EFFECT OF ADMINISTRATION MARTAPURA RIVER WATER ON HISTOPATHOLOGICAL FEATURES OF RAT TESTES

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Abstract - This study aims to prove that there are effect changes in the histopathological features of rat testes administration Martapura river water. The results of the study indicate there are significant changes to enlargement of testicular size (p=0.036), damage to seminiferous tubules (p=0.039) and depletion of seminiferous tubule walls (p=0.000) in rats administration Martapura river water.

Index terms - Martapura river water, testes, seminiferous tubulus, histopathological features

I. INTRODUCTION

Martapura River is a river that is very important for the life of the people of Martapura, South Kalimantan, Indonesia. This river has has been polluted by heavy metal pollution such as heavy metals Pb, Cd, Hg and Fe [1], [2]. Research on the effects of heavy metals such as lead (Pb) on changes in various organs has been carried out. Lead (Pb) is known to cause changes in the testicular organs and purkinje cerebellum cells [3-8]. Another various heavy metal pollutans cadmium causes interference in various organs such as brain[9],[10], kidney[6],[11], liver[12],[13], ovary[11],[14-16] bone and muscle[17].

The mechanism of organ damage due to heavy metals have stated that organ damage to cellular level through changes in cell signal cascade pathways, cell to cell adhesion, damage to protein structure, and oxidative stress [10],[18]. Cellular damage by heavy metals begins with mild injury stage injury occurs due to activation of the autofagi process and non-proliferative repair. If exposure to heavy metal concentrations increases, there will be a mechanism for releasing cell-to-cell bonds, and disruption of cell signaling pathways that have an impact on cell necrosis and apoptosis [19],[2]. The testes are male reproductive organs that produce sperm cells in the seminiferous tubules. The seminiferous tubule is the site of spermatogenesis, in the event of damage to the seminiferous tubules, especially in the tubular wall which contains many spermatogonia cells and spermatocytes with various stages of development, which can result in infertility[21-22]. There are many factors that influence decreased testicular function caused by cell damage. One of the factors is testicular poisoning by heavy metals. Based on research reports it is known that heavy metals can cause testicular germ cell damage in the form of apoptosis and cell necrosis in rodents, banded knife fish, Pseudosciaena crocea (large yellow croaker), and Oreochromis niloticus (Nile tilapia) [23-24].

II. SUBJECT AND METHOD

Animal

Total number of study subjects were 32 rats (Rattus novergicus), males, aged 2-6 months, and weight 250 - 300 g; obtained from BVET Banjarbaru. The study group was divided into 2 groups (16 groups each) namely the control group (given aquades) and treatment (given the river Martapura river) for 30 days. Before the treatment period the mice were acclimatized for 7 days. Mice are maintained according to maintenance standards in standard laboratories. Given a special cage made of plastic boxes with a size of 60 cm x 50 cm x 22 cm given a cage under the form of dry husk and given a lid from the wire each cage is only filled with 4 tails. For cage beds are replaced once a week continuously. Feeded specifically for animals, given an ad libitum drink. Evaluated physical and psychological conditions of mice every day.

Administration of Martapura river water

Rats were administration Martapura river water as a substitute for drinking water ad libitum. Rats were sacrificed on the 31st day and continued the process of making testicular histopathology with HE staining.

Surgery and Testicular Isolation

Rats were sacrificed by anesthesia using pentobarbital sodium drug. After the rat was declared dead, an autopsy of the abdomen was performed to isolate the testicular organ. Testicular organ pieces were immediately fixed by soaking the organ in fixative solution (formol saline) for 24 hours or more.

