# CHAPTER I INTRODUCTION

## 1.1 Research Background

One of the health problems that occur around us is air pollution. Hendrawan *et al*. (2018) stated that sources of air pollution possibly come from various activities, including industry, transportation, and housing, as well as various natural disasters, such as forest fires, volcanic eruptions, and toxic natural gas. Air pollution that comes from various sources, can be in the form of particles of dust, gas, lead, and combustion products called Particulate Matter (PM).

The main component of PM is in the form of carbon particles. There are two terms regarding the terms of these carbon particles, namely black carbon (soot) and carbon black. The terms black carbon and carbon black are already generally used, but they are physically and chemically distinct entities. The term "black carbon" or soot is commonly used to describe carbon particles from unwanted by-products of urban housing, industry, and Diesel Exhaust Particles, with an impure carbon component of less than 60%. The term "carbon black" is used to describe carbon produced for commercial use. Both have a similar main structure, the element carbon (Niranjan *et al*., 2017). Hendrawan *et al*. (2019) stated that people who work as factory workers, motor vehicle repair shops, fuel industry, and in the oil and coal industry receive the highest exposure to carbon black particles. Carbon black pollution from industry has a major effect on air pollution. Worldwide production of carbon black is about 8.1 million metric tons per year, where 90% comes from rubber production, 9% as pigmentation, and the remaining 1% is used as raw material for various types of equipment (Kim *et al*., 2011). Black carbon (soot) is considered an unwanted by-product with carbon component of less than 60%. In contrast, carbon black is produced under controlled conditions in the rubber, printing, and painting industries for commercial use, with a pure carbon content of up to 97% (Watson and Valberg., 2001; Niranjan *et al*., 2017).

Black carbon (soot) and carbon black although mentioned to have differences in structure and production, they are both similar in their effects on health problems. Carbon black is often used in animal-based and in vitro studies to determine environmental toxicity (Kim *et al*., 2011; Niranjan *et al.,* 2017). Carbon black has a size ranging from tens to hundreds of nanometers and belongs to the Ultrafine Particles (UFP). Carbon particles that are less than 100nm in size are a major part of Diesel Exhaust Particles (DEP) and air pollution. UFP can stay in the lungs for a long time. Continued exposure can lead to a buildup of particles in the lungs, leading to the entry of these particles through the alveolar capillaries and followed by their spread throughout the body (Jefrey and Edison, 2013; Sahu *et al*., 2014; Kwon *et al*., 2020).

Further PM exposure also affects women's reproductive health and is closely related to the process of folliculogenesis. Folliculogenesis is a process of follicular development in the ovarian cortex, with two stages, the preantral and antral phases (Rosadi, 2011). The preantral phase consists of two types of follicles, namely primary and secondary follicles, while the antral phase consists of two types of follicles, namely tertiary and de Graaf follicles (Griffin *et al*., 2006). During development, not all follicles will develop and be ovulated. Many of them will experience atresia, namely oocyte cell death, and form atretic follicles (Vidal and Dixon, 2018).

Exposure to carbon black has a negative impact on reproductive health. Luderer *et al*. (2022) explained that the ovaries do not have Germline Stem Cells (GCSs) in postnatal life, so women are born with a limited number of oocytes, which are arranged in the form of primordial follicles. The research of Luderer *et al*. (2022) also concluded that exposure resulted in a reduction in the number of developing follicles, inducing cell death due to DNA damage and increased cell apoptosis. Previous studies on PM exposure on ovarian function and folliculogenesis in mice have shown a decrease in fertility, an increase in the incidence of irregular estrous cycles, and a reduction in the number of follicles (Veras *et al.,* 2009).

Exposure to as carbon black causes an increase in the production of Reactive Oxygen Species (ROS) which affects follicle development and female reproduction (Luderer *et al*., 2022). ROS itself is one of the reactive free radicals produced from mitochondrial activity in the body. Under normal conditions, there is a balance between the production of ROS and antioxidants. ROS in certain levels has benefits for the body, but if it is produced in excess, it can interfere with the body's physiology. Excessive ROS production can lead to oxidative stress in the body which has a negative impact on women's reproductive health. Oxidative stress occurs when the balance between the production of ROS is higher than the antioxidant capacity in the body (Agarwal *et al*., 2005; de Barboza *et al*., 2017).

Okra (*Abelmoschus esculentus*) is known to have many health benefits. The okra plant belongs to the Malvaceae family originating from Africa but has been widely planted in other areas such as Asia, Southern Europe, and America with tropical, subtropical, and warm climates. All parts of this plant have various uses, with the main part often used being the pods. Okra can be used in a variety of dishes, from vegetables to food additives (Durazzo *et al*., 2018). Okra pods and seeds contain flavonoid active ingredients such as procyanidin B2, procyanidin B1, rutin, quercetin, catechin, and epicatechin which function as antioxidants (Khomsug *et al*., 2010).

