Alteration of the Kidney Structure of White Rat after Water Administration from Martapura River

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ABSTRACT

Martapura River is indicated to have been contaminated by heavy metal can adversely affect the kidneys. This study aims to analyze the impact of water consumption of the Martapura River on the microscopic image of white rat kidneys. Microscopic structure of the kidneys studied were the glomerulus, proximal renal tubule, and renal medullary tubule. Research design used a posttest only with control group, with 32 white rats as the subject divided into 2 research groups, namely control group (distilled water) and treatment group (water from Martapura River) ad libitum for 30 days. Analysis of the data was independent t-test at 95% confidence level. The results showed that the number of glomerulus in the control group was less than the treatment group (p = 0.017); the glomerular diameter in the treatment group was smaller than the control group (p = 0.007); the number of proximal renal tubules in the treatment group was less than the control group (p = 0.025); lumen diameter of proximal renal tubules in the treatment group was not significantly different than the control group (p = 0.025); the number of renal medullary tubules in the treatment group was not significantly different than the control group (p = 0.347); and the lumen diameter of the renal medulla tubules in the treatment did not differ significantly compared to the control group (p = 0.015). Therefore, consuming water from Martapura River which contains heavy metals causing damage to the glomerulus, proximal renal tubule, and tubule of the renal medulla.

Keywords: Martapura River water, glomerulus, proximal renal tubule, renal medullary tubule

INTRODUCTION

Martapura or Kayutangi River is a tributary of the Barito River which empties into the city of Banjarmasin and upstream is the city of Martapura, the capital of Banjar Regency, South Kalimantan (Ulmi EL & Amal N, 2017). 59.4% of the people along the Martapura River use river water as a source of water for household needs (Mirwan A & Indrawati R, 2012). Data from the Regional Environmental Agency (BLHD) of South Kalimantan and other studies explain that heavy metals content in the Martapura River exceeds the predetermined quality standard (Mirwan A, 2012; Amalia et al., 2012; Fokus Batulicin, 2011). Heavy metals are dangerous pollutants because they are not easily destroyed in the body and the environment, so they tend to accumulate in organs (Harteman, 2011). According to Robbins and Jannette, heavy metals that enter through the digestive tract will be distributed to tissues and organs, one of which is the kidney which has a major function in the urinary system (Janardani et al., 2018). These changes are related to the metabolism of heavy metal content in the body. Heavy metals in the digestive system then accumulate in the blood. Heavy metals pass through the blood to the kidneys. This will cause a cell adaptive response due to inflammation by heavy metals. Heavy metal ions that accumulate in the kidneys cause the antioxidant enzymes to be inactive, increasing the production of Reactive Oxygen Species (ROS). This increase causes an increase in the formation of free radicals, which triggers oxidative stress in cells (Danusantoso, 2012; Kumar et al., 2010). Cells are hypoxic due to
ROS. Hypoxia causes cell damage with reduced aerobic oxidative respiration (Kumar et al., 2010). The kidney, as one of the vital organs in the body, is most often subject to damage by harmful chemicals (Suhita et al., 2013). This is because the kidneys receive blood flow of 25% of the volume of blood flowing to the heart (Fatimah U, 2013). The kidneys histologically consist of the cortex, medulla, and renal pelvis. Within the renal cortex are the main structures that produce urine known as nephrons. The nephron is composed of the renal corpusculum (combined glomerulus and Bowman's capsule), and the renal tubule (proximal convoluted tubule, Henle's ansa, distal convoluted tubule). Meanwhile, in the renal medulla, there is a duct system in the form of a tubule which functions as a channel that distributes urine to the urinary tract to the next (Kumar et al., 2012; Junquiera et al., 2007). Research on the impact of water pollution by heavy metals has been widely reported. Research by Wahyuni et al. stated that water contaminated with heavy metals causes necrosis of snakehead fish glomeruli (Wahyuni et al., 2017). Khoiriyah et al.'s research on tilapia kidneys also found severe damage such as necrosis and cell swelling in the proximal tubular epithelium (Khoiriyah et al., 2016). In addition, the research of Wagiman et al. also stated that heavy metals can cause abnormalities in the ansa Henle tube of fish in the form of cell swelling and necrosis (Wagiman et al., 2014). Based on these data, this study was conducted to further determine the impact of water consumption of the Martapura River on the microscopic image of the kidneys in Sprague Dawley white rats. This can provide input for the government to make policies related to water pollution in the Martapura river and provide information and education to the public about the correct use of Martapura river water to improve their health status.

