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Published by : Advanced Scientific Research

EDITORIAL BOARD

ISSN 0975-2366
DOI:https://doi.org/10.31838/ijpr/2020.12.03.153

Research Article

Effect of Green Tea (*Camellia sinensis*) Ethanol Extract Administration on The Number of Spermatogenic Cells of Male Mice (*Mus musculus*) Exposed to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin

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Received: 11.03.20, Revised: 14.04.20, Accepted: 03.05.20

ABSTRACT

One of the cause of infertility in male reproduction is contamination of hazard chemical. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is known as most toxic chemical which can impair the reproductive system and cause low count of spermatogenic cells. The purpose of this study was to know the effect of green tea (*Camellia sinensis*) ethanol extract on the number of spermatogenic cells of male mice (*Mus musculus*) exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. This research used 25 male mice which divided into 5 groups of treatment i.eC(-) received aquadest only, C(+). T(1), T(2), and T(3) injected with TCDD 0,14 µg per mice and continued with the treatment. C(+) treated with Epigallocatechin gallate (EGCG) 1,2 mg/kg BW, T(1), T(2) and T(3) given green tea ethanol extract with a dose of 1 mg/kg BW ; 2 mg/kg BW and 4 mg/kg BW respectively orally 0.1 ml for 53 days. After that the mice sacrificied and the testis were taken for making histopathology slides using HE staining. The results showed that there are significant difference ($P < 0.05$) of spermatogenic cells among the treatment group where T(1) with a mean of 100.68 ± 1.91, T(2) with a mean of 136.32 ± 2.33 and T(3) group with an average of 166.84 ± 3.40. Between T(3) and C(-) with a mean of 169.72 ± 2.67 showed there are no significant difference. The conclusion is green tea ethanol extract could maintain the number of spermatogenic cells.

Keywords: Green tea, Spermatogenic cells, TCDD

INTRODUCTION

Cases of infertility in both humans and animals are the cases most often encountered in the field of reproduction. One of the reason is the entry of hazard chemicals that can damage the work of the reproductive system and cause infertility(Mathur andD'Cruz, 2011). The chemicals which are classified as very toxic are 2,3,7,8-tetracholorodibenzo-*p*-dioxin (TCDD) where these materials are not produced commercially but as the result of industrial waste and combustion(Dhanabalan*et al.*, 2013).

TCDD has been found to elicit toxicity by binding to cystosolic aryl hydrocarbon receptor (AhR), enforcing the expression of enzymes like cytochrome P450A1 (CYP450A1), which involved in the production of reactive oxygen species (ROS) (Androutsopoulus, 2009). Range of the dioxin toxicity are quite wide include reprotoxic, neurotoxic, hepatotoxic, immunotoxin, carcinogenic and teratogenic (Hutahaean, 2009). Oxidative stress induced by TCDD leaded to damage in DNA and cause cell death or apoptosis. TCDD is known to reduce spermatogenic cell counts, sperm quality and damage hormone systems, especially androgens (Ishinawa*et al*, 2013). As a result of TCDD pollution, it can increase free radicals in the body and reduce antioxidant action.

Green tea (Camellia sinensis) is known as a tea with high antioxidants. One of its main components is polyphenol, which contains flavonoids. In addition, the green also has catechin, where these compounds act as antioxidants by donating electrons or hydrogen and believed to reduce the effects of free radicals on the male reproductive system (Senanayake, 2013). Green tea is believed could increase the number of spermatogenic cells and improving sperm quality (Mahmoudi, 2018). This study aims to determine whether green tea (Camellia sinensis) ethanol extract can maintain the number

of spermatogenic cells of male mice (*Mus musculus*) exposed to TCDD.

MATERIAL AND METHODS

Preparation of Experimental Animal

This research is experimental research using 25 male mices age 10-12 weeks old. Mice was collected from veterinary center for research experimental animals Surabaya. This research has passed ethical clearence with certificate number 1.KE.166.08.2019. After collected mice were adapted for one week.

