Comparative Morphology of Sperm in The Rat (Rattus novergicus) Exposed to Cadmium(Cd) and Mercury (Hg) with Green Coconut Water (Cocos nucifera L.) as Antidontum

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Abstract: Environmental conditions more polluted by heavy metal Cd and Hg resulted in various effects of toxicity to humans. One of them is an abnormality sperm morphology which can affect the occurrence of infertility. Green coconut water is plants that contain a variety of vitamins, minerals, amino acids and tannins that can be used to remove the toxins in the body. This research aims to know the difference count of the normal sperm and abnormal sperm morphology in rats exposed Cd and Hg with the giving of the Green coconut water. Research carried out experimentally using a Rattus novergicus which exposure two µ g/mL Cd and Hg. Rats were divided into six groups, each of which are exposed to metals Cd, Hg, Cd with green coconut water, Cd with green coconut water, and green coconut water as a control. The research results obtained a p-value for normal sperm count of 0.014, whereas in abnormal sperm count p-value 0.008 so it can be concluded there is a significant difference in the number of normal or abnormal sperm morphology of rat exposed Cd and Hg with the giving of the Green coconut water. The giving of green coconut water helps reduce effects sperm damage due to exposure Cd and Hg.

1 INTRODUCTION

Development of technology today provides convenience in human life. However, the negative impact that occurs is the environmental pollution due to heavy metal contamination. Heavy metals such as mercury (Hg) and cadmium (Cd) is the most abundant heavy metals found in nature, as it is used in industrial activities.

Various research has recently discovered the existence of the pollution of heavy metals in water, soil, air and even on sea life. Research in the area of coastal Subdistrict Medan Labuhan obtained Cd pollution of 0.0029 mg/L, and in Medan Belawan of 0.0042 mg/l. That values above the value of the raw quality of environment of 0.002 mg/L (Indirawati, 2017).

Research by (Komarawidjaja, 2017) in one wetland Bandung obtained the content of Hg of 92.2 mg/L with Hg quality raw value of 25.9 mg/L. Research on one of the quiet sea on the coast of Kenjeran by (Lestari, 2015). Hg metal levels obtained about 0.099 – 0.112 ppm. Hg levels of 0.005 mg/Kg total mercury and 0.0016 mg/Kg methyl mercury toxicity effects can give to humans, while the levels of cadmium that can give effect to toxicity in humans is 0.007 mg/Kg (Badan Standarisasi Nasional, 2009). Exposure heavy metals Hg can improve fetal abnormalities and death when born and may cause Fetal Minamata Disease. In addition, Hg can cause nerve damage, brain damage, cerebral palsy, motor and mental retardation. Hg exposure in men will can cause impotence and libido disorder while in women will cause menstrual disorders (Putranto, 2011). Toxicity of Cd (Cadmium) cause kidney disease, stomach disorders, brittle bones, reduced hemoglobin, pigmentation teeth (Ratmini, 2009).

Mice exposed to mercury were found to have a lower sperm count than mice not exposed to mercury (Heath et al., 2012). Research Martinez (2014), obtained as a result of mercury can lower production,

the number and motility of the sperm and morphological abnormalities in the head and tail of the rat sperm (Martinez et al., 2014).

Cadmium in low doses can damage sperm quality and reduced fertility. In addition, morphological abnormalities also found the presence of either a deformed heads or tails of sperm (Adamkovicova et al., 2016). It is also found in similar research Wang (2017), where the sperm viability and motility are exposed to cadmium decreased significantly (Wang et al., 2017).

Coconut water is a common drink in Indonesia society. Even the type of green coconut is believed to be used for the treatment or antidote to poison. Green coconut is a young coconut that contain more tannin than other kinds of coconut. This substance can be used as parser toxins in the body. In addition, coconut water contains various minerals, vitamins, sugar, and essential amino acids (Candra C et al., 2016).

The research of Zulaikha (2015) against gold mining in workers exposed Selogiri Hg showed an increase in activity of antioxidant enzyme SOD, CAT, glutathione peroxidase (GPx) after consuming coconut water as much as 450 mL/day for 30 days (Zulaikha et al., 2015).

