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Effect of Lime Juice (*Citrus Aurantifolia*) on Blood Urea Nitrogen and Creatinine Levels in Wistar Rats Induced by Gentamicin

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ABSTRACT

Gentamycin is an antibiotic that has the effect of acute kidney failure. Increased levels of BUN and Creatinine can characterize acute kidney failure. Lime juice (Citrus aurantifolia) contains flavonoid compounds as antioxidants that can regenerate kidney function. This study aims to determine the difference in BUN and Creatinine levels before and after being given lime juice. This study uses an experimental study design with a pretest-postest design. In the model mice, gentamicin was induced at a dose of 60 mg/KgBB for 10 days, and then BUN and creatinine levels were checked. This study was divided into 5 groups, including the negative, healthy, and treatment groups 1, 2, and 3, with a dose of lime juice 0.5 ml/KgBB, 1.0 ml/KgBB, and 1.5 ml/KgBB. The results showed that the average change in BUN levels before and after between groups was significant (sig < 0.05), and the average change in creatinine levels before and after between groups was significant (sig < 0.05). This study concluded that the average decrease in BUN levels in treatment group 3 was significant by 7.9 mg/dl, and the average decrease in creatinine levels in treatment groups 2 and 3 was significant by 0.8 mg/dl.

KEYWORDS:
Citrus aurantifolia, Gentamicin, Kidney, BUN, Creatinine

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INTRODUCTION

The kidney is a vital organ that has essential functions for the health of the body. The occurrence of disorders in the kidneys can cause various health problems, including acute kidney failure, chronic kidney failure, kidney stones, nephritis, kidney poly cystitis, and also glomerulonephritis[1]. Kidney disease can cause the kidneys to be impaired in cleaning and filtering waste or toxins from the blood. Risk factors that cause kidney damage include consuming too much alcohol, sugary foods and drinks, foods high in sodium, drugs that exceed average doses, lack of water, and frequent urination[2]. Factors such as Increased blood sugar levels, side effects of medications, and certain diseases also cause kidney damage[3]. The prevalence of chronic kidney failure in Indonesia in 2017 was 0.38% (713,783 people), and 19.33% (2,850 people) who underwent hemodialysis therapy. East Java Province is ranked 9th with a prevalence of 0.29% (75,490 people) suffering from chronic kidney failure and 23.14% (224 people) undergoing hemodialysis therapy [4].

Blood Urea Nitrogen (BUN) is the concentration of urea in serum or plasma that is determined to have nitrogen content. Urea is a non-protein nitrogen compound in the blood. Urea is synthesized by the liver and transported by the blood to the kidneys for excretion. Elevated BUN levels in blood serum are an indicator of kidney damage. Kidney

damage causes a buildup of BUN in the blood so that the glomerular filtration rate decreases [5].

Creatinine is a waste product of protein and creatinine phosphate metabolism in skeletal muscle. The kidneys excrete creatinine from the body in urine. Elevated serum creatinine levels are a biochemical marker of acute kidney damage [5].

Gentamicin is a type of antibiotic for gram-negative bacteria in the aminoglycoside drug group that has a nephrotoxic effect. Gentamicin causes acute kidney injury. Gentamicin will be filtered through the glomerulus and retained in the proximal tubules. It triggers impaired function in the kidneys [6].

Lime is an alternative treatment for kidney disorders. According to Mufida & Puspitasari (2020), using lime juice as a therapy can improve kidney function [7]. Lime fruits contain bioactive flavonoid compounds [8]. Flavonoids are phenolic compounds that have health benefits, including anticancer, antioxidant, anti-inflammatory, and antiviral properties. In cases of kidney damage, flavonoids act as antioxidants for kidney nephroprotection [9].

METHODS

The research method uses an experimental study design with a pretest-posttest group. The male Wistar rats used for this study were aged 2-3 months and weighed 100-200 grams. This research was approved by the ethics committee of Universitas dr. Soebandi with No. 562/KEPK/UDS/VII/2024.

Before starting the study, the rats were adapted for 7 days. The rats are fed and normal drinking water every 2 days. The number of Wistar rats is determined by Federer's formula with a total number of 30 rats. In the model mice, gentamicin was induced at a dose of 60 mg/Kg body weight, and lime juice was received for 10 days.

