

Effect of Tomato Juice (*Lycopersicum esculentum*) on Malondialdehyde (MDA) Levels of *Rattus norvegicus* Testes Exposed to Mosquito Repellent Smoke

Wayan Wariata^{1*}, I Nyoman Bagus Aji Kresnapati², I Gede Angga Adnyana³

¹Department of Animal Science, University of Mataram, Mataram, Indonesia

²Department of Pharmacy, Bumigora University, Mataram, Indonesia

³Department of Medicine, Universitas Islam Al-Azhar, Mataram, Indonesia

*e-mail: wariatawayan@gmail.com

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Abstract: Mosquito coil smoke contains reactive oxygen species (ROS) that may induce oxidative stress and increase malondialdehyde (MDA) levels as a marker of lipid peroxidation and cellular damage. Tomato juice contains lycopene, a natural antioxidant that may help reduce oxidative stress from free radicals. This study aimed to evaluate the effect of tomato juice administration on testicular MDA levels in rats exposed to mosquito coil smoke. This laboratory experimental study used a Completely Randomized Design (CRD) involving 20 male *Rattus norvegicus* rats randomly divided into four groups: negative control, positive control (mosquito coil smoke exposure for 8 hours/day), and two treatment groups receiving smoke exposure combined with tomato juice at doses of 3.81 g/day and 7.62 g/day for 30 days. Testicular MDA levels were measured using the Thiobarbituric Acid Reactive Substances (TBARS) method with a UV-Vis spectrophotometer. Data were analyzed using one-way ANOVA followed by LSD post hoc test with a significance level of $p < 0.05$. The results showed that the positive control group had the highest testicular MDA levels, whereas administration of tomato juice significantly reduced MDA levels in the treatment groups. The greatest reduction was observed in the group receiving 7.62 g/day of tomato juice, with MDA levels approaching those of the negative control group. In conclusion, exposure to mosquito coil smoke increased testicular MDA levels, while tomato juice administration, particularly at higher doses, reduced oxidative stress-induced lipid peroxidation. These findings suggest that tomato juice has potential as a natural antioxidant against oxidative damage from mosquito coil smoke exposure.

Keywords: Lycopene; Malondialdehyde (MDA); Oxidative Stress; Testicular Damage; Tomato Juice.

Introduction

Indonesia has a tropical climate that provides favorable conditions for mosquito breeding, particularly during transitional seasons. Consequently, mosquito-borne diseases such as malaria and dengue fever remain major public health concerns. Various mosquito repellents are widely used in Indonesia, including coils, sprays, ointments, and electric repellents. Among these, mosquito coils are the most commonly used method for preventing mosquito bites, accounting for approximately 48.4% of household mosquito control practices. In addition, data from the Indonesian Basic Health Research (RISKESDAS) 2018, involving more than 56,000 respondents, showed that mosquito prevention methods such as bed nets, repellents, and insecticides are widely used across regions, with varying prevalence rates [1].

Mosquito coils commonly contain active compounds such as dichlorvos, propoxur, pyrethroids, diethyltoluamide, or combinations of these substances. Allethrin, a pyrethroid derivative frequently found in mosquito coils, has been classified as a moderately toxic insecticide that may cause irritation to the eyes, skin, and respiratory tract [2]. Chronic inhalation of allethrin-containing smoke may not only impair pulmonary function but also interfere with hepatic detoxification processes [3]. In addition, mosquito coil

smoke contains Polycyclic Aromatic Hydrocarbons (PAHs), which are recognized carcinogenic compounds and may further increase health risks associated with long-term exposure [4]. Mosquito coil smoke also releases pollutants such as carbon monoxide (CO), volatile organic compounds (VOCs), sulfur dioxide (SO₂), nitrogen dioxide (NO₂), and particulate matter (PM_{2.5} and PM₁₀), all of which contribute to oxidative damage in biological tissues [5].

The compounds found in mosquito coils generate free radicals and Reactive Oxygen Species (ROS). Free radicals are molecules that have unpaired electrons. This can cause free radicals to become highly reactive toward body cells and achieve stability by attacking nearby molecules to obtain electron pairs. Excessive ROS production may damage cellular macromolecules, including DNA, proteins, and lipids, resulting in impaired cellular function and tissue injury. In the male reproductive system, oxidative stress may disrupt spermatogenesis and impair testicular function, potentially contributing to infertility [6]. Oxidative stress-induced ROS also trigger lipid peroxidation in sperm cell membranes, resulting in cellular damage and reproductive dysfunction [7].