This research would like to examine the difference in the number of normal and abnormal sperm morphology in Hundred novergicus by awarding the Green coconut water (Cocos nucifera L.).

2 METHOD

This research method is experimental, with Design Complete/Complete Randomly Random Design (CRD) with the treatment that is the control group, treatment group metals Hg, and Cd, the Group's treatment of metals Hg and Cd which was given green coconut water. The animals to use for this research are a white Rat Rattus novergicus L. weight controlled for all the treatments. Each group has 3 repetitions. The amount of each treatment group consists of 3 mice, so total number of mice used in this study as many as 18 mice.

The awarding of the heavy metals are conducted orally for 4 weeks as much:

1. Hg Group: given the Hg 2 µ g/mL, 3 mL/week;
2. Cd groups : given a Cd 2 µ g/mL by, 3 mL/week;
3. Control group was given a mineral water.
4. Contol group cocos was given green coconut water
5. Hg and cocos Group : given Hg 2 µ g/mL, 3 mL/week and green coconut water instead of mineral water.
6. Cd and cocos Group : given Cd 2 µ g/mL, 3 mL/week and green coconut water instead of mineral water.

After 4 weeks of treatment, the animals to determine with chloroform were placed in the chamber. Then surgery and taken the testes by using tweezers. The testes of mice placed on a measuring cup containing NaCl in order to easily separate the testicles with fat.

After the surgery, then taking of secretion of the cauda epididymis with microscope magnification 400x, cauda epididymis are separated by means of cutting 1/3 part of the kauda epididymis. Then separated from lipid bonding. Cauda epididymis put on a petri dish containing a 1 ml NaCL 0.9%. Then cauda epididymis pressed with fluid secretions slowly to the epididymis out then put in a petri dish and suspended with NaCl 0.9%.

Cauda epididymis of spermatozoa suspensions have been obtained can be used for motility of spermatozoa observations. Identification of morphology of spermatozoa is performed by placing the sperm suspension on glass objects, make preparations applying with glass to other objects forming an angle of 45 ° above, dried and fixation with alcohol during the 3-5 hours, colored with 3% giemsa for 45 minutes, rinsed with the flow water, then dried at room temperature. The observation is done under a microscope with a magnification of 400 and 1000 times plus emersi oil.

Morphology of spermatozoa with 100 count spermatozoa in one viewpoint with repetition 4 times in each sample. According to WHO, the normal spermatozoa morphology consists of the head (caput) which shape is bent like a hook the middle part (middle piece) are short and the tail (cauda) a very long.

3 RESULT AND DISCUSSION

After being given treatment for 4 weeks, mice dissected and carried out observations on the morphology of sperm. Based on the observations, found the presence of sperm morphological abnormalities in mice exposed to heavy metals. The form of the disorder in the form of sperm without heads, without tails, double curved tail, head, and tail of the coil (see figure 1).

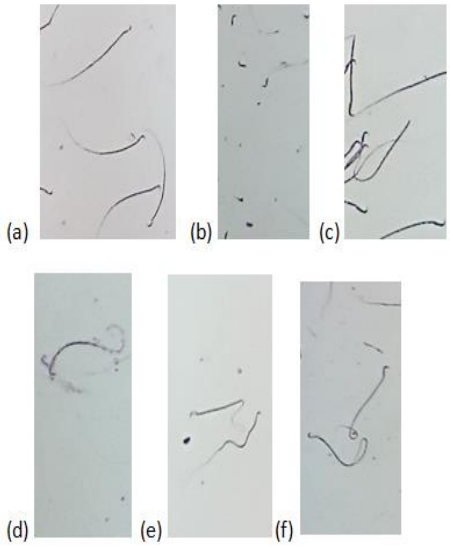

Figure 1. Normal and abnormal sperm morphology with 400x enlargement (a) normal sperm; (b) without tail; (c) without the head; (d) double head; (e) a curved tail; (f) coil tail. The results of calculation sperm morphology with 4 repetitions obtained average number of normal and abnormal morphology sperm on each group's treatment as follows:

