CHAPTER 1 INTRODUCTION

1.1. Research Background

Formaldehyde (FA) is a toxic chemical that has been widely used in industrial and medical settings. It is a colourless, pungent, and irritant compound and is usually found as 37 - 40% solution which is known as formalin (Immaculate and Jamila, 2018). The main routs of formalin exposure in our environment are air, dermal exposure, and also through water. Formaldehyde in the form of formalin can also be administered through food and drinking water naturally and artificially when is used as food preservative. Formalin is used for various purposes, such as preservative and disinfectant. However, because of the fact that it is quickly absorbed from the gastrointestinal tract, this makes formalin a dangerous chemical to be used as a food preservative. (Mamun, et al., 2014).

Formalin as food preservative can prolong food from its decay by protecting against deterioration caused by microorganisms. Some dishonest traders have used formalin in perishable foods to prevent from its decay. However, formaldehyde is also naturally produced as a common metabolic by-product in a wide variety of food items, such as: fruits and vegetables, meats, fish, crustacean and dried mushroom (Nowshad, et al., 2018).

Exposure to formalin causes local irritation; skin sensitization after acute and sub-acute exposure; irritation to the eyes, nose, throat and airway. Chronic exposure to formalin may result in salivation, dyspnea, headache,

insomnia, seizures, and neurodegenerative disorders (Murta, et al., 2016). If formalin consumed at a higher concentration, it can damage the GI tract, kidney, liver, and lungs. When ingested, it also caused an irritant action upon mucous membranes and may cause inflammatory changes in the liver and kidneys. The toxicity caused by exposure to formalin also trigger the production and the release of reactive oxygen species (ROS) (Ramos, et al., 2016).

Studies have shown that the toxicity of formalin also leads to several findings showing histopathological and biochemical alterations in kidney tissue. It was observed that systemic application of formalin impaired glomerular patterns and thickened tubular and glomerular basal membranes (Inci, et al., 2013). A research conducted by E. Bakar (2014) also showed epithelial damage to the parietal area of the glomerulus, mononuclear cell infiltration, loss of integrity, and degeneration of epithelium were observed in both proximal and distal tubules in kidney cortex.

The main mechanism in the nephrotoxicity of formalin is related to the oxidative stress caused by the depleting endogenous antioxidant activity, such as glutathione, superoxide dismutase, and glutathione peroxidase thus leading to high level of reactive oxygen species (ROS). The histopathological alterations may be caused by the degenerative effects of free radicals, such as adverse effects in cellular proteins and DNA (Abdulqader and Mustafa, 2014). This finding suggests that formalin caused

oxidative injury by impairing antioxidant defense mechanisms in the kidneys.

To decrease the toxicity effects caused by formalin, antioxidant is needed to counteract the effect of free radicals. The human body is equipped with a variety of antioxidants (Birben, et al., 2012). However, due to the depletion of endogenous antioxidant caused by formalin the body needs exogenous antioxidant to counterbalance the free radicals. The antioxidant compounds contained in extracts of a plant are thought to be able to inhibit and neutralize oxidation reactions involving free radicals, both exogenous and endogenous (Parwata, 2016).

Cinnamon extract and its different isolated bioactive compounds have been demonstrated to possess a potential source of natural antioxidants exhibiting strong free radicals scavenger activity in in vitro models. Cinnamon polyphenols have been shown to enhance the superoxide dismutase action, improving the scavenging of oxygen free radicals in organisms and protecting the tissues against oxidative stress injury (Da Silva, et al. 2018). This finding leads to evaluation of toxic effects of formaldehyde on kidney of rat (*Rattus norvegicus*) and to investigate the possible protective effects of cinnamon (*Cinnamomum burmannii*) bark extract supplementation during formaldehyde exposure.

1.2. Problem Statements

Can bark of cinnamon (*Cinnamomum burmannii*) extract protect the kidney of rat (*Rattus norvegicus*) from damage due to formalin exposure by oral gavage?

1.3. Theoretical Basis

Human health is threatened to be exposed to reactive toxins that can damage fundamental biomolecules such as DNA and proteins. One of these molecules is formaldehyde, the simplest and one of the most reactive aldehydes (Pontel, 2018). It is usually found as 37 - 40% solution which is known as formalin. Formalin is used for various purposes, such as preservative and as a disinfectant. However, there are some dishonest traders have used formalin as food preservative to prevent food from its decay.

