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Effectiveness of Beetroot Extract (Beta Vulgaris) on Cholesterol Levels and Kidney Histopathology in Aloxan-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a chronic metabolic disease that can cause severe complications, including lipid abnormalities and kidney damage. Beetroot (Beta vulgaris) contains bioactive compounds such as betacyanin and flavonoids with antioxidant and hypolipidemic properties, potentially improving lipid profiles and protecting renal tissues. To evaluate the effectiveness of beetroot extract (Beta vulgaris) on cholesterol levels and kidney histopathological features in alloxan-induced diabetic rats. This true experimental study used a pre-test and post-test only control group design involving 36 male white rats (Rattus norvegicus), divided into six groups: normal, negative control, positive control (metformin), and three treatment groups receiving beetroot extract at doses of 22.5 mg, 45 mg, and 90 mg/200 gBW. Diabetes was induced using alloxan monohydrate 150 mg/kgBW intraperitoneally. The extract was administered orally for 14 days. Cholesterol levels were analyzed using spectrophotometry, while kidney histopathology was examined using Hematoxylin-Eosin (HE) staining. ANOVA test showed significant differences in cholesterol levels among groups ($p < 0.001$). The treatment group receiving 45 mg/200 gBW demonstrated the most optimal cholesterol reduction compared to the negative control. Histopathological examination revealed improvement in glomerular and tubular structures in the treatment groups. Beetroot extract (Beta vulgaris) is effective in reducing cholesterol levels and improving kidney histopathology in alloxan-induced diabetic rats.

KEYWORDS Beetroot (*Beta vulgaris L.*), cholesterol, kidney histopathology, diabetes mellitus, alloxan.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by increased blood glucose levels (hyperglycemia) due to impaired insulin secretion and function. This disease is one of the global health problems with a prevalence that continues to increase every year. People with diabetes have a higher risk of experiencing serious complications, especially in the cardiovascular, kidney, nerve, and other vital organs. Complications that often appear include coronary atherosclerosis, diabetic cardiomyopathy, and myocardial infarction, which contribute greatly to the mortality rate of people with diabetes (Esdaile et al., 2023).

Epidemiological data show a very worrying trend of increasing diabetes cases. In 2019, around 19.3% of the population aged 65–99 years, or equivalent to 135.6 million people, were estimated to have diabetes (Sinclair et al., 2020). Furthermore, in 2021, the global prevalence of diabetes increased to 10.5%, or around 536.6 million people aged 20–79 years. This figure is projected to continue to rise to 12.2%, or around 783.2 million people by 2045 (Sun et al., 2022). This fact confirms that diabetes is one of the main health threats, especially for lower-middle-income countries that have limited resources for its treatment.

In addition to causing hyperglycemia, diabetes mellitus is also known as a metabolic syndrome that is often accompanied by lipid metabolism disorders. This condition is characterized by an increase in total cholesterol, LDL, triglycerides, and decreased HDL levels.

These changes in lipid profiles trigger a greater risk of cardiovascular disease, including coronary heart disease and stroke (Rinjani, Septriana, & Herawati, 2022). Thus, cholesterol control is an important step in preventing further complications in diabetics.

Cholesterol itself is an essential lipid component that functions in hormone synthesis, cell membrane formation, and bile acid production. In blood circulation, cholesterol is carried through lipoproteins consisting of chylomicrons, VLDL, LDL, and HDL. Each has a specific function; for example, LDL is the main carrier of cholesterol to the tissues, while HDL plays a role in transporting cholesterol back to the liver. However, excess cholesterol levels can lead to hypercholesterolemia, leading to atherosclerosis and increasing the risk of cardiovascular disease (Adeloye et al., 2020).

The increasing number of diabetics and dyslipidemia-related complications has prompted research into alternative therapies based on natural ingredients. One of the approaches that attracted attention was beetroot extract (*Beta vulgaris*). Beetroot is known to be rich in vitamins, minerals, and betalain pigments that have high antioxidant activity. Other bioactive compounds in beetroot, such as flavonoids, betacyanin, and phenolic acid, have also been shown to have protective effects against various degenerative diseases (Winanta, Haresmita, & Merilla, 2023a; Thiruvengadam et al., 2024).

Previous studies have shown that beetroot extract can lower cholesterol levels, protect liver function, and play a role in the prevention of atherosclerosis. In addition, the antioxidant effects of betalain in beetroot can minimize cell damage due to oxidative stress that often occurs in people with diabetes. This content provides a scientific basis that beetroot has the potential to be used as an additional or alternative therapy in the management of dyslipidemia in diabetics.

