

CHAPTER INTRODUCTION

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1.1. Research Background

The purpose of food coloring is to attract the consumers in a product that is being sold or marketed. However, some of them use food dyes that do not have a FDSA (Food and Drug Supervisory Agency) distribution permit, and they frequently use synthetic dyes that are typically used as textile dyes (Pamungkas and Nopiyanti, 2014). According to data from the Food and Drug Supervisory Agency (FDSA), the incidence of food poisoning was the highest at 66,7% in 2012, when compared to poisoning from other causes such as drugs, cosmetics, and others. The presence of chemical contamination in the food is one of the causes of food poisoning (Paratmanitya and Aprilia, 2016). According to FDSA's findings, four types of hazardous materials are frequently added to foodstuffs: Rhodamine B, Methanyl Yellow (textile dye), formalin, and borax (Kemenkes, 2011). The results revealed that the most significant discovery on snacks was Rhodamin B (FDSA, 2013).

Rhodamine B is a coloring agent green or purple crystalline powder that is reddish, odorless, and easily soluble in water bright red solution it fluoresces as textile or clothing dyes (Yamlean. 2011). The main route rhodamine B exposure is enters the body primarily through the digestive process (Roosdiana, et al., 2017). It frequently enters the body when combined with food (Sulistina and Martini, 2020). Rhodamine B is commonly used because their composition is more stable and produces brighter colors. Rhodamine B is not intended for

food coloring, but it is frequently misapplied by food manufacturers due to its low cost. However, the use of rhodamine B in food, can be harmful to health (Hidayat, et al., 2016).

If used indefinitely, rhodamine B can cause cancer and stimulate the growth of cancer cells (Alhamedi et al., 2009). The human body is poisoned by rhodamine B. It puts cells and tissues under oxidative stress. Nausea, vomiting, diarrhea, cancer, cardiovascular disease, kidney disease, liver dysfunction, hormonal imbalance, premature birth, decreased immunity, nervous system development disorders, mental health problems, and learning disabilities/cognitive dysfunction. are all caused by Rhodamine B exposure. When large amounts of rhodamine B are exposed in a short period of time, it causes acute poisoning (Sulistina and Martini, 2020). The carcinogenic nature of Rhodamine B is caused by the elements N^+ (nitronium) and Cl^- (chlorine), which are highly reactive and dangerous (Wijaya, 2011).

According to Mayori et al., (2013), giving Rhodamine B in graded doses can increase the percentage of glomerular damage mice. This is due to Rhodamine B, which is toxic and can have a negative effect as the dose increases. Toxic materials, according to Hanifah (2008) in Mayori et al., (2013), will easily cause kidney tissue damage in the form of structural changes and kidney function.

Kidney tubule degeneration is characterized by swollen tubular epithelial cells with or without vacuolation. The cytoplasm appears to be discolored and

fragmented. Necrosis of kidney tubules is an irreversible injury characterized by morphological changes, particularly in the nuclei. For example, pyknotic nuclei have shrunk nuclei that are dark, homogeneous, and round. Karyorrhexis is characterized by nucleus fragmentation and release into the cytoplasm. Tubular necrosis also has sloughed epithelium cells and a thinning/attenuated epithelial layer lining the tubules (Frazier, et al., 2012). Congestion is defined as an excess of red blood cells in dilated capillaries and venules (Mosier, 2017).

Rhodamine B is found in xenobiotic substances that are metabolized in the body by cytochrome P450 to produce free radicals such as ROS (reactive oxygen species). This reduces the activity of Superoxidase Dismutase (SOD), causing oxidative stress and cell injury. SOD enzymes are the most important endogenous antioxidants that can mitigate the effects of oxidative stress. It catalyzes the reaction that converts superoxide to hydrogen peroxide and oxygen. Furthermore, it aids in the protection of body cells and the prevention of inflammation caused by free radicals (Sulistina and Martini, 2020). Radical compounds produced by rhodamine ROS (Reactive Oxygen Species) as a result of toxic substance metabolism can also cause glomerular damage (Singh et al., 2006) and reduce the availability of the body's antioxidant reserves (Ercal et al., 2001).

To reduce the toxicity of Rhodamin B, an antioxidant is required to counteract the effect of free radicals. The human body contains a variety of antioxidants (Birben, et al., 2012). However, since Rhodamin B diminishes endogenous antioxidants, the body requires exogenous antioxidant to

counteract free radicals. Plant extracts are thought to contain antioxidant compounds effective in inhibiting and minimizing oxidation reactions involving free radicals, both exogenous and endogenous (Parwata, 2016).

Origanum vulgare (*O. vulgare*) is a well-known aromatic herb that is widely used as a spice in western diets (Savkovi et al., 2016; Zhang et al., 2014). *O. vulgare* is a Lamiaceae-family Mediterranean herb with anti-carcinogenic, anti-mutagenic, and antimicrobial properties (Kubatka et al., 2016). Ursolic acid, rosmarinic acid, caffeic acid, and carnosic acid are the most important phenolic compounds in *O. vulgare* that have antioxidant properties to protect kidney from oxidative stress and increasing the activity of SOD (Sharifi-Rigi, A., & Heidarian, E, 2019). This discovery motivates researchers to examine the toxicity of Rhodamin B on the kidneys of mice (*Mus musculus*) and to look into the potential protective effects of Oregano (*Origanum vulgare*) leaves extract supplementation during Rhodamin B exposure.

