Gentamicin Nephrotoxicity in Animal Model: Study of Kidney Histopathology and Physiological Functions

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Abstract. The purpose of this study was to analyze abnormal changes in kidney histology and physiology in acute kidney injury animal models with multiple doses of gentamicin. Induction of experimental animals was carried out on 20 rats (Rattus norvegicus) Wistar strain which were divided into 4 groups, group I was a negative control group, groups II, III and IV are gentamicin-induced groups at doses of 30 mg/kg, 40 mg/kg, and 50 mg/kg, respectively. Kidney histopathology were stained with Hematoxylin Eosin (HE), while analysis of serum BUN and creatinine by spectrophotometric method. Data analysis for kidney histopathology was descriptively while for BUN and Creatinine were statistically tested with one way ANOVA. The results of this study showed there was a kidney damage in all gentamicin-induced groups, which is necrosis in the contortus tubule and the Bowman's capsule compared to the negative control group. The group of rats with the higher doses of gentamicin showed a more severe level of histopathological changes, however, BUN and creatinine levels were not significantly different (p> 0.05). This study concludes that gentamicin induction could cause kidney histopathological changes but not kidney physiology.

Keywords: animal model, antibiotic, BUN, creatinin, nephrotoxicity.

1. Introduction
Acute kidney injury is a disease of kidney failure or kidney damage that occurs rapidly with a term of a few hours or a few days. Symptoms that appear in the incidence of acute kidney injury such as abdominal pain, polyuria, oliguria, and others(1). This disease mostly diagnosed by measuring biomarkers level; such as Blood Urea Nitrogen (BUN) and creatinine. However, these biomarker alterations often do not describe the actual condition of the kidneys. Acute kidney injury condition can be seen clearly by observing macroscopically and microscopically of the kidney. Damaged kidneys can be characterized by renal cortex swelling and also necrosis of the cell(2). While the increased levels of BUN and creatinine biomarkers in the blood occur when the kidneys are damaged by around 30% - 59%, accompanied by anemia and lethargy(3). Kidney is known to be able to regenerate, but with a cell damage rate of up to 50%, it will be difficult to restore its physiological
functions normally. This has prompted many researchers to investigate various substances that have potential effect to cause nephrotoxicity in order to reduce the incidence of this disease.

In addition, it is also important to study the mechanism of acute kidney injury by using animal models. Animal models of acute kidney injury occur because of the nephron's damage in the kidneys. In the nephron which consists of the glomerulus, Bowman’s capsule, tubular contortus or Henle arch, cell necrosis will occur. According to Halim, (2014) creating an acute kidney injury animal model takes about one month(4). The material that can be used to induce kidney injury are varied such as medicines. One of the drugs that can cause kidney damage is antibiotics(2). Antibiotics that are often used for creating kidney injury animal models are gentamicin antibiotics. The induction doses of antibiotic to stimulate kidney injury are vary widely. Various studies on nephrotoxic gentamicin have been widely reported such as at low doses of 10 mg/kg BW in a period of months(4), at high doses of 60 mg/kg BW within 10 days(5, 6), doses 100 mg/kg BW within 8 days (7).

Nowadays, the use of gentamicin injection in animals is still often be used, whereas in humans it has begun to be abandoned. The safe dose that is often used in humans is 5-7 mg/kg BW(8), in the following year, it drops to 3-5 mg/kg BW(9). Gentamicin dose which10 times of the safe dose is known to cause toxicity in rat animals. This safe dose alteration may allow in toxic doses changes also, therefore this study aims to analyze the histological and physiological changes of rats as animal models of acute kidney injury due to gentamicin injection in multilevel doses from 30 mg/kg body weight to 50 mg/kg body weight.

2. Material and methods
2.1. Animal Model
Twenty male Wistar strains rats, obtained from de Wistar Bandung were divided into 4 groups, Group I was controlled group with placebo injection, Group II was induced by gentamicin dose of 30 mg/kg BW, Group III was induced gentamicin dose by 40 mg/kg BW, and Group IV was induced by gentamicin dose of 50 mg/kg BW. Animals were euthanized on the 10th day. Histopathology examination was performed on the kidney tissue of rats after euthanizing. The whole animal has complied with the guidelines declared by the institutional animal care and use committee with the registration number No. 954-KEP.

2.2. Gentamicin injection
Each rat received a daily intramuscular injection (between semi membrinosus and semi tendinosus) of gentamicin sulfate (Genta-100®) for 10 days with various doses according to their group.

2.3. Examination of renal histology preparations
The kidney organs were inserted into 10% buffered formalin for making preparations for histopathology. The kidney was made into a histological preparation by tissue transfer into 70% alcohol as a stopping point. Then the tissue was dehydrated with multilevel alcohol with a concentration of 70% for 24 hours, 80% ethanol for 2 hours, 90% ethanol, 95% and absolute ethanol for 20 minutes. Then the organs were soaked in xylol I for 20 minutes and xylol II for 30 minutes so that the organs are clean. Then the filtered tissue and embed in paraffin block 56-58 ºC. Kidney organ sliced to a thickness of 5 microns using a rotary microtome. Each repetition was made 4 incisions at intervals of 10 incisions and placed on the glass of the object that has been given a 70% alcohol solution and then stained with Hematoxylin-eosin (HE) staining(10).