In addition to its effects on lipid profiles, beetroot is also thought to have a protective role for the kidneys. People with diabetes are prone to developing diabetic nephropathy, which is characterized by structural and functional damage to the kidneys due to chronic hyperglycemia. With its antioxidant and anti-inflammatory activities, beetroot extract has the potential to reduce damage to kidney tissue, thereby slowing down the progression of diabetes complications. Therefore, research on the effects of beetroot on kidneys in experimental animals is important to strengthen scientific understanding.

Previous studies have examined the potential benefits of beetroot (*Beta vulgaris*) in managing diabetes-related complications, but gaps remain in understanding its effects on both lipid profiles and kidney histopathology. For instance, Winanta, Haresmita, & Merilla (2023a) demonstrated that beetroot extract could reduce cholesterol levels and provide antioxidant protection in diabetic models, highlighting its potential in cardiovascular risk reduction. However, this study primarily focused on lipid-lowering effects without a detailed evaluation of renal tissue damage. Similarly, Thiruvengadam et al. (2024) reported that beetroot's bioactive compounds, such as betalains and flavonoids, can minimize oxidative stress and cellular damage in diabetes, but their research did not specifically investigate histopathological changes in the kidneys. Both studies provide important evidence of beetroot's therapeutic potential, yet they fail to integrate the dual analysis of lipid profile improvement and kidney protection in a single experimental design.

Based on this background, this study aims to evaluate the effectiveness of beetroot extract (*Beta vulgaris*) in lowering cholesterol levels and assess its effect on the histopathological

picture of the kidneys in alloxan-induced diabetic rats. The results of this study are expected to contribute to the development of alternative treatments based on natural ingredients that are safe, affordable, and have the potential to reduce the risk of diabetes complications, especially in the cardiovascular and kidney systems.

METHOD

This type of research is true experimental research with a post-test only control group design. This design involves two groups, namely the control group and the treatment group. The research includes several stages, ranging from the manufacture of beetroot extract (*Beta vulgaris*), the formation of research groups, preparation and treatment in experimental animals, the induction of alloxan, to cholesterol level testing and the preparation of histopathological preparations for the kidneys of white rats (*Rattus norvegicus*) before and after the administration of beetroot extract.

This research was carried out at the Scholar Laboratory in March-April 2024. The test animal used was a male white rat of the Wistar strain with a body weight of 150–200 grams. The inclusion criteria included healthy rats weighing 150–250 grams, 8–12 weeks of age, and having no anatomical abnormalities. Meanwhile, the exclusion criteria were female rats, rats weighing outside the range of 150–250 grams, less than 8 weeks old or more than 12 weeks, unhealthy, and dead rats before or after treatment.

The study population consisted of an accessible population of male Wistar rats, while the target population was healthy male Wistar rats aged 8–12 weeks. The study sample was healthy male Wistar rats weighing 150–250 grams and aged 8–12 weeks. The selection of male rats was carried out to avoid variations due to hormonal cycles in female rats that can affect metabolism and response to drugs.

The calculation of the number of samples was carried out using Federer's formula to determine the minimum number of samples per group with statistical validity. Based on this calculation, the minimum number of samples for each group is 4 rats. However, the researchers chose to use 6 rats per group, so that with a total of 6 groups, the total number of study rats was 36.

The variables in this study consisted of an independent variable, namely the administration of beetroot extract (*Beta vulgaris*), and a dependent variable, namely cholesterol levels and the histopathological picture of the kidneys of male Wistar rats induced by alloxan. The relationship between these two variables will be analyzed to assess the effectiveness of beetroot extract in lowering cholesterol while providing a protective effect on the kidney organs.

The research instruments used include various laboratory tools such as 3 mL syringes, hematocrit capillaries, spectrophotometers, centrifuges, micropipettes, digital scales, light microscopes, microtomes, and small surgical instruments. Meanwhile, the research materials include white rats of the Wistar strain, cholesterol kit reagents, alloxan monohydrate at a dose of 150 mg/kg BW for diabetes induction, fresh beetroot as the main ingredient of the extract, 96% ethanol solvent, 10% buffer formalin solution, and hematoxylin-eosin (HE) dye for the manufacture of histopathological preparations.

