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Effects of Clove Syrup Therapy on Creatinine, Urea, and Kidney Histology of Diabetes Mellitus Rat

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Abstract

This study aims to determine the effect of clove syrup on creatinine, urea, and kidney histology of diabetic rats induced by streptozotocin. Eighteen male rats were divided into six groups: N, C (-), and C (+) were the control groups, and groups 1.8, 3.6, and 7.2 were the experimental groups. Groups C (-), C (+), 1.8, 3.6, and 7.2 were injected with streptozotocin 45 mg/kg BW in a single intraperitoneal injection. Normal control group or N and negative control group or C (-) were left untreated; positive control group or C (+) were treated with glibenclamide 0.09 mg/kg BW; groups 1.8, 3.6, and 7.2 were given clove syrup with doses of 1.8, 3.6, and 7.2 ml/rat/day respectively for 14 days. The obtained data were analyzed using a one-way ANOVA test, followed by the Duncan test at the significant level of 95% (a = 0.05). Kidney histology was observed by Hematoxylin-Eosin staining using Microscope. The result showed that clove syrup therapy with a dose of 3.6 ml/rat/day significantly decreases glucose, urea, and creatinine levels. Also, it showed that the dose of 3.6 has a regeneration effect to repair the damaged kidney. However, the higher amount of 7,2 ml/rat/day was toxic, causing an increase in blood glucose, urea, and creatinine levels and impairing kidney cells. In conclusion, clove syrup consumption at the right dose gave the best result in decreasing glucose, urea, and creatinine levels and regenerating the damaged kidney.

Keywords: *Clove Syrup; Rat; Diabetes Mellitus; Diabetes Nephropathy; Blood Glucose; Urea; Creatinine; Kidney Histology*.

INTRODUCTION

Diabetes, an endocrinal system disease, is also considered a chronic metabolic disorder (Cole and Florez, 2020). This disease is characterized by hyperglycemia, a condition when the blood sugar level is higher than usual; this often happens in a diabetic patient (Thomas et al., 2019). Hyperglycemia may occur because of defects in insulin secretion, insulin malfunction, or both. This condition has commonly happened in diabetic patients, and it also can be considered a characteristic of diabetes.

Diabetes is also considered an upcoming epidemic that affects almost every country, age, and economic group worldwide. According to the International Diabetic Federation, in 2015, approximately 415 million people worldwide had diabetes, and this number is expected to exceed 640 million in 2040. It estimated that half of this patient was unaware of their condition (Papatheodorou et al., 2018). This unawareness causes some patients not to get the proper treatment in time and may develop into diabetic complications. In diabetes cases, complication commonly happens, whether in diabetes mellitus type I or type II. These complications were responsible for morbidity and mortality in individuals with diabetes.

Diabetes complications are divided into microvascular and macrovascular complications. Microvascular complications consist of neuropathy, nephropathy, and retinopathy diabetes, while macrovascular complications include cardiovascular disease, stroke, and peripheral

artery disease (PAD) (Papatheodorou et al., 2018). One of the most recognizable and common microvascular diabetes mellitus complications was diabetes nephropathy (DN) (Karim et al., 2019). Diabetes nephropathy or diabetic kidney disease refers to the deterioration of kidney function that happens in individuals with diabetes type I or diabetes type II (Sulaiman, 2019). Diabetes patients with nephropathy complications will show a decline in glomerular filtration rate (GFR). Declining glomerular filtration rate will show a progressive increase in other kidney parameters, such as increased serum creatinine and urea levels; this may be considered early signs of diabetes kidney complication (Omoboyowa et al., 2021). If this complication were not treated immediately, it may become kidney failure and develop end-stage renal disease (ESRD), which may cause death to the patient.

Diabetes treatment was considered expensive, so it cannot be accessed by every socio-economic group, especially those with low socio-economic conditions. Because of this reason, another method that is equally effective but less expensive is needed so that it can be accessible to a broader socio-economic group. One of the methods that can be considered is by utilizing natural resources such as certain plants. One of the most common plants used as medicine is cloves.