2.4. Blood Collection in Experimental Animals
Collection of blood in animal models through infraorbital sinus using microhematocrit, then inserted into vacutainer. The formed serum was inserted into a noncoagulant microtube to be analyzed for serum BUN and creatinine.
2.5. Measurement of BUN and Creatinine Levels

BUN and creatinine levels were examined using spectrophotometry (ABX Pentra). The kidney physiology was evaluated using the colorimetric Jaffe method (Creatinine and Urea kits DiaSys diagnostic systems GmbH Germany) to calculate the serum levels of blood urea nitrogen (BUN) and creatinine.

2.6. Statistical Analysis

The observations of kidney histopathology using an electron microscope were then analyzed descriptively. BUN and creatinine data were analyzed quantitatively using the Statistical Package for the Social Sciences program (SPSS, v20). One way ANOVA test was conducted to observe the significant differences between groups (\( \alpha <0.05 \)) and continued with the Tukey test.

3. Results and discussion

3.1. Macroscopic changes in the gentamicin-induced kidney

Gentamicin-induced rats showed clinical symptoms of lethargy, inactivity, and urine macroscopy showed slightly reddish color but feces were normal, consumption of food and drink was still normal. Macroscopically, the size of the kidney of the control and treatment rats was no distinction but differences occur outside the renal mucosa color in the control group (figure 1a) and gentamicin induction group (figure 1b).

![Figure 1](image1.png)

**Figure 1.** The kidneys in negative control rats are bean-shaped and reddish in color like normal kidneys (a), while the kidneys in the gentamicin induction group look paler (b)

3.2. Alteration in renal histology of animal models with gentamicin induction.

Microscopic observation using magnification 100 times and 400 times showed a histopathological change in the gentamicin induction group. Whereas in the control group that was not induced by gentamicin showed no microscopic changes (Figure 2). Spherical glomerulus is composed of simplex endothelial cell capillaries, Bowman’s capsules was a round shape with simplified squamous cells, proximal contortus tubules with a cuboid simplex cellular with microvilli, and distal contortus tubules with a rounded cuboid cell with no microvilli, and the medulla consists of a loop of Henle with rounded simple squamous cell(11).
Figure 2. Kidney histopathology in the negative control group (hematoxylin-eosin staining, 100x and 400x magnification). Glomerulus (arrow) are normal with round shape and solid structure, proximal tubules (arrowhead) with cuboid-round celled and villis.

Microscopic images of rat kidneys with gentamicin 30 mg/kg body weight injection (group I) were seen histopathological changes compared to the negative control group (Figure 3), the Glomerular endothelial cells erode, Bowman’s capsule occurs necrosis and the Bowman’s space becomes more tenuous. The condition of proximal tubular cells was necrosis, which is showed by desquamation of the cuboid simplex epithelial cells with cytoplasm and cell nuclei that disappear. Inflammatory cells in the proximal tubule are found around the tubular epithelial cells characterized by large blackish-purple round cells.

Figure 3. Histopathology of Renal Kidney in Treatment Group I with 30 mg/kg BW induction (hematoxylin-eosin staining, 100x and 400x magnification). (arrow) Glomerulus showed endothelial cell erosion, (arrowhead) Bowman’s capsules showed necrosis and stretching, * Tubule contortus showed necrosis and plenty of inflammation cells.

Histopathological images of rats injected with 40 mg/kg body weight in group II shows a more severe degree of damage compared to the control and treatment group I (Figure 4). the glomerulus were necrosis and erosion of endothelial cells, Bowman's space becomes more tenuous and the epithelial cell nucleus in Bowman’s capsule was lysis. Cuboid simplex epithelial cells in the proximal contortus tubules seen necrosis and epithelial cell desquamation occurs both in the cytoplasm and in the nucleus. Inflammatory cells are often seen around damaged nephrons.

Figure 4. Kidney histopathology in treatment group II Induction of 40 mg/kg BW (hematoxylin-eosin staining, 100x and 400x magnification). Glomerulus (arrow) showed desquamation of endothelial cells, Bowman’s capsule (arrowhead) was found necrosis and Bowman's space becoming stretched, Tubular Contortus (*) were necrosis, and followed by inflammatory cells (circle).
As the gentamicin induction doses increased the kidney damage was seen to be more severe as illustrated in the microscopic images of rats injected with gentamicin 50 mg/kg body weight in group III (Figure 5). Damage occurred in all parts of the nephron i.e. glomerulus, Bowman’s capsule, proximal tubule, and distal tubule. In glomerulus, necrosis in endothelial cells is causing a larger gap in the Bowman’s space area, Bowman’s capsules were seen necrosis in simplex squamous cell wall structure, proximal contortus tubule and distal contortus tubules were seen necrosis or marked by acute tubular necrosis (ATN), simplex squamous cell wall structure was necrosis and appear desquamated in its histological structure. In the tubular sections were seen a lot of mononuclear inflammatory cells in cells undergo necrosis.