Lipid peroxidation is a chain oxidation reaction that also produces free radicals, thus triggering further peroxidation. This peroxidation reaction breaks down the fatty acid chains in the cell membrane into various compounds, producing end products that are toxic to cells,

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including dialdehydes such as Malondialdehyde (MDA), the main lipid peroxidation product. Malondialdehyde (MDA) is an aldehyde derivative that can act as a secondary toxic messenger and trigger oxidative injury, so that MDA levels in the body can be used as an indicator of oxidative stress.

Antioxidant compounds can prevent the oxidation of Polyunsaturated Fatty Acids (PUFAs), thereby preventing the formation of lipid peroxides and the chain reaction of radical compounds [8]. Antioxidants are substances that delay, prevent, or eliminate oxidative damage to target cells. Catalase and peroxidase enzymes work by reducing the rate of initiation of a series of reactions. While superoxide dismutase is a chain-breaking antioxidant that works by capturing free radicals. Vitamin E has been shown to protect lipid membranes from oxidative damage caused by ROS, such as hydrogen peroxide (H_2O_2). In model cell membranes, vitamin E helps maintain membrane stability and prevents structural disruption caused by ROS [9].

Tomatoes (*Lycopersicon esculentum*) are one of the fruits that contain polyphenols, carotenoids, and vitamin C, which can act as antioxidants. Polyphenols in tomatoes are mostly flavonoids, while the dominant type of carotenoid is lycopene [10]. These antioxidant compounds can inhibit the oxidation process that can cause chronic and degenerative diseases [11]. Lycopene is the main carotenoid in tomatoes, which is a strong antioxidant. In vitro studies have shown that lycopene is twice as potent as β -carotene and 10 times more potent than α -tocopherol (vitamin E) in reducing reactive oxygen species. Lycopene can be absorbed directly from tomato juice, tomato sauce, and supplements. Serum lycopene levels have been shown to increase significantly after consumption of tomato products and supplements, accompanied by a decrease in oxidation biomarkers, including serum lipid oxidation [12,13].

Several studies have reported that exposure to mosquito coil smoke may induce oxidative stress and reproductive toxicity via excessive ROS production. Previous investigations have also demonstrated the antioxidant potential of lycopene-rich tomato products in reducing oxidative damage. However, studies specifically evaluating the protective effect of tomato juice against testicular oxidative stress induced by mosquito coil smoke exposure remain limited. In particular, evidence regarding its effect on testicular malondialdehyde (MDA) levels, a marker of lipid peroxidation, remains insufficient. Therefore, this study was conducted to evaluate the effect of tomato juice administration on testicular MDA levels in rats exposed to mosquito coil smoke. This study is expected to provide experimental evidence regarding the potential protective effect of tomato-derived antioxidants against oxidative stress-induced reproductive damage.

Research Methods

Location and Time of Research

This research was conducted from July to September 2025 at the Microbiology and Biotechnology Laboratory of Mataram University. Malondialdehyde (MDA) testes levels were measured at the Immunobiology Laboratory of Mataram University.

Experimental Animals

This laboratory experimental study used 20 healthy male *Rattus norvegicus*, aged 8–12 weeks and weighing approximately 200 g. The animals were obtained from the Experimental Animal Unit of Mataram General Hospital. The inclusion criteria were active, healthy rats without visible anatomical abnormalities. The sample size was determined based on Federer's formula for experimental studies. Rats were randomly assigned to four groups of five rats each.

Prior to treatment, all animals underwent a one-week acclimatization period under standard laboratory conditions with controlled temperature and lighting. Rats were provided standard pellet feed and drinking water ad libitum.

Mosquito coils

The mosquito coils used in this study were commercially available mosquito coils containing 0.56 g of D-allethrin per coil, according to the manufacturer's product information.

Tomatoes

The tomatoes used in this study were Plum tomatoes, obtained from the Cakranegara traditional market in Mataram. Fresh tomatoes were processed immediately before administration to maintain their antioxidant content.

