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Research Article

The Effect of Kebar Grass (*Biophtyum petersianum* Klotzsch) Extract on the Number of Leydig Cells of Mice (*Mus Musculus*) Treated with TCDD

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ABSTRACT

This study aimed to examine the effect of *kebar* grass extract on the Leydig cells number of mice to exposed 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD). These animals were divided into five groups. Twenty five BALB/C mice were used with five groups: Negative Control (C-), Positive Control (C+) with TCDD 7µg/kgBW IP, and treatment groups that treated with TCDD 7µg/kgBW IP and Kebar Grass orally for 53 days were presented T1 0,045mg/gBW/day, T2 0,080mg/gBW/day, and T3 0,135mg/gBW/day. Results showed that TCDD decreased the Leydig cells number, while kebar grass extract was increase the number of Leydig cells exposed by TCCD significantly ($p<0,05$). But the increase in the number of Leydig cells had not yet reached the normal number of Leydig cells.

Keywords: Kebar Grass, *Biophytumpetersianum* Klotzsch, Leydig cells, TCDD, mice

INTRODUCTION

The environmental contaminant, such as industrial processes like incineration of medical wastes, chlorine bleaching of paper and pulp and manufacture of pesticides can be called endocrine-disrupting by Dioxin [1]. The dioxin 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD) has been intensively studied and is known to be the most toxic compound among these compounds [2].Those studies demonstrated that TCDD altered the process of testicular steroidogenesis and caused a reduction of Leydig cell volume and number [3].

TCDD has been reported to induce superoxide anion, lipid peroxidation and DNA damage, Induction of oxidative stress upon exposure to TCDD are considered to be an important mechanism for the toxic effects of TCDD [4]. TCDD increases reactive oxygen species (ROS) in endothelial cells by the induction of CYP1A1 [5]. TCDD toxicity is very dangerous because it causes oxidative stress, therefore we need a compound that can break TCDD toxicity such as antioxidant compounds [6]. Antioxidants defend against excessive ROS levels through enzymatic (superoxide dismutase, catalases, and peroxidases) and non-enzymatic (vitamins, steroids etc.) mechanisms [7].

Antioxidant compounds are found in various plants, one of which is Kebar Grass (*Biophytum petersianum* K) which has a role in protecting against free radical attacks. The usage of kebar grass as a fertile fertilizer had been carried out in Papua Indonesia for a long time [8]. Kebar grass contains three types of chemical compounds flavonoids, saponins, and tannins that have the potential to give effect to the process of spermatogenesis. The administration of 5% kebar grass infusion could significant increase spermatogenesis activity [9]. The active compounds of kebar grass act as antioxidants by inhibiting the transcription of CYP1A1 cytosolic enzymes and steroid dehydrogenase genes through inhibition of AhR, as well as reducing the competitive binding of TCDD to AhR [10].

Research on kebar grass extract so far is not widely known, therefore the researchers wanted to observe the effect of kebar grass extract to the Leydig cell number in mice exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin to observe potential kebar grass in counteracting the toxic effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin. In the present study, we have sought to investigate whether the effect of Kebar grass extract in multiple dosages can increase the number of Leydig cells exposed by 2,3,7,8-tetrachlorodibenzo-p-dioxin.

MATERIALS AND METHODS

This research was conducted at the Laboratory of Experimental Animal Faculty Veterinary Medicine Universitas Airlangga, the preparation of the histopathology was conducted at the Department of Pathology Faculty Veterinary Medicine of Universitas Airlangga. Ethical clearance was obtained from the Faculty Veterinary Medicine Universitas Airlangga.

Materials

This research used 25 male mice *(Mus musculus)* 12 weeks old with the body weights between 25-35 grams obtained from Veterinaria Farma Surabaya. Apparatus that was required, 5groups contain 5 mice, food and drink, per oral needle, 1 cc syringe, scalpel, a petri dish, surgical scissors, and microscope. Materials that have been used were 2,3,7,8-tetrachlorodibenzo-p-dioxin, Kebar Grass extract, distilled water, CMCNa 0.5%, and formalin. The dosage of the 2,3,7,8-tetrachlorodibenzo-p-dioxin which has been used for the rats was 50 µg/KgBW. Then it was converted to the dosage of mice 50 x 0.14 = 7µg/KgBW. The dosage of Kebar Grass extract was by using three different dosage of 0,045 mg/gBW/day 0,08 mg/gBW/day, and 0,135 mg/gBW/day [9].