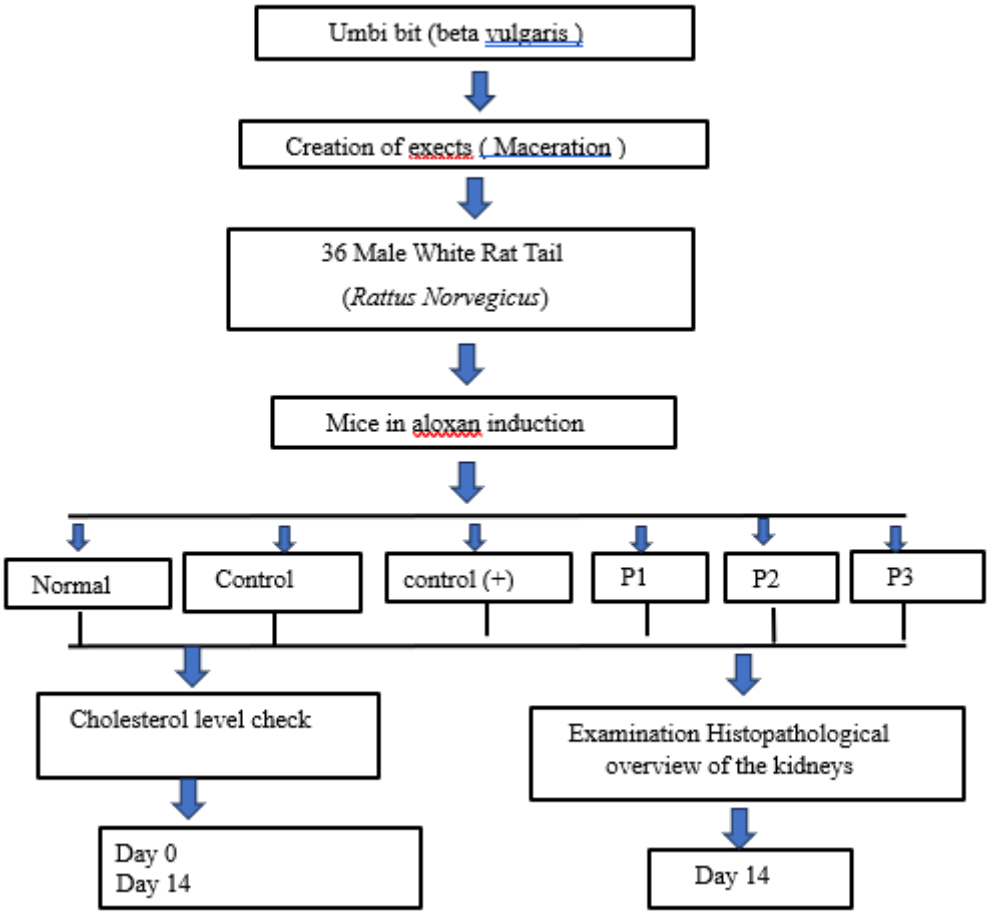

Figure 1. Research Step

Data processing was carried out statistically to evaluate the effect of beet tuber extract (Beta vulgaris) on blood glucose levels, total cholesterol levels, and histopathological picture of the kidneys of white rats (Rattus norvegicus) induced by aloxan. The analysis was conducted using IBM SPSS software version 27. The data normality test was carried out with the Shapiro-Wilk test because the sample count was less than 50, to ensure data distribution. Furthermore, the homogeneity test uses the Levene's Test to assess the similarity of variance between groups. If the data met the assumptions of normality and homogeneity, the One-Way ANOVA parametric test with a 95% confidence level ($\alpha = 0.05$) was used. If there is a significant difference, the analysis is continued with a Post Hoc test. Conversely, if the data are not normally distributed or inhomogeneous, the non-parametric Kruskal-Wallis test is used as an alternative to ANOVA, and if significant differences are found, the analysis is followed by the Mann-Whitney test to determine the different groups.

RESULT AND DISCUSSION

Characteristics of Research Samples

This study used 36 male white rats divided into 6 groups, namely:

1) Normal Group
2) Negative Control Group (aloxan + aquadest)
3) Positive Control Group (aloxan + metformin)
4) Treatment Group 1 (aloxan + beet tuber extract 22.5 mg/200gBB)
5) Treatment Group 2 (aloxan + beet tuber extract 45 mg/200gBB)
6) Treatment Group 3 (aloxan + beet tuber extract 90 mg/200gBB)

All mice were induced by aloxan (except for the normal group) and then given treatment according to their respective groups with a time of 14 days.

