Short-term *Momordica charantia* L. Fruit Concentrated Infusions Therapy on Alloxan-Induced *Rattus norvegicus* Kidney Glomerulus Cells Histology

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Abstract. The aim of this research is to know the effect of short-term bitter melon (*Momordica Charantia* L.) fruit concentrated infusions therapy on the histology of alloxan-induced rat kidney glomerulus. Renal glomerular histology was observed using Hematoxylin-Eosin (HE) test. A therapeutic dose of bitter melon fruit concentrated therapy used was 0.15 mL/200 g BW; 0.30 mL/200 g BW; 0.45 mL/200 g BW; 0.60 mL/200 g BW; 0.80 mL/200 g BW and 1 mL/200 g BW. The results showed that the bitter melon fruit concentrated infusions therapy on alloxan-induced DM rats was able to decrease the Blood Glucose Levels (BGL) of the treated rats in varying degrees. The therapy also successfully repairs the damage kidney glomerulus cells of all rats treated with an average bowman's space improvement of 110% of healthy rat's kidney glomerulus cells. It appears that the mechanism of the bitter melon active substance in reducing the BGL occurs through a different path to its mechanism in repairing damaged glomerulus cells.

Keywords: diabetes mellitus; extract; bitter melon; momordica; hematoxylin; eosin; HE; therapy; blood glucose levels; natural product; alloxan; kidney

1 Introduction

Diabetes Mellitus (DM) is a chronic disease characterized by high blood glucose levels (BGL) condition (hyperglycemia) due to improperly regulating glucose homeostasis. Chronic hyperglycemia leads to over-production of excessive free radicals, which can lead to the generation of oxidative stress. Free radicals can damage the cell membrane, if it continues it will lead to damage the cell membrane system and cell death [1].

Kidneys are the second organs susceptible to the effects of chemicals since these organs receive 25-30% of the blood circulation to be cleaned. So that as filtration organs the likelihood of pathological changes is very high [2]. DM disease can trigger the occurrence of diabetic nephropathy [3], namely complications that occur in fine blood vessels. Damage to the blood vessels leads to glomerular damage that serves as a blood filter in the kidneys. High levels of glucose in the blood will make the kidneys structure change [4]. Some short-term and long-term therapy has been investigated to treat this nephropathy and successfully for both term [5]–[8].

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Bitter melon (*Momordica charantia* L.) fruit water extract contains some classes of carbohydrate compounds, proteins, amino acids, sterols, flavonoids, phlobatannins, terpenoids, glycosides, and saponins [9]. Momordica charantia contains some specific compounds such as albuminoid, charantin, hydroxytryptamine, dyes, vitamins A, B, and C [10]. Some of these compounds have an antioxidant role that can against free radicals.

In this study, the method used to extract bitter melon fruit is an infusion. Infusion is one of the extraction methods using a solvent such as water, oil or alcohol, by allowing the material to remain suspended in the solvent over time at a temperature of 90 ° C without evaporation. This method is very effective to apply to the community because it can only use water as a solvent while still able to maintain its active ingredients.

This study will investigate the ability of bitter melon fruit infusions to repair the glomerular damage of diabetes mellitus suffering rat (DM rat). Rat induced hyperglycemia treated by the alloxan diabetogenic agent. This study aims to determine the effect of concentrated infusions therapy of bitter melon fruit on histologic features of renal glomerulus of DM rat.

2 Materials and Methods

2.1 Material

The material used is the local bitter melon fruit readily harvested, male Wistar strain white rat (*Rattus norvegicus*) 2-3 months weighing ± 200 g, Alloxan (*Alloxan Monohydrate*), 0.9% NaCl, 10% Neutral Buffer Formalin solution, plastics, 70%, 80%, 90% alcohol, absolute alcohol, paraffin, and xylol.

2.2 Preparation of Bitter Melon Fruit Concentrated Infusions

The method of making infusions follows the standard of the Directorate General of POM RI [11], but the simplicia powder is increased to 30 g from the Pharmacopoeia standard of 10 g, to produce concentrated infusions. Concentrated infusions therapy is given daily for 14 days as a fresh solution. The dried bitter melon simplicial powder of 30 g was dissolved in 160 mL of water and then put in an infusion pot. The infusion pot is heated in a water bath for 15 minutes since the temperature reaches 90ºC while stirring occasionally. Filtering is done using a white cotton cloth in a still hot solution. The infusions are made fresh every day when it will be given as therapy.

2.3 Experimental Animal Preparation

The experimental animals to be adapted for ± 3 weeks to a uniform pattern of animal life. Feeding and drinking are done daily in ad libitum. The trial was divided into 8 treatment groups with 4 mice for each treatment group:

KN: Normal control group without treatment
KP: Positive DM control group
KT1: DM group treated with infusions dose 0.15 mL / 200 g BW.
KT2: DM group treated with infusions dose 0.30 mL / 200 g BW.
KT3: DM group treated with infusions dose 0.45 mL / 200 g BW.
KT4: DM group treated with infusions dose 0.60 mL / 200 g BW.
KT5: DM group treated with infusions dose 0.80 mL / 200 g BW.
KT6: DM group treated with infusions dose 1 mL / 200 g BW.