Preparation of TCDD, EGCG and Green Tea Ethanol Extract

TCDD is obtained from sigma (Brand Supelco® catalog number 48599). The dosage used in this research is 7 μg/kg (Jong-soon, 2007). The extract of green tea was made with maceration process using ethanol 96 % The doses for treatment in T(1) group is 1 mg/kg BW, T(2) group is 2 mg/kg BW and T(3) group is 4 mg/kg BW (Sari, 2018). The dose of EGCG for C(+) will be 1,2 mg/kg BW (Mawarti, 2012).

Treatment Procedures

The animal are divided into five group by randomization they areC(-); only administrate with Aquadest. C(+), T(1), T(2),and T(3); received injection of TCDD 7 μg/kg. The next day C(+) group givenEGCG 1,2 mg/kg BW.T(1), T(2), and T(3) administrated green tea ethanol extract with dose 1 mg/kg, 2 mg/kg, 4 mg/kg body weight respectively peroral 0,1 ml per mice for 53 days. After 62 days mice were sacrificed for testis removal and continue with making histopathology slides

Data analysis

Data which obtained by counting the average of spermatogenic cells from 5 seminiferous tubule were analyzed using *Analysis of Varian* (ANOVA), if there is any significant difference will be continue with Multiple Range Test Duncan ($p<0.05$) to determine difference in every group of treatment.

RESULT

The data obtained by counting the average of every spermatogonium, primary spermatocyte, and spermatid from 5 seminiferous tubule in microscope with 400x of magnification. From the result, among treatment group T(3) which exposed with TCDD and given green tea ethanol extract 4 mg/kg BW showed the highest number of cell and there are significant differences among T(1), T(2) and T(3) ($P<0,05$).

Table 1: The average number of spermatogonium, primary spermatocyte and spermatid cells of male mice exposed to TCDD and given green tea ethanol extract (Mean ± S.D)

Treatment	The average number of spermatogonium cells Mean ± S.D	The average number of primary spermatocyte cells Mean ± S.D	The average number of spermatid cells Mean ± S.D
C(-)	$20,80^{c}$± 1,39	$33,84^{c}$ ± 1,40	$115, 08^{d}$ ± 2,93
C(+)	$12,48^{a}$ ± 0,79	$16,20^{a}$ ± 1,3	$65,20^{a}$ ± 2,60
T(1)	$12,88^{a}$ ± 0,57	$18,40^{ab}$ ± 1,01	$69,40^{ab}$ ± 0,90
T(2)	$15,48^{b}$ ± 0,65	$23,64^{bc}$ ± 1,39	$97,20^{c}$ ± 1,80
T(3)	$20,58^{c}$ ± 1,21	$33,32^{c}$ ± 0,91	$112,84^{d}$ ±2,65

Description: Different letter in superscript showed a significant difference ($P<0,05$)

This is also showed in Figure.1 where the spermatogenic cells among the treatment group is increased in gradual. While between T(3) and C(-) group which only given aquadest showed there is no significant differences ($P>0,05$). The number of spermatogenic cells from T(3) are close to C(-) group which also showed in histopathology slides where the cell is spread evenly and density is high. C(+) group which exposed with TCDD and given EGCG 1,2 mg/kg BW showed the lowest number of cell compared to all of group treatment but nearly close to T(1) which exposed with TCDD and given green tea ethanol extract 1 mg/kg BW. The C(+) groups showed lower density and cells are spread unevenly based on histopathology slides picture. Figure bellow is the chart of average number of spermatogenic cells after exposed with TCDD and treated with green tea ethanol extract which we can see there is gradual increase from every group of treatment. T(3) result are close to C(-) result.