Once formalin enters the body, formaldehyde is metabolized into formic acid in the liver then excreted by urine and feces (Morsy, 2018). Studies on the effect of formaldehyde (FA) on the human body and its rapid metabolism, suggest that cellular toxicity due to formalin exposure is because of its metabolism (Faghani, et al., 2014). The metabolism of formaldehyde into formic acid is catalyzed by formaldehyde dehydrogenase (FDH) enzymes after formaldehyde (FA) is taken into body. FDH oxidizes HMSGH (S-hydroxymethylglutathione) to S-formylglutathione, in the presence of NAD as cofactor. This intermediate is further metabolized by *S*-formylglutathione hydrolase (FGH) to yield format and reduced

glutathione (Pontel, 2018). Moreover, it is worth to note that the formaldehyde oxidation is catalyzed by enzymes including NAD-dependant formaldehyde dehydrogenase, in which catalase, peroxidase, and reduced glutathione are required as a cofactor during this reaction (Costa and Teixeira, 2015). Thus, as the FA concentration increases, there will be depletion of glutathione and other endogenous antioxidant, which will increasing FA toxicity (Inci, et al., 2013).

The amount of free radicals increase the intensity of oxidation reaction that will lead to tissue disturbance, because antioxidant defense mechanism has been altered. Both endogenous and exogenous formaldehyde metabolism take place in all tissue and enter the same pathway where it will be eliminated as formic acid and CO_2. However, If formaldehyde is not metabolized, their structure itself can form cross-linkages between protein and single stranded DNA (IARC, 2006). The carbonyl atom which is the electrophilic site, making it easy to react with cellular macromolecules, such as proteins, nucleic acid, and amino acids. DNA damage can also induced by the presence of formaldehyde (Murta, et. al., 2016). This suggests that formaldehyde can cause cellular damage due to the change of free radicals, oxidants, and antioxidant balance. This phenomenon is named oxidative stress. Oxidative stress occurs when the balance between antioxidants and ROS are disrupted because of depletion of antioxidants and accumulation of ROS (Birben, et al., 2012).

Free radicals and oxidants appear in normal physiological process. However, due to formaldehyde toxicity causing the depletion of cellular antioxidant defense mechanism, the free radicals can react easily with nucleophilic sites on membrane cell. Lipid peroxidation also occurred in formalin toxicity, showed by studies conducted by Ramos et al. 2015 and Lima et al. 2015 where MDA level increased after treatment with formalin. Damage caused by lipid peroxidation process changes the structure and the permeability of cell membranes. The cell loss its biomembranes selectivity, as in ion exchange, causing Na^+, H_2O, and Ca^{2+} will easily move into cell. Thus, leads to cell swelling. If the cells are overwhelmed by formalin toxicity and could not restore its homeostasis, necrosis can occur and damaging the tissue (Breshears 2017).

Exogenous antioxidant supplementation is required to counteract the oxidative stress when the endogenous antioxidant is depleted. The antioxidant compounds contained in extracts of a plant are thought to be able to inhibit and neutralize oxidation reactions involving free radicals, both exogenous and endogenous (Parwata, 2016).

Cinnamon (*Cinnamomum burmannii*) has been extensively researched since it has many benefits. Cinnamon bark is one of the most popular herbs utilized as a spice in cooking. Cinnamon plant consists of a variety of compounds, including cinnamic acid, eugenol, and cinnamaldehyde, which are potential antioxidant compounds found in cinnamon methanol extract with the ability to scavenge free radicals (Ribeiro-Santos, 2017; Ervina, et

al., 2016). Cinnamon (*Cinnamomum burmannii*) bark extract has a high content of cinnamaldehyde (68.65%) which is a source of antioxidant compounds with the ability to capture free radicals or radical scavenger. The antioxidant activity of cinnamon extract lies on the chemical structures of cinnamaldehyde, cinnamic acid, and eugenol that have hydroxyl groups (OH) function as hydrogen ion donors that can convert peroxyl radicals into less active free radicals, thus neutralize and stabilize free radicals. Number of OH groups and its position represents the power of scavenging radicals. (Sharma, et al., 2016; Rafita, et al., 2015).

1.4. Aim of Research

To examine the protective effect of cinnamon (*Cinnamomum burmannii*) bark extract on histopathological changes of renal tissue after induced with formalin by oral gavage.

1.5. Outcome of Research

1.5.1. Theoretical Approach

To provide information about the effect of cinnamon (Cinnamomum burmannii) bark extract as an antioxidant to the histopathological changes of renal tissue after induced with formalin which can cause oxidative stress.

1.5.2. Practical Approach

The results of the study are expected to be a reference for further research on the toxicity of formalin and the benefits of cinnamon (Cinnamomum burmannii) bark extract.

1.6. Hypothesis

The administration of cinnamon (*Cinnamomum* burmannii) bark extract can protect the rat (*Rattus* norvegicus) kidney oxidative damage induced by formalin through oral gavage.