Clove (*Syzygium aromaticum*) is a spice plant belonging to the Myrtaceae family; this plant is native to the Indonesian region, especially the Maluku region (Cortés-Rojas et al., 2014). This plant has high antioxidant activity because of its high eugenol content. Apart from eugenol, cloves also have several other compounds that can be utilized. The part of cloves that are most often used as ingredients for medicines and spices is the flower part because clove flowers consist of 10-20% essential oil higher than other clove parts (Pratama et al., 2019). In addition, clove flowers also contain other phytochemicals compounds such as alkaloids, saponins, tannins, and flavonoids (Jimoh et al., 2017). Based on Chaudhry et al. (2013), ethanol extract of clove flowers has a good effect in reducing blood glucose levels in diabetic rats. The lowering blood glucose effect in diabetic rats given clove flower ethanol extract was not much different from the decrease of blood glucose levels in insulin-treated diabetic rats. In addition, the research of Akila et al. (2018) states that cloves are also considered effective in repairing damaged kidney conditions on diabetic rats.

On this basis, it is safe to say that cloves can treat kidney damage in diabetes patients by formulating into syrup. After all, the syrup is a popular product in the community because it has a sweet taste and is liquid texture, making it easier to absorb. For clove syrup itself, it is made using the main ingredient, namely cloves, with the addition of cinnamon and honey, where each of these ingredients has its antioxidant activity. So, it is believed that clove syrup has the potential to treat kidney damage in diabetes patients. Therefore, this study aims to determine the effect of clove syrup therapy on creatinine, urea, and kidney performance of diabetic rats.

MATERIALS AND METHODS

Site and Time

This research was conducted in the Zoology Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon, Maluku, Indonesia. This research lasted two months, from December 2020 to February 2021.

Animals

The animal model used in this research was 18 males *Rattus novergicus* rats with an average body weight of ± 200 gr. Before being used as animal models, all rats were acclimatized for 21 days. Then the rats were divided into six groups, which in each group consisted of 3 rats.

Making of Clove Syrup

The preparation of clove syrup is done in the following way, 12.70 gr of clove are boiled with 9 gr of cinnamon (which have been mashed) in 120 ml of water for 15 minutes to a maximum volume of 50 ml. After that, the water is filtered and let still, and then 12 ml of honey is added.

Making Diabetes Mellitus Model Rats

Streptozotocin induction was performed to make the diabetes mellitus rats' model. First, the rats were fasted for 10 hours before being injected with streptozotocin. In this study, streptozotocin was injected at a 45 mg/kg BW dose, dissolved using citrate buffer with a pH of 4.5. After streptozotocin solutions were made, and then intraperitoneal injection of streptozotocin was performed. Then, three days after the injection, blood glucose levels were measured to determine which rats indicated diabetes mellitus. Based on the results of these measurements, rats were taken to be used in this experiment; the criteria for the rats used were those with fasting blood glucose levels > 126 mg/dL.

Treatment of Diabetes Mellitus Rats

Eighteen male rats were divided into six groups: normal control group or N, negative control group or C (-), and positive control group or C (+) were the control groups, and groups of rats that were given clove syrup with doses of 1.8 ml/rat/day or 1.8, groups of rats that were given clove syrup with doses of 3.6 ml/rat/day or 3.6, and groups of rats that were given clove syrup with doses of 7.2 ml/rat/day or 7.2 were the experimental groups. Groups C (-), C (+), 1.8, 3.6, and 7.2 are injected with streptozotocin 45 mg/kg BW in a single intraperitoneal injection. Normal control group or N and negative control group or C (-) were left untreated; positive control group or C (+) were treated with glibenclamide 0.09 mg/kg BW; groups 1.8, 3.6, and 7.2 were given clove syrup with doses of 1.8, 3.6, and 7.2 ml/rat/day respectively for 14 days.

Measurement of Blood Glucose Levels

Blood glucose measurements were carried out using a glucometer, once every seven days, with the following procedure: the rats were fasted 10 hours before blood glucose levels were measured. After that, the rat's blood was drawn, which was done by inserting a needle in the tail vein until the blood came out, then touching a drop of blood to the strip. When the strip container is filled with blood, the device will start measuring the blood glucose level. The results of measuring blood glucose levels are read 10 seconds after the blood enters the strip.

Measurement of Urea and Creatinine Levels

Urea and Creatinine measurements were carried out using a spectrophotometer with the Jaffe method. This measurement was done on the last day of the study or day 14, before the rats were necropsied.