Hematoxylin Eosin (HE) Staining

Starting with the deparafinization process by means of preparations that are ready to be soaked first in a solution of xylol I and xylol II for 2 minutes. Continued the hydration process in an alcohol solution with a decreasing concentration of concentration, namely from 100% -95% -90% -80% -70% for 2 minutes each. Then the preparation is incubated in Hematoxylin Harris solution for 15 minutes, then rinsed in running water for 5-7 minutes. Preparations are dipped in ammonium or 0.4%

carbonate lithium quickly as much as 3-10 x, then checking color differentiation under a light microscope. The preparation is rinsed again in running water for 1 minute. The preparation is dipped in lithium carbonate solution so that the preparation is bright blue. Preparations are rinsed in running water for 10-20 minutes. The preparation is incubated in 1% eosin for 2 minutes. The process of dehydration is continued by dipping the preparation into alcohol with a concentration that increases slowly, each for 2 minutes. The preparations were soaked in xylol I and xylol II solution for 2 minutes each. Continued sealing and covered with a cover glass. The preparation is ready to be observed under a light microscope.

Measurement of testicular size and microscopic analysis of seminiferous tubules

Measuring the size of the testes using a ruler (units of cm). Microscopic analysis of seminiferous tubules using the binocularmicroscop [Nikon] and software [Image Raster], with a 10 µm and 100 µm scale and magnification of 10 x.

Statistical analysis

The results of the research data were analyzed by independent t-test to evaluate alteration in body weight and testes in the control and treatment groups. In this study using a 95% confidence interval and significant data if p value <0.05.

III. RESULT

Alteration in rat body weight control and treatment can be seen in table 1.Morphological alterations from both control and treatment groups can be seen in Table 1 and Figure 1.

Table 1. Morphological alterations from both control and treatment groups

Alteration on testes	Control	Treatment	P value
Testicular size(cm)	2.493±0.58	2.893±0.62	0.036
Damage to the seminiferous tubules (%)	9.38±11.09	75.75±23.79	0.039
Seminiferous tubule wall thickness (mm)	123.12±43.38	95.72±12.29	0.000

