Nephroprotection Of PIGEON PEA (*Cajanus cajan (* Linn.) Huth) Against Gentamycin-induced Nephrotoxicity In White Male Rats Wistar Strain (*Rattus novergicus)*

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ABSTRACT

Pigeon pea (*Cajanus cajan* (Linn.) Huth) is a family of plant species, *Leguminoceae* can provide Nephroprotection based antioxidant effect. The purpose of this study was to determine the activity of pigeon pea as Nephroprotection on white male rats Wistar strain (*Rattus novergicus*) with the induction of gentamicin were seen from the levels Creatinine, Blood Urea Nitrogen and a picture of rat kidney histopathology. This research is the true experimental use of white male rats Wistar strain were divided into 5 groups randomly. The first group was normal control, group II negative control (gentamicin 60 mg/kg rats i.p and CMC 1% orally), Group III, IV and V (pigeon pea dose of 100 mg/kg, 200 mg/kg and 400 mg/kg rats in CMC 1% orally and then 2 hours after administration of the test dose followed by induction of gentamicin 60 mg/kg rats intraperitoneal) for 7 days. The results of Statistical analysis showed that pigeon pea can lower creatinine levels ($p < 0.05$) and may protect the kidney cell necrosis. But there is no effect on the level BUN $p=0.795$. The effectiveness of Nephroprotection was best produced in dose II (200 mg/kg) because it can reduce creatinine levels and repair necrosis cell 19.608

Keywords : Pigeon pea, creatinine, Blood Urea Nitrogen (BUN), Nephroprotection

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1. BACKGROUND

The working units of the kidney are individual nephrons; each nephron is formed by a glomerulus, which filters fluid from the blood, and a tubule, which modifies the filtrate through the resorption of water and some solutes and the secretion of other solutes. The concentration of the final product, urine, depends on hydration, obligate solute excretion, osmotic load, and other factors. In humans, the total number of nephrons is set at birth and declines over time as a result of injury and aging (Anders, H.J., Davis, J.M., Thurau, K, 2016)

Pigeon pea are one of the family plant species *Leguminoceae.* The use of Pigeon pea by the community is still not optimal, people only use Pigeonpea as vegetables or just made vegetables. Pigeonpea have a myriad of health benefits, including antidiabetic (S Ariviani et al, 2018), hepatoprotector, and antibacterial (R. Raveena, R. Premalatha and A. Saranya, 2016).

During the last few decades a large number of compounds have been isolated from C. cajanan and some of them have got excellent biological activities, such as Antibacterial Activity, Hypocholesterolemic Effects, Antidiabetic Activity, Neuroactive Properties, Hepatoprotective Effects, Antioxidant, Anticancer Activity (Pal, D., Mishra, P., Sachan, N., Ghosh, A., 2011).

To date, few studies have evaluated the antioxidant activity of pigeon pea.

Therefore, this study aimed to investigate Nefroprotector activity of pigeon pea in vitro, by assessing the effects of pigeon pea extracts on gentamycin-induced kidney damage.

2. METHODS

Tools

Blender (*Maspion),* sifter (size 80 Mesh) electric scales (*Ohaus),* oral sonde (*Europlex),* 1 mL and 5 mL syringes, beaker glass(*Pyrex),* test tube (*Pyrex),* pipette, stir bar,funnel, *aluminum foil,* Steam Cup (*Pyrex),* blender (*Philips),* drinking water bottles, filter paper, capillary pipes, effendrop tubes, centrifugator, photometer (*intermha-168),* heparin tubes, 1000 micron pipettes and 10 - 100 microns.

Material

The ingredients used in this research are Pigeonpea, gentamicin inj, ammonia 10%, hydrogen chloride (HCl), sodium hydroxide (NaOH), Mg metal, FeCl3, reagents *Mayer* reactor *Dragendroff,* reactor *Liberman-Buchard,* anisaldehyde, ether, amyl alcohol, vanillin, creatinine reagent kit (DiaSys), ureum reagent kit (*DiaSys),* 5% picric acid, glacial acetic acid and 40% formalin.

