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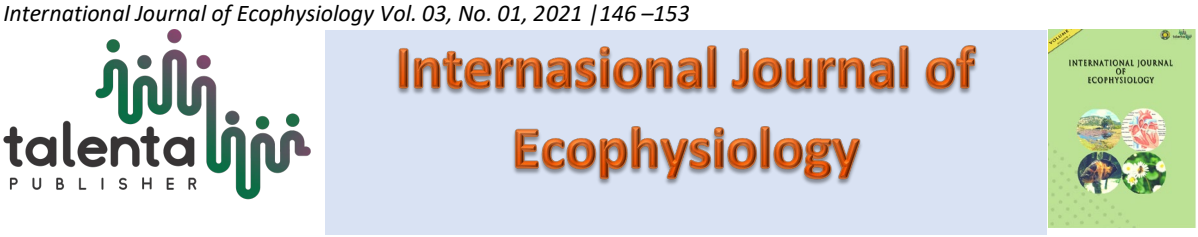

The Effect Of Giving Nano Herbal Biwa Leaf (Eriobotrya japonica Lindl.) Against Histological Description Of Kidney Rat That Induced By Alloxan

Syafruddin Ilyas[1]*, Salomo Hutahaean[1], Isnaini Nurwahyuni[1], Wardah Sawitri Polem[2],Herwina Francisca[2], Lailatun Nisfa[2], Olvita Mayani[2],.

[1]Biology Departement, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan. Jl. Bioteknologi No. 1 Kampus USU, Padang Bulan, Medan, Indonesia.
[2]Student of Biology Departement, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan. Jl. Bioteknologi No. 1 Kampus USU, Padang Bulan, Medan, Indonesia.

Abstract. Diabetes mellitus is a metabolic disease characterized by hyperglycemia due to abnormal insulin secretion and insulin action. Uncontrolled diabetes mellitus will cause various organ complications, one of which is diabetic nephropathy or kidney failure. The aim of this study was to determine the effect of nano herbal of biwa leaves (Eriobotrya japonica) on the structure of gromerulus, renal necrosis, proximal tubular constriction, kidney weight and kidney discoloration. This study uses an analytic study with a true experimental design in male rats (Rattus norvegicus) consisting of five treatments with each of six replications. This study consisted of a negative control group that was only given ad-libitum feed, a positive control injected with alloxan, and two groups injected with alloxan and given a 250 mg/kg and 500 mg/kg biwa nanoherbal. It was concluded that the nanoherbal of biwa leaves is effective enough to reduce blood glucose levels and is safe for consumption by diabetics because it does not damage kidney cells.

Keyword: Diabetes, Nanoherbal, Alloxan, Biwa, Kidney

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1 Introduction

Diabetes mellitus is a metabolic disease characterized by hyperglycemia due to insulin secretion and insulin action [1]. According to WHO data the number of diabetics in Indonesia is ranked 6th in the world, uncontrolled diabetes mellitus will cause various complications, long-term damage, dysfunction and failure of various organs.

Kidney damage (DM) is also called diabetic nephropathy. Diabetic nephropathy (ND) is a complication of diabetes mellitus which is included in microvascular complications, which are complications that occur in small (small) blood vessels. This is because there is damage to the smooth blood vessels in the kidney. Damage to blood vessels that cause glomerular damage that

**Corresponding author at: Departement of Biology, Faculty Mathematics and Natural Science, Universitas Sumatera Utara, Medan, Indonesia

E-mail address: syafruddin6@usu.ac.id

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works as a blood detector. High levels of sugar in the blood will make the kidney structure change so that its function is disrupted.