1.2. Problem Statements

Is oregano (*Origanum vulgare*) leaves extract capable of protecting the kidneys of mice (*Mus musculus*) from damage due Rhodamine B exposure which is characterized by degeneration, necrosis and congestion of vessels?

1.3. Theoretical Basis

According to WHO (2000), Rhodamine B is hazardous to human health due to its chemical properties and heavy metal content. Rhodamine B contains chlorine (Cl) compounds. Chlorine is a dangerous and reactive halogen compound. In the body, rhodamine B is metabolized to produce reactive chlorine compounds, which attempt to achieve stability by binding to other compounds. In the body, chlorine compounds can activate the cytochrome P450 (CYP450) system and reduce the activity of endogenous and antioxidant enzymes (Roosdiana, et al 2017).

Halogen compounds are free radical compounds with a high reactivity to achieve body stability by binding to compounds in the body and forming new radical molecules or Reactive Oxygen Species (ROS). Rhodamine B, which enters the body through the diet and is absorbed by the intestinal epithelium, has the potential to boost ROS productivity. ROS can disrupt DNA synthesis, damage the lipid layer on cell walls, and disrupt blood vessels, prostaglandin production, and other enzymes in the body (Werdhasari, 2014). The reaction between ROS and unsaturated fats in cells, which produces peroxidase compounds known as lipid peroxidases, which have the potential to cause damage to cell membranes, so that the enzymes contained in cells enter the bloodstream very reactive and can bind to DNA in the cells, causing cell death (Mahdi, et. al., 2019).

Free radicals can cause structural and functional changes. Excessive Rhodamine B exposure can result in oxidative stress. Oxidative stress occurs

when the activity of oxidants (free radicals) exceeds that of antioxidants. Under stress, the body engages in a homeostatic process that activates the production of ROS in order to maintain the continuity of cell activity. (Arimbi dkk., 2021; Muliarta dkk, 2009; Rismawati, 2015).

Kidney tubule degeneration is characterized by swollen tubular epithelial cells with or without vacuolation. The cytoplasm appears to be discolored and fragmented. Necrosis of kidney tubules is an irreversible injury characterized by morphological changes, particularly in the nuclei. For example, pyknotic nuclei have shrunk nuclei that are dark, homogeneous, and round. Karyorrhexis is characterized by nucleus fragmentation and release into the cytoplasm. Tubular necrosis also has sloughed epithelium cells and a thinning/attenuated epithelial layer lining the tubules (Frazier, et al., 2012). Congestion is defined as an excess of red blood cells in dilated capillaries and venules (Mosier, 2017).

When the endogenous antioxidant is depleted, exogenous antioxidant supplementation is required to counteract oxidative stress. Plant extracts are thought to contain antioxidant compounds that can inhibit and neutralize oxidation reactions involving free radicals, both exogenous and endogenous (Parwata, 2016).

Antioxidants work to eliminate or neutralize free radicals (Zuhra et al, 2018). *Origanum vulgare* L. (Lamiaceae) (OV), also known as oregano, is a popular flavoring herb all over the world. *Origanum vulgare* is a fragrant plant native to Asia, most notably Iran. It is used to treat respiratory conditions, hypoglycemia,

and leukemia (Sheibani et al., 2010). The most abundant components of oregano aqueous extract, rosmarinic acid and origanol A and B, are antioxidative (Kulisic et al., 2007; Matsuura et al., 2003). SOD levels in the kidneys can be increased by *O. vulgare* leaf extract. Phenolic compounds stimulate the activity of nuclear factor erythroid 2-related factor 2 (Nrf2), which plays a role in the synthesis of cellular antioxidants such as SOD (Deck et al., 2018; Pang et al., 2016). It has been demonstrated that *O. vulgare* leaf extract contains various phenolic compounds with antioxidant properties capable of destroying oxidative agents (Kaurinovic et al., 2011). Furthermore, phenolic compounds function as antioxidants (Aloulou et al., 2012; Dipti et al., 2003).

1.4. Aim of Research

To investigate the protective effect of oregano (*Origanum vulgare*) leaves extract on histopathological changes in kidney tissues induced by rhodamine B through use of oral gavage.

1.5. Outcome of Research

1.5.1. Theoretical Approach

To provide information on the effect of oregano (*Origanum vulgare*) leaves extract as an antioxidant on the histopathological changes in kidney tissues affected by rhodamine B.

1.5.2. Practical Approach

The research findings are intended to serve as a basis for future research on the advantages of oregano (*Origanum vulgare*) leaves extract and the toxicity of rhodamine B.

1.6. Hypothesis

Oral gavage of oregano (*Origanum vulgare*) leaves extract can protect the kidney of mice (*Mus musculus*) from damage on histopathological changes of kidney caused by rhodamine B.