Sample Collection and Determination

Pigeon pea were collected from the Sendang hamlet farmer group, Dadapayu Village, Semanu District, Gunung Kidul Regency, DI Yogyakarta Province and the determination was carried out at the Faculty of Biology FMIPA Padjadjaran University (UNPAD).

Simplisia Processing

Pigeon pea are cleaned of dirt that sticks to the peanut skin, then washed with running water. Beans are soaked for 24 hours then heated to dry under the sun covered with black cloth. After dry then pollinated by blending.

Phytochemical Screening

Phytochemical screening is done by testing the presence of alkaloid compounds, flavonoids, saponins, polyphenols, tannin, steroids, triterpenoid, quinon, monoterpenes and sesquiterpenes.

The high antioxidant activity of the 50% ethanol extracts correlated with higher content of total phenolics (13.1 mg g^{-1}), flavonoids (4.7 mg g^{-1}), and anthocyanin (2.9 mg g^{-1}), compared to those present in aqueous (10.8, 2.4, and 1.0 mg $^{g-1}$, respectively) and 25% ethanol extracts (11.4, 2.8, and 1.6 mg $^{g-1}$, respectively) (Lai, Yi. Et all, 2012)

Test The Activity Of Pigeon Pea

Animal Preparation

Total of 25 rat acclimatized with the environment for 7 days and given normal treatment, and each cage given chaff.

Grouping and Treatment of Test Animals

Grouping and treatment of test animals as follows:

a. The first group as a normal control, rats are only given food and drink.
b. The second group as negative control were mice induced gentamicin 60 mg / kg BW in intraperitonial mice and CMC 1% in orally
c. The third group as the first dose test is mice Pigeonpea were given 100 mg / kg body weight in 1% po CMC and 2 hours after administration of dose I was induced gentamicin 60 mg / kg body weight by Intera.
d. The fourth group as a test dose II, rats Pigeonpea were given 200 mg / kg body weight in 1% po CMC and 2 hours after administration of dose II induced gentamicin 60 mg / kg body weight by IP.
e. The fifth group as the test dose III are mice Pigeonpea were given 400 mg / kg body weight rat in CMC 1% po and 2 hours after administration of dose III induced gentamicin 60 mg / kg body weight rat by IP.

The treatment was carried out for 7 days and on the 8th day the creatinine and BUN levels were measured and surgery was performed for histopathological testing.

Creatinine and BUN levels

Urea levels were tested using the urease method, while creatinine levels were examined using *Jaffe reaction.*

Kidney histopathology

Left and right kidneys were fixed in 10% formalin, dehydrated in alcohol of ascending grades and embedded in paraffin. Sections of 5-lm thickness were cut and stained with hematoxylin and eosin (H&E). The slides were examined under light microscope by a pathologist not aware of the identity of slides being examined (Fouad, Amr., Qutub, Hatem., Melhim, Walid., 2016).

3. RESULTS AND DISCUSSION

Phytochemical Screening of Pigeonpea

From the results of phytochemical screening of Pigeonpea containing flavonoids, polyphenols, saponins, quinones, triterpenes, monoterpenes and sesquiterpenes. These results are in line with previous studies which stated that the simplicia powder of Gude bean skin contains flavonoids, saponins, quinones, polyphenols, and triterpenoids.

Table. 1 Phytochemical Screening Results

Compound Group	Results
Alkaloids	-
Flavonoids	+
Saponin	+
Polyphenols	+
Tannin	-
Steroids	-
Triterpenoid	+

Monoterpenes and Sesquiterpenes	+
Kuinon	+

Note: (+) detected there are compounds
(-) detected no compounds

The presence of flavonoids and polyphenols in the simplicia of Pigeonpea can be used as an antioxidant and used as a nephroprotector. In previous studies, the activity of Gude bean powder with DPPH method obtained IC50 values of 70.08 mg / ml. These flavonoids and polyphenol compounds contained in the Pigeonpea will protect kidney cell components from free radicals produced by gentamicin by donating H atoms to free radicals so that they can inhibit and neutralize oxidation reactions.

