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Research Article
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Effect of cinnamon oil (*Cinnamomum burmannii*) on the histological kidney of male diabetic rats (*Rattus norvegicus*)

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ABSTRACT

Background: Chronic systemic disease known as diabetes mellitus is defined by elevated blood glucose levels and problems with fat, carbohydrate, and protein metabolism. In Indonesia, the cinnamon species found include *Cinnamomum burmannii*, which also has hypoglycemic activity. The primary antioxidant chemicals found in *C. burmannii* bark extract are polyphenols, which include tannins and flavonoids.

Aim: The antidiabetic activity of cinnamon essential oil (*C. burmannii*) against streptozotocin (STZ) was examined in this study.

Methods: STZ (45 mg/kg BW) was administered intraperitoneally as a single dose. Twenty male rats were employed in this investigation. The rats were divided into five groups: treatment 1 (P1) was administered with STZ and provided with 100 mg/kg BW; treatment 2 (P2) was administered with STZ and provided with 200 mg/kg BW; and treatment 3 (P3) was administered with STZ and provided with 400 mg/kg BW. The negative control group (K−) was not treated with STZ and was treated with 1% Tween 80. For 14 days, the medication was administered daily.

Results: The essential oil of cinnamon may lessen glomerulosclerosis, tubular necrosis, tubular degeneration, and glomerular necrosis. The kidney P3 treatment (400 mg/kg BW) produced negligible effects.

Conclusion: It is possible to create cinnamon essential oil as an herbal antidiabetic medication by lowering the degree of kidney cell damage.

Keywords: *Cinnamomum burmannii*, Essential oil, Kidney, Streptozotocin, Antidiabetic.

Introduction

Chronic systemic disease known as diabetes mellitus (DM) is defined by elevated blood sugar levels and problems with the metabolism of fats, carbohydrates, and proteins (American Diabetes Association, 2018). DM occurs due to abnormalities in insulin secretion, insulin function, or both. Types 1 and 2 DM are the two categories into which DM is separated. Type 1 DM is caused by autoimmune disorders in the body that cause damage to pancreatic β cells, so insulin production is insufficient and the body experiences insulin deficiency. Type 2 DM is caused by the body's inability to use insulin or insulin resistance (American Diabetes Association, 2010). High blood sugar levels

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increase the production of reactive oxygen species (ROS) because they impair natural antioxidant enzymes like superoxide dismutase and increase the levels of advanced glycation end products (Khalid *et al.*, 2022). Oxidative stress plays a major role in the occurrence of diabetic nephropathy, which is one of the complications of hyperglycemia in the body. In people with DM, high blood glucose levels can cause progressive kidney damage, both in tubule cells and glomeruli, leading to chronic kidney disease (Krishan and Chakkarwar, 2011).

Related research exploring active substances in plants has been conducted. Among them, several plant species have been found to have antidiabetic activity that can lower blood glucose levels or repair pancreatic β cells. In Indonesia, the cinnamon species found include *Cinnamomum burmannii*, which also has hypoglycemic activity (Pulungan and Pane, 2020). Cinnamon plants are species of the genus *Cinnamomum* and the Lauraceae family. They are woody plants commonly known as spices (Rao and Gan, 2014). Cinnamon contains low-fat content and has antibacterial, antifungal, antiviral, antioxidant, anticancer, and blood pressure-lowering properties (Nabavi *et al.*, 2015). Eugenol and cinnamaldehyde compounds have potential as antidiabetic and antibiofilm (Olszewska *et al.*, 2020). The primary antioxidant chemicals found in *C. burmannii* bark extract are polyphenols, which include tannins and flavonoids.

These antioxidants can suppress the formation of ROS, which plays a major role in kidney tubule and glomerular damage (Ervina *et al.*, 2016). However, despite the abundance of literature related to antidiabetes, the use of cinnamon essential oil has been found to be very little related to kidney damage prevention. Furthermore, the primary components of cinnamon essential oil are chemicals called cinnamaldehyde and eugenol, which may have the ability to influence the α-glucosidase enzyme and be developed into an antidiabetic agent (Stevens and Allred, 2022). Based on these findings, researchers aimed to investigate the effect of cinnamon essential oil on the histopathology of the kidneys of streptozotocin (STZ)-induced white rats. Disclosure of results from experimental animals will strengthen our understanding of the field of veterinary medicine, especially kidney histopathology.