Mean Cholesterol Levels Before and After Treatment

Table 1. Mean Cholesterol Levels Before and After Treatment

Variable	Mean	SD	Minimum	Maximum
Cholesterol level before treatment	82.64	25.45	36	135
Cholesterol level after treatment	82.39	16.61	36	119

Table 1. shows that the mean cholesterol level before treatment was 82.64 mg/dL (SD = 25.45), whereas the mean cholesterol level after treatment was 82.39 mg/dL (SD = 16.61).

Table 2. Mean Cholesterol Levels in Each Group Before and After Treatment

Group	Mean Before (mg/dL)	Mean After (mg/dL)	Change
Normal	63.37	81.67	Increased
Negative control	52.00	92.17	Increased
Positive control	96.83	80.00	Decreased
Treatment 1	86.17	68.50	Decreased
Treatment 2	110.67	82.33	Decreased
Treatment 3	87.00	89.67	Increased

Table 2. shows that the mean cholesterol level in the normal group increased from 63.37 mg/dL to 81.67 mg/dL after treatment.

In the negative control group, cholesterol levels increased from 52.00 mg/dL to 92.17 mg/dL. In contrast, the positive control group showed a decrease from 96.83 mg/dL to 80.00 mg/dL. The treatment groups also demonstrated reductions: treatment 1 decreased from 86.17 mg/dL to 68.50 mg/dL, and treatment 2 decreased from 110.67 mg/dL to 82.33 mg/dL. Meanwhile, treatment 3 showed a slight increase from 87.00 mg/dL to 89.67 mg/dL.

Normality Test

Table 3. Normality Test

Variable	p-value	Distribution
Cholesterol before treatment	0.338	Normal
Cholesterol after treatment	0.107	Normal

Table 3. indicates that both pre- and post-treatment cholesterol levels were normally distributed ($p > 0.05$).

ANOVA Test

Table 4. Homogeneity and ANOVA Test

Variable	Levene Statistic	p (Homogeneity)	F	p (ANOVA)
Cholesterol levels before and after treatment	2.27	0.073	7.74	<0.001

Table 4. shows that the homogeneity test produced a Levene statistic of 2.27 with p = 0.073 (p > 0.05), indicating homogeneous variances. The ANOVA test yielded F = 7.74, p < 0.001, demonstrating a statistically significant difference in cholesterol levels before and after treatment. Therefore, the treatments administered had a significant effect on cholesterol levels.

Table 5. Post Hoc Test

Group	Compared With	Mean Difference	Std. Error	Sig.	95% CI (Lower–Upper)
Normal	Negative control	-21.667	13.174	0.577	-61.74 – 18.40
	Positive control	35.333	13.174	0.109	-4.74 – 75.40
	Treatment 1	36.167	13.174	0.095	-3.90 – 76.24
	Treatment 2	46.833*	13.174	0.015	6.76 – 86.90
	Treatment 3	15.833	13.174	0.832	-24.24 – 55.90
Negative control	Positive control	57.000*	13.174	0.002	16.93 – 97.07
	Treatment 1	57.833*	13.174	0.002	17.76 – 97.90
	Treatment 2	68.500*	13.174	<0.001	28.43 – 108.57
Treatment 2	Normal	-46.833*	13.174	0.015	-86.90 – -6.76
	Negative control	-68.500*	13.174	<0.001	-108.57 – -28.43

(*Significant at p < 0.05)

Table 5. indicates that there was a statistically significant difference in mean cholesterol levels between the normal group and treatment 2 ($p = 0.015$), and between the negative control group and the positive control ($p = 0.002$), treatment 1 ($p = 0.002$), and treatment 2 ($p < 0.001$).

Histopathological Overview of Rat Kidneys

Description Histopathological picture

Histopathological picture of rat kidneys was observed in the right and left kidneys of each treatment group. The examination was performed using a light microscope with 100x and 400x magnification after the tissue was fixed and stained with hematoxilin eosin (HE). This histopathological observation aimed to assess the toxic effects of aloxan induction as well as the protective potential of beet tuber extract on the kidney tissue of male white rats (Rattus norvegicus) of the Wistar strain.

Histological changes observed include:

- Fat degeneration: accumulation of fat in kidney cells, indicating metabolic damage of cells.
- Congestion: dilation and increased blood volume in the capillaries, as a sign of impaired circulation.
- Necrosis: death of kidney cells characterized by hom1ogen cytoplasm and loss of the nucleus.
- Infiltration of inflammatory cells: the entry of immune cells into the kidney tissue, as a sign of an inflammatory response.