Methods

Experimental animals were obtained using simple random sampling procedure. There are five treatment groups (5 mice each group): C (-) was treated with a placebo, C (+) was exposed by TCCD 7μg/KgBW single dose intraperitoneal injection. T (1) group was exposed by TCCD 7μg/KgBW single dose intraperitoneal injection and treated by Kebar grass extract was given orally at a dosage 0,045 mg/kg BW/day. T (2) was exposed by TCCD 7μg/KgBW single dose intraperitoneal injection and treated by kebar grass extract was given orally at a dosage 0,080 mg/kg BW/day. T (3) was exposed by TCCD 7μg/KgBW single dose intraperitoneal injection and treated by Kebar grass extract was given orally at a dosage 0,135 mg/kg BW/day. The treatment were conducted for 53 days consecutively. The testicular examination was conducted by hematoxylin and eosin (HE) staining (Hematoxylin Staining for Millicell®-HA, Merck, Germany) and observed from five different fields by using a light microscope with 400x magnification (Olympus® CX-41). Data analysis was done by using the ANOVA test. Then continued with the Duncan test to determine differences in each group. Data were analyzed by using the Statistical Analysis Software program (SPSS) version 22.

RESULTS

Table 1. showed a decrease in the mean of Leydig cells number in the positive control group (C+) compared to negative control (C-). Furthermore, in groups T1, T2, and T3 there was a gradual increase in Leydig cells number. The increase in Leydig cells number that occurred in all treatment groups reached the highest size in the T3 group, which was 24.24. The result of the effect Kebar grass extract on the number of Leydig cells exposed to TTDC by calculating the normal Leydig cells in the mice's testes listed in Table 1.

Table 1: Amount of Leydig cells in each group.

Groups		Amount of Leydig cells (Mean ± SD)
C (-)	Placebo	$43,04^{e} \pm 3.36$
C (+)	exposed TCCD 7μg/KgBw single dose intaperitoneal injection	$4,08^{a} \pm 0.78$
T (1)	TCCD 7μg/KgBw single dose intaperitoneal injection and Kebar grass extract at a dosage 0,045 mg/gBw/day	$11,8^{b} \pm 2.31$
T (2)	TCCD 7μg/KgBw single dose intaperitoneal injection and Kebar grass extract at a dosage 0,080 mg/gBw/day	$16,2^{c} \pm 4.44$
T (3)	TCCD 7μg/KgBw single dose intaperitoneal injection and Kebar grass extract at a dosage 0,135mg/gBw/day	$24,24^{d} \pm 13.83$

Description: Different superscript in the same column indicate significant differences ($P<0.05$).

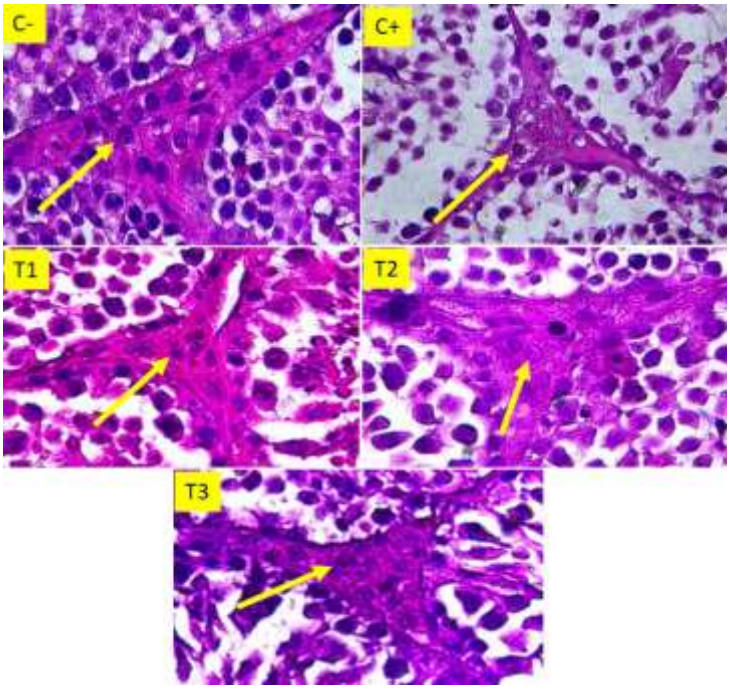

Fig.1: Overview of testicular microscopic image (Hematoxylin Eosin staining; magnification of 400x; Olympus® CX-41). Image of group C (-) indicates a normal Leydig cells. Image of group C (+) which induced by TCDD showed most decrease of number Leydig cells. Image of group T (1) showed improvement of Leydig cells. Image of group T (2) many cells showed normal shape of Leydig cells still look some cells died. Image of group T (3) showed the most normal cells between another treatment groups.

