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Original Article

Nephrotoxicity and Kidney Fibrosis Due to Gentamicin in Wistar Rats

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Abstract

Background : Gentamicin is an aminoglycoside used as a treatment for various infections. One of the side effects reported on the use of gentamicin is nephrotoxic. However, there are still many uses of gentamicin that have not been precisely indicated. This study was aimed to analyze the nephrotoxic effects leading to renal fibrosis due to gentamicin induction in Wistar rats.
Methods : This research is an in vivo experimental study, pre- and post-test control group design, conducted in September and October 2022 at the Animal House Laboratory of the Faculty of Medicine, Sriwijaya University, and the Palembang Health Laboratory Center. There were 4 treatment groups: Group I, placebo; Group II, gentamicin-induced (GIG I) at 80 mg/kgBW; Group III, gentamicin-induced (GIG II) at 120 mg/kgBW; and Group IV, gentamicin-induced (GIG III) at 240 mg/kgBW. Gentamicin was administered intraperitoneally for 7 days, to 8 Wistar rats per group. Blood was taken from all Wistar rats in each group on days 0, 3, 7, and 14. The results of the study were tested for normality with the Shapiro-Wilk test and homogeneity with the Levene's test. The ANOVA test and the Post-Hoc test were used to conduct the analysis.
Results : Induction of gentamicin in the GIG I and GIG II groups was significant in increasing the mean creatinine and urea levels on day 0 and day 14 of treatment ($p<0.05$). In the GIG III group there was a 50% mortality in experimental animals showing a Lethal dose of 50 (LD50) at that dose.
Conclusion : GIG I and GIG II have significant nephrotoxic effects in increasing creatinine and urea levels which lead to renal fibrosis.

Keywords : Gentamicin, Renal Fibrosis, Nephrotoxic, Wistar Rat

INTRODUCTION

Nephrotoxicity is defined as kidney injury or decreased kidney function[1] caused directly or indirectly by drugs[2] and other chemicals.[3] Drug-induced nephrotoxicity can affect the vascular, glomerulus, and renal tubules.[4] Some antibiotics are nephrotoxic that cause acute renal injury (AKI)[5] and chronic kidney disease (CKD) which leads to renal fibrosis.[3,6] The class of antibiotics that cause nephrotoxicity and renal fibrosis is the aminoglycoside group.[6]

Gentamicin is one example of an aminoglycoside[7] used to treat infections caused by gram-negative bacteria.[8] Gentamicin can cause nephrotoxic adverse effects in the majority of patients. Nephrotoxicity due to gentamicin also involves an inflammatory role through cell infiltration, increased cytokine production and capillary hyperpermiability.[9] The inflammatory response that was originally thought to be a defense mechanism will contribute to kidney failure.[10]

Initial inflammation of the kidneys then increases cystacin-c and inflammatory mediators in the kidneys. Increased cystacin-c and inflammatory mediators, which further increases the expression of intercellular adhesion molecule-1 (ICAM-1) and P-selectin from endothelial cells,[11] resulting in increased attachment of inflammatory cells especially netrophil cells. This leads to an increase in reactive oxygen species (ROS), leading to cell necrosis and increasing creatinine and ureal levels which are used as biomarkers to assess kidney damage.[3]

The kidneys are known to be able to regenerate, but with a cell damage rate of up to 50%, it will be difficult to restore the physiological function of the kidneys. This has prompted many researchers to investigate various drugs, especially antibiotics, that have the potential effect of causing nephrotoxicity. Gentamicin can have nephrotoxic effects, which can result in renal fibrosis, a disorder known as end-stage renal disease (ESRD). Numerous investigations have shown that gentamicin is nephrotoxic and can result in renal fibrosis, for example, at low doses of 10 mg/kg body weight over the course of several months[2], at high doses of 60 mg/kg body weight within 10 days, doses of 100 mg/kg body weight within 8 days.[12] Toxicity was known to occur at gentamicin doses ten times the safe dosage in Wistar rats. Administration of gentamicin 100 mg/kgBW i.p. to wistar rats for 8 days showed a toxicity effect on the glomerulus and renal tubules.[13]