Each of these pathological changes is scored based on the degree of damage as follows:

Score 0: No histopathological abnormalities (normal tissue)

Score 1: Light damage (focal)

Score 2: Moderate damage (multifocal)

Score 3: Heavy damage (diffused)

The assessment was carried out in five fields of view for each kidney, both right and left. The average scores of the two kidneys were used as the final score of each individual, then averaged back to group size to be analyzed descriptively and compared between groups.

Interpretation of Scoring

Based on the results of the effectiveness test by giving a combination of beet tubers with a certain dose to kidney repair in diabetic mice induced by aloxan, it can be seen as follows:

H&E-colored histological photographs, observed at 400× magnification. The blue arrow marks the degeneration of tubule cells, the green arrow marks the infiltration of inflammatory cells in the interstitial space.

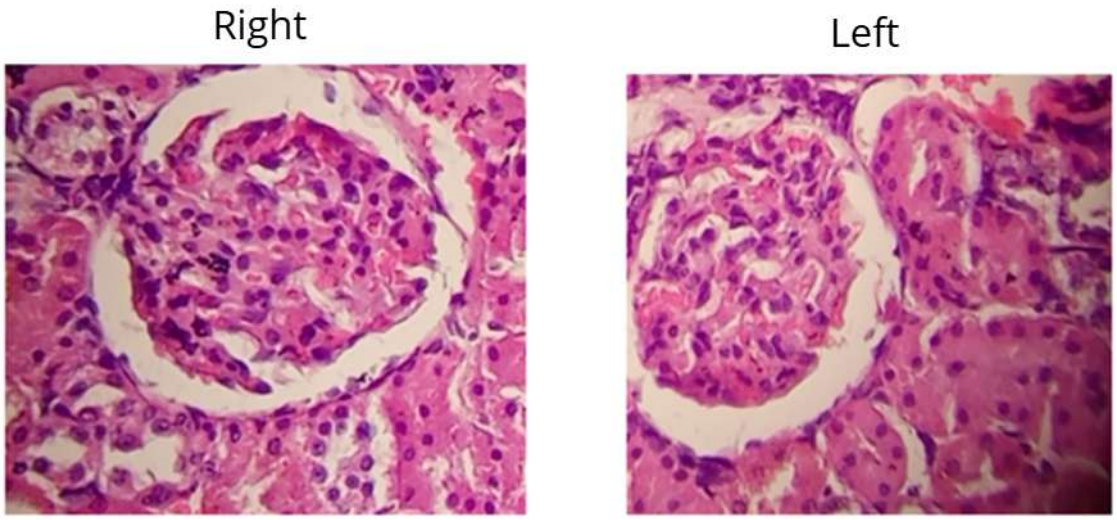

Figure 1. Histopathology of the kidneys – Normal Group.
Remarks: The glomerular and tubule structures appear intact; no degeneration, congestion, necrosis, or infiltration of inflammatory cells were found. Histopathology score = 0 (no damage)