MATERIALS AND METHODS

The research design used was true experimental laboratory research with a posttest only design with the control group (Dahlan SM, 2012). The research subjects were white (Rattus novergicus) Sprague Dawley strain, male, 2-6 months old, and weighing 250-300 g. The number of samples of the study was 32 rats (determined by Federer's formula). The research group was divided into two groups, namely the control group which was given ad libitum distilled water and the treatment which was given water from the Martapura River ad libitum (Yuliana et al., 2018).

Rat obtained from BVET Banjarbaru; Martapura River water; neutral buffer formalin 10% I, II, and III; ethanol 70%, 80%, 96%; xylol I, II and III; liquid paraffin I and II; distilled water; hematoxylin harris; eosin 1%; and gummy. Bottles, Perkin Elmer 5100 PC atomic absorption spectrophotometer, beaker glass, filter paper, water bath, hotplate, mouse cage, Ohaus analytical balance, gloves, masks, minor surgical instruments, syringes, raster image software, optical camera, binocular light microscope, scalpel, base mold, tissue cassette, slide glass, cover glass and microtome.

Identification of water characteristics

Martapura River water was taken from the waters behind the Darussalam Martapura Islamic Boarding School using a grab sampling technique in August 2019. An examination of the water characteristics of the Martapura River was carried out by the Banjarbaru Center for Environmental Health and Disease Control Engineering (BBTKLPP) using the atomic absorption spectrophotometric method (Torowati et al., 2008).

Research procedure

Starting from the treatment process on experimental animals provided that the animals used are healthy, male, 2-6 months old, and weighing 250-300 g. Then the acclimatization process was carried out for one week. The experimental animals were randomly grouped into a control group and a treatment group. The implementation of this research was carried out for 30 days (Yuliana et al., 2018). After the 31st day, the rats were sacrificed, and the kidneys were taken and made histological preparations stained with HE.

Observation of Renal Histological Preparations

In the initial stage, the histological preparations were examined with a binocular
light microscope, then the best histological preparations were taken of the white rat kidney (Rattus norvegicus) with the criteria of glomerular shape, proximal tubule, and intact medullary tubule. Micro photos were made using an optilab camera in jpeg format with a magnification of 100x and 400x, then observed using raster image computer software. The results obtained were numerical data.

**Data Analysis**

The data obtained were tested for normality with the Shapiro-Wilk test. Then proceed with the Levene test for homogeneity. The analytical test used was the independent t-test (95% confidence level).

**RESULTS**

Based on the results of the study, to find out whether there were differences in the microscopic picture of the kidneys of rats in treatment group and control group can be seen in Table 1 and Figure 1-6.

<table>
<thead>
<tr>
<th>Alteration in the kidney’s microscopic features</th>
<th>Control</th>
<th>Treatment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of glomerulus renal</td>
<td>4.19 ± 0.77</td>
<td>4.14 ± 0.74</td>
<td>0.017*</td>
</tr>
<tr>
<td>Glomerular diameter</td>
<td>89.51 ± 6.71</td>
<td>81.29 ± 9.27</td>
<td>0.0007*</td>
</tr>
<tr>
<td>Number of proximal tubules</td>
<td>122.35 ± 21.12</td>
<td>94.12 ± 18.74</td>
<td>0.000*</td>
</tr>
<tr>
<td>The lumen diameter of the proximal tubule</td>
<td>26.78 ± 3.19</td>
<td>25.38 ± 1.91</td>
<td>0.142</td>
</tr>
<tr>
<td>Number of tubules in the medulla</td>
<td>157.89 ± 3.04</td>
<td>148.41 ± 2.54</td>
<td>0.347*</td>
</tr>
<tr>
<td>Number of tubules in the medulla</td>
<td>54.36 ± 7.93</td>
<td>47.97 ± 5.94</td>
<td>0.015*</td>
</tr>
</tbody>
</table>

* t-test independent significance

Figure 1. Description of the number of a glomerulus control group (A) and treatment (B). The black arrow is a countable glomerulus; the red circle is the glomerulus that cannot be counted because the glomerulus is fragmented, the boundary between the parietal stratum and the stratum visceral is not visible, and the glomerular structure is not intact; the glomerulus atrophy due to adhesions; HE staining. Magnification 100x.
Figure 2. Description of glomerular diameter for control (A) and treatment groups (B). HE staining. Magnification 400x.

Figure 3. Description of the number of proximal tubules of the group (A) control and treatment (B). The black arrow is countable proximal tubules; HE staining. Magnification 100x.

Figure 4. Microscopic analysis of proximal renal tubular diameter on control (A) and treatment groups (B). HE staining. Magnification 400x.