Materials and Methods

The type of research is an experimental study (True experimental) with a post-test random control approach. The research was conducted at the Experimental Animal Laboratory, Faculty of Medicine, Airlangga University, Surabaya for the maintenance of experimental animals and treatment. The manufacture of cinnamon essential oil was carried out at PT Hiptasari, and the manufacture and observation of histopathology preparations of white rat kidneys were carried out at the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Airlangga University. This research was conducted from August 2019 to April 2020.

The research began with the production of cinnamon essential oil (*C. burmannii*) by steam distillation without washing. Adaptation of the white rats for 7 days was then induced with 0.5 ml of STZ at a dose of 45 mg/kg BW administered intraperitoneally. In this study, the essential oil doses used were 100, 200, and 400 mg/kg BW. Kidney organ removal was performed after 14 days of treatment, and preparation of kidney preparations (Ding *et al.*, 2011). The examination results in the form of Tubular epithelial degeneration scores, tubular epithelial necrosis, glomerular necrosis, and glomerulosclerosis were tested using the Kruskal–Wallis test followed by the Mann–Whitney test. The statistical analysis in this study used the Statistical Program of Social Sciences (Ding *et al.*, 2011).

Result

In this study, a semi-qualitative assessment (scoring) was carried out according to the modified Klopfleisch (2013) method. The results of the microscopic examination of the kidneys are presented in Table 1.

Tubular hydropic degeneration

In the kidney preparation of group K, a normal picture of the renal tubules of white rats was observed, whereas, in group K+ induced with STZ, the histopathology of the kidneys of white rats experienced degeneration in the tubular epithelium, especially in the proximal area (Fig. 1). The statistical test, the Kruskal–Wallis test, was used to analyze renal tubular cell degeneration

Table 1. Sample examination results.

Groups	Tubular necrosis (Mean ± SD)	Tubular degeneration (Mean ± SD)	Glomerular necrosis (Mean ± SD)	Glomerulosclerosis (Mean ± SD)
K−	0.70^{a} ± 979	0.45^{a} ± 510	1.20^{a} ± 1.508	0.55^{a} ± 510
K+	7.10^{d} ± 1.021	3.55^{e} ± 510	7.90^{d} ± 1.021	3.70^{d} ± 470
P1	3.60^{c} ± 1536	2.95^{d} ± 605	4.60^{b} ± 11536	2.55^{c} ± 999
P2	2.80^{bc} ± 1.152	1.75^{c} ± 716	4.20^{c} ± 1.196	1.70^{c} ± 801
P3	2.50^{b} ± 1.469	0.85^{b} ± 587	4.10^{bc} ± 1.210	1.05^{b} ± 686

Note: a,b,c,d,e, different superscripts in the same column indicate significant differences ($p < 0.05$).

in STZ-induced white rats. The results indicated a significant difference ($p < 0.05$).

The highest hydropic degeneration score of tubular cells was observed in the K+ group (3.55 ± 510) and the lowest cell degeneration score was observed in the K group (0.45 ± 510). In the treatment group using cinnamon essential oil (*C. burmannii*), a P3 cinnamon essential oil dose of 400 mg/kg BW had the lowest tubular cell degeneration score (0.85 ± 587) followed by P2 (1.75 ± 716) and P1 (2.95 ± 605). Based on the Mann–Whitney test between groups presented in the table, each group was significantly different.

Tubular cell necrosis

Kidney preparations in the K group showed normal renal tubules, whereas in the K+ group induced with STZ, the histopathology of the kidneys of white rats showed necrosis in the tubules, especially in the proximal area (Fig. 2).

A statistical test of tubular necrosis score assessment using the Kruskal–Wallis test yielded significant differences ($p < 0.05$) in the data analysis. The highest score of renal cell tubular necrosis was obtained in the K+ group (7.10 ± 1.021). The smallest tubular necrosis score was obtained in the K− group (0.70 ± 979). P3 showed the lowest tubular necrosis value (2.50 ± 1.469) from the P2 (2.80 ± 1.152) and P1 (3.60 ± 1536) groups. Based on the Mann–Whitney test presented in Table 1, K− had a significant difference with groups P1, P2, P3, and K+. The K+ group exhibited a significant difference compared with groups P1, P2, P3, and K−. Group P1 differed significantly from P3, K−, and K + but not significantly from P2. Group P2 showed a significant difference between groups P1 and K, and K+ was not significantly different from group P3.