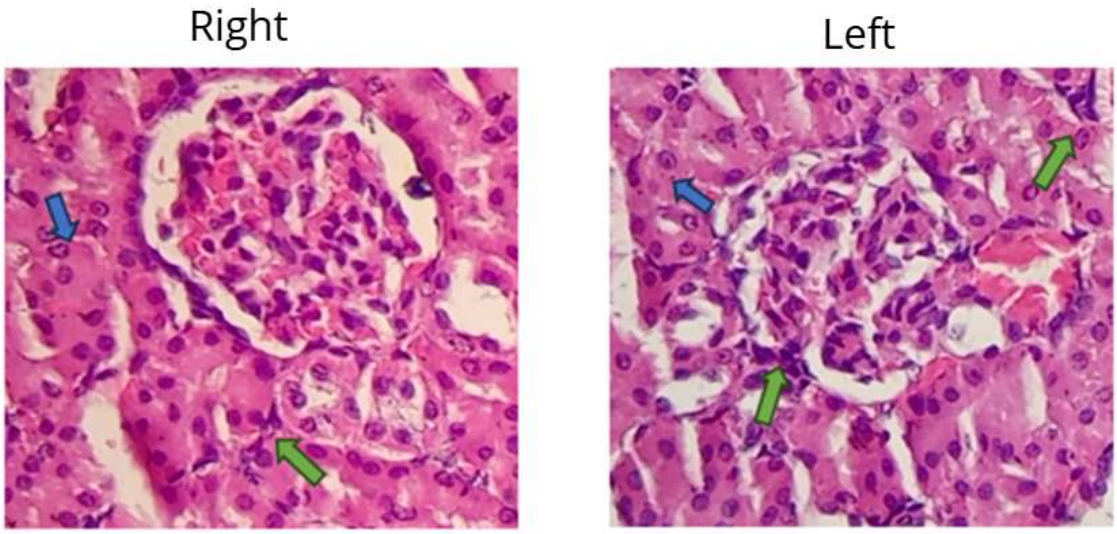

Figure 2. Renal histopathology – K1 (Negative Control/allocant + aquadest).
Remarks: Tubule cell degeneration (blue arrow) and interstitial inflammatory cell infiltration (green arrow) are observed in many areas. Multifocal damage pattern with medium degrees. Score = 2.

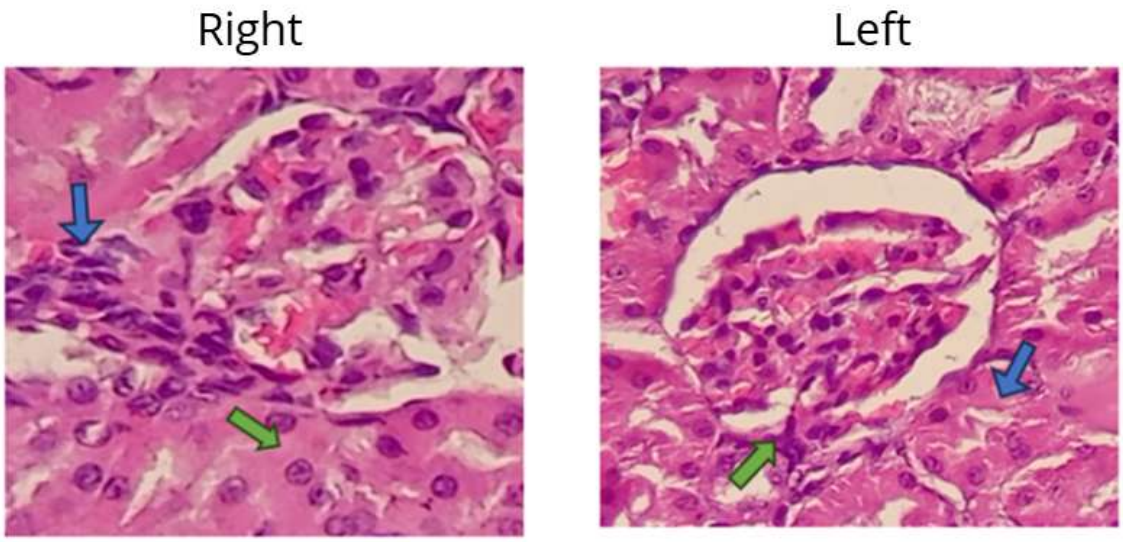

Figure 3. Renal histopathology – K2 (Positive Control/metformin).
Description: The lesions are milder than K1. Tubule cell degeneration (blue arrow) and inflammatory cell infiltration (green arrow) are still present but limited (focal). Score = 1 (mild).