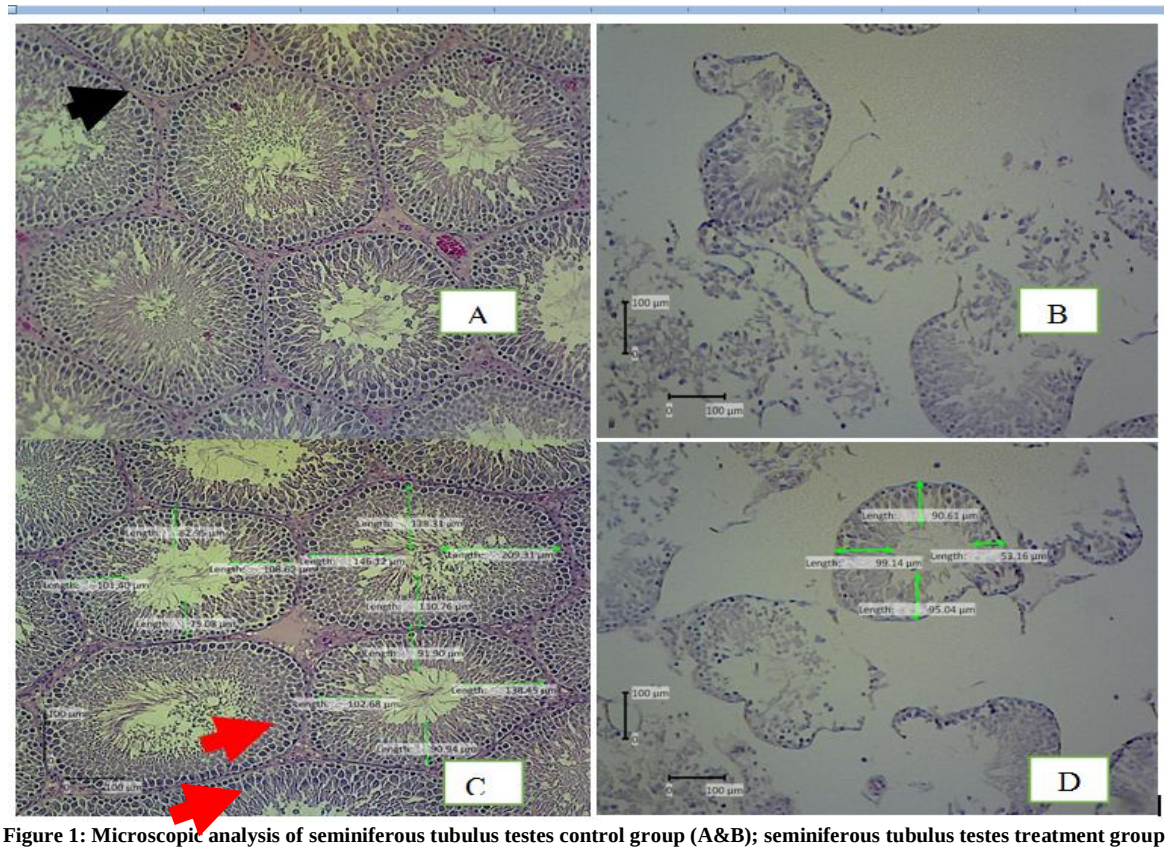


Figure 1: Microscopic analysis of seminiferous tubulus testes control group (A&B); seminiferous tubulus testes treatment group (C&D); black arrow (normal seminiferous tubule); red arrow (damage seminiferous tubule); (HE stain, binocular camera with 10 µm and 100 µm with magnification of 10 x)

DISCUSSION

The testes function to produce male germ cells in the seminiferous tubules [21],[22]. These organs can experience various disorders that can lead to a decrease in function because it is an organ that is susceptible to damage due to heavy metals contaminating river water.Testicular damage in the study results was marked by enlargement of testicular size (**Table 1**). These conditions indicate the process of cell damage that begins with cell degeneration conditions include hydropic degeneration, fatty degeneration, swollen degeneration and vacuole degeneration.][26],[27]. This is consistent with the study of Vergilio CS et al (2015) which shows that heavy metal exposure can cause changes in size testis [22].

Martapura River water adminstration also causes cell and tissue damage in the form of damage to seminiferous tubules and depletion of seminiferous tubule walls in rats(**Table 1 and Figure 1**). Ayano Hirako et al (2017)said damage to seminiferous tubules and depletion of seminiferous tubule walls in rats testes due to the process of necrosis and apoptosis cause by heavy metal . This is in accordance with the research of Teiichiro Aoyagi et al. (2002); N Shiraishi et al (2001), FW Santos et al (2004), Wu X et al (2017); which statement that heavy metals cause a decrease in the number of germ cells in the seminiferous tubules and intetitial tissue proliferation [20],[28-30]. [31]. There are several pathomechanism theories mentioning cell damage in organs due to heavy metals. Exposure to heavy metals with low concentrations causes the process of oxidative stress, changes in the cascade pathway of cell signals, changes in cell adhesion to cells and damage to protein structure. The mechanism of cell damage through the pathway of oxidative stress because heavy metals such as iron, mercury, copper, nickel, cadmium and arsenic can be explained because of the ability of these molecules to form Reactive Oxygen Species (ROS). Reactive Oxygen Species causes cell damage through a mechanism of decreasing lipid bilayer and changes in DNA[32]. The accumulation of ROS in tissues characterized by the formation of active species such as hydroxyl radicals ($^{\bullet}OH$), hydrogen peroxidase (H_2O_2) and single oxygen (o=o) will cause disruption of balance between oxidants - antioxidants. The active species will act with biomolecules such as proteins, nucleic acids, and lipids which cause lipid peroxidation, protein denaturation, and DNA mutations which result in changes in the normal activity of cells, tissues, organs and even the physiological system of the body [10],[18],[33]. At the beginning exposure to heavy metals in cells will cause mild injury stage injury that occurs due to activation of the autofagi process and non-proliferative repair. If exposure to heavy metal concentrations increases, there will be a mechanism for releasing cell-to-cell bonds, and disruption of cell signaling pathways that have an impact on cell necrosis and apoptosis [19],[20].

CONCLUSION

The conclusion of the study shows that the administration of Martapura river water can cause enlargement of testicular size, damage to seminiferous tubules and depletion of seminiferous tubule walls in rats

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