In a study conducted by Awodele O. *et al*., it was determined that induction of gentamicin 80 mg/kgBW for 7 days caused necrosis and fibrosis of the kidney due to the progression of kidney damage; specifically by examining the nephrotoxicity effect that leads to renal fibrosis by analyzing the parameters of urea and creatinine.[14] Therefore, a research is required to evaluate kidney function parameters such as urea and creatinine in order to analyze the nephrotoxic effect causing renal fibrosis in Gentamicin-induced Wistar rats.

METHODS

Preparation of Experimental Animals

This research is an experimental research conducted in vivo with a pre and post test control group design. The research sample was Wistar rats weighing 150–200 grams with a rat age of 12–16 weeks.

The research was carried out in September October 2022 at the Animal House Laboratory, Faculty of Medicine, Universitas Sriwijaya and Palembang Health Laboratory Center. Before the study, all rats were allowed to adapt for 7 days in stainless steel cages with a minimum required volume of 500 cm^2 for two rats and a minimum required cage height of 20 cm with a room temperature of 22±1°C, 12 hours of light-dark cycles, and given ad libitum access to drinking water and food.

The sample was taken by simple random sampling and divided into four groups with 8 mice per group. Group I placebo, given aquadest; group II gentamicin-induced (GIG I) of 80 mg/kgBW; group III induced gentamicin (GIG II) of 120 mg/kgBW; group IV induced gentamicin (GIG III) of 240 mg/kgBW. Wistar rats were induced for 7 days intraperitoneally.

Nephrotoxic Effects Assessment of Gentamicin and Statistical Analysis

All Wistar rats in each group were taken blood samples from the retroorbital plexus in the eyes of mice on days 0, 3, 7, and 14. Blood samples are checked for creatinine and ureum levels by a measurement procedure using a clinical spectrophotometer.

Data analysis processing using IBM SPSS Statistic 26 application. The homogeneity and normality of the data were examined. Nephrotoxic effect is determined by Paired T test. Differences in nephrotoxic effects are determined by the Independent T test. The dose match between gentamicin and placebo was determined by PostHoc Test.

A certificate of research ethics has been obtained from the Medical and Health Research Ethics Commission (KEPKK) of the Faculty of Medicine, Sriwijaya University with certificate number 281-2022.

RESULTS

This study is an invio experimental study using 8 wistar rats each group (32 wistar rats). Group 1 (placebo), II (gentamicin 80mg/kgBW), group III (gentamicin 120mg/kgBW) and group IV (gentamicin 240mg/kgBW). In group IV, gentamicin injection 240 mg / kgBW on day 2, caused the death of 6 rats which means death in > 50% of experimental animals (Lethal Dose 50 / LD 50) at a dose of 240 mg / kg BW.

TABLE 1
Inter-Group Weight Homogeneity Test

Rat group	Rat body weight Mean ± SD (gram)	p value
Placebo group	158.57 ± 6.502	0.198
GIG I (80 mg/kgBW)	164.33 ± 2.958	
GIG II (120 mg/kgBW)	179.33 ± 2.291	
GIG III (240 mg/kgBW)	168.67 ± 9.709	

*Levene Test, p = 0.05

TABLE 2
Homogeneity Test of Creatinine and Urea Levels Before Inter-Group Treatment Intervention

Rat group	Treatment group	Mean ± SD (gram)	p value
Creatinine level	Placebo group	33.29 ± 2.431	0.290
	GIG I (80 mg/kgBW)	31.88 ± 1.387	
	GIG II (120 mg/kgBW)	32.86 ± 2.494	
Ureum level	Placebo group	7.18 ± 1.447	0.356
	GIG I (80 mg/kgBW)	5.88 ± 0.975	
	GIG II (120 mg/kgBW)	6.71 ± 1.573	

*Levene Test, p = 0.05

Homogeneity Test

The homogeneity test was conducted to determine the homogeneity of the data on the body weight of rat before treatment. In the levene test, rat body weight was obtained, p value = 0.198 ($p > \alpha$) which showed no difference in the mean body weight of rat before treatment between treatment groups. Thus, the body weight of the rats had homogeneous data variance (Table 1).

