CHAPTER I

INTRODUCTION

1.1 Background of the Study

Hepatic diseases are deadly health problems that cause deaths globally. In 2010, a report from the Global Burden of Disease project estimated that around 2 million deaths each year in the world are associated with liver diseases. Acute hepatitis, cirrhosis, liver cancer, and alcohol-associated liver diseases are more common, contributing to 4% of the deaths associated with liver diseases. This percentage is expected to rise due to lifestyles with little or without physical activities, over nutrition and several factors such as exposure to harmful chemicals (Xiao *et al*., 2019). Other liver diseases and illnesses include non-alcoholic fatty liver disease, alcohol-related liver disease, drug-induced liver injury, and hepatic failure (Abdelhafez *et al*., 2018). Intrinsic and extrinsic factors such as being born with metabolic defects, malnutrition, viral infection, exposure to toxins (Saravanan *et al*., 2013) may produce reactive nitrogen/oxygen species (RNS/ROS) facilitating liver diseases and illnesses. The liver is a vital organ responsible for the detoxification of poisonous materials such as xenobiotics. It plays various roles in metabolism, secretion of wastes, and elimination of superfluous materials from the body (Akhzari *et al*., 2019), hence it is the most affected organ by toxins (Abdelhafez *et al*., 2018).

Natural and artificial manufactured food additives are used to increase and maintain food safety, texture, taste, and nutrient value, their applications were approved by the U.S. Food and Drug Administration. Although they are beneficial, synthesized additives may cause some adverse effects (Hassan *et al*., 2009). One of the synthesized food additives is sodium nitrite ($NaNO_2$) which can be present in vegetables, cured meats, and fish. Human activities such as agriculture which involves the use of nitrogenous fertilizer to increase crop productions, improper handling and maintaining wastes increase the probability of $NaNO_2$ exposure to humans. Sometimes drinking water contains $NaNO_2$ beyond the accepted level of 1 part per million (ppm) (Ansari and Mahmood, 2016). Nitrite has various functions, ranging from physiological, immunological to neurological at a low physiological amount (0.45-23 μM). In the body, $NaNO_2$ can be interconverted into various kinds of nitrogen molecules including nitric oxide (NO). Nitric oxide has vital functions in the body such as improving gastrointestinal health by facilitating gastric flow, maintaining the integrity of the gastric epithelium and muscular barrier (Ansari *et al*., 2017), preventing the white blood cells from adherence to the endothelium as well as lessening the risk of cardiovascular diseases and improving the pulmonary health. In a clinical setting, $NaNO_2$ is used for vasodilation, bronchodilation (Ansari and Mahmood, 2016) as well as an antidote in cyanide poisoning (Bhattacharya and Flora, 2015).

In the food industry, $NaNO_2$ is used as a color fixative agent in meat, fish and poultry products to improve flavor, delay rancidity by preventing fat oxidation and inhibit the growth of microorganisms such as *Clostridium botulinum* that cause botulism (Sherif and Al-Gayyar, 2013; Ansari *et al*. 2017).

Despite the health benefits of $NaNO_2$ at low concentrations, the compound is detrimental at high concentrations due to its ability to form free radicals that can oxidize various biomolecules (Akhzari *et al*., 2019) such as protein, lipid, and deoxyribose nucleic acid (DNA). Chronic exposure to $NaNO_2$ even at low doses can cause cancer (Hassan *et al*., 2009). Chronic and high concentrations exposure to $NaNO_2$ can cause death mainly due to methemoglobinemia, the immediate effect of $NaNO_2$ toxicity. Other effects of $NaNO_2$ exposure are hepatotoxicity, birth defects, problems in the respiratory tract, damage in the nervous system as well as dysregulation of inflammatory responses, nephrotoxicity, impairment of the reproductive system, disturbance of the endocrine system, growth retardation and tissue injuries (Hassan *et al*. 2009; Akhzari *et al*. 2019; Atila Uslu *et al*., 2019).

Sodium nitrite can be converted into nitrosonium ions (Özen *et al*., 2014) after it is consumed and enter in the erythrocytes by anion carriers or diffusion (Ansari and Mahmood, 2016) that later can react with amine and amides to form nitrosamines and nitrosamide at low pH (less than 2) and high temperature (Ansari *et al*., 2017). The nitroso compounds such as N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), and N- nitrosomorpholine (NMOR) (González Delgado *et al*., 2018) are more detrimental than the nitrite itself and NO (Özen *et al*., 2014). For example, N-nitrosodimethylamine is hepatotoxic causing fibrosis and cancers (González Delgado *et al*., 2018). The nitroso compounds are carcinogenic and can induce oxidative stress because of their ability to generate free radicals (Atila Uslu *et al*., 2019). The nitrosonium ions can also react with the heme group of the hemoglobin to form methemoglobin (Ferric hemoglobin) (Özen *et al*., 2014) hence causes hypoxia (Al-Rasheed *et al*., 2017).