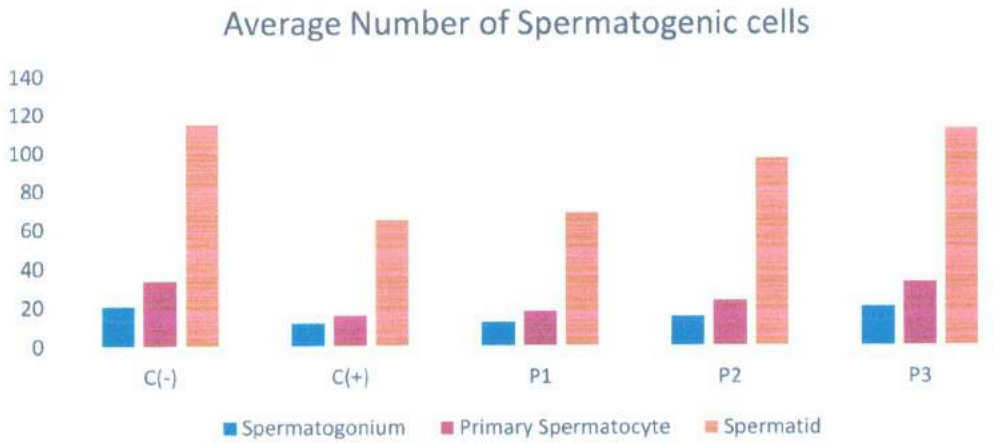

Fig.1: Chart of average number of spermatogenic cells after exposed with TCDD and treated with green tea ethanol extract.

DISCUSSION

TCDD is persistent and most toxicant substance in environment. It has broad spectrum toxicities including reprotoxicity. The male reproductive system known as most sensitive end point of TCDD exposure (Fukuzawa*et al.*, 2004). It is known that TCDD cause disruption in spermatogenesis process, decrease of sperm parameter and increase free radical in testis which leads to infertility (Ishinawa*et al.*, 2013). The mechanism where TCDD increase the number of free radical in testis is through the aryl hydrocarbon receptor (AhR) bond which binds together with Aryl hydrocarbon Receptor Nuclear Translocator (ARNT) (El-Gerbed*et al.*, 2015).

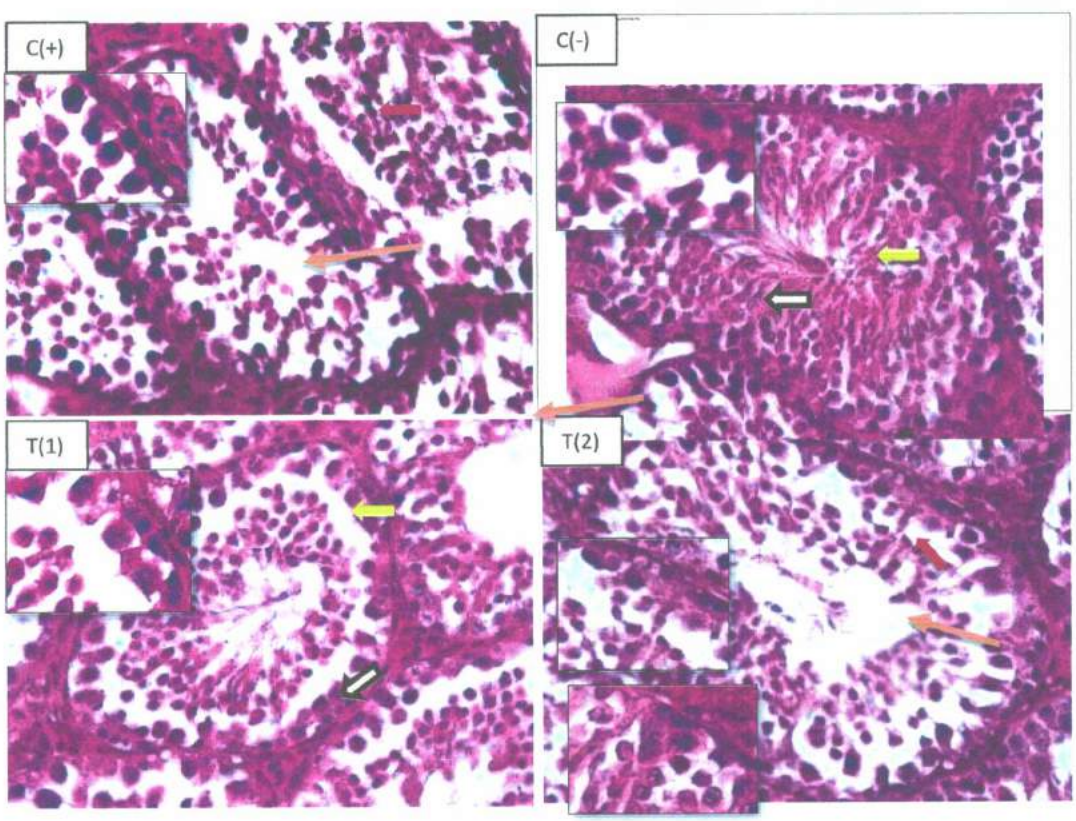

Fig.2: Histopathology slides of Testis with HE staining observed in micro-scope using 400*x* of magnification