Homogeneity tests were also performed to determine homogeneity in creatinine and ureum levels before treatment (day 0). The results of statistical tests with levene test statistical tests obtained rat creatinine levels p = 0.290 ($p > \alpha$) and rat ureum levels p = 0.356 ($p > \alpha$) which showed that there was no difference in the mean creatinine and ureum levels of rats before treatment between treatment groups (Table 2).

Normality Test

Table 3 showed the results of the normality test of serum creatinine and ureal levels before treatment in each group with the Shapiro Wilk test, it was found that serum creatinine and ureum levels before treatment were normally distributed ($p > \alpha$) with a value of $\alpha = 0.05$.

The nephrotoxic effect of gentamicin in the group on creatinine and ureum levels can be seen using the statistical test Paired T-Test. Table 4 shows the mean creatinine levels before treatment and day 14 of treatment in the placebo, GIG I and GIG II groups showed significant differences in mean creatinine levels.

Table 5 shows that the mean ureum levels on day 0 and day 14 after induction in the placebo group showed no difference in mean ureal levels, while GIG I and GIG II showed significant differences in mean ureal levels,

Intergroup Nephrotoxic Effects on Creatinine and Ureal Levels

The nephrotoxic effect of gentamicin in the group on creatinine and ureal levels can be seen using the statistical test Paired T-Test. In Table 4 which shows the mean creatinine levels before treatment (day 0) and day 3 of treatment in the placebo group there is no difference in the mean creatinine levels, while in GIG I and GIG II show a significant difference in mean creatinine levels.

In Table 4 also showed mean ureum levels before treatment (day 0) and day 3 after induction in placebo, GIG I, and GIG II groups there were differences in mean ureum levels.

Table 6 demonstrates that the placebo group had no significant differences in mean ureum levels on day 0 and day 14 of treatment, whereas GIG I and GIG II had significant differences in mean ureum levels. Table 5 also

TABLE 3
Normality Test on Creatinine and Ureal Levels between Intervention Groups

Rat group	Treatment group	p value
Creatinine level	Placebo group	0.873
	GIG I (80 mg/kgBW)	0.739
	GIG II (120 mg/kgBW)	0.484
	GIG III (240 mg/kgBW)	0.220
Ureum level	Placebo group	0.755
	GIG I (80 mg/kgBW)	0.093
	GIG II (120 mg/kgBW)	0.577
	GIG III (240 mg/kgBW)	0.388

* Shapiro Wilk test, p = 0.05

TABLE 4
Nephrotoxic Effects of Gentamicin on Creatinine Levels at Day 0 and Day 14 between Intervention Groups

Rat group	Creatinine level		p value
	day-0	day-14	
Placebo group	33.29 ± 2.43	38.28 ± 1.32	0.301
GIG I (80 mg/kgBW)	31.88 ± 1.38	227.07 ± 3.43	0.000
GIG II (120 mg/kgBW)	32.86 ± 2.49	405.81 ± 0.57	0.003

*Paired T test, p = 0.05

TABLE 5
Nephrotoxic Effects of Gentamicin on Creatinine and Ureum Levels on Day 0 and Day 3 between Intervention Groups

Rat group	Creatinine level		p value	Ureum level		p value
	Day-0	Day-3		Day-0	Day-3	
Placebo group	33.29 ± 2.43	34.38 ± 2.10	0.301	7.18 ± 1.44	9.94 ± 2.76	0.022
GIG I (80 mg/kgBW)	31.88 ± 1.38	33.21 ± 1.65	0.000	5.88 ± 0.97	6.39 ± 1.00	0.000
GIG II (120 mg/kgBW)	32.86 ± 2.49	34.98 ± 2.55	0.003	6.71 ± 1.57	8.80 ± 0.99	0.010

*Paired T test, p = 0.05

shows that the mean ureum levels on day 3 and day 7 of treatment in the placebo, GIG I, and GIG II groups showed differences in mean ureum levels.