Glomerular necrosis

The results of the K− group preparation showed normal glomeruli, whereas the K+ group showed necrosis in the glomeruli, which was because the K+ group was induced with STZ without administering Cinnamon essential oil (Fig. 3).

A significant difference ($p < 0.05$) was found in the data analysis from the statistical test of the renal glomerular necrosis score assessment using the Kruskal–Wallis test. The K+ group had the highest glomerular necrosis

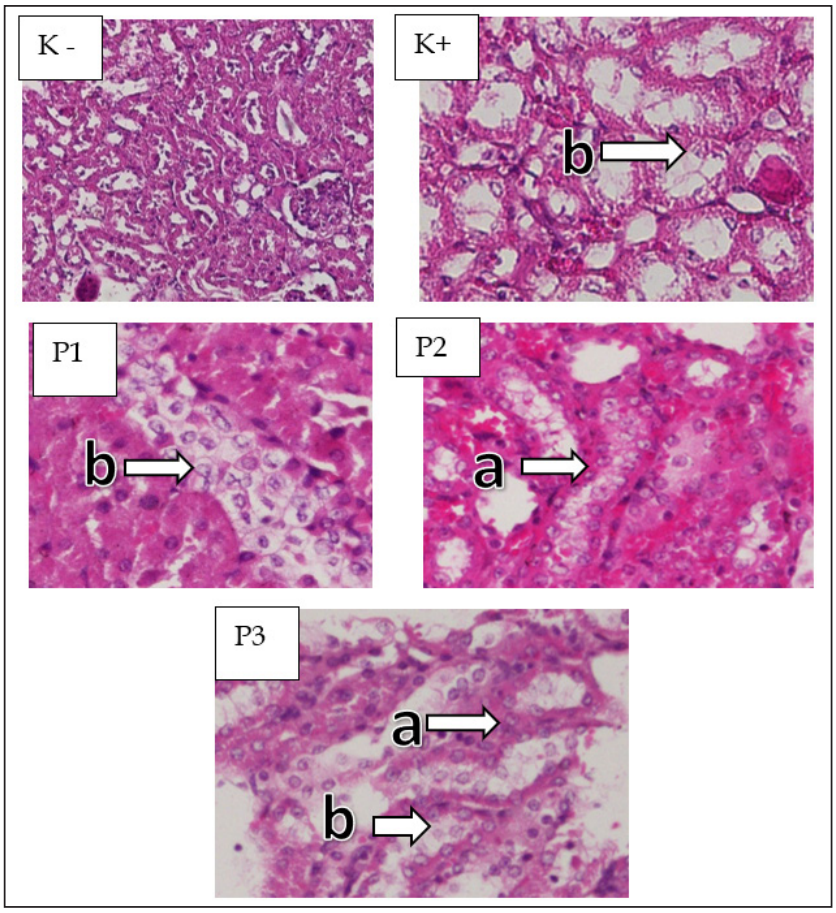

Fig. 1. Microscopic image of degeneration of white rat kidney cells (H&E staining with 200x magnification) groups: K, K+, P1, P2, and P3. (a) normal cells (b) hydropic degeneration.

