherolon spermatogenic and Leydig cellcounts of white rats (*Rattus norvegicus*) exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin. Ovozoa: J Anim Reprod. 10: 74-9.

Majzoub A, Agarwal A, Esteves SC. 2017. Antioxidants for elevated sperm DNA fragmentation: a mini review. Transl Androl Urol. 6: S649-S653.

Majzoub A, Agarwal A. 2018. Systematic review of antioxidant types and doses in male infertility: Benefits on semen parameters, advanced sperm function,

assisted reproduction and live-birth rate. Arab J Urol. 16: 113-24.
Makhlouf M, Saad Eldien HM, Zagloul D, Abu Dief E, Elhaliem NA. 2008. The Effect of Lead Acetate on Testiscular Structure and Protective Effect of Vitamin E in Adult Albino Rat. Egypt. J Histol. 31: 406-18.
Mardatillah M, Wurlina W, Yudaniayanti IS, Plumeriastuti H, Primarizky H, Hamid IS. 2022. Moringa oleifera leaf extract restored the diameter and thickness of the seminiferous tubules of rat (Rattus norvegicus) injected with gentamicin. Ovozoa: J Anim Reprod. 11: 15-21.
Martí R, Roselló S, Cebolla-Cornejo J. 2016. Tomato as a Source of Carotenoids and Polyphenols Targeted to Cancer Prevention. Cancers (Basel) 8: 58.
Mendelová A, Fikselová M, Mendel L. 2013. Carotenoids and lycopene content in fresh and dried tomato fruits and tomato juice. Acta Univ Agric Silvic Mendel Brun. LXI: 1329-37.
Munteanu IG, Apetrei C. 2021. Analytical Methods Used in Determining Antioxidant Activity: A Review. Int J Mol Sci. 22: 3380.
Ng CP, Hashim NH, Adli DSH. 2014. Effects of Nigella sativa (Habbatus sauda) Oil and Nicotine Chronic Treatments on Sperm Parameters and Testis Histological Features of Rats. Evid Based Complement Alternat Med. 2014: 218293.
Ramaswamy S, Weinbauer GF. 2015. Endocrine control of spermatogenesis: Role of FSH and LH/ testosterone. Spermatogenesis 4: e996025.
Salawu EO, Adeeyo OA, Falokun OP, Yusuf UA, Oyerinde A, Adeleke AA. 2009. Tomato (Lycopersicon esculentum) prevents lead-induced testicular toxicity. J Hum Reprod Sci. 2: 30-4.
Sassykova LR, Aubakirov YA, Sendilvelan S, Tashmukhambetova ZK, Faizullaeva MF, Bhaskar K, Batyrbayeva AA, Ryskaliyeva RG, Tyussypova BB, Zhakupova AA, Sarybayev MA. 2019. The Main Components of Vehicle Exhaust Gases and Their Effective Catalytic Neutralization. Orient J Chem. 35: 1-10.
Shahid M, Pourrut B, Dumat C, Nadeem M, Aslam M, Pinelli E. 2014. Heavy-metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. Rev Environ Contam Toxicol. 232: 1-44.
Shan G, Tang T, Zhang X. 2009. The Protective Effect of Ascorbic Acid and Thiamine Supplementation Against Damage Caused by Lead in the Testes of Mice. J Hazhong Univ Sci Technol Med Sci. 29: 68-72.
Thilakarathna SH, Rupasinghe HP. 2013. Flavonoid bioavailability and attempts for bioavailability enhancement. Nutrients 5: 3367-87.
Thoreux-Manlay A, Le Goascogne C, Segretain D, Jégou B, Pinon-Lataillade G. 1995. Lead affects steroidogenesis in rat Leydig cells in vivo and in vitro. Toxicology 103: 53-62.
Wang B, Wang B, Lv B, Wang R. 2022. Impact of Motor Vehicle Exhaust on the Air Quality of an Urban City. Aerosol Air Qual Res. 22: 220213.
Xu W, Guo G, Li J, Ding Z, Sheng J, Li J, Tan W. 2016. Activation of Bcl-2-Caspase-9 Apoptosis Pathway in the Testis of Asthmatic Mice. PLoS One 11: e0149353.
Yu Y, Yu L, Zhou X, Qiao N, Qu D, Tian F, Zhao J, Zhang H, Zhai Q, Chen W. 2020. Effects of acute oral lead exposure on the levels of essential elements of mice: a metallomics and dose-dependent study. J Trace Elem Med Biol. 62: 126624.
Zirkin BR, Papadopoulos V. 2018. Leydig cells: formation, function, and regulation. Biol Reprod. 99: 101-11.