Table 7 shows that there was no difference in mean creatinine levels on day 7 and day 14 after induction in the placebo group, whereas GIG I and GIG II showed significant differences in mean creatinine levels. Table 6 also demonstrates that there was no difference in the placebo group's mean urea levels on the seventh and fourteenth days after treatment, whereas GIG I and GIG II exhibited significant differences in mean urea levels.

Table 8 demonstrates that there was a significant difference between the placebo, GIG I, and GIG II groups' mean creatinine levels before and after induction. Table 7 demonstrates that there was no difference in the placebo group's mean urea levels before and after treatment, whereas GIG I and GIG II exhibited significant differences in their mean urea levels.

TABLE 6
Nephrotoxic Effects of Gentamicin on Creatinine and Ureal Levels at Day 3 and Day 7 between Intervention Groups

Rat group	Creatinine level		p value	Ureum level		p value
	Day-3	Day-7		Day-3	Day-7	
Placebo group	34.38 ± 2.10	34.15 ± 3.79	0.873	9.94 ± 2.76	8.03 ± 2.14	0.003
GIG I (80 mg/kgBW)	33.21 ± 1.65	156.01 ± 1.31	0.000	6.39 ± 1.00	21.19 ± 1.23	0.000
GIG II (120 mg/kgBW)	34.98 ± 2.55	228.36 ± 2.21	0.000	8.80 ± 0.99	34.04 ± 1.84	0.000

*Paired T test, p = 0.05

TABLE 7
Nephrotoxic Effects of Gentamicin on Creatinine and Urea Levels on Day 7 and Day 14 between Intervention Groups

Rat group	Creatinine level		p value	Ureum level		p value
	Day-7	Day-14		Day-7	Day-14	
Placebo group	34.15 ± 3.79	38.28 ± 1.32	0.058	8.03 ± 2.1	7.57 ± 0.63	0.650
GIG I (80 mg/kgBW)	156.01 ± 1.31	227.07 ± 3.43	0.000	21.19 ± 1.23	40.39 ± 3.99	0.000
GIG II (120 mg/kgBW)	228.36 ± 2.21	405.81 ± 0.57	0.000	34.04 ± 1.84	47.00 ± 1.88	0.000

*Paired T test, p = 0.05

TABLE 8
Nephrotoxic Effects of Gentamicin on Creatinine and Urea Levels on Day 0 and Day 14 between Intervention Groups

Rat group	Creatinine level		p value	Ureum level		p value
	Day-0	Day-14		Day-0	Day-14	
Placebo group	33.29 ± 2.43	38.28 ± 1.32	0.005	7.18 ± 1.44	7.57 ± 0.63	0.610
GIG I (80 mg/kgBW)	31.88 ± 1.38	227.07 ± 3.43	0.000	5.88 ± 0.97	40.39 ± 3.99	0.000
GIG II (120 mg/kgBW)	32.86 ± 2.49	405.81 ± 0.57	0.000	6.71 ± 1.57	47.00 ± 1.88	0.000

*Paired T test, p = 0.05

Differences in Nephrotoxic Effects Between Groups on Creatinine Levels

The Independent T-Test statistical measure can be used to identify differences in creatinine levels between groups. Table 9 demonstrates a significant difference in creatinine levels between the placebo group and GIG I and GIG II (p) and significant difference in creatinine levels between GIG I and GIG II.

Differences in Nephrotoxic Effects Between Groups on Ureum Levels

Differences between groups on urea levels with the Independent T-Test in Table 10 showed that there were significant differences in urea levels after treatment in the placebo group compared to GIG I and GIG II ($p<\alpha$) and there were significant differences in mean urea levels after treatment in GIG I compared to GIG II.

