CHAPTER 1

INTRODUCTION

1. 1. Project Background

For decades, mental disorders have continued to be a mystery about us, humans. Amongst other mental disorders, Major Depressive Disorder (MDD) is the most common. With more than 300 million people affected worldwide, MDD is projected to earn the highest rank as a cause of burden of disease worldwide by 2030 (Malhi & Mann, 2018). People from different walks of life can be affected by this disorder, regardless of their demographics. Thus, makes it a global public health priority to care and prevent MDD (Shrivastava, Shrivastava, & Ramasamy, 2017). According to the World Health Organization (WHO), MDD is characterized by sadness, loss of interest or pleasure (anhedonia), feelings of guilt, disturbed appetite, feelings of tiredness and poor concentration (WHO, 2017). It can affect performance and ability to do daily tasks for at least 2 weeks. Therefore, increase in burden and diminished quality of life. Patients diagnosed with depression have higher mortality risk which is associated with physical illness, family burden, self-harming behaviours, and cardiovascular disease (Cranford, Eisenberg & Serras, 2009; Serras, Saules, Cranford, & Eisenberg, 2010; Lépine & Briley, 2011). Typically, MDD correlated with the increase in mortality risk through suicide. As it contributes to almost 800.000 people die each year due to suicide (WHO, 2017).

Management of MDD generally aims for the remission of symptoms. The management of depression has been centered around clinical antidepressants with varying outcome. Generally, MDD treatment aims to increase serotonin availability by inhibiting serotonin transporter or receptor (such as 5-HT) of the monoaminergic neurotransmitter system (Pitsillou *et al*., 2020). Although its wide, long-term use and side effects such as sexual disorders, weight gain and withdrawal effects remain a hurdle (Cartwright, Gibson, Read, Cowan, & Dehar, 2016). At the moment, numerous clinical antidepressants are a mainstay for

the management of depression coupled with psychological treatment. Unfortunately, despite after 50 years of research, antidepressant agents still bear side effects and its mechanism of action is still not fully understood (Behr et al., 2012; Krishnan & Nestler, 2011).

While the pathophysiology of MDD is still elusive, the condition of oxidative stress is widely seen as a risk factor for depression. Thus, restoration or increasing the antioxidant levels in MDD patients are seen as a possible treatment option. By which the antioxidants can help the body fight against ROS. Known antioxidants such as ascorbic acid has been proven to be able to reverse behavioral alteration in the effect of induced stress in animal models (Moretti et al., 2012). This antidepressant-like activity is also found from natural origins, such as observed in young barley leaf extract (Yamaura et al., 2012). Lentils (*Lens culinaris*) are part of legumes from the family of Fabaceae. It is consumed in parts of the world, mainly Europe, the Middle East, Africa and South Asia. Studies have shown that the lentils reserve the highest antioxidant activity and the highest concentration of phenolic compounds (Xu, Yuan, Chang, 2007). Thus, its use as a health-promoting supplement as an antioxidant might be considered (Zou, Chang, Gu, & Qian, 2011; Ganesan & Xu, 2017).

Another problem posed in this research is that depression is a psychiatric illness in humans and not animals. It is problematic to model psychiatric illness, such as MDD, in mice under laboratory setup since diagnosing MDD in mice is troublesome (Liu et al., 2018). Diagnosing MDD has always been reliant on the teamwork that the patient and doctor established. Doctors talks to patients and in turn the patient use depression scale based on a self-rating to describe their condition. The inability of researchers to communicate through language with rodents means that the aforementioned approach is virtually impossible. Moreover, the varieties and the source of stressors in human society cannot be mimicked for rodents (Liu et al., 2018). Therefore, physical stressors are applied to rodents to in an attempt induce depression instead (Liu et al., 2018).

Depression is related with the condition of stress (J. Wang et al., 2019). Stress interferes with the nervous system and it is positively correlated with the onset of depression (Kioukia-Fougia et al., 2002; P. Xu et al., 2017). Chronic restraint stress (CRS) is a type of stress paradigm that can produce persistent depressive-like symptoms in murine models (J. Wang et al., 2019). This method has been established as a widely utilized model of depression in mice (Q. Wang et al., 2017). Typically, there is behavioral changes or symptoms exhibited by the rodent as a consequent of the physical stress introduced to the rodent. In light of this, there are numerous behavioral assessment have been developed that can be utilized to identify and validate whether a mouse has anhedonia or depressed; such as forced swimming test (FST) and sucrose preference test (SPT) (Liu et al., 2018; Son et al., 2019; Tanti & Belzung, 2010; Q. Wang et al., 2017).

This study would like to evaluate the phytochemical properties and antioxidant activity of *Lens culinaris* extract. This is done to investigate whether lentils is an attractive prospect in combating oxidative stress. Aside from that, in regards to animal models, it is required to have an appropriate trigger to induce depression in mice that model depression. In this study, the trigger wherein physical chronic restrain was used upon mice. By which, it would then be validated with two behavioral analyses of FST and SPT.

1. 2. Research Objective

This study aims to evaluate the antioxidant activity of *Lens culinaris* extract and validate mice stress paradigm using chronic restraint stress. Listed below are several objectives that is created to achieve the set aim:

- **a.** To investigate the phytochemical properties of *Lens culinaris* extract through qualitative and quantitative approach;
- **b.** To evaluate the antioxidant activity of *Lens culinaris* extract;

c. To validate model of depression in murine models by inducing them with chronic restraint stress (CRS). Two behavioral analyses of forced swimming test (FST) and sucrose preference test (SPT) are used to validate the CRS stress paradigm.

1. 3. Research Scope

The study revolves within the field of biomedical sciences. More specifically, in the research of mental disorders and its prospective treatment that is the main interest of this study. The study will be performed in the Department of Biomedicine of Indonesia International Institute of Life Sciences (i3L).

The scope of work of this study can be broken down into three main activities with subsequent details, which are:

a. Plant extraction of *Lens culinaris*

- Plant extraction of *Lens culinaris* using maceration procedure and purification;
- Screening and determination of phytochemical content. This step will be carried out with help from *Lembaga Ilmu Pengetahuan Indonesia* (LIPI);
- Analysis of antioxidant capacity of *Lens culinaris* extract using DPPH assay.

b. Depression model validation in mice

- Conditioning of mice to chronic restraint stress (CRS) to induce stressful environment/ depression;
- Observation of behavioral analysis in mice using two methods: (1) forced swimming test (FST) and (2) sucrose preference test (SPT). These methods are used to model stress response to threat and anhedonia respectively;
- Perform ANOVA statistical analysis to validate FST and SPT results.