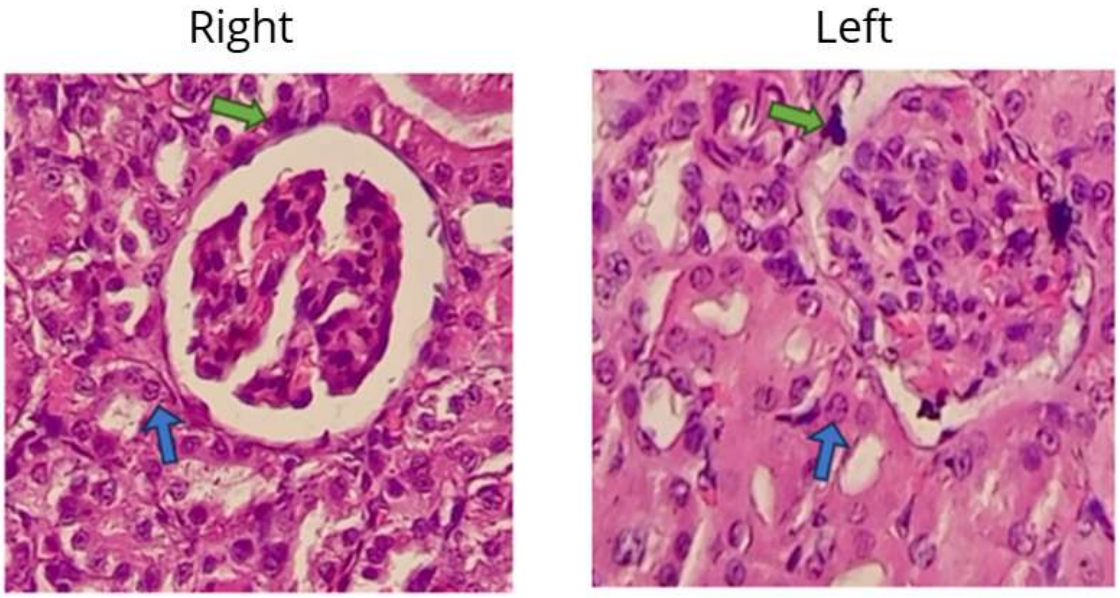

Figure 4. Renal histopathology – P1 (Treatment 1; beet tuber extract 22.5 mg/200 gBB).
Remarks: Compared to K2, there is noticeable improvement: degeneration (blue arrow) and infiltration of inflammatory cells (green arrow) is less and is limited. The degree of damage remains mild (focal). Score = 1. These findings suggest an initial protective effect of the extract.

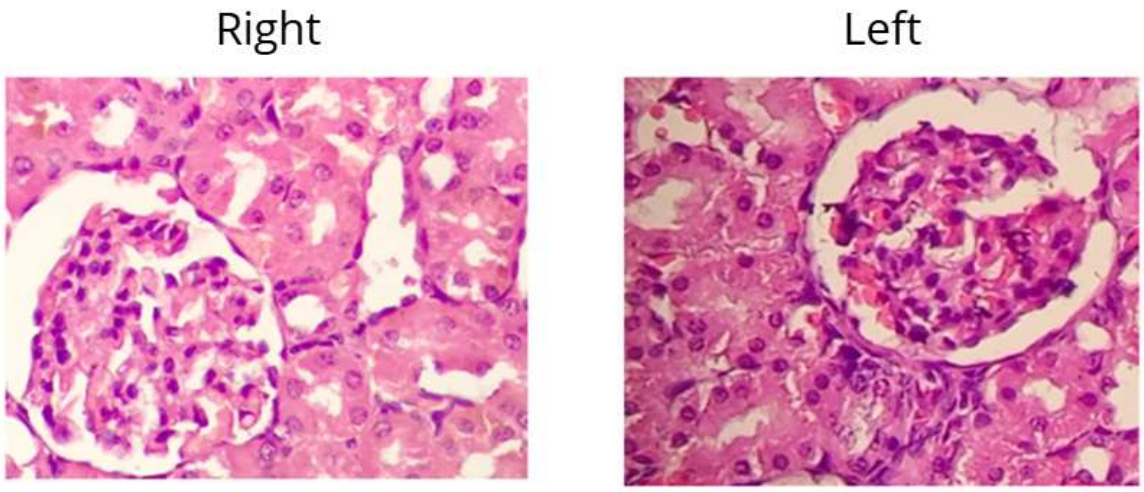

Figure 5. Renal histopathology – P2 (Treatment 2; beet tuber extract 45 mg/200 gBB).
Remarks: Further improvements compared to P1: degenerative changes (blue arrows) are less frequent and the range of distribution is narrower; The infiltration of inflammatory cells (green arrows) is also reduced. Damage remained in the mild (focal) category with the lowest severity among the treatment groups. Score = 1. These findings are in line with biochemical data showing a decrease in cholesterol levels in the P2 group.

