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Kebar grass (*Biophytum petersianum K.*) The effect inmaintaining mice (*Mus musculus*) sperm quality exposed to dioxin

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ABSTRACT

Kebar grass contains active antioxidants and potential vitamins to neutralize TCDD toxicity. Prove that Kebar grass extract in various dosage can maintain viability, motility, and sperm concentration of male mice exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. This study was an experimental laboratory study with five groups consists of Negative Control (C-), Positive Control (K +) with TCDD exposure of 0.7μg/Kg BW IP single dose. The treatment group was given oral Kebar grass extract for 53 days treatment 1 (T1) with a dose of 0.045 mg / g BB, Treatment 2 (T2) with a dose of 0.08 mg/g/BW, and Treatment 3 (T3) with a dose of 0.135 mg/g/BW. The data of motility, viability, and concentration of spermatozoa obtained were analyzed using One Way Anova test and Duncan Multiple Range Test. Administration of Kebar Grass Extract at a dosage of 0.135 mg/g/BW showed a significant difference between the control group and the treatment group ($p<0.05$). Exposure to TCDD in C+ decreased motility ($13 \pm 6.70\%$), viability ($28 \pm 19.35\%$), and concentration (0.87 ± 0.64 cells / mm^3) of sperm significantly compared to C-. The administration of Kebar grass extract can maintain motility ($74 \pm 5.47\%$), viability ($76 \pm 2.72\%$), and concentration (2.50 ± 0.69 cells/mm^3) spermatozoa in the T3 group with a dose of 0.135mg/g/BW. Kebar grass extract is effective for maintaining the quality of mice sperm from damage due to exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin.

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INTRODUCTION

One of the many pollutant compounds is a group of Persistent Organic Pollutants (POPs) which can interfere with the ecosystem. There are twelve banned POPs, referred to as "dirty dozen," due to their persistence and toxicity to the biotic elements of the ecosystem such as dioxin compounds (Baqar *et al.*, 2017). 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have reprotoxic properties which means can cause intoxication in the reproductive system, especially the male reproduction. Dioxin exposure can cause a decrease in spermatozoa concentration

from 22.19 million cells to 13.10 million spermatozoa per mm^3 of semen (Latchoumycandane and Mathur, 2002). Because there are low concentrations (parts per trillion—ppt; ng/g) of the seven dioxins, 10 PCDFs, and 12 dioxin-like PCBs of concern in humans, a relatively small additional exposure from work, environment, or food cannot always be detected (Schecter *et al.*, 2006). Research conducted by Choi *et al.* (2008) proved that administration of TCDD in mice induced testicular histology changes, an imbalance of spermatogenesis, an increase in estradiol, and a decrease in testosterone.

Kebar grass (*Biophytum petersianum K.*) is a plant originating from Kebar sub-district, Papua. This plant is not widely known yet, but in Papua empirically used as a fertility enhancer. Kebar grass contains phytochemical elements such as alkaloid, saponin, tannin, glycoside and flavonoid (Baaka *et al.*, 2017). Research conducted by Lefaan (2014) showed that kebar grass given to male mice was proven to increase spermatogenesis activity in mice. Sembiring and Darwati (2016) states that kebar grass contains flavonoids and calcium, which are useful for fertility. Research on kebar grass has not been widely carried out. Therefore it is necessary to investigate the effect of kebar grass extract on male mice sperm quality of that have been exposed to TCDD or 2,3,7,8, -tetrachlorodibenzo-p-dioxin.

MATERIALS AND METHODS

Sample and Population

The sample of this study was male mice aged three months with the certified ethical clearance. A total of 25 male mice were randomized into five groups contained four replications and one correction factor for each group. The treatment group was Negative Control (C-) with aqua dest, Positive Control (C+) with TCDD exposure 0.7μg/KgBW IP and aqua dest, Treatment 1 (T1) with TCDD andKebar Grass Extract 0.045 mg/g/BW, Treatment 2 (T2) with TCDD and Kebar Grass Extract 0.08 mg/Gb/W, and Treatment 3 (T3) with TCDD and Kebar Grass Extract 0.135 mg/g/BB. TCDD treatment was given once single dose IP injection while the treatment of Kebar Grass Extract is given orally for 53 days.

Material Preparation

Kebar grass was processed into 70% ethanolic extract. A total of 350 grams of crushed and dried Simplicia, macerated with 70% ethanol solvent (ratio 1:10) in a tube for 76 hours, then filtered and the pulp was macerated two times with the same treatment. The collected macerate was evaporated using a rotary evaporator at a temperature of 30-40^{0}C to form a thick extract. This thick extract is put into a bottle and stored in a refrigerator (Claudya *et al.*, 2016).