2.4 Diabetes Mellitus Rat Preparation

Diabetes Mellitus rat prepared by injecting alloxan in its intraperitoneal region on fasted condition. Alloxan solution prepared by homogeneously dilute of 896 mg alloxan p.a. in 0.9 % NaCl solution until the solution volume was 28 mL. The alloxan solution dose used was 32 mg/200 g BW [12], [13]. Blood Glucose Levels (BGL) measurements were performed using a DR Glucometer through rat tail ends little cutting. The rat will be determined as positive for DM if BGL is above 200 mg/dL.

2.5 Kidney Organ Harvesting

The rats were dissected after putting to death by neck dislocation. The kidneys are removed and rinsed in water, then immersed in 10% Formalin Neutral Buffer solution in a sealed container for further analysis.

2.6 Preparation of Kidney Organs

The kidney organs were graded dehydrated using an alcohol of concentrations of 70%, 80%, 90%, absolute alcohols I, II every 2 hours respectively. Then cleared off by xylol and molded using paraffin so they were cast in paraffin blocks and then stored in a refrigerator. The paraffin blocks are then sliced as thick as 5-6 μm using a microtome. The slice results were floated in warm water at 60 ° C to stretch for the tissue not to fold. The preparations were then lifted and placed in an object glass for Hematoxylin and Eosin (HE) staining [14], [15].

2.7 Hematoxylin-Eosin (HE) Stain for Renal Histologic Test

Preparation was observed under an electron microscope with a magnification of 40x. The observed part is the Bowman's space of glomerulus. Bowman's space improvement is calculated using the percentage formula:

$$BS\ Percent\ of\ improvement = ((b)) / ((a)) \times 100\% \quad (1)$$

where
Average KT = (Average KT1 to KT6) / 6
(a) = (Average KP) - (Average KN)
(b) = (Average KP) - (Average KT)

3 Result and Discussions

This study uses an in vivo test. The rats are conditioned for hyperglycemic by alloxan induction. Alloxan is a substance that works to increase blood glucose levels so that the rats suffer from Diabetes Mellitus (DM). The alloxan induction was done 2 times because in the first induction there were still some rats that its BGL have not reached 200 mg/dL so it has not determined as DM-positive. BGL measurements were performed on day-0 i.e. before alloxan induced and the day after alloxan induction. Day-1 is the day when all the rats have been tested as positive for DM. When all the rats were positive DM then given bitter melon fruit concentrated infusions therapy for 14 days. On the 15th day, the rats were dissected and the kidney organ was taken to be observed.

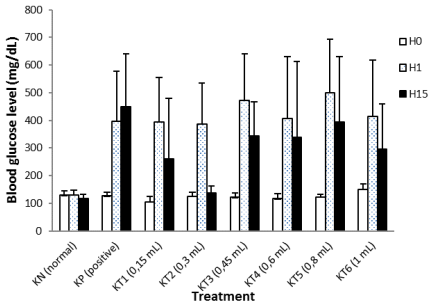
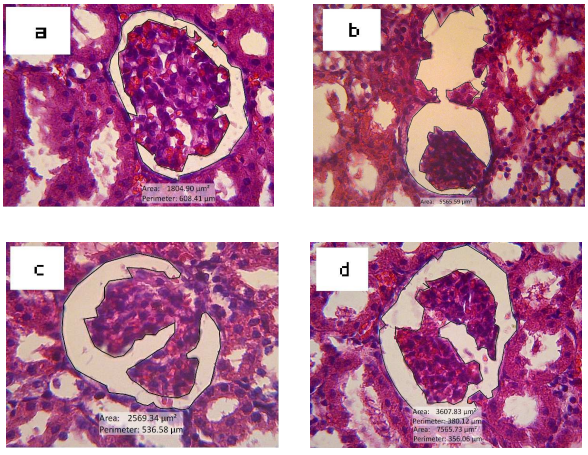


Figure 1. Rat blood glucose levels before treatment (H0), after injection of alloxan (H1), and after therapy (H15).

Before any treatment, all rats were in the same condition it had healthy rat's blood glucose levels below 200 mg/dL. The BGL condition of all rats after alloxan induction was DM-positive with a BGL value of above 400 mg/dL. After bitter melon fruit concentrated infusions therapy for 14 days were given, the rat's BGLs decreased substantially. The greatest decrease in BGL occurred in rat with bitter melon fruit concentrated infusions therapy at a dose of 0.3 mL. While the DM-positive rats without therapy, until day-15 have a steadily increasing BGL until reach an average value of 450 mg/dL.

