

Effect of Green Tea (*Camellia Sinensis*) Extract Administration on Sperm Quality of Mice (*Mus Musculus*) Exposed To 2,3,7,8-Tetrachlorodibenzo-P-Dioxin

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ABSTRACT

The aim of this research is to analyze the effect of green tea extract on motility, viability, and sperm concentration of mice exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The study used 25 male mice of BALB/c strain with an average weight of 20 gram were used in this research. TCDD was given single dose 7 µg/Kg BW via intraperitoneal injection while green tea extract was given orally for 53 days at a dose as follows: 1, 2, and 4 mg/Kg BW/day. Mice semen were collected then its motility, viability, and concentration were checked. Sperm quality were highest in mice exposed to TCDD and given green tea extract at a dose 4 mg/Kg BW/day than those mice exposed to TCDD and given green tea extract at the lower dose. The sperm motility, viability, and concentration mean were sequentially: $72.57 \pm 5.20\%$; $76.48 \pm 5.61\%$; 6.97 ± 0.11 million/mm³. In conclusion, administration of green tea extract at a dose of 4mg/Kg BW/day could maintain sperm motility, viability and concentration from a damage caused by TCDD.

Keywords: green tea extract, motility, viability, concentration, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

INTRODUCTION

Increased environmental pollution is an inevitable consequence of modern human life. One of the pollutants that have high toxicity is 2,3,7,8-tetrachlorodibenzo-p-dioxin or commonly called TCDD. TCDD is the most toxic compound based on derivation of toxic equivalent factors (TEF) [1]. TCDD is stable and fat soluble so that it can accumulate in body tissues, and its concentration multiplies at a higher level in the food chain [2]. TCDD is toxic to the endocrine, reproductive, and carcinogenic properties [3]. TCDD induces the production of free radicals or Reactive Oxygen Species (ROS) excess in the body causing oxidative stress, which will further damage various components in the cell so that its activity is disrupted [4]. Previous studies showed that fertility in male mice exposed to dioxin decreased, due to a disruption in the process of spermatogenesis in the form of decreased spermatogenic cell numbers and diameter of seminiferous tubules [2]. Free radicals generated by TCDD can increase the production of cytochrome C which will increase apoptosis in cells [5].

Green tea (*Camellia sinensis*) is known as a potential source of antioxidants that are beneficial to health. Flavonoid compounds in green tea have been shown to have anti-carcinogenic properties, anti-allergenic properties, anti-sclerotic properties, and their antioxidant activity is much higher than other compounds [6]. Flavonoids can contribute hydrogen or electron groups to free radicals and break the chain reactions in them so that cell damage can be prevented [7]. Previous studies have shown that the catechin content in green tea can balance the production of anti-apoptotic proteins so that it can prevent the death of mice testicular germ cells due to X-ray radiation [8].

MATERIAL AND METHODS

This research received ethical clearance number: 1.KE.166.08.2019 released by Animal Care and Use Committee, Universitas Airlangga, Faculty of Veterinary Medicine. The study used 25 BALB/c *Mus musculus* were randomly divided into five groups consisting of 5 animals in each. TCDD used for this study were 7 µg/kg BW at a single

dose. T1, T2, and T3 groups received 1, 2, and 4 mg/Kg BW/day of Camellia sinensis ethanolic extract orally for 53 days. While the control group C received 1,2 mg/kg BW of epigallocatechin-3-

gallated dissolved in aquades as vehiculum. After 53 days of treatment, the left testicles were collected and the sperm quality were examined under microscope with 400x magnification.

RESULTS

Table 1. Motility, viability, and concentration of mice sperm mean results after 53 days of treatment

Treatment	Mean \pm SD		
	Motility (%) \pm SD	Viability (%) \pm SD	Concentration (million/mm ³) \pm SD
C-	70.83 ^c \pm 9.93	73.92 ^c \pm 8.03	7.23 ^e \pm 0.04
C+	32.22 ^a \pm 2.71	35.48 ^a \pm 2.77	3.01 ^a \pm 0.05
T1	36.03 ^a \pm 2.37	39.92 ^a \pm 4.87	3.11 ^b \pm 0.04
T2	50.61 ^b \pm 6.41	54.03 ^b \pm 4.54	4.77 ^c \pm 0.06
T3	72.57 ^c \pm 5.20	76.48 ^c \pm 5.61	6.97 ^d \pm 0.11

Different superscript indicated significant differences (p<0,05)

C- group received aquades for 53 days

C+ group received 1,2 mg/Kg BW of epigallocatechin-3-gallated dissolved in aquades for 53 days

T1 group received 1 mg/Kg BW/day of Camellia sinensis ethanolic extract orally for 53 days.

T2 group received 2 mg/Kg BW/day of Camellia sinensis ethanolic extract orally for 53 days.

T3 group received 4 mg/Kg BW/day of Camellia sinensis ethanolic extract orally for 53 days.

Based on table, there was a significant decrease in the C + group compared to C- for all observed parameters. The results of spermatozoa quality test have the same pattern on motility and viability, while the spermatozoa concentration appears to have a significant difference (p <0.05) in each treatment group. In the C + group to the T3 group experienced a gradual increase, with a T3 result that was insignificant different from the C- group.

DISCUSSION

After entering the body TCDD will bind AhR (Aryl hydro carbon Receptor) in the cytoplasm then move to the nucleus. TCDD and AhR complex will bind to Aryl hydro carbon Receptor Nuclear Translocator (ARNT) thereby increasing cytochrome production through stimulation of the cytochrome P450 gene [9]. This complex will also bind to the Dioxin Response Element (DRE) in the target DNA which will induce several enzymatic systems to produce free radicals in the body [10]. Free radicals or excessive Reactive Oxygen Species (ROS) in the body will cause oxidative stress, which will further damage various

components in the cell so that its activity is disrupted [11]. Male reproductive organs are very susceptible to ROS because they contain high unsaturated fatty acids, including testes and epididymis [12]. Fenton's reaction that occurs due to TCDD exposure will cause lipid peroxidation and DNA damage resulting in necrosis [1]. In addition, lipid peroxidation also produces Malondialdehyde (MDA) and 4-hydroxynonenal (HNE) which can increase caspase activation and release of cytochrome C. Cytochrome C is an expression in the mitochondrial pathway to activate programmed cell death or apoptosis. Apoptosis and necrosis then affected the decrease in sperm motility, viability, and concentration.

The low quality of spermatozoa in the C + group given epigallocatechin-3-gallate can be caused by the absence of others active ingredient content which also helps the antioxidant process. The active substances in pure herbs generally work synergistically to provide beneficial effects, so that if only one of the active substances is taken, the activity cannot be as maximal as pure extracts [13]. Although epigallocatechin-3-gallate occupies 59% of all catechins in green tea, the presence of other catechins contained in green tea such as EGC, ECG, and EC also plays a role and synergizes with each other to carry out antioxidant activities.

The catechins contained in green tea extract will form complexes with metal ions such as Cu and Fe, thereby preventing the Fenton's reaction from initiating free radicals. Green tea contains flavonoids and vitamin C at 0.28% per 100 g [14,15] which can act as free radical scavenger by donating electrons to ROS so that their reactivity is reduced.

CONCLUSION

The conclusion of this study is the administration of green tea extract of 4mg/Kg BW / day for 53 days can maintain the motility, viability, and spermatozoa concentration of mice exposed to TCDD

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article.

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