Preparation and Observation of Diabetes Mellitus Rat Kidney

The rats were sedated using chloroform, then necropsied to isolate the kidney. After that, the kidney was weighed. Then the isolated organ is fixed using formalin and then rehydrated using graded alcohol with a concentration of 30%, 50%, 60%, 70%, 80%, and 96%. Furthermore, paraffin immersion is carried out, and the organ is cut with a microtome within the size of 3-4 microns. Then staining was carried out using hematoxylin and eosin. The histological results were observed using a light microscope with a magnification of 1000x.

Data Analysis

The measurements of blood glucose, urea, and creatinine levels were obtained and then analyzed by Analysis of Variance (ANOVA) and continued with the Duncan test at the significant level of 95% (a = 0.05) using SAS software.

RESULTS

Clove Syrup Therapy Effect on Decreasing Blood Glucose Level

The results showed that blood glucose levels at the beginning, before being injected with streptozotocin and clove syrup, were not significantly different ($P>0.05$) for all groups. This proves that all groups are still in average condition. Meanwhile, the results showed an increase in blood glucose level in the group of rats that were injected with streptozotocin, compared to the normal group (N) ($P<0.05$). This proves that streptozotocin has a diabetogenic effect that causes the increase of blood glucose levels in the rat (Table 1).

After 14 days of treatment, groups of rats that were treated with clove syrup showed significant changes, especially in the groups 1.8 and 3.6, which was significantly different from the negative control group or C (-) ($P<0.05$), while the positive group control or C (+) was not significantly different from this groups ($P>0.05$). Based on this result, it can be said that glucose level in the rat's blood can be lowered until it reaches normal, which means that regular consumption of clove syrup can reduce glucose levels. Also, it showed that clove syrup has the same effect as glibenclamide in regulating blood glucose levels. However, giving clove syrup as much as 7.2 ml/rat/day can cause an increase in blood glucose levels, indicating that this dose of clove syrup had no effect anymore; this was due to the accumulation of excess phytochemicals that became toxic to the body.

Clove Syrup Therapy Effect on Decreasing Serum Urea and Creatinine Level

The changes in urea and creatinine levels of diabetic rats after being treated for 14 days are presents in Table 2. Results showed that urea levels of diabetic rats after being treated with a 3.6 ml dose of clove syrup have the effect of lowering the urea levels until reaching normal. This means that group 3.6 can lower urea levels until meeting the same condition as the normal control group or N ($P>0.05$), compared to the group given 7.2 ml ($P<0.05$), which

shows a toxic effect. Meanwhile, the results also showed that the group of rats that given clove syrup with a dose of 3.6 ml/rat/day shows a decrease in creatinine levels that lower than N or those given clove syrup 1.8 ml/rat/day ($P<0.05$) but experienced a similar decrease in the C (+) group ($P>0.05$).

Table 1. Changes in blood glucose levels on rats after being made diabetic and treated for 14 days.

Days	Treatment ($\bar{x} \pm$ SD)					
	N	C (-)	C (+)	1,8	3,6	7,2
Pre stz	$82,3 \pm 6,65^a$	$92,6 \pm 5,50^a$	$81,6 \pm 3,05^a$	$79,3 \pm 12,85^a$	$74,6 \pm 12,50^a$	$82,3 \pm 13,52^a$
Post stz	$87,6 \pm 6,02^a$	$164,6 \pm 3,05^b$	$157,3 \pm 11,84^b$	$161,3 \pm 10,59^b$	$157,3 \pm 10,06^b$	$165,0 \pm 10,44^b$
Day 7	$84,3 \pm 10,78^a$	$212,0 \pm 12,0^b$	$117,6 \pm 9,29^c$	$120,0 \pm 6,55^c$	$129,0 \pm 7,0^c$	$127,0 \pm 4,58^c$
Day 14	$91,6 \pm 7,37^a$	$224,3 \pm 12,50^b$	$83,3 \pm 7,02^a$	$89,6 \pm 9,01^a$	$101,0 \pm 7,0^a$	$133,3 \pm 18,82^c$

Pre stz = Before streptozotocin injection, Post stz = After streptozotocin injection, Day 7 = 7 days after treatment, Day 14 = 14 days after treatment.
Read from left to right. Different superscript letters in one row showed significantly different results ($P<0.05$) between treatment groups.