In the digestive tract especially at the intestine, nitrite is rapidly absorbed into the bloodstream. When foreign toxic materials absorbed from the gastrointestinal tract are taken to the liver to be detoxified, this exposes the liver to higher amounts of free radicals, thus the organ is more vulnerable to oxidative damages and injuries than any organ in the body (Akhzari *et al*., 2019). In the liver, $NaNO_2$ causes oxidative stress, a source of liver diseases and disorders as it is known as the crucial mediator of cellular degradation that ultimately leads to necrosis (Özen *et al*., 2014). During oxidative stress, mitochondria become permeable to release pro-apoptotic factors and caspase-3 activations that destroy the hepatocytes. The oxidative stress causes the releases of various cytokines including transforming growth factor (TGF)-β1 and tumor necrosis factor (TNF)-α from the liver cells (Abdelhafez *et al*., 2018). Tumor necrosis factor (TNF)-α has a primary role in influencing the release of other cytokines to increase the rate of cellular destruction. Transforming growth factor (TGF)-β1 is the topmost influencer of various liver diseases as it can affect all stages of liver disease progression from the initial to the final stage (cirrhosis). Transforming growth factors can cause fibrogenesis by activating the stellate cells of the liver and change them into myofibroblasts. Generally, the effects of oxidative stress in the liver are cellular inflammation and tissue damage that cause liver dysfunction (Akhzari *et al*., 2019).

During normal metabolic processes, the body produces free cellular radicals. If they are in excess, the free radicals can disturb the normal redox state of the cells hence cause damage to some biomolecules. For example, through lipid peroxidation, free radicals can affect the integrity of the cell membrane and also cause mutation through DNA modification. The body has a biological antioxidants system (endogenous antioxidants defense system) made up of enzymatic materials including glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) (Abdelhafez *et al*., 2018; Akhzari *et al*., 2019), glutathione reductase (GR) (Akhzari *et al*. 2019), and non-enzymatic substances such as ascorbate, tocopherols, carotenoids, bilirubin and uric acid which protect the body from the damage of free radicals by balancing the oxidants and the antioxidants (Abdelhafez *et al*., 2018). An antioxidant prevents oxidative stress by donating its electron to the oxidant (Husen *et al*., 2019) hence terminate a chain reaction initiated by the oxidants. In some conditions such as when toxic materials including $NaNO_2$ enter into the body, the endogenous antioxidant system fails to function properly resulting in oxidative stress. Oxidative stress is associated with damage of cellular membranes, hepatocellular necrosis, depletion of glutathione (Abdelhafez *et al*., 2018), a decrease in total serum protein (TSP) (Alqasoumi, 2012) and an increase of serum liver enzymes which are alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) (Abdelhafez *et al*., 2018).

Various approaches have been used for a long time to treat liver disorders such as the use of vaccines, corticosteroids, (Alqasoumi, 2012) drugs, and liver transplantation (Abdelhafez *et al*., 2018). The approaches have some limitations including serious side effects when they are used for a long time and have restricted ability to produce the desired impacts (Alqasoumi, 2012; Abdelhafez *et al*., 2018), hence alternative approaches should be searched to treat and solve the problem. The uses of natural products such as plant compounds and their derivatives have shown promising results to solve liver problems because they are less toxic and have little or no side effects (Alqasoumi, 2012; Wahyuningsih *et al*., 2018; Abdelhafez *et al*., 2018; Hayaza *et al*., 2019). One of the plants that can be used as an alternative to synthesized medicine in treating and prevent the development of liver diseases due to xenobiotics and chemical toxic is red okra.

