# CHAPTER 1 INTRODUCTION

## 1.1 Background

Environmental pollution is currently a problem that has become a major government concern. Pollutant waste becomes more uncontrollable due to an increase in number of industrial factory. Heavy metals are generally the most dangerous pollutant waste that can enter animal, plant, and human tissues via diet, inhalation, and direct exposure. Lead being the most dangerous natural heavy metal imposes metabolic dysfunction in organ system (Das and Pal, 2017).

Lead is a bluish-gray metal that is naturally found in earth in small amounts and can be found in the environment (Gupta, 2007). Lead has low melting point and active chemical properties, so it is commonly used to coat metals from corrosion. Lead can be found in all parts of our environment such as the air, the soil, and the water. Burning fuel is one source of lead that will be settled to the ground and absorbed by plants. Therefore, food can be a cause of lead exposure (Khan *et al*., 2008). Lead contaminants can also enter the food chain from air exposure, and then destroy functional organs.

Exposure to lead leads to an elevation of reactive oxygen species (ROS) and lipid peroxidation, and suppresses antioxidant activity such as glutathione (GSH) (Monteiro *et al.,* 1991). Oxidative stress has occurred by inducing ROS such as superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxyl radical ($OH^-$), peroxy radical ($LOO^-$) and by disrupting the active side of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Hsu and Guo, 2002). According to study conducted by Sharma and Singh (2014) administration

lead orally at a dose of 150 mg/kg BW in Swiss-albino mice for 40 days significantly decreasing superoxyde dismutase (SOD) and catalase (CAT). In addition, according to Xu *et al*., (2008) giving lead orally at a dose of 100 mg/kg BW for 28 days can cause DNA damage through the production of ROS and malondialdehyde (MDA).

The kidney is one of main target organs of lead toxicity (Craan *et al*., 1986). Nephrotoxic is one of the effects of ROS which can interfere renal physiology. This is because the kidneys have the ability to absorb during filtration or reabsorption and as a place for accumulation of divalent metal ions (Rana *et al*., 2018). According the same researchers, lead can react with junction cells and can change the structure of epithelial cells so that the lumen of the proximal tubule in the kidney shrinks as a result of oxidative stress.

Clinical trials have demonstrated that lead poisoning can cause renal failure. Renal failure is a medical condition in which kidney is no longer has function. Urea and creatinine are released from the body through the kidneys at normal levels, increasing level of urea and creatinine in blood serum indicates dysfunction of kidney. Blood urea nitrogen (BUN) and creatinine levels in blood serum measurements are biochemical parameters that can be used to measure the state of kidney function. Blood urea nitrogen is the amount of urea concentration in blood serum. Urea is produced from ammonia in the liver and is the final product of protein metabolism. Creatinine is the result of creatine metabolism in muscles so that the amount of creatinine represents muscle mass and stability in healthy individuals (Henry, 2001).

Lead toxicity that causes oxidative stress can be prevented by administering antioxidants. Antioxidants can be grouped into two types namely enzymatic antioxidants and non-enzymatic antioxidants (Flora, 2011). Enzymatic antioxidants are antioxidants that are produced endogenously in cells in the form of enzymes or proteins, while non-enzymatic antioxidants are antioxidants that are not composed of proteins, for example carotenoids, flavonoids, vitamins, minerals, *quercetin*, and others. Sources of natural non-enzymatic antioxidants are found in fruits, vegetables, nuts, and seeds (Flora, 2009). According to study conducted by Hu *et al.*, (2014), phenolic compounds such as polyphenols which are included in plant secondary metabolites exhibit antioxidant reactions and prevent the formation of health problems caused by ROS.

The use of drugs as protector is needed to reduce negative effects of free radicals due to heavy metal exposure such as lead. Drugs with antioxidants contained are able to inhibit oxidation process and neutralize free radicals to prevent health problem. Considerations of the advantages of natural resource as drugs are now common. Currently, available drugs for metal toxicity therapy are from chelating agents. In point of fact, chelating drugs have many adverse effects such as divalent metal ion loss and liver or renal dysfunction and often fail to improve renal injury induced by Pb (Flora and Pachauri, 2010).