Creatinine and Ureum Level Checking

The ureum is one of the common signs used to estimate *Glomerulus Filtration Rate (* GFR), but urea examination is only as a supporting examination due to several reasons including the fact that the urea level is not only influenced by kidney function but also by its production which comes from protein and urea intake is also reabsorbed by tubules. Experimental research has explained the mechanism of GM-induced nephrotoxicity in the form of induction of renal oxidative stress by gentamycin, as shown by a reduction in kidney glutathione, and an increase in lipid peroxidation. Oxidativ stress and nephrotoxicity are demonstrated in many experimental animal models (Kamal, S, 2015). Creatinine is more accurate in knowing kidney function than urea because creatinine is produced from muscle at a constant level and is almost completely filtered on the glomerulus (Khan. R., et al., 2012). The method for determining ureum is to measure nitrogen. Then, the ureum examination results are converted to BUN. The results of creatinine and urea examination that were converted to BUN can be seen in Table 2.

Table 2. Creatinine and BUN examination results

Group	Results	
	Creatinine levels (mg / dl)	**BUN content (mg / dl)**
Normal	1.448 ± 0.383	30.701 ± 17.868
Negative	2.24 ± 0.764	34.007 ± 17.237
Dose Test I	2.032 ± 0.264	31.952 ± 10.322
Dose Test II	1.64 ± 0.243	29.711 ± 13.182
Dose Test III	1.62 ± 0.223	23.509 ± 6.570

From Table 2 it can be seen that administration of gentamicin induction can increase creatinine and BUN levels, where the negative group of creatinine and BUN levels is greater when compared to the normal group.

Likewise in the test group doses 1, 2, and 3 produced creatinine and BUN levels were smaller than the negative group. This happens because creatinine and BUN levels will increase along with decreased glomelurus filtering ability because the release of creatinine and BUN in the body is inhibited.

Statistical Analysis of Creatinine Examination Results

The test results were analyzed using SPSS version 16.0 including normality, homogeneity and ANOVA tests. Normality test results with *Shapiro-Wilk* homogeneity with *levene test* the data are homogeneous (p = 0.229). Test *One-way Anova* showed a significant difference with p = 0.045 (p <0.05). To find out which treatment group had the same or different activities, the LSD test was continued. There was a significant difference between the normal group (p = 0.008), test dose II (p = 0.038), and dose (p = 0.032) against the negative group. This means that doses II and III provide effective and significant Nephroprotection activity compared to the negative group. This means that dose II and dose III are able to restore creatinine levels close to normal.

Statistical Analysis of BUN Examination Results

BUN levels obtained were then tested for normality by testing *Shapiro-Wilk,* the results obtained that the five treatment groups were normally distributed (p> 0.05). In the homogeneity test with *levene test* the results obtained that the data are homogeneous (p> 0.063). Test results *One-way Anova* there was no significant difference (p> 0.05). There are no differences in results because the amount of urea in the blood is determined by dietary protein and the ability of the kidneys to excrete urea

Histopathological Results of Renal Kidney

Histopathology done to observe the effect of toxicity and protection of test doses on the kidney organ microscopically. From Table 3 it can be seen that the administration of gentamicin induction of 60 mg / kg body weight / day can cause damage to kidney cells by 38.935% compared to normal controls.