Figure 2 shows that the glomerulus of DM rat (KP) has been damaged. The glomerulus undergoes considerable damage and decreases in the number of cells, resulting in a broadening of the Bowman's space to reach 5866.232 µm2 (see Table 1). The Glomerular cell damage rate in diabetic rats was twice that of healthy rat (KN). The presence of samples with mild or moderate necrosis in the normal control (KN) and treatment control (KT) group in Figure 1 was normal due to the aging process and cell death that physiologically occurs in all normal cells. Every cell in a body will always experience aging, which ends with cell death and is replaced by new cells through the regeneration process [16], [17]. In addition, the influence of psychological condition affected by the surrounding environment and different hypersensitivity reactions in each rat can also affect the aging process.



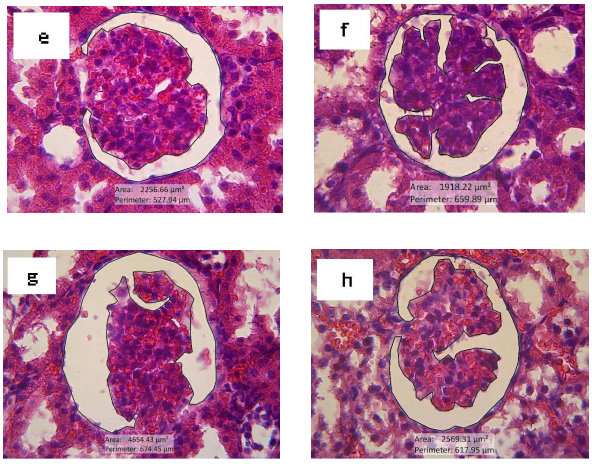


Figure 2. Rat's kidney glomerulus cells histology of each treatment. (a) KN, (b) KP, (c) KT1, (d) KT2, (e) KT3, (f) KT4, (g) KT5, (h) KT6.

Table 1 shows that there is a difference between health rat (KN), diabetic rat (KP), and treated rats (KT) Bowman's space. The average of Bowman's space in health rat was 2947,215 µm2. KP shows the average of the Bowman's space has widened to 5866.232 µm2. KT1, KT2, KT3, KT4, KT5, and KT6 have a wide narrowing of the Bowman's space back. The narrowing Bowman's space indicates a repairing health glomerulus cells. The six therapeutic doses did not show significant differences in glomerular cell repairing degree. The average ability of bitter melon fruit concentrated infusions therapy in improving the rat Bowman's space was 110% of the health one. The BGL data after the rats were treated by bitter melon fruit concentrated infusions shows a tendency that it is not in line with the improvement degree of glomerular cells. The improvement of glomerular cells in treating rats occurs to almost perfectly with an average value of 110% at all therapeutic doses. Glomerular cell returns to a healthy state with a value of the Bowman's space approaching the mean value of a healthy one. While the value of rat BGLs after therapy in different doses showed varying degrees of decreasing values. This indicates that the repairing mechanism of glomerular cells occurs through a different path to the mechanism of decreasing blood glucose levels.

Table 1. Bowman's Space Average Of All Rat Kidneys

Group	Bowman's Space (μm^2)
KN (health)	2947.215
KP (DM-positive)	5866.232
KT1	2642.015
KT2	2368.635
KT3	2540.445
KT4	2269.607

KT5	3470.090
KT6	2527.930

Chronic hyperglycemia conditions encourage free radical production excessively. The formation of excess free radicals in diabetes triggers a decrease in antioxidant content and tissue damage. Antioxidants serve as the body's defense against free radicals that induce oxidative stress and reactive oxygen compounds in plasma and cells so that cell damage does not occur. Some active ingredients in concentrated infusions of bitter melon fruit are antioxidants, namely vitamin C (ascorbic acid) and flavonoids. Vitamin C (L-Ascorbic Acid) is a natural compound that is a powerful antioxidant and free radical binder but not enzymatic [18], [19]. Vitamin C will work extracellularly, which is to reduce the superoxide radical produced in the process of glucose autoxidation and nitric oxide synthesis. When superoxide radicals are excessive, there will be a reaction with nitric oxide producing a cytotoxic peroxynitrite radical. Inhibition of the formation of peroxynitrite radicals will maintain the vasodilation function of blood vessels played by nitric oxide. In endothelial cells, ascorbic acid affects the nitric oxide synthase enzyme so that superoxide radicals as byproducts of nitric oxide formation can be suppressed and antioxidants and free radicals will be balanced [21]. Due to the balance of antioxidants and free radicals will effect on repair glomerulus. Bitter melon fruit infusions also contain flavonoids. Polyphenol compounds, especially flavonoids, are thought to play a role in the inhibition of lipid peroxidation because they have the ability to capture free radicals as a free radical scavenger. Flavonoids donate an H atom of hydroxyl phenolic groups when reacting with free radicals. As an antioxidant, flavonoid compounds will react with free radicals resulting in stable products and reduced cellular damage [22].

4 Conclusion

The concentrated infusions therapy of bitter melon fruit on alloxan-induced DM rats was able to decrease the Blood Glucose Levels (BGL) of the treated rats in varying degrees. The therapy also successfully repairs the damage kidney glomerulus cells almost perfectly. It appears that the mechanism of the bitter melon active substance in reducing the BGL occurs through a different path to its mechanism in repairing damaged glomerulus cells.

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