Shui and Peng (2004) reported about 70% of the antioxidant effect of okra in the form of quercetin. Quercetin can reduce oxidative stress conditions and induce antioxidant enzymes in the body (Kobori *et al*., 2015). The ability of quercetin as an antioxidant is 4.7 times greater than vitamin C (Adelakun *et al*., 2009), and can provide biological protection against endogenous ROS and other exogenous carcinogenic substances (Granado-Serrano *et al.*, 2012). Quercetin as a natural ingredient has a beneficial effect on the female reproductive system. Apart from being a natural antioxidant, quercetin can also act as a phytoestrogen and promote a healthy follicle population and hormone secretion in the ovaries. Quercetin also increases the number of secondary and antral follicles due to its positive effect in inhibiting the formation of follicle atresia (Rashidi *et al*., 2021).

The antioxidant effect in okra is expected to prevent the production of ROS in the body exposed to carbon black. Therefore, the authors wanted to observe the antioxidant effect of okra in preventing oxidative stress caused by exposure to carbon black and maintaining the number of preantral and antral follicles in the ovaries.

### 1.2 Problem Statement

Based on the above background, the problem that can be formulated is : can okra (*Abelmoschus esculentus)* pods ethanol extract administration be able to maintain the number of preantral and antral follicles of mice (*Mus musculus*) exposed by carbon black.

## 1.3 Research Purpose

This study aims to determine the effectiveness of Okra (*Abelmoschus esculentus*) pods ethanol extract in maintaining the number of preantral and antral follicles of mice (*Mus musculus*) exposed by carbon black.

## 1.4 Research Benefits

### 1.4.1 Theoretical benefits

Providing scientific information related to the development of research on okra (*Abelmoschus esculentus*) pods ethanol extracted in various doses can prevent the negative effects of carbon black exposure on the folliculogenesis process.

### 1.4.2 Practical benefits

The results of this study are expected to provide information on the antioxidant effectiveness of okra (*Abelmoschus esculentus*) in preventing the negative effects of exposure to Carbon Black on female reproduction and provide a new reference for herbal remedies that have antioxidant abilities.

## 1.5. Theoretical Base

Exposure to carbon black particles from the environment can enter the body through inhalation from the air (Lee *et al*., 2011). Carbon black particles are then deposited in the lungs and some very small particles (ultrafine particles) will translocate into the blood circulation slowly and continuously (Hendawan *et al*., 2017). Kim *et al*. (2014) explained that carbon black particles can settle on blood cells or non-specific organs and stimulate the innate immune response in the body. Carbon black particles enter the lungs and then interact with lung epithelial cells and alveolar macrophages. These cells will secrete pro-inflammatory cytokines, which then circulate in the bloodstream. Continuous exposure results in systemic disorders of the body (Niwa *et al*., 2008). Exposure to carbon black also results in disruption of cell mitochondrial metabolism. These metabolic disorders are caused by mitochondrial dysfunction and will increase the production of Reactive Oxygen Species (ROS). Carbon black exposure also increases the production of Reactive Nitrogen Species (RNS). The release of ROS and RNS will increase the release of inflammatory responses such as IL-6 and TNF-α. The expression of IL-6 and TNF-α can affect the integrity of cell membranes, causing a decrease in cell viability, resulting in cell death (Niranjan *et al*., 2017; de Almeida Rodolphoa *et al*., 2021).

Hendrawan et al. (2018) stated that ultrafine particles deposited in the lungs can increase the production of free radicals. ROS is one of the reactive free radicals produced in the body. Certain levels of free radicals are beneficial for the body, but excess production can cause physiological disturbances in the body (Giriwijoyo, 2007). The imbalance between the production of free radicals and their elimination by the body's protective mechanisms can result in oxidative stress (de Barboza *et al*., 2017). ROS can induce the activation of caspase-3 which can cause cell death (Madyawati *et al*, 2015). Caspase-3 is the main caspase that functions in the regulation of cell apoptosis (Widjiati *et al*., 2020). The increased expression can cause degenerative diseases (Anggraini *et al*., 2018). Luderer *et al*. (2022) stated that caspase-3 activation can cause the death of the granulosa cells surrounding the oocyte. The granulosa cell death also affects the oocyte death.

Antioxidants are chemical compounds produced in the body and have the ability to reduce the reactivity of free radicals (Sayuti and Yenrina, 2015). Higher

ROS production than antioxidants in the body can cause oxidative stress (Agarwal *et al*., 2005). One source of natural antioxidants is okra (Abelmoschus esculentus). Khomsug *et al*. (2010) explained that okra fruit contains various flavonoids that can function as antioxidants in it, and one of them is quercetin. Shui and Peng (2004) stated that at least 70% of the antioxidant activity of okra comes from the quercetin content. Research by Granado-Serrano *et al*. (2012) and Kobori *et al*. (2015) showed that quercetin can interact with the body's immune system, induce antioxidant enzymes, and Glutathione (GSH). The mechanism that occurs against ROS was described by Xu *et al*. (2019), where $O^{2-}$ (one of the oxygen-free radical compounds) is captured by Superoxide Dismutase (SOD), and converted into $H_2O_2$. The $H_2O_2$ compound then reacts with GSH to produce non-toxic $H_2O$. GSH which has reacted with $H_2O_2$ will be oxidized to GSSG (Oxidized glutathione disulfide). GSSG is then reduced by GSH reductase and re-forms GSH.

**1.6 Hypothesis**

The hypothesis proposed is that the administration of okra (*Abelmoschus esculentus)* pods ethanol extract can maintain the number of preantral and antral follicles of mice (*Mus musculus)* exposed by carbon black.