*Ocimum sanctum* is a plant originally from India that easily found in environment. Holy basil has natural antioxidant which is beneficial to human and animal health. Natural antioxidant compound that contained in holy basil are phenolic compound (tocopherol, phenolic acid, flavonoid), nitrogen compound

(chlorophyll derivates, amino acid, alkaloid, and amine), and beta carotene (Basith, 2016). Beta carotene in holy basil has ability to prevent cell damage (Cui *et al.*, 2012).

*Ocimum sanctum* is still uncommon for medical use in our society. Antioxidant substances contained in holy basil leaves are expected to protect kidney from damage due to exposure of lead acetate. Therefore, this study tries to examine the effect of holy basil (*Ocimum sanctum*) leaf extract as nephroprotector using serum creatinine level and blood urea nitrogen (BUN) level as indicator of kidney function.

## 1.2 Problem Statement

Based on the background, the problem statement is whether extracted holy basil (*Ocimum sanctum*) leaf can decrease serum creatinine level and blood urea nitrogen (BUN) level of mice (*Mus musculus*) due to exposure of lead acetate?

## 1.3 Research Purpose

The purpose of this research is to prove the effect of *Ocimum sanctum* leaf extract as nephroprotector towards serum creatinine level and blood urea nitrogen (BUN) level of mice (*Mus musculus*) exposed by lead acetate.

## 1.4 Aim of Research

### 1.4.1 Theoretical aim

The theoretical aim is to give explanation about the effect of *Ocimum sanctum* leaf extract as nephroprotector in decreasing serum creatinine levels and blood urea nitrogen (BUN) levels of mice (*Mus musculus*) exposed by lead acetate.

### 1.4.2 Practical aim

Result of this research is expected to give information and knowledge about how to protect organs from oxidative stress due to lead acetate exposure by giving natural antioxidant *Ocimum sanctum*.

## 1.5 Theoretical Base

Lead is an element that can increase the formation of free radicals and reduce antioxidants in the body causing damage to an organ. Mechanism of lead toxicity is oxidative stress. Oxidative stress is an imbalance amount between production of ROS and elimination of ROS by an antioxidant system (Garcia-Nìño and Pedraza-Chaverri, 2014). Lead induced overproduction of ROS are superoxide radicals, hydrogen peroxide, hydroxyl radicals, and lipid peroxides causing oxidative stress (Senapati *et al.*, 2001). Reactive oxygen species will change the composition of membrane lipids resulting in changes in integrity, permeability, and organ function. Oxidative stress has been proposed as a possible mechanism involved in lead induced nephrotoxicity (Jia *et al*., 2012).

Lead poisoning significantly increases ROS and lipid peroxidation, and decreases antioxidants in the kidney (Yurekli *et al.,* 2009). Cell damage due to imbalance in production and elimination of ROS can be prevented by using antioxidants. The herbal antioxidants have been reported to provide protection against lead induced oxidative stress and emerged as a potential therapeutic to prevent free radical generated damage in the body (Sudjarwo *et al*., 2019). According to Mishra *et al.,* (2007) that the main contents of *Ocimum sanctum* which function as antioxidants are ascorbic acid (vitamin C), tocopherol (vitamin

E), beta carotene, beta-sitosterol, eugenol, palmitic acid, ursolic acid, phenolic (flavonoids, phenolic acids), and nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amine). The antioxidants presented by vitamin C and vitamin E as demonstrated by Tian *et al.,* (2005) can significantly reduced renal oxidative stress. Furthermore, the vitamin treatment effectively increased glomerular filtration rate (GFR) and renal plasma flow, markedly decreased renal damage, and decreased arterial pressure. Other than that, the use of substances containing antioxidants can prevent the occurrence of factors that can lead to cell damage.

**1.6 Hypothesis**

According to problem statement and purpose of this research, hypothesis of this research is: Holy basil (*Ocimum sanctum*) leaf extract can decrease serum creatinine level and blood urea nitrogen (BUN) level of mice (*Mus musculus*) exposed by lead acetate.