

CHAPTER 1 INTRODUCTION

1.1 Research Background

Infertility is the inability of a sexually active couple to achieve spontaneous pregnancy in one year (WHO, 2000). Approximately 15% couples do not achieve pregnancy within one year and seek medical treatment for infertility (Jungwirth *et al.*, 2015). Infertility is a reproduction disruption which can be experienced both man and woman (Anzila *et al.*, 2017).

Environment contamination might disrupt reproduction system causing infertility. Many potentially hazardous chemicals have been released into the environment and their exposure to both human and wildlife has become inevitable (Mathur and D'Cruz, 2011). A dangerous environmental agent which released can disrupt reproduction function especially testis, the male gonads, which are the site of spermatogenesis and androgen production (El-Gerbed, 2015 ; Rebourcet *et al.*, 2010). Defect in spermatogenesis process has been stated as one of the cause of male infertility.

One of most toxic chemical agent is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the by-products of various industrial processes (i.e. bleaching of paper, pulp and pesticides) and combustion activities. TCDD and its congeners are extremely resistant to metabolic degradation and are persistent in both environmental and biological systems.

The major route of human exposure to TCDD is through food consumption like fish, meat and dairy products (90%) because of its high lipophilicity and bioaccumulative in fatty tissue (Jong-soon *et al.*, 2007). Other routes where TCDD entered to body are from inhalation, drinking water, soil ingestion and skin absorption (Marinković, 2010)

TCDD toxicities may be seen in both males and females (El-Gerbed *et al.*, 2015). TCDD, as an endocrine disruptors is known to impair testicular function and causing infertility (Aly, 2011). It is transported from the external environment to the adipose tissue or liver through the plasma lipids, and TCDD binds to the aryl hydrocarbon receptor (AhR) (Calkonski *et al.*, 2014). AhR is ligand-activated nuclear transcription factor which mediates cellular response in term of expression regulation of a large number of genes (Marinkovic *et al.*, 2010).

TCDD-induced expression of cytochrome P4501A1 enzyme causing inflammation process and induce the generation of reactive oxygen species (ROS) which can cause apoptosis in germ cells and decrease the sperm count and sperm quality leading to infertility. ROS is one of free radicals form which can lead to oxidative stress. It is found that oxidative stress caused by TCDD can inhibit secretion of Testosterone Hormone by damaging the endoplasmic reticulum and mitochondria of Leydig cell. Inhibition in this hormone secretion could interference spermatogenesis process and cause decreased of spermatogenic cells (Dhanabalan *et al.*, 2013).

Oxidative stress is exist when the amount of antioxidant is low and free radical is higher. In normal body free radical is controlled by the antioxidant and should be in small number. Naturally inside the body cell there are various antioxidant both enzymatic and non-enzymatic and its function as cell organelles defense system from the effect of free radical damage (Sinaga, 2016). Some antioxidant can be produced from nature product such as spices, herbs, fruits, vegetables and plants.

Camellia sinensis is the same plant species which white, black and green tea are derived. The various types are classified on the basis of their processing and the associated oxidation and fermentation, green tea is minimally oxidized and non-fermented tea (Ahmed *et al.*, 2013). Green tea is known to have good antioxidant and anti-inflammatory properties (Ahmed,*et al.*, 2013).

The active constituents in green tea are powerful antioxidants called polyphenols. Green tea polyphenols acts as free radical scavengers and destroy it to protect male reproduction system against oxidative stress. It has strong capacity in neutralizing ROS and regulate DNA damage in order to improve fertility. These polyphenols downregulate the inflammation process (Rahman, 2018).

Among the polyphenols in tea, there is a family of compound called the flavonoids. Flavonoids are basic phenolic compounds in green tea responsible for antioxidant activities such as neutralization of free radicals that are formed in the process of metabolism (Horzic *et al.*, 2009). These flavonoids contain a substance called catechins. Major catechins present in green tea are epicatechin (EC),

epigallocatechin gallate (EGCG), epigallocatechins (EGC), and epicatechin gallate (ECG). Beside that catechin component in green tea is also known to have good reproductive effects (Idowu, 2017). Green tea also known to have vitamin A, K, E and lots of minerals which essential for body health.

Recent research have approved that green tea can significantly increase serum testosterone and showed restoration of seminiferous tubules through spermatogenesis in rats which treated with lead acetate (Hassan *et al.*, 2016; Jassem *et al.*, 2008). It is also believed that green tea can prevent excessive generation of free radicals in sperm as an antioxidant properties of green tea (Sheteifa and Morsy, 2014). Based on the description above, it can be used as a basis for research on the effect of green tea (*Camellia sinensis*) extract on the number of spermatogenic cells of male mice (*Mus musculus*) exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

1.2 Problem Formulation

From this background problem, obtained problem formulation that is

Is green tea (*Camellia sinensis*) ethanol extract could maintain the number of spermatogenic cells (spermatogonia, spermatocyte and spermatid) of male mice (*Mus musculus*) exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)?

1.3 Aim of Reseach

The aim of this research is to know the effect of green tea ethanol administration with various doses could maintain the number of spermatogenic cell

(spermatogonia, spermatocyte and spermatid) in male mice (*Mus musculus*) exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

1.4 Research Benefit

1.4.1 Theoretical benefit

To give scientific information about green tea (*Camellia sinensis*) extract administration could maintain the number of spermatogenic cell (spermatogonia, spermatocyte and spermatid) in male mice (*Mus musculus*) exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

1.4.2 Practical benefit

To give information about green tea (*Camellia sinensis*) extract property as an antioxidant to reduce free radical sources include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in male reproduction

1.5 Theoretical Base

Testis is important organ in male reproductive system. It has two main function which are producing testosterone hormone and producing spermatozoa (Johnson, 2013). Environmental factors can cause infertility by defecting the DNA and semen quality. Nutritional deficiencies and oxidative stress originated from pesticide, industrial chemical, and smoke exposure can increase infertility risks (Rahman, 2018).

One of the most toxic industry chemical released in environment which can cause infertility is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCDD is believed as

carcinogenic compound and endocrine disruptor. TCDD was found on body through food consumption which contaminated, water pollution, soil pollution or air pollution and this compound are lipophilic and bioaccumulate and biomagnify in the food chain (Lind *et al*, 2009). Inside the body, TCDD will binds to an aryl hydrocarbon receptor (AhR)-dependent manner (Yoshioka and Tohyama, 2019). This bound suppress expression of cytochrome P4501A1 enzyme which involved in ROS production, and it has been found to decrease the male fertility which known as the most sensitive target of TCDD (Dhanabalan *et al.*, 2013).

Green tea (*Camellia sinensis*) is known as non-fermented tea and minimally oxidize where it makes green tea to have high level of antioxidant. The biggest antioxidant compound found in greentea is flavonoid which contain catechins. This antioxidant compound could maintain spermatogenic cells (Mahmoudi, 2018). Green tea also believed to have vitamin A and vitamin E which also known as antioxidant. Antagonistic properties toward TCDD toxicity owned by vitamin E also know could increase spermatogenic cell counts in seminiferous tubule (Wati, 2014).

1.6 Research Hypothesis

Hypothesis of this research is green tea (*Camellia sinensi*) extract administration with various dose could maintain the number of spermatogenic cells (spermatogonia, spermaocyte and spermatid) in male mice (*Mus musculus*) exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.