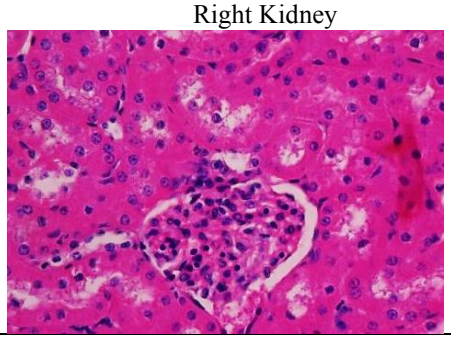
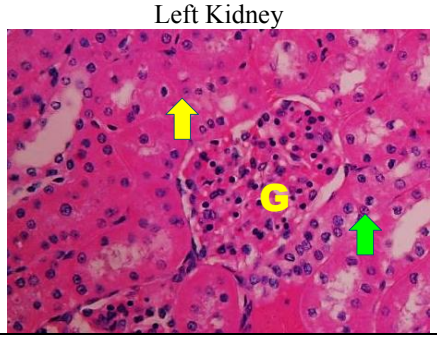
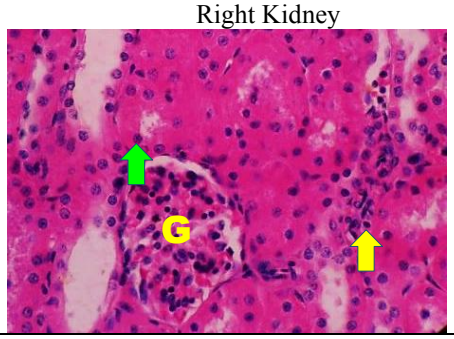
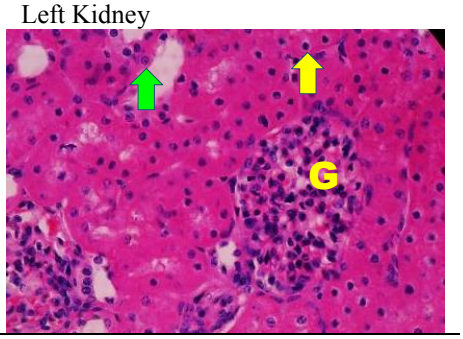
Table 3. Histopathological Analysis Results

NO	Group	Per 1000 cells		Percentage of protection
		Normal Cell	Cell Necrosis	
1	Normal	891 ± 5.657	109 ± 5.657	-
2	Negative	821.5 ± 6.364	178.5 ± 6.364	-38.935 *
3	Dose I	838.5 ± 2.121	161.5 ± 2.121	9.524
4	Dose II	856.5 ± 3.536	143.5 ± 3.536	19.608
5	Dose III	848.5 ± 4.950	151.5 ± 4.950	15.126

Information :
* = No protection against cell damage

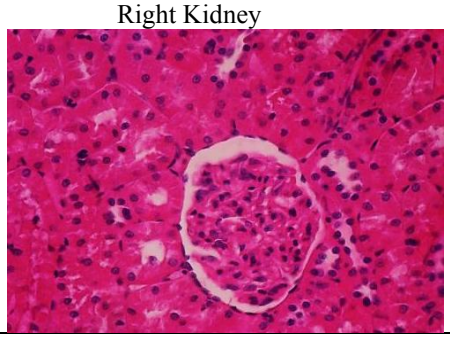
Pigeon pea can provide protection against gentamicin induction, seen from the precent protection of all test doses, either teat doses 1 (100 mg/kg body weight), dose 2 (200 mg/kg body weight), and dose 3 (400 mg/kg body weight) can provide protection. The highest oxidize percentage of protection was seen at dose 2 with as much protection 19, 608%. That means it shows that the administration of Pigeonpea can provide protection against gentamicin induction due to the pigeonpea contains antioxidant compounds.

Antioxidants are defined as compounds that can protect cell the danger of reactive oxygen free radicals. Where antioxidants are very easily oxidized, so free radicals will these antioxidants and protect other melocules in cell from damage caused by oxidation by free radicals or reactive oxigen.