Description: Spermatogonium; = primary spermatocyte; = Spermatid

The activated AhR induces expression of various genes with xenobitotic response elements in their enhancer regions, such as the gene for cytochrome P450 (Mohammadiet *al*, 2019). The expression of CYP450 enhance the inflammatory process. TCDD is known to be interfered with physiological signaling of the AhR , led to cell-specific changes in gene transcription and cell differentiation and thereby producing toxic effect (Esseret *al.*, 2005).

TCDD also cause lipid peroxidation. Lipid peroxidation is a process of oxidative degradation of polyunsaturated fatty acids that result in impaired membrane structure and function (Goel *et al.*, 2005). Spermatogenic membrane cells are known to be sensitive with lipid peroxidation because the membrane cells contain polyunsaturated fatty acid which is the target of lipid peroxidation. The cause of lipid peroxidation are increased level of ROS and decrease level of antioxidant which leads into oxidative stress. Oxidative stress known to cause cell death (Shintaninngrumet *al.*, 2019)

TCDD also considered as most potent endocrine disruptor agent where it promotes degeneration of Leydig cell by damaging the smooth endoplasmic reticulum or mitochondria of the Leydig cell. Thereby it is interfering the androgen synthesis especially testosterone and LH. Low level of testosterone and LH will interference the spermatogenesis process and leads to low count of spermatogenic cells (El-Gerbedet *al.*, 2015).

The control positive group which exposed with TCDD and treated with epigallocatechin gallate (EGCG) 1,2 mg/kg shown result close to treatment 1 group which exposed with TCDD and treated with green tea ethanol extract 1 mg/kg. EGCG has been well established polyphenol from green tea and is widely used against oxidative damage, because of its antioxidant ability to repress the free radical mediated inflammation (Thangapandiyan, 2015).

It is known that EGCG could reduce the testicular damage caused by oxidative stress with a significant restoration from the testis weight and the number of spermatogenic cells. The potent free radical scavenging activity of EGCG was mainly attributed to the presence of hydroxyl group. It was confirmed that the more of hydroxyl groups present in the catechin could exhibit more free radical (Thangapandiyan, 2015).

Treatment 3 group which treated with TCDD and given green tea ethanol extract at dose 4 mg/kg BW is known to be most effective and showed result nearly close to control negative group which were not treated with TCDD. This is showed that green tea ethanol extract could maintain the number of spermatogenic cells by protecting the cell from free radical caused by TCDD (Mahmoudi, 2018). Green tea contains polyphenols which acts as antioxidant. Flavonoids is the major polyphenols in green tea which contains catechin. The catechin compound owned by green tea extract believed can suppress ROS generation caused by oxidative radical that resulted in prevention of cell death (Susilowatiet *al.*, 2018).

Green tea catechin also act as antioxidant by donating electrons and hydrogen atoms from their phenolic hydroxyl group, therefore stabilize lipid free radicals as a result inhibiting lipid peroxidation caused by TCDD. Another way of green tea help to attack oxidative stress is by scavenge oxygen and help inducing antioxidant enzyme to regenerate endogenous antioxidant (Senanayake, 2013). Minerals and vitamin owned by green tea also deactivate free radicals, protect the cellular membrane against the oxidative stress effect caused by lipid peroxidation (Rahman, 2018). This has proven that green tea ethanol extract can suppress free radicals effect and maintain spermatogenic cell number. In this research the effective dose of green tea ethanol extract is 4 mg/kg body weight.

CONCLUSION

The conclusion from this study are TCDD exposure can cause low count of spermatogenic cells andthe green tea ethanol extract administrationwith dose 4 mg/kg BW for 53 days could maintain the number of spermatogenic cells after exposed with TCDD.

ACKNOWLEDGMENT

The author would like to thank for Faculty of Veterinary Medicine, UniversitasAirlangga for the providing the facilities and funding this research. The authors hope this script can be usefull for futher research and finding about green tea or TCDD.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

SOURCE OF FUNDING

This research uses independent funds.