DISCUSSION

In group C (-), there were more normal Leydig cells than Leydig cells which occurred necrosis. Contrary to the C (+) group exposed to TCDD, the normal number of Leydig cells experienced a significant decrease. On histopathological features of Leydig cells exposed to TCDD given various doses of kebar grass extract increased significantly compared to the C (+) group. C(+) group that exposed by TCDD 7μ/KgBW single dose intraperitoneal injection proved that TCDD exposure affected the number of Leydig cells due to highly toxicity (Figure 1). The results of the research in this group similar with [3] that hazardous chemicals can cause antiandrogenic or estrogenic disorders. Antiandrogenic disorders can cause infertility by suppressing androgen production in Leydig cells, reduce the number of Leydig cells, induce Leydig cell apoptosis, or increase aryl hydrocarbon (AhR) to bind to TCDD thereby blocking androgen activity [11].

TCDD ligands, Arylhidrocabon Receptor (AhR), and Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT) will bind to certain DNA, namely Dioxin-Responsive Enhancer Elements (DRE), the bond will change the expression of various genes including cytochrome P450 in the nucleus [10]. Increased cytochrome P450 can affected on Leydig cells through a receptor called Androgen Receptor (AR) which plays a role in the maturation of the steroidogenesis pathway [12]. Cytochrome P450 bond with Androgen Receptor (AR) is known to increase Reactive Oxygen Species (ROS) production, so that it can trigger an increase in superoxide radicals which could cause membrane lipid peroxidase [13]. However, an increase in cytochrome P450 results in the formation of cytochrome P450 bonds with AR which could reduce the action of AR on Leydig cells and increase ROS formation in cells so that death occurs in Leydig cells [14].

Furthermore, in groups T1, T2, and T3 there was a gradual increase in Leydig cells number. The treatment group that exposed by TCDD single dose 7μ/KgBW intraperitoneal injection and treated by kebar grass extract at dosage of 0.135 mg/kg BW/day had the most significant number of normal Leydig cells was 24.24 when compared with T (1) and T (2) that treated with kebar grass extract 0,045 mg/gBw/day and 0,080 mg/gBw/day. This shows that kebar grass extract can increase the number of Leydig cells that exposed by TCDD.

Kebar grass (*Biophytum petersianum* Klotzsch) was a plant belonging to the family group Oxalidaceae found in Kebar District, West Papua Indonesia [15]. Kebar grass contains flavonoids, retinol, tocopherol and saponins [8]. Flavonoids that had a primary antioxidant function because they were free radical acceptors so they could inhibit free

radical chain reactions in lipid oxidation that could prevent membrane damage [16]. Polyphenol compounds such as flavonoids work by changing H_2O_2 into H_2O and O_2 [17]. The content of retinol (vitamin A) in Kebar grass functions as an antioxidant. Beta carotene works by reacting with free radicals and causing free radicals to become stable. Beta carotene (retinol) works together with tocopherol contained in Kebar grass and ascorbic acid (vitamin C). Furthermore ascorbic acid which turns into radicals is stabilized by the natural antioxidant glutathione [6]. The content of tocopherol (vitamin E) in Kebar grass prevented free radicals by giving H atoms from hydroxyl groups to lipid peroxyl radicals. Radicals formed from alpha-tocopherol would be stabilized through electron delocation in the aromatic ring [18]. Vitamin E can reduce TCDD-induced AhR activation so that it can be used as prevention and treatment of acute or chronic TCDD intoxication [12]. This can be strengthened by research conducted by [19] which proved that vitamin E has an antagonistic effect on TCDD toxicity in the process of spermatogenesis. Saponins included in the steroid group compound in an acidic stomach will break the sugar portion, so that it can have an effect for increasing the in free sterols. These sterol compounds are the basic ingredients of testosterone (pregnenolone) [20]. From this explanation it can be seen that saponins can increase testosterone levels which accelerate the Leydig cells number.

In this study, there was a significant decrease in the positive control group (C+) in Leydig cells number when compared to negative controls (C-) due to TCDD exposure. Whereas in T1, T2, and T3 there was a significant increase in Leydig cells number each group. This proves that the administration of kebar grass extract at a dose of 0.045mg/g BB/day in group T1 can significantly increase Leydig cells number. In the T2 and T3 groups there was a significant increase in Leydig cells number when compared with positive controls. The three treatment groups differed significantly with C- which proved that all three doses cannot maintain the Leydig cells number. An increase in the number of Leydig cells had not yet reached the normal number of Leydig cells. Higher doses and longer doses can be used to achieve optimal results. The implication of oxidative stress in the etiology of several chronic and degenerative diseases suggests that antioxidant therapy represents a promising avenue for treatment. In the future, a therapeutic strategy to increase the antioxidant capacity of cells may be used to fortify the long term effective treatment [21].

CONCLUSION

Based on research that has been done, it can be concluded that the Kebar Grass extract could increase amount of Leydig cells which were exposed by 2,3,7,8-tetrachlorodibenzo-p-dioxin, although the increase in Leydig cells had not reached a normal amount (C-).

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

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