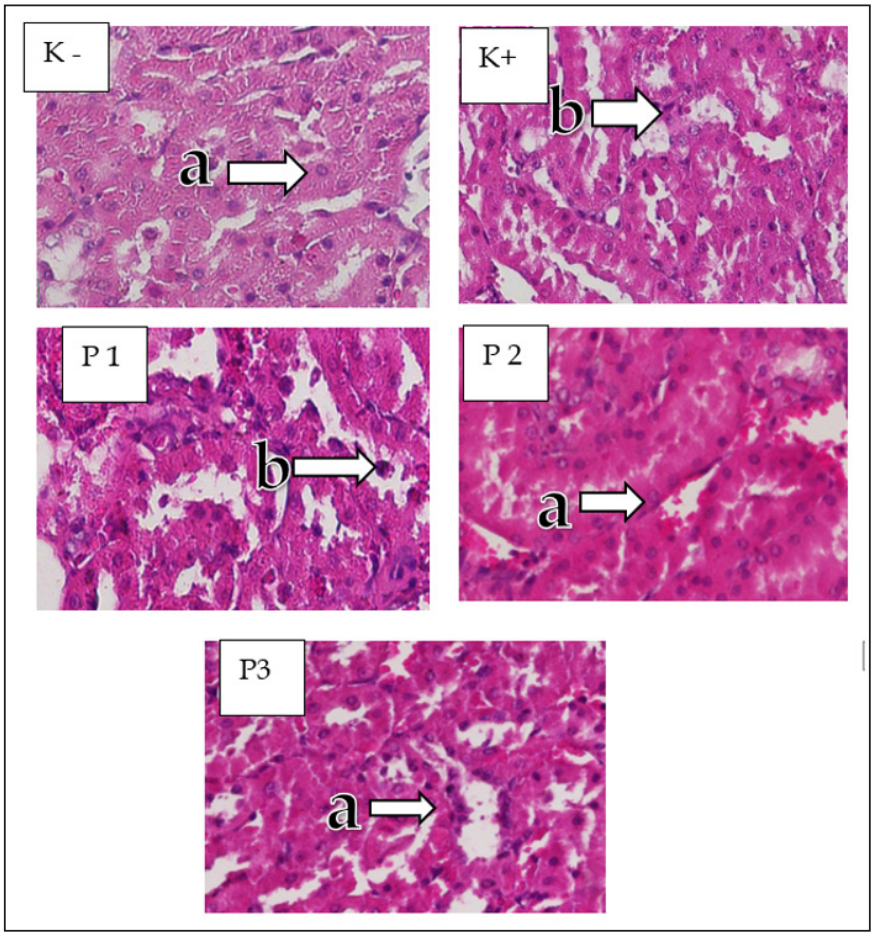

Fig. 2. Microscopic image of tubular cell necrosis. Renal cortex (H&E staining with 200x differed significantly frommagnification) K−, K+, P1, P2, and P3 groups. (a) normal peritubular cells (b) tubular cell necrosis.

score (7.90 ± 1.021), whereas the K group had the lowest glomerular necrosis score (1.20 ± 1.508). In the cinnamon essential oil treatment group, P1 had the highest glomerular necrosis score (4.60 ± 11536), whereas P2 had a glomerular necrosis score (4.20 ± 1.196) and the lowest glomerular necrosis value in the cinnamon essential oil treatment group was P1 (4.10 ± 1.210). Based on the Mann–Whitney test analysis to determine the differences in each group, as presented in Table 1 shows that K− has a significant difference with P1, P2, P3, and K+. The K+ group was significantly different from the P1, P2, P3, and K groups. Group P2 differs considerably from K−, K+, and P1 but not significantly from P3, whereas treatment group P1 differs significantly from groups K−, K+, and P2 but not significantly from P3.

Glomerulosclerosis

In the kidney preparations of the K group, a normal picture of the kidney glomerulus was observed, whereas in the K+ group induced with STZ, a histopathological picture of the kidneys of white rats experiencing glomerulosclerosis was shown (Fig. 4).

A significant difference ($p < 0.05$) was found in the data analysis from the statistical test of glomerulosclerosis score assessment using the Kruskal–Wallis test. The K+ group had the highest glomerulosclerosis necrosis score (3.70 ± 470), whereas the K group had the lowest glomerular necrosis score (0.55 ± 510). In the cinnamon essential oil treatment group, P1 had the highest glomerulosclerosis score (2.55 ± 999), whereas P2 had a glomerulosclerosis score (1.70 ± 801) and the lowest glomerulosclerosis value in the cinnamon essential oil treatment group was P3 (1.05 ± 686). Based on the Mann–Whitney test analysis to determine the differences in each group, as presented in Table 1, it shows that K has a significant difference with P1, P2, P3, and K+. There was a substantial difference between the K+ group and the P1, P2, P3, and K− groups. Group P3 was considerably different from K−, K+, P1, and P2, whereas the P1 treatment