The administration of clove syrup showed a significant change in urea and creatinine levels. The group that was given clove syrup 3.6 ml had the most significant differences with C (-) group ($P<0.05$), also different from the group treated with 1.8 ml clove syrup compared to the group that was given 3.6 ml ($P<0.05$), while C (+) was not significantly different from the group given 3.6 ml ($P>0.05$). This result shows that the decrease in urea and creatinine levels in the blood of rats reached normal, based on rat's normal standard levels for urea which were 12.3 - 24.6 mg/dL, while creatinine was 0.2 - 0.8 mg/dL. This means giving clove syrup a dose of 3.6 ml/rat/day can reduce urea and creatinine levels and have the same effect as the administration of glibenclamide on C (+). However, giving 7.2 ml of clove syrup for 14 days showed an increasing effect in urea and creatinine levels, stating that the clove syrup dose had no effect anymore; this was due to the accumulation of excess phytochemicals that became toxic.

Table 2. Changes in urea and creatinine levels on diabetic rats after being made diabetic and treated for 14 days.

Parameter (mg/dL)	Treatment ($\bar{x} \pm$ SD)					
	N	C (-)	C (+)	1,8	3,6	7,2
Urea	$24,3 \pm 0,57^a$	$57,6 \pm 1,52^b$	$26,0 \pm 0,1^a$	$29,0 \pm 0,1^c$	$25,3 \pm 0,57^a$	$71,0 \pm 1,73^a$
Creatinine	$0,68 \pm 0,01^a$	$0,94 \pm 0,01^b$	$0,56 \pm 0,005^c$	$0,63 \pm 0,02^d$	$0,56 \pm 0,01^c$	$0,9 \pm 0,02^b$

Pre stz = Before streptozotocin injection, Post stz = After streptozotocin injection, Day 7 = 7 days after treatment, Day 14 = 14 days after treatment.
Read from left to right. Different superscript letters in one row showed significantly different results ($P<0.05$) between treatment groups.

Kidney Cells Photomicrograph of *Rattus norvegicus* Diabetes Mellitus using Hematoxylin-Eosin Staining

The kidney histopathology results of diabetes mellitus rats using hematoxylin-eosin staining showed that there was damage on C (-) or a group of rats that were made diabetic by injecting streptozotocin 45 mg/kg without any other treatment (Figure 1b). C (+) or group of diabetic rats treated with glibenclamide (Figure 1c), group of diabetic rats given clove syrup

1.8 ml/rat/day (Figure 1d) and a group of diabetic rats given clove syrup 3.6 ml/rat/day (Figure 1e) shows that those groups can prevent damage and even regenerate kidney cells on diabetes mellitus rats. While the group of diabetic rats given clove syrup 7.2 ml/rat/day (Figure 1f) showed that giving this dose of clove syrup caused damage to the rat kidney cells, this was due to the accumulation of increased phytochemical content, which became toxic.