Biwa leaf (Eribotrya japonica) (Rosaceae) is a tropical plant that is often found in the content of biwa leaf (Eriobotrya japonica (Thunb.) Lindl.) Contains triterpenes, essential oils, flavonoids, tannins, megastigmane glycosides, and sesquiterpenes [2]. The benefits of biwa leaf plants are used as anti-inflammatory drugs, hypoglycemia, antioxidants, anti-tumor, antiviral, cytotoxic and antimutagenic. Dried biwa leaves are also commonly used in traditional Chinese medicine.The manuscript including the graphic contents and tables should be between 4 to 10 pages. The manuscript is written in English. The Standard English grammar must be observed. The title of the article should be brief and informative and it should not exceed 12 words. The keywords are written after the abstract, where the manuscript consists of two abstract section, which are conveyed in English and Indonesian language.

2. Material and Methods

This study used healthy and fertile rats (*Rattus norvegicus*) and were 8-11 weeks old, weighing 150-200 g, healthy, fertile as many as 30 birds. The rats were obtained from the North Sumatra Animal Disease Investigation Center Medan and divided into treatment and control groups. Rats were given food and drink in an adlibitum. Rats were injected with alloxan at a dose of 150 mg / kg to raise blood sugar levels of rats until diabetes. After that, blood sugar levels are checked on days 1, 7 and 14 days.

The material used in this study was the leaves of the biwa (*Eriobotrya japonica* (Thunb.) Lindl.) Obtained from the Gundaling area of Berastagi subdistrict, Karo Regency, North Sumatra Province. Then the biwa leaf (*Eriobotrya japonica* (Thunb.) Lindl.) Is processed into nano herbs at LIPI Cibinong.

Five groups of rats, each of which six received the following treatment schedule:

Group I: Normal control.

Group II: Controls treated with Alloxan (120 mg / kg, peritoneal injection / i.p).

Group III: Alloxan (120 mg / kg.ip) + nano biwa leaf herbs (250 mg / kg, orally / p.o)

Group IV: Alloxan (120 mg / kg.ip) + nano biwa leaf herb (500 mg / kg, p.o)

Group V: Alloxan (120 mg / kg.ip) + Standard drug (Glibenclamide) (5 mg / kg, p.o). Nano herbal extracts of biwa leaf and standard drug glibenclamide (5 mg / kg) are given with a gavage needle.

Group I functioned as a normal control, which received physiological saline (0.9%) for 14 days. Group II to Group V were diabetic control rats. Group III to Group V (which previously received alloxan) were given fixed-dose biwa leaf herbal nano (250 mg / kg, po), (500 mg / kg, po) and

standard drug Glibenclamide (5 mg / kg) for 14 consecutive days participate. Rats were dissected on day 14 and kidneys were taken and for histological preparations by the paraffin and hematoxylin methods. Eosin staining (HE) to observe gromerulus damage, necrosis and narrowing of the tubules in the kidney.

3. Result and Discussion

3.1.1 The color and shape of kidney

Based on the observations of the color and shape of the kidneys of male rats (*Rattus norvegicus*) after giving biwa leaf nano herbal (*Eriobotrya japonica* lindl.) can be seen in Figure 3.1.1

Figure 3.1.1. Image of right and left kidneys of male rats (Rattus novergicus) from the negative (-), positive (+) control group, P3: the group given nano biwa extract (Eriobotrya japonica) at a dose of 250 mg / kgBB, P4: group given nano biwa extract (Eriobotrya japonica) at a dose of 500 mg / kgBB, P5: the group given glibenclamide at a dose of 5 mg / kg.

The treatment showed that there were differences in color between the treatment group and the control group. The extract treatment group showed a dark red kidney color, because blood flow to the kidneys was normal and there was an adequate supply of oxygen from the lungs. While the treatment group showed a slightly pale red kidney color, because blood flow and oxygen supply were inhibited and the effect of giving alloxan to the treatment group caused damage to the renal glomerulus.

The red color in the kidneys may be caused by high volume of blood flow in the kidneys. The kidneys are known to receive blood flow as much as 22% of the total volume of blood pumped by the heart [4].