Table 1. Results of the average number of Normal and Abnormal Morphology on each group's treatment

No.	Treatment	Sperm	
		Normal	Abnormal
1	CD 1	124	176
2	CD 2	30	270
3	CD 3	80	180
4	CD CCS 1	127	173
5	CD CCS 2	155	145
6	CD CCS 3	120	151
7	HG 1	89	211
8	HG 2	41	259
9	HG 3	80	221
10	HG CCS 1	239	61
11	HG CCS 2	232	85
12	HG CCS 3	220	74
13	CCS 1	262	38
14	CCS 2	208	92
15	CCS 3	250	51
16	KONTROL 1	290	10
17	KONTROL 2	267	33
18	KONTROL 3	210	28

If the calculation result displayed in a graph will appear as figure 2.

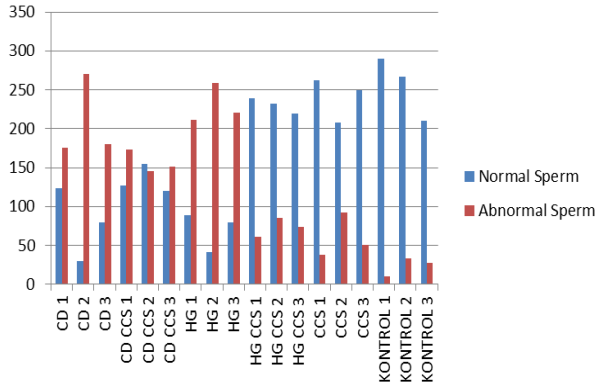

Figure 2. A graph of the average number of normal and abnormal sperm in rat.

Based on the tables and charts, it can be seen that the number of abnormal sperm morphology in rat exposed to metals Hg and Cd more than rat exposed to Cd and Hg are given a green coconut water. The results support the research by Adamkovicova (2016), where the cadmium exposure changes the morphology of sperm and reduced sperm fertility. Cadmium specifically interfere with sertoli cells in tubule seminiferus causing failure in spermatogenensis. Damage to the tubules seminiferus causes abnormalities in the early development of sperm. In addition, exposure to low-dose cadmium can affect steroid hormone involved in the reproductive process. Decrease in the number and quality of sperm are associated with a decrease in the amount of testosterone and oxidative damage resulting from suppressed antioxidant enzyme activity (Adamkovicova et al., 2016).

Hg exposure can reduce the activity of creatine kinase in human sperm by an apparently competitive inhibition, possibly through displacement of Magnesium (Mg) in this enzyme. Cretaine kinase has a important role in supplying energy homeostasis sperm, so the reduced activities of creatine kinase can interfere with the function of sperm (Ghaffari and Motlagh, 2011).

Martinez et al (2014) that examines the impact of exposure to mercury for 60 days against sperm, getting results that exposure to mercury can be decrease sperm quality and disrupt the balance of LH hormones. High mercury exposure and for a long time can lower testosterone, LH, FSH and prolactin. Mercury can cause oxidative stress on organs in the body. One form of mercury toxicity in reproductive organs is the occurrence of hypospermatogenesis. It indicated an increase in fat in the testes as well as the presence of peroxide reduction of antioxidant enzymes activities in the reproductive organs due to

exposure to the heavy metal mercury (Martinez et al., 2014). Cd exposure in the short term against the male rats found the existence of a significant decline in sperm motility. This is caused because there are disturbances in calcium concentration related to important processes in spermatogenesis (Wang et al., 2017).

The results of the Zhou research show the harmful effects of exposure to cadmium on quality sperm. Sperm exposed to cadmium could not do against ovum fertilization. Exposure to cadmium in high doses may interfere with the absorption of calcium. Calcium is one of the components that are present at the tip of the sperm that help the entry of sperm into the egg cell membranes to tejadi conception (fertilization) (Zhao et al., 2017). Heavy metals such as Cd and Hg is a form of free radicals that can cause the occurrence of damage to cells in the body. Most free radicals in biological systems of the body are a type of derivative of oxygen/oxygen reactive species (ROS).