Conformity Test of Gentamicin Induction on Creatinine Levels in Wistar Rats

The results of the dose compatibility tests for GIG I, GIG II, and placebo were analyzed using the PostHoc Test (Tukey), and it was revealed that GIG I and GIG II had distinct nephrotoxic effects than the placebo group in increasing creatinine levels (Table 11).

TABLE 9
Comparison of the Nephrotoxic Effects of Gentamicin on Creatinine Levels after Intervention (Day-14)

Group	Comparison	p value
Placebo group (38.28 ± 1.322)	GIG I (227.07 ± 3.435)	0.000
	GIG II (405.8 ± 0.579)	0.000
GIG I (227.07 ± 3.435)	Placebo group (38.28 ± 1.322)	0.000
	GIG II (405.8 ± 0.579)	0.000
GIG II (405.8 ± 0.579)	Placebo group (38.28 ± 1.322)	0.000
	GIG I (227.07 ± 3.435)	0.000

*Independen T test, p = 0.05

TABLE 10
Comparison of the Nephrotoxic Effects of Gentamicin on Ureum Levels after Intervention (Day-14)

Group	Comparison	p value
Placebo group (7.57 ± 0.635)	GIG I (40.39 ± 3.991)	0.000
	GIG II (47.00 ± 1.886)	0.000
GIG I (40.39 ± 3.991)	Placebo group (7.57 ± 0.635)	0.000
	GIG II (47.00 ± 1.886)	0.000
GIG II (47.00 ± 1.886)	Placebo group (7.57 ± 0.635)	0.000
	GIG I (40.39 ± 3.991)	0.000

*Independen T test, p = 0.05

Conformity Test of Gentamicin Induction on Ureum Levels in Wistar Rats

The results of the dose conformity tests for GIG I, GIG II, and placebo using the PostHoc Test (Tukey) revealed that GIG I and GIG II had distinct nephrotoxic effects than the placebo group in increasing ureum levels (Table 12).

DISCUSSION

This study aims to assess the nephrotoxic effect that leads to renal fibrosis by induction of gentamicin doses of 80 mg/kgBW, 120 mg/kgBW, 240 mg/kgBW. Nephrotoxic effects seen from creatinine and urea levels. The results of statistical analysis showed that all doses of gentamicin increased creatinine and urea levels ($p<0.05$). Gentamicin increased urea and creatinine levels at doses of 80 mg/kgBW, 120 mg/kgBW, 240 mg/kgBW.

At the time of the study, six rats induced by gentamicin 240 mg/kgBW died within 24 hours after the first dose was administered. This shows that a dose of 240 mg/kgBW of gentamicin has a toxic effect on >50% of experimental animals in GIG III, and has reached a Lethal Dose of 50 (LD50).The results of 240 mg/kgBW gentamicin induction contradicted previous studies conducted by Awodele O., *et al* which showed that rats induced by 280 mg/kgBW gentamicin for 14 days had a nephrotoxic effect and showed renal fibrosis due to progressive kidney damage but did not show any death in rat. Differences in research results reaching death in >50% of experimental animals (LD50) can be caused by differences in age in each experimental animal.[15] The value of creatinine and ureum in the early stages of an individual's life tends to be higher due to growth, development, and high metabolic processes. Along with increasing age and maturity until aging, this value will decrease due to the ongoing process of high metabolism. In this study, however, creatinine and urea levels increased due to gentamicin's toxic effect. Consequently, the nephrotoxic effect varies according to the age of rats.[16,17]

According to The Kidney Disease: Improving Global Outcomes (KDIGO), the increase in serum creatinine in AKI conditions is up to 1.5 times the initial value in the first 7 days. An increase in creatinine levels twofold from normal can be used as an indication of a 50% decrease in kidney function and a three-fold increase in creatinine levels can be used as an indication of a 75% decrease in kidney function. Based on the results of the