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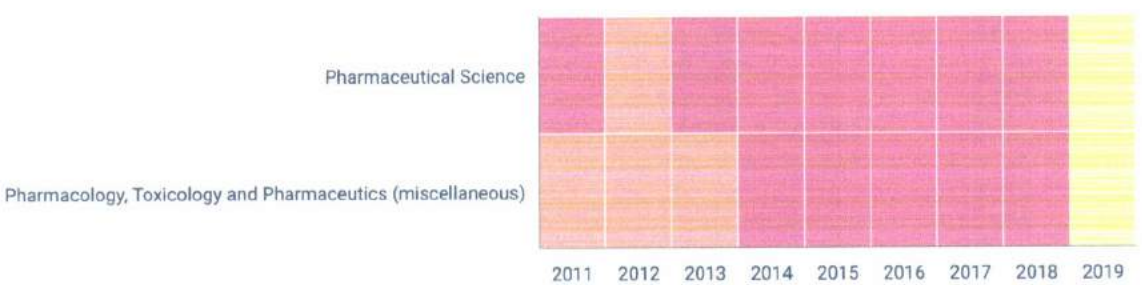

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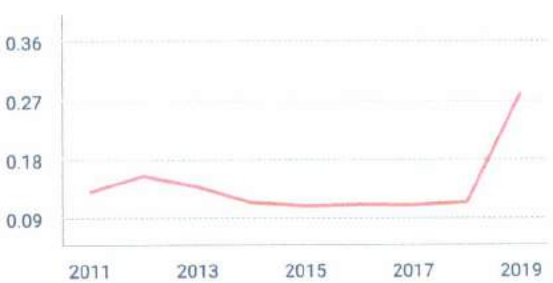

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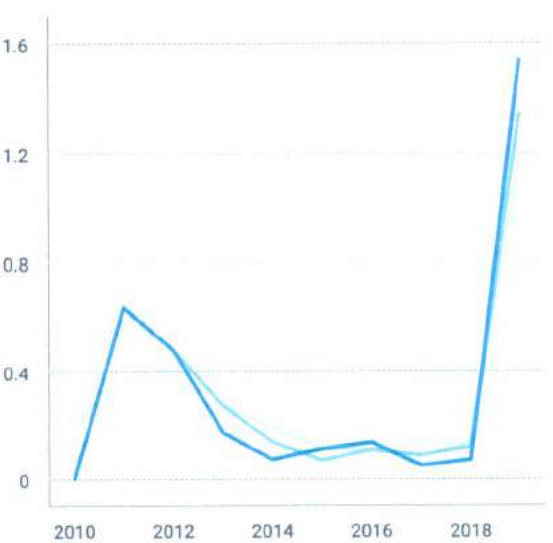

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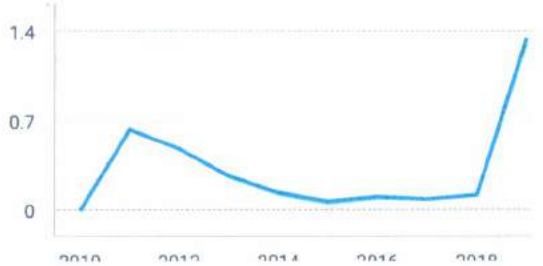

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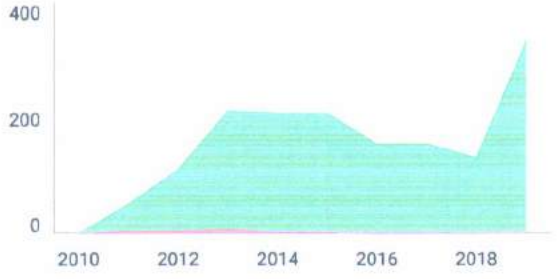

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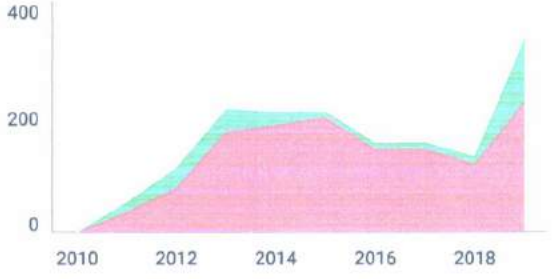

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