Damage to the kidneys can be seen from the shape of the kidneys. The shape of the kidney affects the size and weight of the kidney. The size of the kidney that changes to become bigger is usually associated with a widening of the pelvicalices due to obstruction in the distal part which causes hydronephrosis, which forces the renal cortex and causes cortical thinning. Reduced kidney size usually occurs due to chronic processes, such as prerenal disorders [5].

3.1.2 The Histology of kidney

Observation of changes in renal histology was carried out microscopically using histopathological preparations with HE (Haematoxylin-Eosin) staining from the kidneys of

diabetic rats from each treatment. The results of the observations made on the description of diabetic rat kidney and biwa leaf nano herbal can be seen in Figure 4.2.2

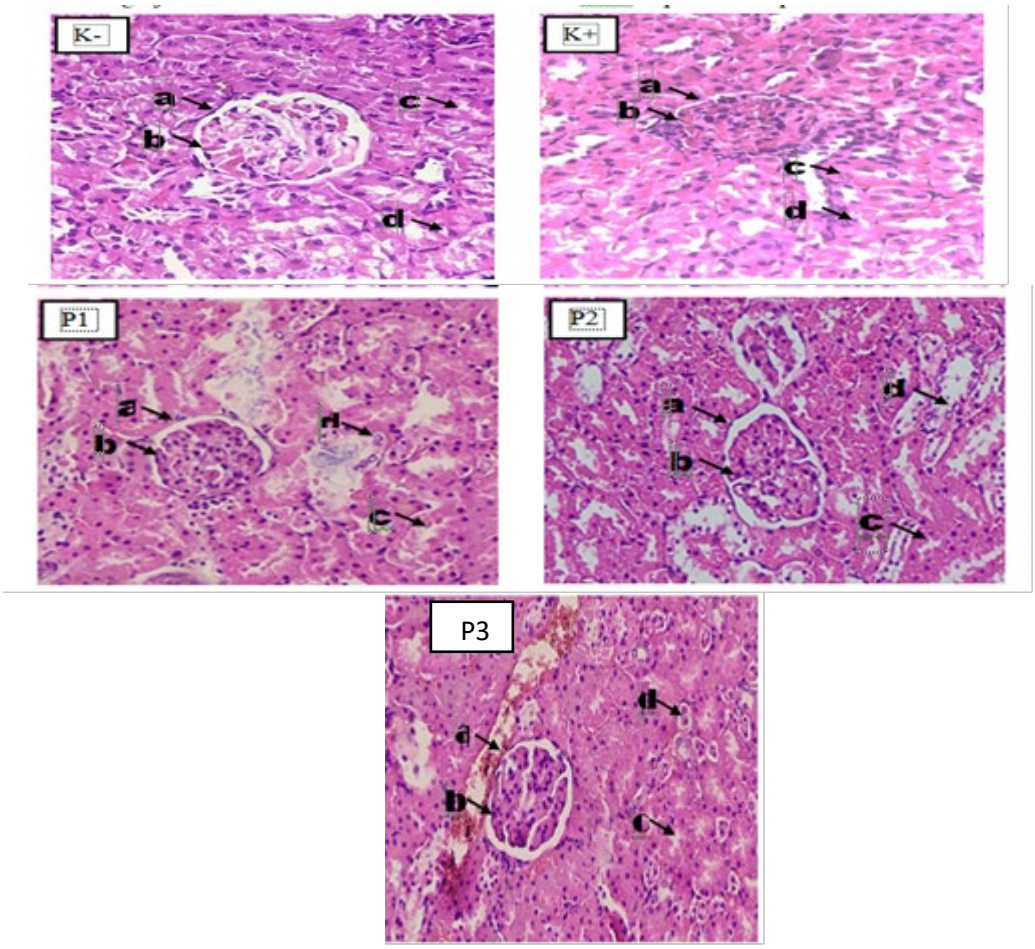

Figure 3.1.2 shows the histology of the kidney. K (-): Negative Control, K (+): Positive Control, P3: Nano extract of biwa leaf herb (*Eriobotrya japonica*) at a dose of 250 mg / kgBB, P4: Nano extract of biwa leaf herb (*Eriobotrya japonica*) at a dose of 500 mg / kg kgBB, and P5: Glibenclamide at a dose of 5 mg / kgBW. a) Bowman's capsule b) Gromerulus, c) Necrosis, d) Narrowing of the tubule.

