

CHAPTER 1 INTRODUCTION

1.1. Background

CCl_4 or known as carbon tetrachloride is substance that does not occur naturally and more likely to released into the environment by human activities that the utilization controlled and practically banned by the Montreal Protocol (MP) because it's ozone-depleting substance (Sherry *et al.*, 2018). Based on National Institute of Occupational Safety and Health Recommended Exposure Limit (2014), the maximum airborne concentration that nearly individuals could be exposed up to one hour without experiencing or developing irreversible or other serious health effects are around 2 ppm (12.6 mg/m^3). Even it's controlled and banned, some researcher found that the emissions of carbon tetrachloride came from the usage and production of *chlorine* in industrial and domestic uses e.g paper bleaching and disinfection also found in dry-cleaning textile laundries, in fire extinguishers, and as a precursor of refrigerants or rocket propellant (Sherry *et al.*, 2018; and Teschke, 2018).

CCl_4 is well known as a hepatotoxic industrial solvent and frequently used for experimental reasearch to study the pathogenesis of liver injury because it can induce free radical in our body (Scholten *et al.*, 2015). Free radicals are the products of normal cellular metabolism (Phaniendra *et al.*, 2015). Free radicals is a unstable atom or molecule because of their number of electron either it is too low or to high that makes them attached to other compound easily to gain stabitily and it makes them has highly reactive (Howard, 2018)

This radical is major cause of lipid peroxidation and damage the unity of biomolecules including lipids, protein, and DNA because the excess production of Reactive Oxygen Species (ROS) called oxidative stress (Phaniendra *et al.*, 2015). Reactive Oxygen Species (ROS) generally produced in eukaryotic cells by mitochondria and little amount of Reactive Oxygen Species (ROS) produced in endoplasmic reticulum, plasmatic, and nuclear membranes (Krumova and Cosa, 2016).

The main product of lipid peroxidation are lipid hydroperoxides and the secondary products are malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4-HNE). Malondialdehyde known as secondary product from lipid peroxidation that more mutagenic but stable compared than other aldehyde component (Gaschler and Stockwell, 2018; and Anggraeni *et al.*, 2017). This is why malondialdehyde mostly used as a biomarkers of lipid peroxidation.

Beetroot originated from Asia and Europe used for food, beverages, and traditional medicine to treat constipation, gut and join pain, and anti-dandruff from 8th century (Barbarykin *et al.*, 2019; and Chhikara *et al.*, 2019). Current pharmacology shows that beetroot extract has antihypertensive and hypoglycaemic activity as good as antioxidant activity (Barbarykin *et al.*, 2019). Beetroot contain several type of antioxidant, phenolics, carotenoids, betalains, ascorbic acid, and nitrate (Clifford *et al.*, 2015). Antioxidant categorized as a substance can reduce the oxidative stress in our body (Goodarzi *et al.*, 2018), and have diverse physiological role in the body (Yadav *et al.*, 2016). Antioxidant helps converting the radicals to be less reactive species.

Betalains is one of major compound in beetroot that has red pigment and has hepatoprotective effect against free radical (Clifford *et al.*, 2015). Because of that benefits, herbal extract used for restore the body function, especially rich in antioxidant. Antioxidant balancing what the free radical damaged.

1.2. Problem Statement

Does Beetroot (*Beta vulgaris L.*) extract able to protect liver by decreasing malondialdehyde (MDA) level on rat induced with CCl_4 ?

1.3. Theoretical Basis

National Academic Press (2014) explained that the exposure of carbon tetrachloride most likely through inhalation, ingestion, and dermal. The absorption by lungs around 50% up to 80% and removed by exhaled after converted directly to carbon dioxide around 4%. The absorption through dermal is rapid than inhalation. While the absorption through ingestion could be more rapidly absorbed if the diet contain high of fat and if the gut contain alcohol. When the exposure cease, carbon tetrachloride is distribute to all organs and tissues to blood perfusion and lipid contain.

When the blood carry chemical substance like CCl_4 , it will metabolize in liver by activating cytochrome P450 then produce two very reactive radicals, trichloromethyl radical (CCl_3) and trichloromethylperoxyl radical (CCl_3OO) (Chávez-Morales *et al.*, 2017). Trichloromethyl radical is a result from homolytic cleavage of the carbon-chlorine bond and react with fatty acid in the endoplasmic reticulum. While the trichloromethylperoxy radical more reactive than trichloromethyl radical and react to unsaturated membrane lipids. These radical

both resulting lipid peroxidation that leads into cellular degeneration (National Academic Press, 2014).