Figure 5. Description of the number of tubules of the renal medulla of the control (A) and treatment groups (B). The black arrow is countable renal medulla tubule. HE staining. Magnification 100x.
DISCUSSION

Microscopic features of the glomerulus

Table 1 showed a significant decreased in the mean number of renal glomerulus in white rats, namely 89.51 ± 6.71 in the control group and 81.29 ± 9.27 in the treatment group; with a value of P = 0.017. These results indicated a significant reduction in the number of glomeruli in the treatment group compared to the control group. This data was also corroborated by the results of observations on renal histological preparations in Figure 1 which shows the difference in the number of glomeruli between the study groups. The decreased in the number of glomeruli in the treatment group was indicated by a picture of the boundary between glomerular podocyte cells and stratum parietal epithelial cells, Bowman's capsule was not clear so that the spatial space appeared widened and some even disappeared. This was probably the result of the inflammatory response of cells around the glomerulus due to toxic substances. In addition, the appearance of Bowman's capsule in the treatment group appeared to be atrophic and partly fragmented. The provision of water from the Martapura River containing heavy metals for a long time was thought to have caused this situation. These results are in line with research conducted by Ratnaningsih which showed that high Cd levels caused a change in the image of the white rat kidney in the form of the boundary between glomerular podocytes and stratum parietal epithelial cells, Bowman's capsule was not clear and even disappeared.

Cd causes inflammation of the glomerular membrane gap, causing an adaptive response in the form of leakage of red blood cells from the glomerular capillaries and infiltration of inflammatory cells in the glomerulus (Ratnaningsih et al., 2016). The inflammatory response also causes adhesions between the glomerulus and Bowman's capsule. Glomerulus that experiences atrophy due to this adhesion cannot perform optimal glomerular function (Fahrimal et al., 2016). Glomerulus with unclear boundaries, structures that experience fragmentation and atrophy due to adhesion cannot be calculated on the variable number of glomeruli in this study so that there is a reduction in the number of glomeruli in the treatment.

In Table 2, it is also known that there was a decrease in the mean glomerular diameter of the renal white rats, namely 89.51 ± 6.71 in the control group and 81.29 ± 9.27 in the treatment group; with a P-value = 0.007. These results indicate a shortening of the glomerular diameter in the treatment group. This data is collaborated by the results of observations on renal histological preparations in Figure 2 which shows the difference in the size of the glomerular diameter between the study groups. The theory that underlies a shortening of the glomerular diameter is due to atrophy of the glomerular capillary endothelial cells in response to cell inflammation due to toxic substances. Puspitasari's research states that the shortening of the glomerular diameter was thought to occur due to atrophy of the glomerular capillary endothelial cells (Puspitasari, 2015). It was suspected that the shortening of the glomerular diameter was due to chronic consumption of the Martapura river water which contains heavy metals. This result was in line with the research of Widyaningrum et al who observed that...
exposure to the toxic substance HgCl₂ for six weeks caused glomerular cell atrophy so that the glomerular diameter shortened.

**Microscopic Features of the Proximal Renal Tubule**

The results of research observations on the proximal renal tubule showed a significant decrease (p-value = 0.000) with the mean number of proximal renal tubules of white rats, namely 122.35 ± 21.12 in the control group and 94.12 ± 18.74 in the treatment group (Table 2). Figure 3 shows a decrease in the number of proximal tubules in the treatment group. The decrease in the number of proximal tubules was thought to be due to toxic substances that cause cell swelling and congestion in the proximal tubules. This results in stretching the space between the tubules. This result was in line with Sari's research (2010) which stated that the cause of the decrease in the number of proximal renal tubules can be due to Pb exposure, which was also identified in the water content of the Martapura River used in this study.

The proximal renal tubule is the part most frequently damaged due to exposure to nephrotoxic substances. Active transport systems for ions, organic acids, low molecular weight proteins, peptides, and heavy metals mostly occur in the proximal tubule, causing accumulation and proximal tubular toxicity which ultimately results in proximal tubular damage. The loose epithelium of the proximal tubule facilitates the entry of various components into the proximal tubular cell. This was thought to be something that contributes to damage to the proximal renal tubule.

The results of research observations on the lumen diameter of the proximal renal tubule showed a tendency to narrow the lumen diameter, namely 26.78 ± 3.19 in the control group and 25.38 ± 1.91 in the treatment group, although not significantly different from the control group (p = 0.142) (Table 2 and Figure 4). This was in line with Khoiriyah et al's research which states that there is a cause of narrowing of the proximal tubular lumen is Cd (Khoiriyah et al., 2016). Sari's research also found an image of the proximal tubule with swelling of the tubular epithelial cells which caused a narrowing of the lumen due to Pb administration (Sari DH, 2010). Cell swelling is an important disorder associated with reversible injury due to failure of the energy-dependent / energy-dependent ion pump on the plasma membrane which results in the cell being unable to maintain and fluid homeostasis, causing Na buildup in the cell and K excretion from the cell. The final result is the addition of iso-osmotic water (Khoiriyah et al., 2016).