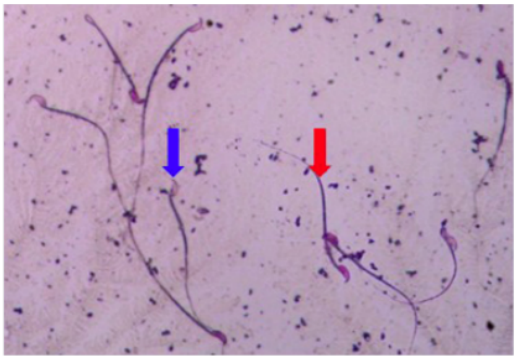

Figure 1: Viability of mice spermatozoa collected. The blue arrow shows the life spermatozoa while the red arrow shows the dead spermatozoa

Determination of Dosage

TCDD was given to mice through intraperitoneal at a dose 0.7μg / KgBW TCDD exposure in mice after being converted from rat dose according to the previous study carried out by Choi *et al.* (2008). Lipophilic TCDD dissolved in corn oil Geyer *et al.* (1993) and injected with a volume of 0.1ml / mice (Wati *et al.*, 2014). Kebar grass extract is given in 3 dosages. The lowest dosage is 0.045 mg / Gb/W, and the highest dosage is 0.135 mg/g/BW. The middle dose of 0.08 mg/g/BW is obtained from the calculation of the dose interval.

Data Collection Technique

After the termination of mice, the sperm was taken through cutting the left. Right cauda epididymis then dissolved it in 0.5ml of NaCl 0.9%, To see the sperm motility of the cauda epididymis solution, one drop was taken using a pipette and viewed on a glass slide object for witnessed the movement of the spermatozoa in the Nikon Eclipse E-100 microscope with 100x magnification. Determination of sperm motility is done by looking at individual progressive movements. Assessment of sperm viability was done by making a slide with Eosin Negrosin staining. The percentage of live spermatozoa expresses the results in each sample.Semen is dripped on the glass object using an ose. Eosin Negrosin staining is dropped use another ose, and then both are mixed. A mixture of semen with eosinnegrosin is made smeared with the tip of the other object until it is obtained spread along the surface of the object's glass. The samples were observed under a

Table 1: The Result of Sperm Quality of Mice

Treatment Group	Mean ± SD		
	Sperm Motility	Sperm Viability	Sperm Concentration
C-	68 ± 8.36^{b}	83 ± 10.70^{b}	2.62 ± 0.21^{b}
C+	13 ± 6.70^{a}	28 ± 19.35^{a}	0.87 ± 0.64^{a}
T1	51 ± 30.65^{ab}	63 ± 31.24^{ab}	1.43 ± 0.60^{a}
T2	54 ± 30.49^{ab}	58 ± 32.53^{ab}	3.06 ± 0.71^{b}
T3	74 ± 5.47^{b}	76 ± 2.72^{b}	2.50 ± 0.69^{b}

light microscope. Negrosin increases the contrast between the background and sperm heads, making sperm easier to visualize. Eosin stains only the dead sperm, turning them a dark pink, whereas live sperm appears white (Agarwal *et al.*, 2016).

The Spermatozoa Concentration was seen using the Neubauer Improved counting chamber (Sarbishegi *et al.*, 2017). Cauda epididymal solution was taken to reach a scale of 0.5 using the erythrocyte pipette, then taking 2% eosin stain until the pipette was filled to a scale of 101 and shaken. Then it was dripped on the counting board, covered by a thin slide cover glass and observed on a microscope with 400x magnification in the area of five big square (Harahap *et al.*, 2017). Analysis of the results was using One Way Anova test.

RESULTS AND DISCUSSION

The results of the research data were first tested for normality by saphiro wilk test. Furthermore, the results of sperm quality test data include motility, viability and concentration of spermatozoa mice can be seen in Table 1 and Figure 1.

Different superscripts in the same column indicate a significant difference($p<0.05$). Negative Control (C-) with aquadest, Positive Control (C+) with TCDD exposure 0.7 μg/KgBW, Treatment 1 (T1) with TCDD andKebar Grass Extract 0.045 mg/gBW, Treatment 2 (T2) with TCDD and Kebar Grass Extract 0.08 mg/gBW, and Treatment 3 (T3) with TCDD and Kebar Grass Extract 0.135 mg/gBB.

When TCDD enters the body and interacts with cells, TCDD diffuses through the plasma membrane and binds to aryl hydrocarbon receptor(AhR), the chaperone proteins are released, andthe (AhR-agonist) complex binds to the transcription factor AhR nuclear translocator protein (Arnt) (Sorg, 2014). TCDD, AhR, and ARNT ligand translocation processes activate phase 1 and 2 gene coding enzymes such as cytochrome p450 enzymes (CYP1A1, CYP1A2, and CYP1B1), Glutathione transferase and NAD(P)H: quinone oxidoreductase 1 and aldehyde dehydrogenase 3 Das *et al.* (2017). Furthermore, the cytochrome p450 enzyme affects the cell components in male reproduction such as Leydig cells and spermatogenic cells which play a role in decreasing sperm quality. With impaired regulation of testosterone and ABP in Leydig cells, it can reduce sperm motility due to incomplete maturation of cells in the epididymis. In this study, it was clear that there was a significant decrease in the positive control group compared to negative controls both from the data of motility, viability, and concentration of spermatozoa as a result of TCDD exposure. Besides, TCDD is known to bind to Androgen Binding Protein (ABP), resulting in endocrine interference (Wati *et al.*, 2014).