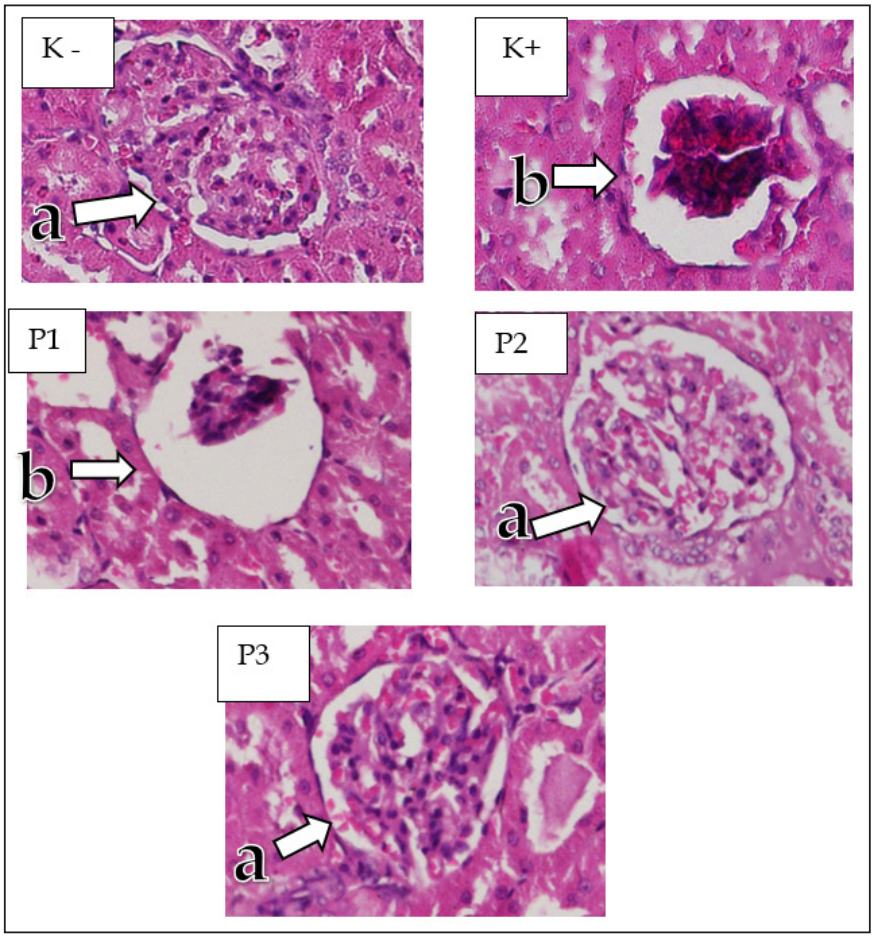

Fig. 3. Microscopic image of glomerular necrosis. Kidney cortex of white rats (H&E staining with 200x magnification) groups: K, K+, P1, P2, and P3 (a) Normal Glomerulus (b) Glomerular necrosis.

group was significantly different from groups K−, K+, and P2, but not from P2.

Discussion

In this study, administration of STZ 45 mg/kg BB resulted in a significant increase in blood sugar levels in the rats. The lowest fasting blood glucose levels were observed in the control group (normal), whereas the positive control and treatment groups had the highest fasting blood glucose levels. The findings of this investigation are consistent with those of Ghasemi and Jeddi's (2023) research, and STZ administration to white rats (*Rattus norvegicus*) can cause hyperglycemia 84 hours after intraperitoneal injection. Rats are considered hyperglycemic if their blood glucose levels are >135 mg/dl (Ali and Ali, 2022). Hydropic degeneration occurs due to disruption of the cell membrane, resulting in fluid entering the cytoplasm and vacuoles. In this study, the average renal tubular hydropic degeneration was significantly different between the treatment and control groups. This indicates the presence of acute diabetic nephropathy in the renal tubules, which results in a decrease in the mechanism of kidney function. Accumulation of fluid in the cytoplasm occurs because of decreased cell function in balancing fluids. Hyperglycemia due to STZ administration increases intravascular ROS production, which affects cell permeability and leads to the formation of hydropic degeneration lesions in the renal tubules (Nakamoto *et al.*, 2014). Necrosis is cell death due to injury. In this study, the average results of renal tubular necrosis showed a significant difference between the negative control group (normal) and positive control groups, indicating that the renal tubules and glomeruli are very susceptible to cell death due to high blood glucose. Hyperglycemia due to STZ induction can increase oxidative stress and cause inflammatory reactions that trigger the release of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α). TNF-α is the main inducer of renal microvascular inflammation and plays a role in the progression of tubular and glomerular damage