**Microscopic Features of the Renal Medullary Tubules**

The observations of the results of the next study were changes in the number of renal medullary tubules. The results showed that there was a tendency to decrease the mean number of tubules in the renal medulla of white rats, namely 157.89 ± 30.44 in the control group and 148.41 ± 25.42 in the treatment group. However, the decrease in the number of medullary tubules was not statistically significant (P-value = 0.347) (Table 2). Microscopic changes in the form of a decrease in the number of tubules in the medulla in the treatment group can be seen in Figure 5. The decrease in the number of tubules of the renal medulla occurred due to the swelling of the medullary tubular cells because of reversible injury. This was in line with the research of Wagiman et al., which states that heavy metals can cause abnormalities in Henle fish such as cell swelling and necrosis (Wagiman et al., 2014). As a result of cell swelling, the number of medullary tubules seen in one field of view was less in the treatment group. In addition, the number of medullary tubules decreases as well as possible due to congestion in the medullary tubules, a condition in which there is excessive accumulation of blood in the blood vessels.

The results of research observations on the lumen diameter of the medullary tubule found that there was a significant narrowing/shortening (P-value = 0.015) in the lumen diameter with an average size of the medullary tubular lumen diameter of 54.36 ± 7.93 in the control group and 47.97 ± 5.94, treatment group (table 2). Figure 6 shows the damage that occurred in the medullary tubule which was characterized by narrowing of the medullary tubule lumen, in the treatment group it was seen that the tubular lumen was narrower than the control group. The results of
these observations are in line with the research of Wagiman et al., who stated that heavy metals can cause abnormalities in fish Henle ansa such as swelling of cells and necrosis. The tubular lumen narrows due to epithelial swelling and is filled with detached cells and other debris (Wagiman et al., 2014). Edema is indicated by the condition of the renal tubular lumen which is narrowing due to the size of the tubular epithelial cells that are enlarging so that the inter-tubules will be stretched. Cell swelling occurs because the electrolyte charge outside and inside the cell is unbalanced (Anderson PS, 1995).

Based on the results of observations on the structure of the kidneys (glomerulus, proximal renal tubule, and tubule of the renal medulla), it was found that there were differences in the microscopic picture between the control group and the treatment group. This difference indicates that there was an indication of damage to the kidney structure in the experimental group of mice that were given the Martapura River water as their daily drinking water for a long period. This condition was strongly related to the presence of heavy metal content in the Martapura river water even though the levels do not exceed the water quality standard (table 1) (Permenkes, 2017). However, because it was consumed for a long time, there was an accumulation of heavy metals in the body. This situation was related to the metabolism of heavy metal content in the body. This was in line with Safitri's research which states that the long duration of exposure to consuming heavy metal Cd even though at low concentrations continuously will still cause a disturbing effect on the kidneys (Safitri FZ, 2015).

Heavy metals in the digestive system then accumulate in the blood. Heavy metals pass through the blood to the kidneys. This will cause a cell adaptive response due to inflammation by heavy metals. Heavy metal ions that accumulate in the kidneys cause the antioxidant enzymes to be inactive, increasing in the production of ROS. This increase causes an increase in the formation of free radicals, which triggers oxidative stress in cells. Cells are hypoxic due to ROS. Hypoxia causes cell damage with reduced aerobic oxidative respiration (Kumar et al., 2016). Free radicals cause a pathological response in the form of reversible or irreversible cell damage. The disorder associated with reversible injury to cells is cell swelling with a microscopic appearance of vacuole degeneration. Continuous and prolonged exposure to toxic substances will result in cells leading to irreversible injury and cell death. The transition from reversible lesions to irreversible lesions through a process of increasing cell swelling, swelling and damage to lysosomes, damage to cell membranes and changes in cell nucleus chromatin which will end in necrosis (Iranti et al., 2017). This is confirmed by Ressang's research in Fahrimal et al which explains that severe glomerular damage makes the peritubular vascular system disrupted and has the potential to drain toxic substances into the tubule, while severe tubular damage due to increased intraglomerular pressure will cause glomerular atrophy (Fahrimal et al., 2016).

**CONCLUSION**

Based on the research results, it can be concluded that administration of Martapura River water in the long term tends to cause damage to the kidney structure, namely damage to the glomerulus, proximal renal tubule and tubule of the renal medulla. This was thought to be due to the heavy metal content in the Martapura River water which accumulates in the kidneys and caused an increase in free radicals which triggered oxidative stress on kidney cells which damaging to the kidney structure.

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