Equipment and Materials

The equipment used in this study included experimental animal cages, treatment cages, gastric tubes, mosquito coil containers, digital scales, 100 mL measuring cylinders, filter cloths, EDTA tubes, spectrophotometers, centrifuges, water baths, injection syringes, blenders, Trichloroacetate (TCA) solution, Thiobarbiturate (TBA) solution, 1,1,3,3-tetraethoxypropane (TEP), and 0.01 N HCl.

Procedure for Exposure to Mosquito Coil Smoke

This procedure is a modification of research conducted by Zubaidi (2021) [14]. A *Rattus Norvegicus* cage is prepared and filled with sufficient rice husks. Food and water are provided inside the cage. A cage is prepared for exposure to mosquito coil smoke. The rats are given an adaptation phase. During this phase, the treated and control rats are divided into four groups (five rats each) and placed in cages. The rats are left to live in the cages for one week without treatment. The cages are regularly cleaned, and the rats are fed pellets twice daily and provided with drinking water. The rats, acclimatized for one week, are exposed to mosquito coil smoke every eight hours from 8:00 AM to 4:00 PM for 30 days.

Procedure for making and administering tomato juice

This procedure is a modification of research conducted by Zulfa (2006) [15]. Tomato juice is made by blending small pieces of tomato in a blender. The tomato dosage is based on the lycopene dose in humans. According to research by Makker et al. (2009) [16], the lycopene dose

for an adult human weighing an average of 70 kg is 40 mg/day, so the daily lycopene requirement per kg of human body weight is $40/70 = 0.57$ mg. The daily lycopene requirement in mice can be calculated by converting the human lycopene requirement to the mouse requirement, assuming an adult male white mouse weighs 0.2 kg and that 100 g of tomato contains 3 mg of lycopene [17]. So, the daily tomato dose for adult mice is $0.2 \times 0.57 \times 100/3 = 3.81$ g/day for mice in treatment 1 and 7.62 g/day for mice in treatment 2. Tomato juice was administered to mice at 3 mL per treatment group via gastric tube.

Blood collection procedure through the heart

The blood collection procedure involves first anesthetizing the white rat with chloroform until it becomes unconscious. After the rat is unconscious, it is dissected using a scalpel, starting from the abdominal skin opening to the diaphragm. The surgery must be performed carefully to avoid injuring the rat's heart. The diaphragm is then opened, freeing the heart area to draw blood with a 3 cc syringe. The collected blood is transferred to an EDTA tube. To obtain the plasma, the blood is centrifuged for 15 minutes at 4000 rpm. This is then examined with a UV-VIS spectrophotometer.

Malondialdehyde (MDA) Examination Procedure with the Thiobarbituric Acid-Reactive Substance (TBARS) Test

Testicular Malondialdehyde (MDA) was measured using Thiobarbituric Acid-Reactive Substances (TBARS) using a colorimetric method with the addition of Thiobarbituric Acid (TBA). One gram of testis was finely ground. Then, 500 μ l of 0.9% NaCl was added and homogenized. The homogenate was collected and transferred to an Eppendorf tube. The supernatant was then centrifuged at 8,000 rpm for 20 minutes. The supernatant from 100 μ l of *Rattus Norvegicus* testis was transferred to a test tube, added with 1 mL of 7.5% TCA and 2 mL of 0.67% TBA, then heated in a boiling water bath at 95°C for 15 minutes. The mixture was then cooled to room temperature for 10 minutes. A chilled mixture of *Rattus norvegicus* testes, TBA, and TCA was centrifuged for 10 minutes at 5000 rpm. The supernatant was collected and read using UV-Vis spectrophotometry at 532 nm to obtain absorbance values. MDA levels were calculated using a linear regression equation on a standard curve [18].

Data Analysis

This study was an experimental laboratory study with a Completely Randomized Design (CRD). Data analysis employed one-way ANOVA and the Least Significant Difference (LSD) test in SPSS 26.

Results and Discussion

A study was conducted on 20 male *Rattus norvegicus* aged 8-12 weeks and weighing 200 g to determine Malondialdehyde (MDA) levels in the *Rattus norvegicus* testes after exposure to smoke from mosquito coils of brand X and to tomato juice. The results of the examination of Malondialdehyde (MDA) levels can be described as follows:

Standard curve

The effect of tomato juice on MDA levels in *Rattus Norvegicus* testes exposed to mosquito coil smoke was determined by measuring MDA levels using the Thiobarbituric Acid-reactive Substance (TBARS) method, which is based on UV-Vis spectrophotometer reaction examination. The analytical method was previously validated by measuring linearity, demonstrating that it met the requirements. The results of the linearity test are shown in Figure 1.