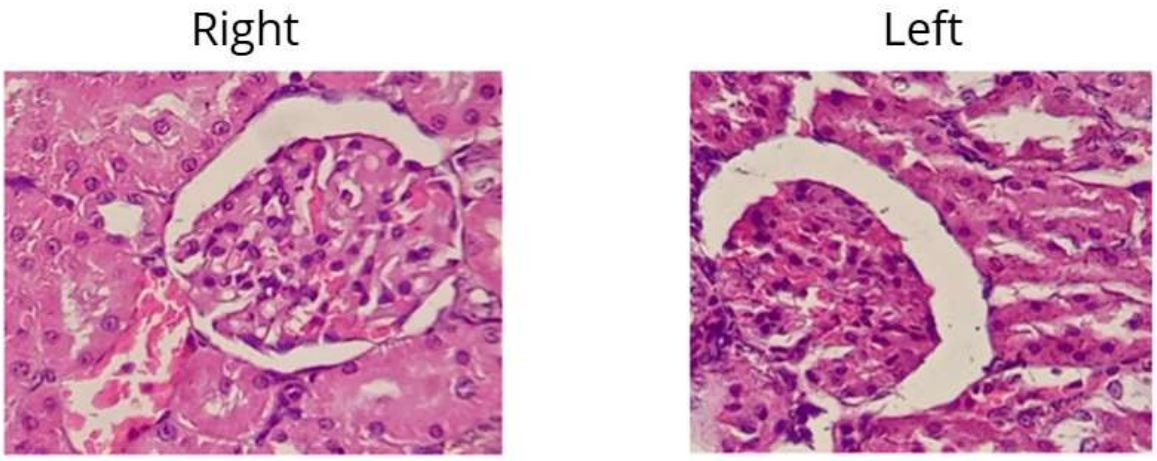

Figure 6. Renal histopathology – P3 (Treatment 3; beet tuber extract 90 mg/200 gBB).

Remarks: In treatment group 3, histological improvement was observed, but not as good as in the P2 group. Tubule cell degeneration (blue arrow) and inflammatory cell infiltration (green arrow) were still found in greater numbers than P2, although still milder than negative controls. The pattern of damage is mild focal, with a histopathological score = 1. This shows that increasing the dose does not provide significant additional improvements, and even tends to be less effective than P2.

Overview of Renal Histopathology – Discussion

The results of the renal histopathology examination showed that there was a variation in tissue changes between treatment groups. In the normal group, the kidney tissue appeared intact with normal glomerular and tubule structures without any signs of degeneration, necrosis, or inflammatory cell infiltration (score 0). This is in accordance with normal physiological conditions without exposure to aloxant or other toxic treatments.

On the other hand, in the negative control group (aloxan + aquadest), histopathological damage was found with a score of 2 in the form of tubular degeneration, congestion, and inflammatory cell infiltration. This is in line with the mechanism of aloxant toxicity which produces free radicals and oxidative stress so that it damages the kidney cell membrane and triggers necrosis and inflammation. (Szkudelski, 2020)

In the positive control group (aloxan + metformin), the histopathological damage that appeared was milder (score 1) than in the negative control group. These findings suggest a partial protective effect of metformin on the kidneys. Pharmacologically, metformin not only lowers blood sugar levels, but also has antioxidant and nephroprotective effects through activation of AMPK pathways that can suppress oxidative stress and kidney fibrosis (Kim, 2016).

In the treatment group with beet tuber extract, there was a variation in the degree of tissue damage. Treatment group 1 (22.5 mg/200 gBB) and treatment group 3 (90 mg/200 gBB) still showed mild to moderate tissue damage, while treatment group 2 (45 mg/200 gBB) showed a better histopathological picture with lower scores. This indicates that in moderate doses beet tuber extract provides an optimal protective effect on kidney tissue. The protective mechanism is thought to come from the content of betayanins, flavonoids, and polyphenols that are antioxidant and anti-inflammatory, so that they are able to suppress oxidative stress and repair damage to kidney tissue. (Al-Harbi et al., 2024) Thus, the results of this study support the potential of beet tuber extract as a nephroprotective agent, especially at moderate doses. However, high-dose treatment does not always provide greater protection, possibly due to excessive metabolic effects or exceeding the optimal therapeutic dose range.

CONCLUSION

Based on the results of this study on the effectiveness of beetroot extract (*Beta vulgaris* L.) on cholesterol levels and kidney histopathology in alloxan-induced diabetic white rats (*Rattus norvegicus*), the following conclusions can be drawn:

1. Alloxan induction in rats led to an increase in total cholesterol levels and caused histological alterations in the kidneys, including degeneration, congestion, necrosis, and inflammatory cell infiltration.
2. Administration of beetroot extract significantly reduced total cholesterol levels ($p < 0.001$). The treatment group receiving a dose of 45 mg/200 gBW (Treatment 2) showed the most effective reduction compared to other groups.
3. Histopathological observations of the kidneys in the group treated with a moderate dose of beetroot extract (45 mg/200 gBW) revealed milder tissue damage compared to the negative control group, indicating a potential nephroprotective effect of the extract.

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