In Figure 3.1.2, it can be seen that there are histological differences in the kidneys between the positive control group that was only given alloxan and the treatment group that was given biwa leaf nanoherba. In the treatment group, cell regeneration occurred in kidney histology.

The amount of blood flow to the kidneys causes kidney exposure to materials circulating in the circulatory system which is quite high, so that toxic materials will easily cause damage to kidney tissue in the form of changes in the structure and function of ren [6].

3.2 The weight of kidney

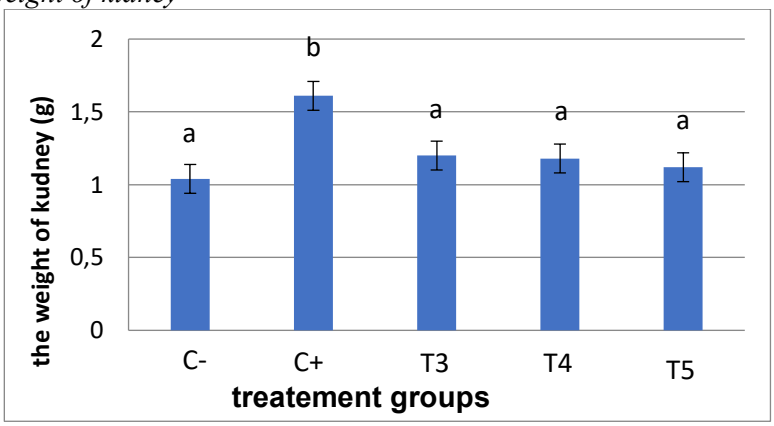

Figure 3.2 *Right and left kidney weight graphs of male rats (Rattus novergicus) from the negative (-), positive (+) control group, P3: the group given nano biwa extract at a dose of 250 mg / kgBB, P4: the group given nano biwa extract at a dose of 500 mg / kgBB, P5: the group given glibenclamide at a dose of 5 mg / kgBB.*

The results of statistical tests showed that biwa leaf nano herbal (*Eriobotrya japonica* lindl.) Had no effect on kidney weight. It is proven that the biwa leaf nano herbal (*Eriobotrya japonica* lindl.) Is not toxic so it does not affect kidney weight.

Changes in the histological structure of the kidneys are certainly influenced by the number of compounds in the body such as compounds in biwa leaf nanoherba but it does not affect kidney weight in mice, even in the highest doses of treatment. Kidney weight depends on the response of the body and metabolism of the rats [7]. The kidneys have the ability to regenerate kidney cells themselves. This regeneration ability replaces damaged cells and keeps the kidney's weight fixed [8].