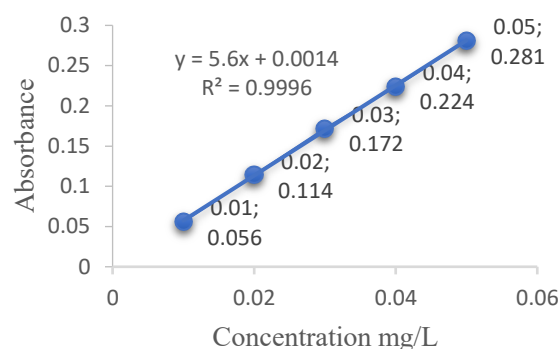


Figure 1. Malondialdehyde (MDA) standard curve

Figure 1 shows a linear or proportional relationship between concentration and absorbance, as indicated by $r > 0.9995$, thus meeting the acceptance criteria and indicating that the method's performance over the measured concentration range was very good.

Malodialdehyde (MDA) Levels

Malondialdehyde (MDA) is a product of lipid oxidation by Reactive Oxygen Species (ROS) and is used as an indicator of oxidative damage [19]. The results of the Malondialdehyde (MDA) testicular examination of *Rattus Norvegicus* exposed to mosquito coil smoke and tomato juice are presented in Table 1 and Figure 2. Mean and standard deviation of Malondialdehyde MDA levels of *Rattus norvegicus*.

Table 1. Malondialdehyde MDA levels

Treatment	Mean MDA Level (mg/L) Mean $\pm$ SD
Negative Control	0.019 $\pm$ 0.002
Positive Control	0.041 $\pm$ 0.003
Treatment 1	0.024 $\pm$ 0.001
Treatment 2	0.013 $\pm$ 0.001

Based on Table 1, the highest average MDA testes levels of *Rattus Norvegicus* testicular tissue were found in the positive control group (0.041 ± 0.003), while the group with the lowest average MDA levels was in treatment group two (0.013 ± 0.001). In the negative control group, the average MDA levels of the testicles were (0.019 ± 0.002), and in treatment group one, the average MDA levels were (0.024 ± 0.001). The results of the ANOVA test showed a significant difference in the effect of tomato juice on the Malondialdehyde (MDA) levels of *Rattus Norvegicus* testicular tissue exposed to mosquito coil smoke ($p < 0.05$).

The distribution of data for each group is shown as a bar chart in Figure 2.

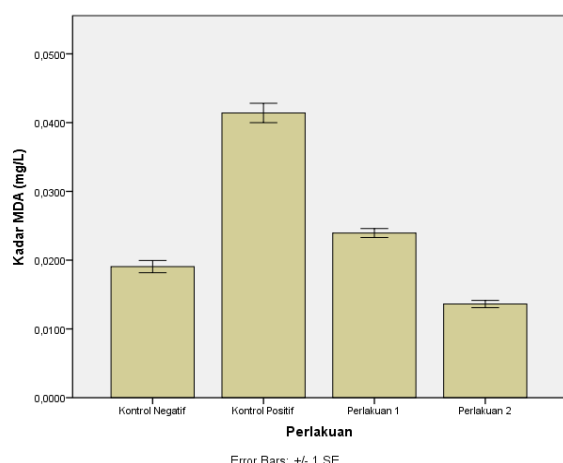


Figure 2. Bar chart of Malondialdehyde (MDA) levels in *Rattus norvegicus* testes

The increase in MDA levels in *Rattus Norvegicus* testes is due to mosquito coil smoke containing chemicals such as Carbon Dioxide (CO₂), Carbon Monoxide (CO), Nitrogen Oxides, Ammonia, and Methane, which can interact with cells in the body and generate free radicals. These chemical compounds enter the body via inhalation and bind to the alveolar membrane in the lungs. Gas exchange in the lungs results in the bloodstream being transported throughout the body. This is in accordance with research, which shows that giving mosquito coil smoke can cause damage to lung cells [5,20]. In addition, chronic exposure to allethrin-based mosquito coil smoke induces significant biochemical and histological damage in mice, affecting both blood parameters and the organization of lung and liver tissues [21].