Trichloromethylperoxyl radical is one of lipid radicals that initiates a chain reaction and transforms polyunsaturated fatty acid into lipid hydroperoxide. Lipid hydroperoxide are very unstable chain so it could easily decompose to secondary products such as aldehydes and malondialdehydes (Ozougwu, 2016). The hydroperoxyl radical formed because superoxide anion at low pH by abstracting a hydrogen atom from side-chain methylene carbon and then reacts with oxygen. Peroxidation of lipids disturb the integrity of the cell membranes and lead to rearrangement of membrane structure and it called oxidative stress (Phaniendra *et al.*, 2015; and Ozougwu, 2016).

Beetroot is a well-known as a source of antioxidant and bundle of bioactive compound for health benefits. One of antioxidant compound in beetroot is betanin. Betanin is one of Betalains antioxidant compound that has purple-red colored that mostly used as a food coloring. Betanin claimed as a effective inhibitor of lipid peroxidation and lowered the reactive radical targeting cell membranes based on research of Chhikara *et al.* (2018).

Betanin can activated nuclear factor erythroid 2-related factor 2 antioxidant response element (Nrf2-ARE) from the cytosol to the nuclear compartment (Da Silva *et al.*, 2019). The Nrf2-ARE is resulting from separation between kelch-like ECH-associated protein 1 (Keap1) and Nrf2 through protein kinase-like endoplasmic reticulum kinase (PERK) that phosphorylate Nrf2 (Zhang *et al.*, 2016).

The dissociated Nrf2 is transferred to the nucleus to dimerizes with the Maf proteins (Maf-F, Maf-G, and Maf-K) and then binds to antioxidant response element (ARE) of phase II genes (Zhang *et al.*, 2016). After that, Nrf2-ARE translates detoxification enzymes such as glutathione synthetase (GSS), glutathione reductase (GR), glutathione peroxidase (Gpx), thioredoxin (TRX), thioredoxin reductase (TRR), catalase (CAT), superoxide dismutase (SOD), and peroxiredoxin (PRX) to prevent oxidative stress (Da Silva *et al.*, 2019; Indumathi *et al.*, 2017; and Da Silva *et al.*, 2019).

Besides, betanin has chemical structure called the hydroxyl ortho position. This makes phenolic and cyclic amine groups not interacting with lipid radical and tend to form a relatively stable radical (Ahmadian *et al.*, 2018). The antioxidant activity of betalains is activated NF- κ B pathways to regulate and strengthen the inflammatory respons that mediates with interfering pro-inflammatory signaling pathways (Clifford *et al.*, 2015).

The antioxidant activity of betanin shown can reduce the level of malondialdehyde and restoring endogenous antioxidant (Da Silva *et al.*, 2019). Malondialdehyde (MDA) is the result from the peroxidation of polyunsaturated fatty acid (PUFA) and has been widely used as a biomarker for lipid peroxidation. MDA can be metabolized by the enzyme or can react on cellular and tissular protein or DNA to form substances that damaged in biomolecular (Ayala *et al.*, 2014).

Based on studies, this research will do the intraperitoneal inject with CCl₄ and beetroot extract via peroral routes as a hepato-protector in the liver tissue of

male rat. The reason why this research use intraperitoneal routes because of the rat has good survival rates, ease performance, excellent reproducibility, and more safety (Scholten *et al.*, 2015). Based on the explanation in Da Silva *et al.* (2019), the biological efficacy of betanin during short supplementation was evaluated in the present study, because the most research evaluated the effect in vivo around 30 days or longer. This research will conduct 21 days only to know the hepatoprotector effect of beetroot.

1.4. Research Purpose

Purpose of this research is:

To find out the hepatoprotective effect on Beetroot (*Beta vulgaris L.*) extract by decreasing malondialdehyde (MDA) level on rats induced with CCl₄.

1.5. Benefits of Research

1.5.1. Theoretical Benefits

To find out the hepatoprotective effect on Beetroot (*Beta vulgaris L.*) extract by decreasing malondialdehyde (MDA) level on rats induced with CCl₄.

1.5.2. Practical Benefits

Can be a guideline and to inform the readers the benefits of beetroot to maintain health from free radicals exposure in daily life.

1.6. Hypothesis

Beetroot (*Beta vulgaris L.*) is able to protect rat's liver by decreasing the level of malondialdehyde induced with carbon tetrachloride.