Oxidation reactions involving free radicals in the body can damage the cell membranes of normal and damage DNA composition so that it can result in the occurrence of mutations. DNA damage which can lead to occurrence of diseases or abnormalities in the body, including sperm morphological deformities (Parwata, 2015). Oxidation at one of the bases of DNA constituents that is Guanosine into 8-hydroxy-2-Deoxy-Guanosine (8-OHdG), could be a hint of the existence of DNA damage due to free radicals. Guanosine can experience normal metabolism is hydroxylation in response or because of the pollution of heavy metals and chemical substances that are carcinogenic (Parwata, 2015). In addition, the spermatozoa in mammals have a polyunsaturated fatty acid that much so susceptible to ROS. As a result, the unsaturated fatty acids can undergo degradation and loss of integrity of the strutural needed for survival and motility of sperm (Martinez et al., 2014).

Furthermore, the observations are analyzed by using the test analysis Kruskall-Wallis. SPSS results show the value of the p value for the amount of normal sperm morphology is 0.014, whereas p value for the number of abnormal sperm morphology is 0.008. The value of less than 0.05, so in this study, there is a significant difference in the number of sperm morphology of rat exposed to heavy metal Cd and Hg with the giving of the Green coconut water. The number of abnormal sperm in the rat exposed to the metal without awarding a green coconut water more than the mice given the Green coconut water. Exposure of cadmium and mercury in high doses for 4 weeks against rat resulted in damage or deformities on the sperm cells.

Biologically, the body produces endogenous antioxidant enzymes, namely in the form of superoxide dismutase (SOD), catalase (CAT) and glutation peroxidase (GPx). However, if free radicals quite a lot in the body exceeds the amount of antioxidants that will happen oxidative stress. Endogenous antioxidant require additional antioxidants from outside the body to neutralize and inhibit the occurrence of oxidation reactions involving free radicals (Parwata, 2015).

Green coconut water contains amino acids, among others is glutamic acid, arginine, lysine, leusin, proline, aspartic acid, alanin, histidin, fenilalanin, serine, cysteine and tyrosine, which can protect the body from free radicals.Content of amino acids in green coconut water can increase the antioxidants SOD, CAT and GPx in the body, thus being able to suppress free radicals from outside. Derivatives of phenol compounds contained in green coconut water is able to inhibit the enzyme that produces the work of ROS forming khelat with metal that trigger the formation of ROS, oxidative stress so that it can be prevented. It can be seen in rats fed coconut water has a number of morphological normal sperm more than mice not given the coconut water in conditions of equally polluted heavy metal Cd and Hg. (Zulaikha et al., 2015)

Consumption of 450 mL/day of green coconut water during 30 days increases the antioxidant enzyme SOD, CAT, GPx and reduce lipid peroxidation on workers who are exposed to mercury. (Zulaikha et al., 2015)

The content of L-arginine on the Green coconut water can be used to reduce the free radicals, increase antioxidant activity and inhibit the concentration of fat. L-arginine do to detoxify heavy metals, increases towards the enzyme SOD activity. In addition, in the Green coconut water contained high amounts of vitamin C that can be used as an antioxidant. Selenium content of green coconut water can strengthen the activity of GPx enzymes as antioxidants (Zulaikha et al., 2015).

Green coconut water consumption can increase endogenous antioxidant enzymes activity in the body, so that it is able to suppress the side effects that occur if the body is exposed to heavy metals such as Cd and Hg.

4 CONCLUSIONS

The results of this study showed a difference in the count of normal and abnormal sperm in mice that

were exposed to heavy metal Cd and Hg with the giving of the Green coconut water, where mice given the Green coconut water abnormal sperm count less. Green coconut water consumption may reduce toxicity effects on sperm from exposure to heavy metal Cd and Hg.

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