In motility data, Treatment 1, Treatment 2, and Treatment 3 showed a gradual increase from the positive control group. Kebar grass is a plant that contains high calcium which can increase sperm motility. The administration of Kebar grass extract orally at a dose of 0.045 mg/g BW/day in the treatment group 1 could not significantly improve sperm motility due to TCDD exposure, so it showed a 51% rate. Likewise the Treatment Group 2 with the administration of Kebar grass extract at a dose of 0.08 mg/g BW per oral which showed the figure was not much different from the Treatment 1 group, which was 54%. Treatment group, 3 with the treatment of Kebar grass extract dose of 0.135 mg/g BW per oral, was the treatment group which showed the results of increased sperm motility after exposure to TCDD. Compared to the positive control group, treatment group 3 showed a 74% progressive rate of sperm movements.

The decrease in sperm viability is caused by the number of spermatozoa that die as a result of TCDD exposure. Excessive expression of cytochrome p450 can cause spermatogenic cell death. According to Zhou *et al.* (2017), TCDD gradually increased mRNA and protein levels of AhR and CYP1A1, in addition to the enzymatic activity. Mitochondrial activity and the mitochondrial membrane quality were also significantly attenuated, and ROS levels

were elevated. Membrane lipid peroxidase is a process of oxidation reactions that derived unsaturated fatty acids into malondialdehyde (MDA) (Catalá and Díaz, 2016).Spermatozoa cell membranes are rich in unsaturated fatty acids which are very susceptible to membrane lipid peroxidase reactions which can result in damage to sperm cells and cause death in sperm cells. Death Spermatozoa cells show red colour due to dyes that penetrate the membranes of the spermatozoa head, which are damaged while living spermatozoa show a bright colour as in Figure 1.

The results showed that the administration of Kebar grass with a dose of 0.045 mg/g BW/day did not provide a significant increase in the viability of treatment group 1 when compared to the positive control group with a percentage of viability of 62.8%. Significant changes occurred in the Treatment group 3 of the Positive Control group with 76.43% viability. Proves that the administration of Kebar grass at a dose of 0.135 mg/g BW/day can maintain mice sperm viability exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Kebar grass has various antioxidant properties, namely flavonoids, saponins and tannins (Lefaan, 2014). In this study, Kebar grass dose of 0.135 mg/g BW successfully maintained the viability of spermatozoa of mice after exposure to TCDD. Flavonoids in the Kebar grass can exhibit antioxidant properties through chelating with transition metals, primarily Fe(II), Fe(III) and Cu(II), which participate in reactions generating free radicals (Malesev and Kuntic, 2007). Kebar grass contains vitamins such as Vitamin E, and Vitamin A. Vitamin E functioning as a chain-breaking antioxidant was reported to protect cellular membranes against ROS, for example through defending polyunsaturated fatty acids (PUFAs) from auto-oxidation (Mutalip *et al.*, 2018). The entry of vitamin E into the cell can occur through a receptor mediation process or through a process assisted by lipoprotein lipase where vitamin E is released from the chylomicrons and VDL in cells. Intracellular transport of tocopherols requires intracellular tocopherol binding proteins. Vitamin E primarily located in the cell and organelle membranes where it can exert its maximum protective effect, even when its concentration ratio may be only one molecule for every 2,000 phospholipid molecules (Rizvi and Shania, 2014).

The administration of Kebar grass with Saponin content has the potential to increase testosterone through the formation of pregnenolone precursors which are composed of free sterols produced by the breakdown of saponin sugar groups. Excessive increase in testosterone can directly affect Sertoli cells to secrete inhibin which will act directly towards the anterior pituitary. Testosterone can aromatize to estradiol, which exerts negative feedback on the hypothalamus and pituitary gland (Majzoub and Sabaneghjr, 2016) because, in the hypothalamus, there are androgen and estrogen receptors (Tilbrook and Clarke, 2001).The decrease that occurs due to negative feedback is not too significant according to the results of statistical analysis. Comparison between the concentration of spermatozoa in group 3 and group 2 also had insignificant differences. This shows that Kebar grass has the potential to maintain the concentration of spermatozoa in male mice exposed to TCDD.

CONCLUSION

The administration of Kebar Grass Extracts dose 0.135 mg/g/BW can maintain viability, motility, and sperm concentration of male mice exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Kebar grass extract was effective for maintaining the quality of mice sperm from damage due to exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin.

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Conflict of Interest

Authors declare that they have no conflict of interest.

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