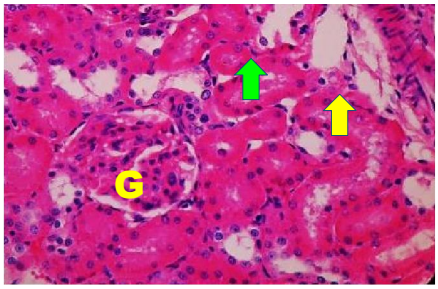
No	Histopathological Results Magnification 40x
1 Normal Control	Right Kidney  Left Kidney 
2 Negative Control	Right Kidney  Left Kidney 

3 Dose Test I

Right Kidney

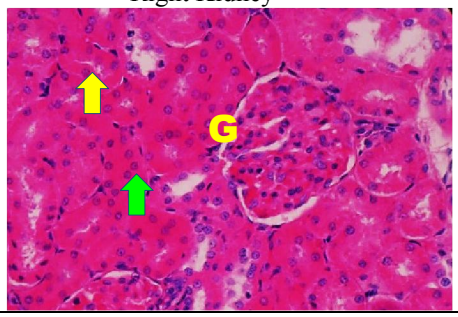


Left Kidney

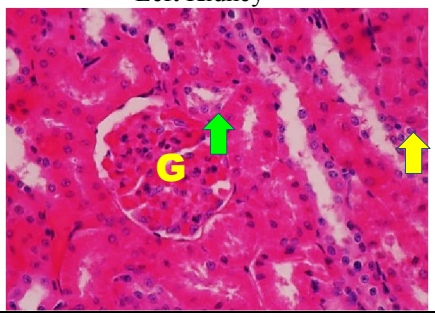


4 Dose Test II

Right Kidney

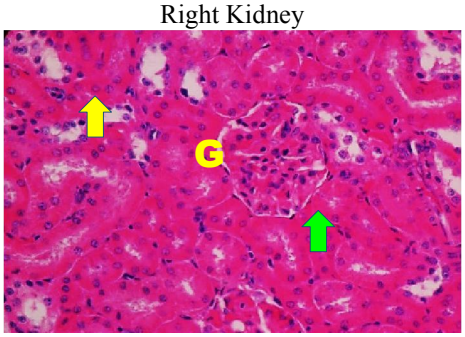


Left Kidney

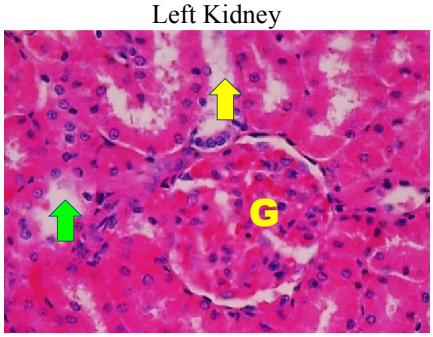


5 Dose Test III

Right Kidney



Left Kidney



G = Glomerulus
⇧ = Necrosis cell (The tubules appear enlarged, the epithelial cells swell, the granular cytoplasm, and the cell nucleus disappear).
⇧ = Normal cell

Gentamicin is classified as an aminoglycoside antibiotic that is known to be toxic to the kidneys. One of the consequences of this nephrotoxic agent is damage to the kidney tubules. From Figure 1 we can see necrosis cells with enlarged tubules, the cells epithelium swell, granular cytoplasm, and nucleus cell disappear.

The process of damage that occurs due to gentamicin induction involves three things, namely oxidative stress, inflammation or inflammation and necrosis (Mahmoud et al, 2017).

4. CONCLUSION

Based on the results of testing the activity of Pigeonpea (*Cajanus cajan (*

Linn.) Huth) against gentamicin-induced white male wistar rats, gude beans have nephroprotector activity. This can be seen from the decrease in creatinine levels and a decrease in the number of cell necrosis in the kidney tubules. But there is no effect on levels Blood Urea Nitrogen (BUN). The best protective effect was produced by the test group dose II (200 mg / Kg BB Rat) with cell necrosis improvement of 19.608%.

5. SUGGESTION

Nephroprotector activity testing needs to be done using Gude bean extract, toxicity, and research needs to be done on the content of specific compounds that work as nephroprotectors.

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