Reactive oxygen species (ROS) attack the testicular membrane primarily through lipid peroxidation, in which ROS such as superoxide anion, hydrogen peroxide, hydroxyl radicals, and peroxynitrite react with polyunsaturated fatty acids (PUFAs) in sperm and testicular cell membranes [22]. Because testicular membranes contain high concentrations of PUFAs, they are highly susceptible to oxidative damage, resulting in the formation of toxic byproducts such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), which impair membrane fluidity and integrity [23]. This membrane disruption negatively affects sperm motility, viability, and fertilization capacity [24]. ROS also oxidize membrane proteins and thiol groups, leading to dysfunction of ion transport systems and enzymatic activity within sperm cells [25]. Furthermore, excessive ROS damages mitochondrial membranes, reduces ATP production, and enhances further ROS generation, thereby amplifying oxidative stress and inducing DNA fragmentation and apoptosis in germ cells [26]. Recent evidence also suggests that ROS combined with iron accumulation can trigger ferroptosis, an iron-dependent cell death pathway characterized by severe lipid membrane destruction in Sertoli, Leydig, and germ cells [27].

The level of Malondialdehyde (MDA) testes in *Rattus Norvegicus* in treatment 1 (P1), given tomato juice with a concentration of 3.81 g, decreased compared to the positive control, but the effect given was not equivalent to the

negative control. This shows that tomato juice at 3.81 g is less effective at reducing MDA levels in rats exposed to mosquito coil smoke, whereas treatment 2 (P2), given tomato juice at 7.62 g, effectively reduced MDA levels to levels approaching the negative control. Based on the results of the Least Significant Difference (LSD) test, there was a significant difference between the two treatment groups with positive and negative controls ($p < 0.05$). The decrease in testicular MDA levels occurred because tomato juice contains polyphenol compounds, carotenoids, and vitamin C, which can act as antioxidants. Polyphenols in tomatoes are mostly flavonoids, while the dominant type of carotenoid is lycopene [10]. The antioxidant content in tomato juice can help normalize Malondialdehyde (MDA) levels to near negative control (K-) due to exposure to free radicals from mosquito coil smoke. In accordance with research that found tomato juice can significantly reduce MDA levels [28].

Lycopene is a potent natural antioxidant carotenoid that can reduce reactive oxygen species (ROS) by directly scavenging free radicals and enhancing the body's endogenous antioxidant defense system. As one of the strongest singlet oxygen quenchers among carotenoids, lycopene neutralizes excessive ROS such as superoxide anions, hydrogen peroxide, and hydroxyl radicals before they can damage cellular components (29). Lycopene also inhibits lipid peroxidation in cell membranes, thereby protecting polyunsaturated fatty acids (PUFAs) in sperm and testicular cells from oxidative damage [30]. In addition, lycopene enhances antioxidant enzyme activities such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), which further help eliminate excess ROS and maintain redox balance [31–33]. Through these mechanisms, lycopene reduces oxidative stress, preserves mitochondrial function, prevents DNA damage and apoptosis, and ultimately protects testicular tissue and sperm quality from ROS-induced injury.

The body has a defense system to fight the effects of free radicals in the form of natural antioxidants produced by the body, including the enzyme Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glutathione reductase and catalase, but under conditions of high oxidative stress, this system may become insufficient and require exogenous antioxidants from food [34]. Research found that lycopene in tomatoes can reduce Malondialdehyde (MDA) levels [28]. Other studies also demonstrate the hepatoprotective effects of lycopene through antioxidant mechanisms. However, these effects may vary depending on dosage, research model, and physiological conditions (e.g., acute versus chronic alcohol consumption) [35,36].

Conclusion

The results of this study showed that exposure to mosquito coil smoke increased Malondialdehyde (MDA) levels in the testes of *Rattus norvegicus*, indicating oxidative stress and lipid peroxidation. Administration of tomato juice at a dose of 7.62 g/day significantly reduced MDA levels, suggesting its protective effect against oxidative damage. This effect is likely due to the antioxidant compounds present in tomatoes, particularly lycopene and vitamin C. Therefore, tomato juice has the potential to serve as a natural

antioxidant to reduce testicular oxidative stress induced by mosquito coil smoke exposure.

Author's Contribution

W. Wariata: contributed ideas and research methodology. I.N.B.A. Kresnapati: contributed to the collection of research data. I.G.A. Adnyana: contributed to processing statistical data (SPSS) and journal revision.

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