TABLE 11
Conformity test of the Nephrotoxic Effects of Gentamicin on Creatinine Levels

Variable	Placebo group	GIG I (80 mg/kgBW)	GIG II (120 mg/kgBW)
Placebo group	–	0.000	0.000
GIG I (80 mg/kgBW)	0.000	–	0.000
GIG II (120 mg/kgBW)	0.000	0.000	–

*PostHoc Test (Tukey), p = 0.05

TABLE 12
Conformity test of the Nephrotoxic Effects of Gentamicin on Ureum Levels

Variable	Placebo group	GIG I (80 mg/kgBW)	GIG II (120 mg/kgBW)
Placebo group	–	0.000	0.000
GIG I (80 mg/kgBW)	0.000	–	0.000
GIG II (120 mg/kgBW)	0.000	0.000	–

*PostHoc Test (Tukey), p = 0.05

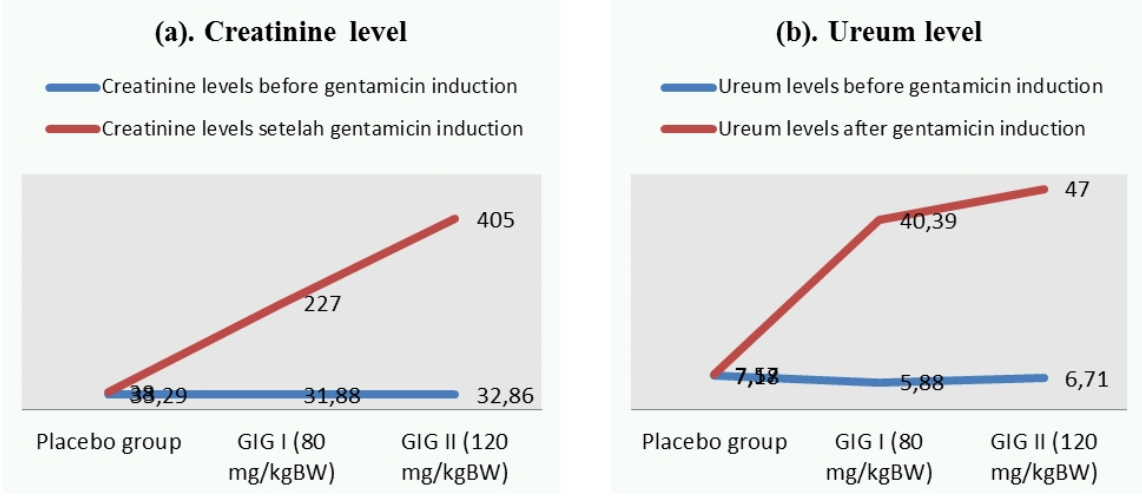

Figure 1. Nephrotoxic effects of gentamicin on the kidney
(a). Creatinine level (b). Ureum level

study, by assessing the mean comparison of creatinine levels on day 0 and day 14, it increased up to seven times in GIG I, and 12 times in GIG II. This indicates a decrease in kidney function up to stage III due to the induction of GIG I (80 mg/kgBW) and GIG II (120 mg/kgBW). Comparison was also seen in the mean urea level on day 0 and day 14, which increased up to seven times in GIG I and GIG II. In this condition, the kidney has led to a state of renal fibrosis and ends in a state of End Stage Renal Disease (ESRD).[18]

Gentamicin induction exerts a toxic effect on the renal tubular epithelium, the vascular system, and the renal glomeruli. The accumulation of gentamicin in the proximal tubule causes the transport of protein and cation molecules (megalin and cubilin complexes) in the proximal tubule by endocytosis. Gentamicin binds to membrane phospholipids and causes a condition called phospholipidosis. When the concentration of gentamicin in the GIG I group (80 mg/kgBW), GIG II (120 mg/kgBW), and GIG III (240 mg/kgBW) in the endosomal structure exceeds the threshold, the cell membrane and its contents will be disrupted which then exits into the cytosol. Gentamicin in the cytosol then binds to mitochondria and activates the intrinsic pathway of