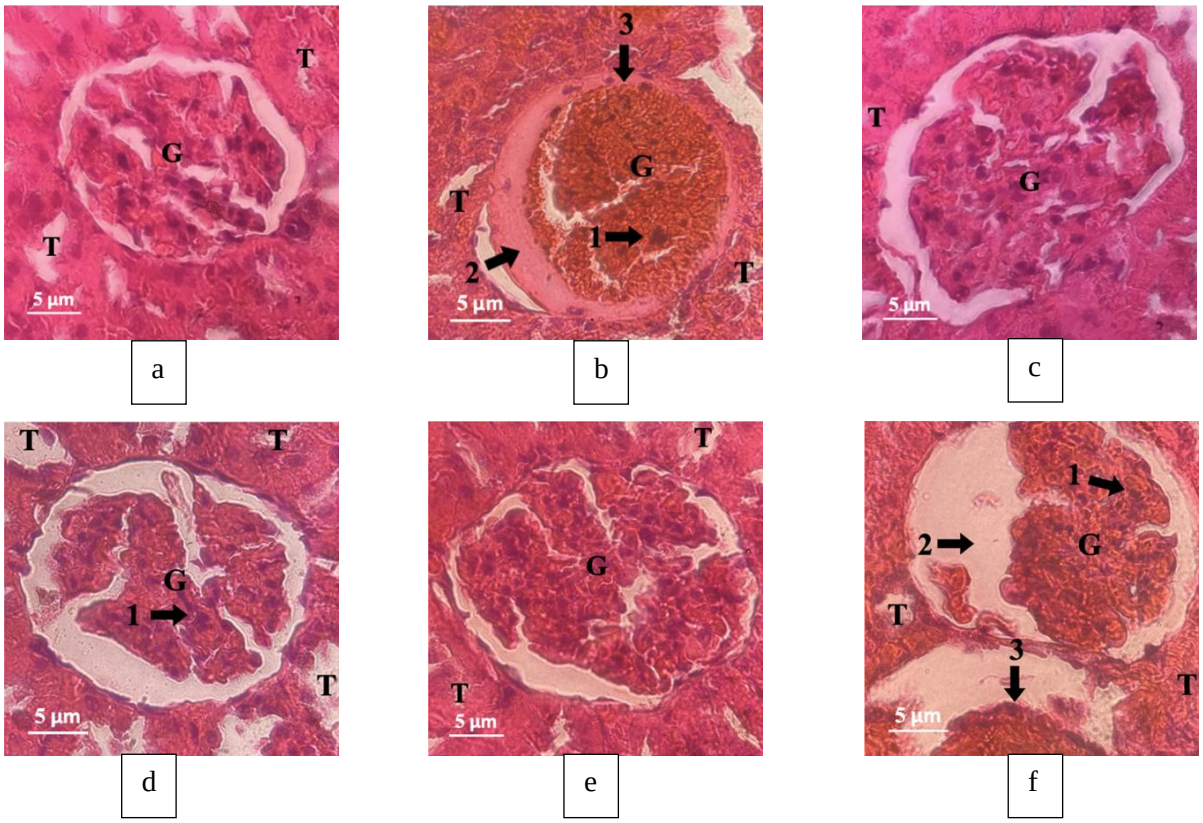

Figure 1. Cross-sectional photomicrograph of rats (*Rattus norvegicus*) kidney cells with hematoxylin-eosin staining, microscopically observed at a magnification of 1000x, Bar: 5 µm, a. normal rat group, b. group of rats made diabetic, c. group of rats made diabetic and then treated with glibenclamide 0.09 mg/rat/day, d. group of rats made diabetic and then given clove syrup 1.8 ml/rat/day, e. group of rats made diabetic and then given clove syrup 3.6 ml/rat/day, f. group of rats made diabetic then given clove syrup 7.2 ml/rat/day. Note: G. glomerulus, T. tubules, arrows (➞) indicate 1. necrosis, 2. glomerular atrophy, 3. Hemorrhage.

DISCUSSION

In this study, increased blood glucose, urea, creatinine level, and impairment of kidney cells resulted from streptozotocin injection. Streptozotocin is a substance that has diabetogenic properties, so it is often used to induce diabetes in animal models; the diabetogenic nature of streptozotocin is caused by damage to pancreatic cells (Furman, 2015), as a result of the formation of highly reactive free radicals that can cause damage to cell membranes, proteins, and deoxyribonucleic acid (DNA). With damage to the pancreatic insulin cells, the insulin secretion process is also disrupted, causing impaired regulation of glucose in the blood and cause hyperglycemia.

The hyperglycemic state that occurs affects the workload of the glomerulus as a blood filter becoming heavier. The accumulation of glucose in the glomerulus causes an increase in osmotic pressure, resulting in necrosis of the glomerular cells. Damage to glomerular cells also results in damage to renal tubular cells; this is because the reabsorption function of certain substances that the glomerulus must do does not occur so that these substances will be in direct contact with the proximal tubular epithelium, this results in renal tubular cell necrosis (Asrifa et al., 2017). In addition, the damage that occurs in kidney cells in this study is related to oxidative stress experienced by diabetic rats. Firdaus (2017) states that the manifestation of DM can cause various cell damage, which indicates the accumulation of free radicals. Excessive free radicals cause lipid peroxidation, a reaction between free radicals and polyunsaturated fatty acids in cell membranes that result in cell damage (Attia et al., 2008 in Wigati et al., 2018).

Damage to kidney cells, especially glomerular atrophy, disrupts the filtration process that occurs in the kidneys, namely by reducing the ability to filter blood. If there is a decrease in the blood's filtration ability, some protein can come out with the urine or accumulate in the tubules because they have escaped the filtration process. Accumulation in the tubules can cause damage to the renal tubules, which will disrupt the reabsorption and secretion processes. The disrupted secretion process causes substances that are not needed by the body cannot be excreted appropriately and continue to amass in the blood (Hasnisa et al., 2015). This is why some metabolic waste substances can be found in the blood, as evidenced by the high levels of urea and creatinine in the negative control group, which can be seen in table 2. Cell damage experienced by the kidneys can cause damage to kidney function by decreasing the glomerular filtration rate. A decrease in the glomerular filtration rate causes substances that should be filtered in the kidneys not to be filter, thereby increasing metabolic waste substances in the blood, including an increase in urea and creatinine levels (Aji et al., 2019).