3.3 Percentage of Proximal Tubule Narrowing

Percentage of tubular narrowing between each treatment can be seen in Figure 3.3

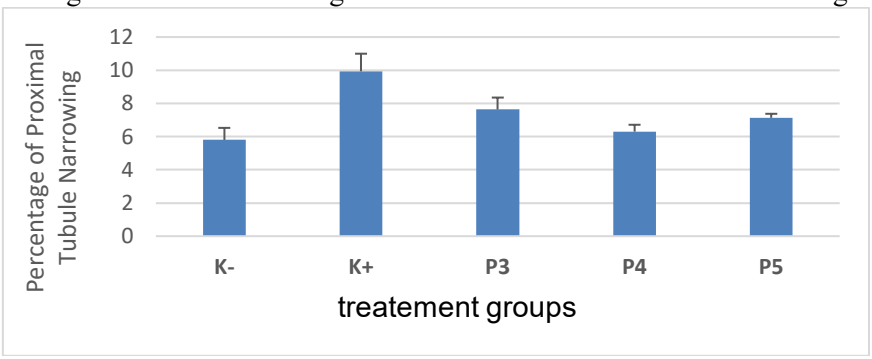

Figure 3.3 The percentage of narrowing of the proximal tubule of the kidney (*Eriobotrya japonica*) K (-): Negative Control, K (+): Positive Control, P3: Nano extract of biwa leaf herb (*Eriobotrya japonica*) at a dose of 250 mg / kgBB, P4: Nano extract of biwa leaf herb (*Eriobotrya japonica*) at a dose of 500 mg / kg kgBB, and P5: Glibenclamide at a dose of 5 mg / kgBW.

Based on Figure 4.3, it can be seen that the percentage of proximal tubular narrowing in the kidneys of male rats (Rattus norvegicus) between each control group and treatment group experienced a statistically significant difference ($p < 0.05$), where the highest percentage of proximal tubular narrowing was found in the positive control group showed significant differences with negative controls.

The proximal tubule is the part that is most often damaged due to exposure to nephrotoxic substances. The predisposing factor that causes proximal tubular cells to be easily damaged is the role of the proximal tubule which reabsorbs 60% -80% of glomerular filtration. The loose epithelium of the proximal tubule facilitates the entry of various components into the tubular cell [9].

3.4 Percentage of Renal Necrosis

Percentage of Renal Necrosis between each treatment can be seen in Figure 3.4

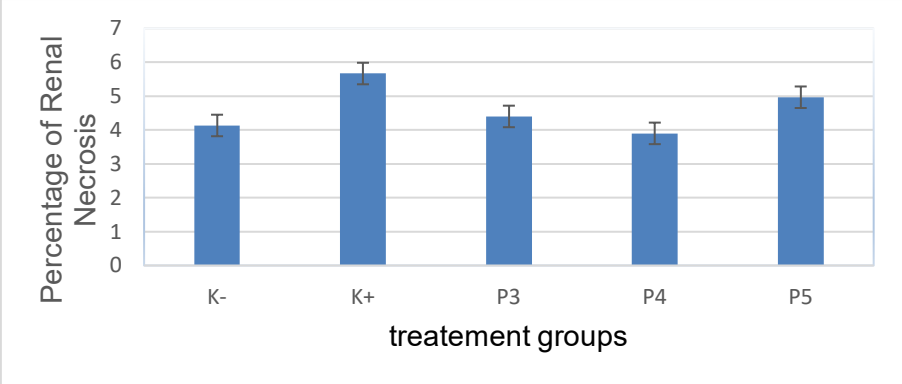

Figure 3.4 Percentage of Renal Necrosis of the kidney. K (-): Negative Control, K (+): Positive Control, P3: Nano extract of biwa leaf herb (*Eriobotrya japonica*) at a dose of 250 mg / kgBB, P4: Nano extract of biwa leaf herb (*Eriobotrya japonica*) at a dose of 500 mg / kg kgBB, and P5: Glibenclamide at a dose of 5 mg / kgBW.

Based on Figure 4.4, it can be seen that the percentage of kidney necrosis narrowing in male rats (Rattus norvegicus) between the control group and the treatment group was statistically significant ($p < 0.05$), where there was a significant difference between the positive control group and the negative control group. Meanwhile, in group P2 there was a decrease in the number of kidney necrosis.

The occurrence of necrosis in the kidney after giving excessive compounds can cause adverse effects on renal blood flow. The high blood flow to the kidneys, causes various drugs, and chemicals in the systemic circulation, to be delivered to the kidneys in large quantities and cause damage to the kidneys [7].

Tabel 3.4 The histopathological damage level of rats' kidneys after administration of biwa leaf nanoherba with different doses and treatment duration.