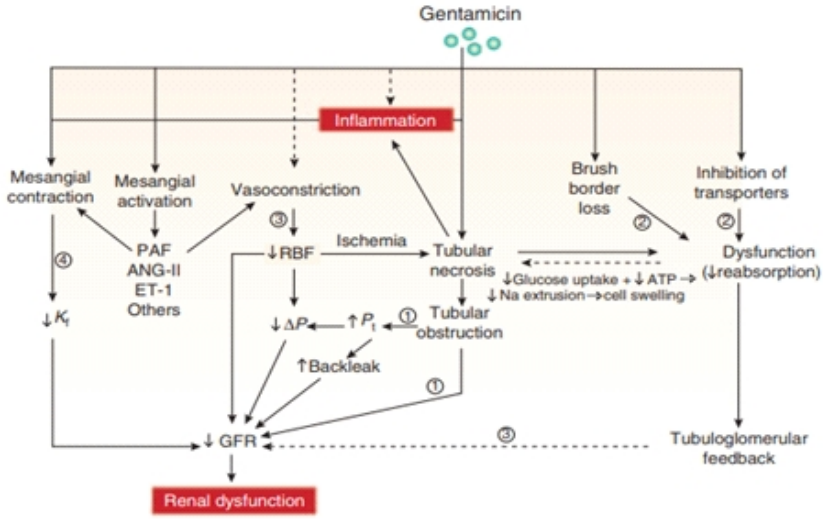

Figure 2. Integration of Gentamicin Nephrotoxicity Mechanisms[9]

apoptosis, affects the respiratory pathway, increases hydroxy radicals and superoxide anions resulting in oxidative stress in cells, and results in inhibition of Adenosine Triphosphate (ATP) production. Gentamicin also binds to mitochondria and causes an increase in Bax level (Bcl 2 binds to protein X) 4 through inhibition of proteosomal degradation. Increased Bax levels cause activation of a protease enzyme called cathepsin which can induce cell death.[11] Gentamicin also inhibits protein synthesis in the endoplasmic reticulum causing the translation process to be disrupted. Ultimately, there is activation of the Calcium-Sensing Receptor (CaSR) with gentamicin which causes apoptosis in kidney tubular cells and indicates cell death.[4] Nephrotoxicity due to gentamicin also involves an inflammatory role through cell infiltration, increased production of cytokines and capillary hyperpermeability. Inflammation is a major factor in the occurrence of kidney damage due to ischemia and nephrotoxicity.[9]

Inflammation due to the toxic effects of gentamicin plays a role in the fibrotic response of kidney tubular cells which causes the progression of kidney disease towards CKD. Continuous injury to the kidney leads to accumulation of scar tissue in the tubular epithelial cells.[19] In renal fibrosis, the tubular epithelial cells lose their polarity and transform into mesenchyme in the process of Epithelial-to-Mesenchymal Transition (EMT).[20] Tubular cells lose tubular markers, such as E cadherin and zonula occludens-1 and express mesenchymal proteins, such as α-SMA and vimentin.[21] TGF-β induces changes in the morphology of the renal tubular epithelium by increasing expression of collagen types I and IV and fibronectin[22] and increasing matrix production.[23] The contractile nature of scar tissue will cause dysfunction so that fibrosis can end in end-stage renal failure.[24,25] This study proves that a dose of 80mg/kgBW can cause nephrotoxicity which leads to kidney fibrosis. In this study, histopathological features of the kidneys of Wistar rats were not shown, however, data on increased levels of urea and creatinine indicated impaired kidney function in the form of nephrotoxicity leading to renal fibrosis.

CONCLUSION

Gentamicin doses of 80 mg/kgBW (GIG I) and 120 mg/kgBW (GIG II) substantially raised the levels of ureum and creatinine, indicating that gentamicin induction result in nephrotoxicity and kidney fibrosis in Wistar rats.

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