![Kidney Histopathology in Group III Induction gentamicin with 50 mg/kg BW (hematoxylin-eosin staining, 100x and 400x magnification). Desquamation of endothelial cells Glomerulus (arrow), Bowman’s capsule (arrowhead) showed necrosis, and Bowman’s spaces become distinct. Necrosis occurs on Tubular Contortus (*)](image)

By giving high doses of gentamicin injection can cause damage to the nephron structure characterized by necrosis and desquamation of epithelial cells in the glomerulus, Bowman’s capsule and tubular which will impact on impaired kidney function of glomerular filtration and reabsorption and secretion in the kidney tubular. The main aspect of gentamicin nephrotoxicity is tubular cytotoxicity. Animal therapy with gentamicin can be related to apoptosis(12) as well as tubular epithelial cell necrosis(13). Gentamicin causes cytotoxicity in cells where it can be accumulated. In the kidney, they are usually proximal tubular epithelial cells(14) while distal tubular cells and collecting ducts are significantly less affected by cytotoxic effects(15). The drug is then transported to the lysosome, the Goldzi device, and the endoplasmic reticulum(16). Gentamicin binds to the phospholipid membrane, changing its function and causing a condition known as phospholipidosis in humans(17) and experimental animals(18). Lysosomal phospholipidosis is caused by disorders of the phosphatidylinositol signaling pathway(19), reduction of phospholipid turnover and their accumulation in the plasma membrane.

Phospholipidosis is directly related to gentamicin toxicity(20). However, similar changes were detected in other cell types exposed to gentamicin but without significant damage and cell death. When Gentamicin concentrations in endosomes reach a certain level, the membrane is disrupted and its contents including gentamicin are released into the cytoplasm(21). In the cytoplasm, gentamicin acts on mitochondria both directly and indirectly(22) and activates the intrinsic apoptotic pathway, breaks the respiratory chain, decreases ATP synthesis and causes oxidative stress by creating superoxide anions and hydroxyl radicals(23) resulted in cell death. When present in large numbers, cathepsin causes massive proteolysis which results in necrosis, especially in the lack of ATP. In the endoplasmic reticulum, gentamicin inhibits protein synthesis, interfering with the accuracy of translation and modification of post-translational proteins(24). This results in endoplasmic reticulum stress and apoptotic activation by caspase 12 and calpain(25).

The results of this study are similar to the results of research conducted by Lintong (2012). Administration of gentamicin for laboratory rats at a dose of 10-20 mg/kg body weight has caused...
characteristic and marked changes in the lysosomes of proximal tubular epithelial cells (6). These changes are tubular dysfunction: detached of the brush border and release of lysosomal enzymes; decrease reabsorption of protein, calcium, magnesium, potassium, and glucose; phospholipiduria, and cast excretion. Gentamicin administration at doses above 40 mg/kg body weight quickly induces necrosis in the renal cortex accompanied by impaired renal dysfunction.

3.3. No Significant Changes in BUN and Creatinin in the gentamicin group

One way ANOVA results showed no significant difference between treatments (p> 0.05). Therefore, the Tukey test was not continued. The average BUN and creatinine level in blood serum can be seen in table 1.1.

| Treatment Group | BUN (mg/dL) Mean ± SD | creatinine (mg/dL) Mean ± SD |
|----------------|-----------------------|-----------------------------|
| Negative Group | 16.00 ± 1.51a         | 0.45 ± 0.09a                 |
| Group I        | 16.48 ± 2.79a         | 0.55 ± 0.15a                 |
| Group II       | 25.00 ± 6.87a         | 1.88 ± 1.19a                 |
| Group III      | 30.70 ± 6.40a         | 1.80 ± 1.39a                 |

According to Johnson (2014), BUN levels in rats range from 10-21 mg / dL (26). Whereas acute kidney injury produces BUN levels 10 times its normal levels (27). Under normal circumstances, BUN is the result of protein metabolism that occurs in the liver. This substance will be channeled through blood vessels and discharged through the urination system in the kidneys so that BUN levels in the blood vessel will be found a small amount. From the mean value of each group that has been obtained, it appears that there is no significant difference within the groups, but there is a tendency to increase in line with the increasing doses of gentamicin.

One way ANOVA test results on creatinine samples showed no difference between treatments (p> 0.05). The average value of the control group and the treatment group I was still within the normal range of white rat creatinine levels, which is 0.2 - 0.8 (26). Current standard tests to determine the status of kidney function was by measuring serum creatinine and urine production. However, the initial measurement of serum creatinine may not reflect the extent of the injury as the accumulation of creatinine always lags behind injury events (28). The increase in serum creatinine can indicate damage to the kidneys but has limited ability to determine the damage in the early stages (29). According to Nguyen & Devarajan (2008), creatinine levels begin to increase after kidney damage reaches 50% (30), so it can be concluded that the damage of the kidney that observed is still below 50%.

4. Conclusion

Based on the results of this study it can be concluded that renal histopathological changes due to gentamicin injection for 10 days occurred in all gentamicin groups, but these abnormality did not appear on BUN and Creatinine serum. Because of the delay biomarker in this study, we suggested to add another variable to observe the acute kidney damage.

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