Treatment Group	Level damage	Explenation
K-	Normal	Normal kidney cells
K+	Advanced	Cell degeneration +++, hemorrhagic +++, necrosis +++
P1	Medium	Cell degeneration ++, hemorrhagic +, necrosis +
P2	Easy	+ Cell degeneration, hemorrhagic +
P3	Medium	Cell degeneration ++, hemorrhagic ++, necrosis ++

Information:
- : normal
\+ : Cell damage reaches 25% in eight fields of view
++ : Cell damage reaches 50% in eight fields of view
+++ : Cell damage reaches 75% in eight fields of view

3.5 Size of the glomerulus

Size of the glomerulus can be seen in Figure 3.5

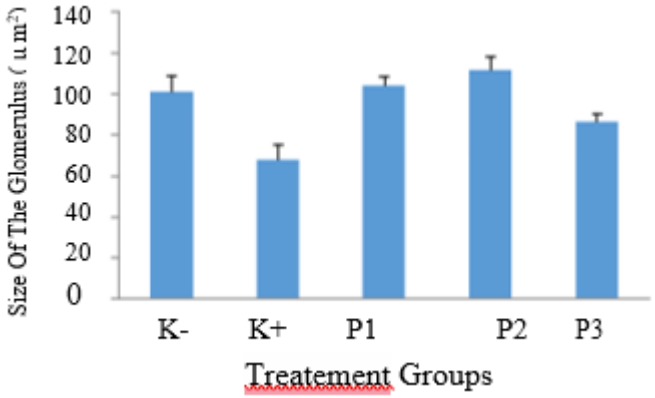

Figure 3.5 *Size of the glomerulus of the kidney.* K (-): Negative Control, K (+): Positive Control, P3: Nano extract of biwa leaf herb (*Eriobotrya japonica*) at a dose of 250 mg / kgBB, P4: Nano extract of biwa leaf herb (*Eriobotrya japonica*) at a dose of 500 mg / kg kgBB, and P5: Glibenclamide at a dose of 5 mg / kgBW.

Based on Figure 3.5, it can be seen that the renal glomerular diameter of male rats (*Rattus norvegicus*) between the control group and the treatment group was statistically different. In the P1 and P2 treatment groups there was an increase in the average glomerular area. This is because the nanoherbal content of biwa leaves can repair damage to the gromerulus.

Renal glomerular narrowing is damage caused by edema, inflammation and proliferation of the Bowman's capsule epithelium, resulting in narrowing of the Bowman's space. When there is an increase in capillary permeability and filtration in the glomerulus, plasma proteins and red blood cells can leak from the glomerulus so that the glomerular filtration membrane is damaged and there is swelling and edema in the Bowman's space which can cause the Bowman's space to narrow [10].

Table 3.5 The level of histopathological damage to the kidneys of white rats after administration of biwa leaf nanoherba which was characterized by gromerulus damage (in scoring).

Treatment Group	Level damage	Explenation		
		Enlargement of the Glomerulus	Narrowing of the capsular space	Erythrocyte granules
K-	Normal	Normal	visible cell nucleus	Round shape
K+	Advanced	+++	+++	+++
P1	Medium	++	++	++
P2	Easy	+	+	+
P3	Medium	++	+	+

Information:
- : normal
+ : Cell damage reaches 25% in eight fields of view
++ : Cell damage reaches 50% in eight fields of view
+++ : Cell damage reaches 75% in eight fields of view

4. Conclusion

Nano Herba of biwa leaves (*Eriobotrya japonica* Lindl.) Has a significant effect on changes in kidney morphology. Nano Herba of biwa leaves Is able to trigger the regeneration of kidney histological cells to overcome and reduce the percentage of proximal tubular narrowing and the percentage of necrosis in the kidney and trigger gromerulus cell regeneration which causes an increase in the area of the kidney gromerulus. The most effective dose of Nano Herba of biwa leaves (*Eriobotrya japonica* Lindl.) was the dose of 500 mg / kg (P2 group).

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