The rats were divided into 5 groups, namely K+ received aquadest, healthy group; K- received gentamicin+0 mg/Kg of lime juice; P1 received gentamicin+0.5 ml/Kg of lime juice; P2 received gentamicin+1.0 ml/Kg of lime juice; P3 received gentamicin+1.5 ml/Kg of lime juice.

BUN and creatinine levels were measured from serum specimens using Selectra Pro-S. 3 ml of blood specimen was collected from the sinus orbitalis and then centrifuged at 3000 rpm for 10 minutes to obtain serum.

BUN and creatinine levels that have been collected are then tested for normality using Shapiro-Wilk ($n<50$) and homogeneity testing using the Lavene test. If the data is normal ($sig>0.05$) and the data is homogeneous ($sig>0.05$), the data is analyzed using parametric One-way ANOVA followed by the post hoc test. If it is not, data will be analyzed using nonparametric Kruskall-Wallis. The data result was carried out with a confidence level of 95% and a $sig<0.05$.

RESULT AND DISCUSSION

Tabel 1. One-way ANOVA results of creatinine levels before and after lime juice administration. Creatinine levels in the K- treatment group from 0.84 ± 0.04 mg/dl to 0.87 ± 0.04 mg/dl (sig 0.012 < 0.05). The creatinine level in the K+ group was from 0.62 ± 0.05 mg/dl to 0.64 ± 0.06 mg/dl (sig 0.009 <0.05). The P1 treatment group showed a decrease in creatinine levels before and after lime juice administration from 0.61 ± 0.08 mg/dl to 0.53 ± 0.08 mg/dl (sig 0.013 < 0.05). In group P2, creatinine levels decreased from 0.55 ± 0.06 mg/dl to 0.48 ± 0.06 mg/dl (sig 0.000 <0.05). In the P3 group, creatinine levels decreased from 0.50 ± 0.05 mg/dl to 0.42 ± 0.03 mg/dl sig 0.001 < 0.05).

Table 1. Creatinine levels before and after treatment between groups

Groups	Creatinine levels (mg/dl) Before		After		*sig*
K-	0,84±	0,04	0,87±	0,04	0,012
K+	0,62±	0,05	0,64±	0,06	0,009
P1	0,61±	0,08	0,53±	0,08	0,013
P2	0,55±	0,06	0,48±	0,06	0,000
P3	0,50±	0,05	0,42±	0,03	0,001

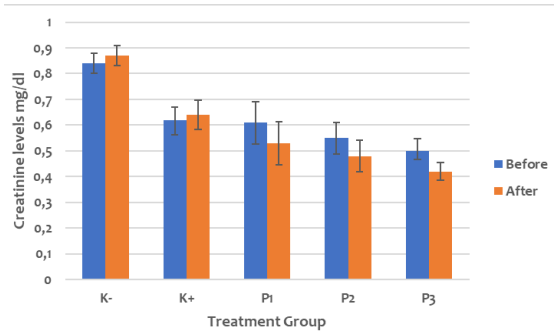

Figure 1. The differences in Creatinine levels before and after treatment between groups

A trend of difference creatinine levels before and after administration of lime juice is shown in Figure 1. Creatinine levels in the K- and K+ groups showed a slight rise after treatment compared to before the treatment. In groups P1, P2, and P3, creatinine levels showed a decline after treatment compared to before the treatment.

Table 2. The *Post-Hoc Tukey test* creatinine levels before and after treatment between groups

Groups	Creatinine ±STD levels (mg/dl)
K+	0.02±0.01a
K-	0.03±0.02ab
P1	0.08±0.04bc
P2	0.07±0.01c
P3	0.08±0.02c

Different letters show significant differences (sig < 0.05)

Table 2. shows the mean decrease before and after administration of lime juice between groups. Groups K+, K-, P1, P2, and P3 showed significantly different mean decreases in creatinine levels. The mean decrease in creatinine levels in groups P2 and P3 was not significantly different. The highest mean decrease in creatinine levels in groups P2 and P3 was 0.08 mg/dl.