Based on the result, the clove syrup treatment group of 1.8 and 3.6 reduced blood glucose levels; the content of secondary metabolites caused this. Nindatu et al. (2021) stated that clove syrup contains alkaloids, flavonoids, terpenoids, phenols, saponins, and tannins. Among the phytochemical compounds in clove syrup, alkaloids and flavonoids are believed to have anti-diabetic effects. The anti-diabetic effect of alkaloids and flavonoids has two mechanisms, namely intra-pancreatic and extra-pancreatic. The intra-pancreatic mechanism mentioned is the ability of the compound to improve the condition and function of the kidneys. In contrast, the extra-pancreatic mechanism is the ability of compounds to suppress the rate of glucose absorption.

Apart from alkaloids and flavonoids, clove syrup also contains saponins. This compound has an inhibitory or inhibitory effect on the α-glucosidase enzyme. The α-glucosidase enzyme is an enzyme found in the small intestine that functions to convert disaccharides into glucose; with the effect of α-glucosidase inhibitors owned by saponins, glucose absorption occurs in the small intestine can be inhibited (Fiana and Oktaria, 2016). This same effect can also be found in flavonoids.

Secondary metabolites contained in clove syrup also could indirectly reduce urea and creatinine levels. The decrease in urea and creatinine levels in diabetic rats can occur due to the content of secondary metabolites with antioxidant activity. The phytochemical content of

alkaloids, flavonoids, phenols, saponins, and tannins in clove syrup contains antioxidant properties. Antioxidants are a class of compounds that have the function of warding off free radicals; in other words, antioxidants can kill foreign substances that cause cells to become damaged so that the body's cells can regenerate (Handani et al., 2015). An imbalance between free radicals and antioxidants in the body can cause oxidative stress, damaging cell structure, and function. This damage is caused due to the reactivity of free radicals in looking for electron pairs (Novianti, 2015). For this reason, in a period of hyperglycemia that causes high free radicals, an adequate intake of antioxidants is needed to compensate for free radicals in the body. When free radicals have bound and reach stability, free radicals cannot interfere with other molecules. After antioxidants capture excess free radicals, cells damaged by free radicals have the opportunity to regenerate (Wirawan, 2018). With the regeneration of the damage that occurs can be overcome, the impaired glomerular filtration rate due to damage to kidney cells will return to normal so that the accumulated metabolic waste substances can be excreted out. Besides that, flavonoids also work as diuretics to increase the glomerular filtration rate (Tandi et al., 2017).

However, it was seen that 7.2 or the group given clove syrup at a dose of 7.2 ml/rat/day showed a continuous increase in blood glucose levels from the seventh to the fourteenth day. The blood glucose level of this group on the seventh day was 127 mg/dL and then increased to 132 mg/dL on the fourteenth day. This increase can occur because the dose given is too high. Sasmita et al. (2017) stated that excessive doses could lead to the accumulation of phytochemical compounds, which can be detrimental. The toxic nature of the excess phytochemical compounds in the body can damage body cells, one of which is pancreatic cells. Damaged pancreatic cells cause insulin to be unable to secrete properly, causing blood glucose levels in the body to increase again. Another finding showed damage to kidney cells in diabetic rats treated with 7.2 ml of clove syrup. This suggests that excessive clove syrup can also cause damage to kidney cells. The content of secondary metabolite compounds in this dose of clove syrup is too excessive, causing damage to kidney cells.

Due to kidney cell damage, the glomerular filtration rate also decreases so that metabolic waste substances that should be excreted through urine cannot be excreted properly, causing them to accumulate in the blood. This leads to the measurement result of urea and creatinine can be seen as high. This is in line with the results of research that showed urea levels of 71.0 mg / dL and creatinine of 0.9 mg / dL; these levels were considered high compared to normal control group (N) rat or normal urea and creatinine standards in rats.

CONCLUSION

The administration of clove syrup at a dose of 3.6 ml/rat/day effectively reduced urea and creatinine levels and repaired the kidney histology of diabetes mellitus rats. Meanwhile, administration of clove syrup at a dose of 7.2 ml/rat/day had a toxic effect that can cause an increase in urea and creatinine levels and impairing kidney cells.

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