Okra, (*Abelmoschus esculentus* (L.) Moench) is a plant in the Malvaceae family that produces flowers. It is also known as ladies' finger, bamyah, bhindi, gumbo, bamia, bamieh, kacang, dharos or bendi (Esmaeilzadeh *et al*., 2020) depends on the region in the world where it is available. The pod of red okra can be eaten raw or cooked. Various parts of the red okra plant have benefits in the body. Fresh okra heal constipation, leucorrhea, spermatorrhea, diabetes, and jaundice, the mucilage can cure diarrhea, dysentery, gastric ulcer (Alqasoumi, 2012; Ortaç *et al*., 2018) and syphilis (Saravanan *et al*., 2013) and when mucilage of okra mixed with a ripe banana can be used to treat colitis, cystitis, hepatitis, and jaundice. The polysaccharides from okra can improve body immunity to *Staphylococcus aureus* (Wahyuningsih *et al*., 2018). Okra is also known to have strong polyphenols and flavonoids that act as antioxidants (Majd *et al.*, 2019; Chaemsawang *et al*., 2019) which could scavenge free radicals and decrease oxidative stress in the cells. Also, anthocyanin is a flavonoid compound that is responsible for the blue, purple and red color in leaves and fruits (Irshad *et al*., 2018) and it has antioxidant potential (Roy *et al*., 2014; Irshad *et al*., 2018; Hutabarat *et al*., 2019). Red okra contains anthocyanin (Irshad *et al*., (2018), that may be a reason for the purple okra to have a higher phenolic content, antioxidant capacity and quercetin content more than the green okra in the studies of Anjani, *et al*. (2018) and Nabila *et al.* (2018). The availability of flavonoids in okra can induce the activation of the nuclear factor-E2-related factor 2-antioxidant response element (Nrf2-ARE). This activation induces transcription genes to encode antioxidant enzymes such as SOD and CAT (Muriel and Gordillo, 2016). Superoxide dismutase enzymes bind to $O_2^{\cdot}$ and change it to hydrogen peroxide (H_2O_2), while the CAT enzyme converts H_2O_2 to oxygen and water. The reactions decline the level of the free radicals and their propagation, therefore, results in a decline or stops the effects of oxidative stress (Hassan *et al*., 2009).

Despite the finding explained, research on antioxidant and hepatoprotective activities of red okra pods (*A. esculentus* L.) ethanolic extracts on *Mus musculus* that has been exposed to $NaNO_2$ has never been done. So, this study is aiming at determining the antioxidant and hepatoprotective activity of red okra pods (*A. esculentus* L.) ethanolic extracts on *Mus musculus* that have been exposed to $NaNO_2$.

1.2 Formulation of the Problem

Based on the background explained the following research questions were formulated:

1. Is there any effect of the dose of ethanol extract of red okra pods (*A. esculentus L.*) on the activity of CAT and SOD enzymes in the hepatocytes of $NaNO_2$-exposed mice?
2. Is there any effect of the dose of ethanol extract of the red okra pods (*A. esculentus* L.) on the activity of AST, ALT, and ALP enzymes in the blood serum of $NaNO_2$-exposed mice?
3. Is there any effect of the dose of ethanol extract from red okra pods (*A. esculentus* L.) on the levels of total serum protein (TSP) of $NaNO_2$- exposed mice?
4. Is there any effect of the dose of ethanol extract of red okra pods (*A. esculentus L.*) on the structure of the liver tissue (percentage of normal, necrotic and swollen hepatocytes as well as inflammation) of $NaNO_2$-exposed mice?
5. What is the relationship between the independent and dependent variables in this study?

1.3 The Objective of the Study

1. To find out the effect of the dose of ethanol extract of red okra pods (*A. esculentus* L.) on the activity of CAT and SOD enzymes in the hepatocytes of $NaNO_2$-exposed mice.
2. To investigate the effect of the dose of red okra pods ethanol extract (*A. esculentus* L.) on the activity of AST, ALT, and ALP enzymes in the blood serum of $NaNO_2$- exposed mice.

3. To determine the effect of ethanol extract dosage from red okra pods (*A. esculentus* L.) on the levels of total serum protein (TSP) of $NaNO_2$-exposed mice.
4. To assess the effect of the dosage of ethanol extract of red okra pods (*A. esculentus L.*) on the structure of the liver tissue (percentage of normal, necrotic and swollen hepatocytes as well as inflammation) of s $NaNO_2$-exposed mice.
5. To determine the relationship between the independent and dependent variables in this study.

1.4 Significance of the Study

1.4.1 Theoretical significance

The results from this study are expected to provide scientific information about the antioxidant and hepatoprotective activity of red okra (*A. esculentus L.*) in the physiological battle against oxidative stress due to $NaNO_2$ in animals.

1.4.2 Practical significance

Based on this research, red okra pods can be used as an antioxidant and a hepatoprotective agent to fight against oxidative stress and tissue damage caused by the $NaNO_2$ and other related chemicals. From the information obtained from the study, people are expected to include the red okra in their daily meal to fight against oxidative stress caused by $NaNO_2$ and other chemicals. Furthermore, the food industry is expected to use the results of the present study in planning to use red okra pods as one of the crucial food supplements.