Table 3. Creatinine levels before and after treatment between groups

Groups	BUN levels (mg/dl) Before		After		*Sig*
K-	30,7±	3,8	33,1±	3,6	0,002
K+	19,7±	0,7	21±	0,9	0,006
P1	28,7±	2,7	22,3±	3,5	0,035
P2	26,4±	2,1	20,4±	4,6	0,039
P3	26,3±	1,6	18,5±	1,3	0,003

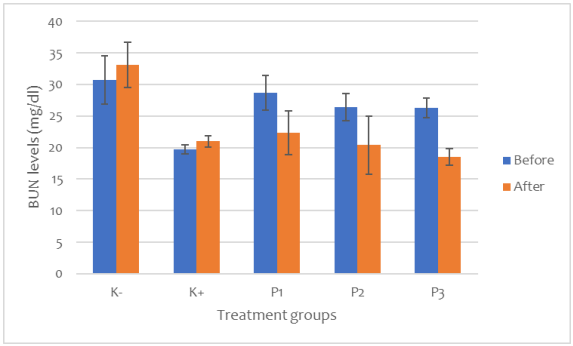

Figure 2. The differences in BUN levels before and after treatment between groups

Tabel 3. One-way ANOVA results of BUN levels before and after lime juice administration. BUN levels

in the K- treatment group from 30.7 ± 3.8 mg/dl to 33.1 ± 3.6 mg/dl (sig 0.002 < 0.05). The BUN level in the K+ group was from 19.7 ± 0.7 mg/dl to 21.0 ± 0.9 mg/dl (sig 0.006 <0.05). The P1 treatment group showed a decrease in BUN levels before and after lime juice administration from 28.7 ± 2.7 mg/dl to 22.3 ± 3.5 mg/dl (sig 0.035 < 0.05). In group P2, BUN levels decreased from 26.4 ± 2.1 mg/dl to 20.4 ± 4.6 mg/dl (sig 0.039 <0.05). In the P3 group, BUN levels decreased from 26.3 ± 1,6 mg/dl to 18.5 ± 1,3 mg/dl (sig 0.003 < 0.05). BUN levels in the K- and K+ groups showed a rise after treatment compared to before the treatment. In groups P1, P2, and P3, BUN levels showed a decline after treatment compared to before the treatment.

Table 4. The *Post-hoc Tukey test* BUN levels before and after treatment between groups

Groups	BUN ±STD levels (mg/dl)
K+	1.3±0.5a
K-	2.4±0.6ab
P1	6.4±4.5ab
P2	6.0±4.4ab
P3	7,9±2,7b

Different letters show significant differences (sig < 0.05)

Table 4. shows the mean decrease before and after administration of lime juice between groups. Groups K+, K-, P1, P2, and P3 showed significantly different mean decreases in BUN levels. The mean decrease in BUN levels in groups K-, P1 and P2 was not significantly different. The highest mean decrease in BUN in groups P3 was 7.9 mg/dl.

Creatinine dan BUN levels in the healthy group tended to be lower than those of other groups. It is because the healthy group has not received any treatment. BUN and creatinine levels increased during gentamicin induction, which shows that it has a nephrotoxic effect on the kidneys [10]. The normal levels of BUN in wistar rats is 15-21 mg/dl while the normal levels of creatinine in wistar rats is 0.2-0.8 mg/dl [11]. Nephrotoxicity occurs when the small blood vessels that filter blood and waste in the kidneys are damaged, allowing proteins to escape filtration and be excreted with urine [12].

The results of the study showed that the administration of lime juice was able to reduce the levels of BUN and creatinine in gentamicin-induced wistar rats. The highest decrease in BUN and creatinine levels was in group 3 with a lime juice volume of 1.5 ml/KgBW. It shows the highest flavonoid content at that dose. It shows the effect of flavonoid compounds, which can repair kidney function [13]. Flavonoids have a nephroprotective effect by increasing antioxidants, reducing levels of Reactive oxygen species (ROS), reducing oxidative stress through the role of mediator of the Nrf2 antioxidant response [14]. On the other hand, citric acid in lime juice also helps regenerate kidney function so that BUN and creatinine levels decrease [15]. Flavonoids bind vitamin C levels in cells and protect cells from damage caused by free radicals (ROS) [16].

CONCLUSION

Based on the results of the study, it can be concluded that the highest average decrease in BUN levels of 7.9 mg/dl was obtained in treatment group 3 with the administration of lime juice volume of 1.5 ml/Kg and the highest average decrease in creatinine content of 0.8 mg/dl was obtained in

treatment group 2 and treatment 3 with the administration of lime juice volume of 1.5 ml/Kg.

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