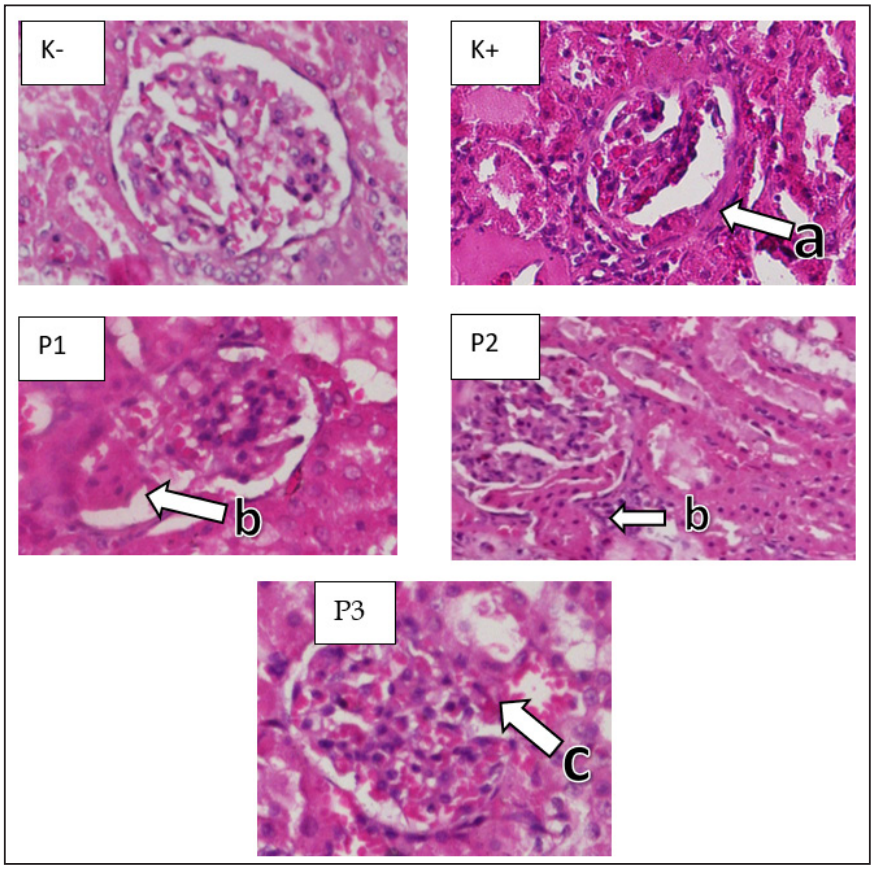

Fig. 4. Microscopic image of glomerulosclerosis. Renal cortex of white rats (H&E staining with 200x magnification) groups: K, K+, P1, P2, and P3 (a) fibrosis in the parietal layer (b) focal glomerulosclerosis (c) (synechiae) adhesions between the glomerulus and the parietal layer.

(Ramseyer and Garvin, 2013). Tubular and renal glomerular necrosis results in blood unfiltration, which results in the body's metabolic waste not being able to be removed. If cell death in the tubules and glomeruli continues, chronic disease and kidney failure will occur.

Glomerulosclerosis in diabetic nephrotes is caused by the accumulation of extracellular matrix in the interstitial mesangial space of the renal glomerulus (Qian *et al.*, 2008). The most abundant matrix proteins finglomerulosclerosis are collagen types I, III, IV, and fibronectin (Bülow and Boor, 2019). The results of the study showed that glomerulosclerosis was still in its early stages, and the glomerular nodules that formed were small. This result proves that STZ at 45 mg/kg BW can trigger the formation of glomerulosclerosis.

Cinnamon is an herbal plant commonly used as a natural diabetes medication. Cinnamon has a flavonoid compound, namely methyhydroxy chalcone polymer (MHPC) (Qureshi *et al.*, 2019). MHCP has the ability like insulin, namely to activate glycogen synthesis, increase glucose uptake, activate insulin receptor kinase, and inhibit insulin receptor dephosphorylation (Wang *et al.*, 2022).

Treatment group P1 cinnamon attrition oil dose of 100 mg/kg BW had the highest tubular degeneration, tubular necrosis, glomerular necrosis, and glomerulosclerosis values compared with groups P2 and P3. This is because the amount of antioxidants at that dose cannot balance the amount of ROS in the kidneys of streptozootocin-induced white mice. In group P3, we observed low values of tubular degeneration, tubular necrosis, glomerular necrosis, and glomerulosclerosis compared with group P1 and no significant difference with group P2, so the use of cinnamon essential oil at a dose of 400 and 200 mg/kg BW has antioxidant properties that can inhibit the formation of oxidative stress in the kidneys, but the higher the dose of cinnamon essential oil given, the higher the antioxidant properties produced to ward off free radicals.

The primary components of cinnamon essential oil are eugenol and cinnamaldehyde, which have antibacterial and antioxidant properties (Behbahani *et al.*, 2020). According to research by Hayward *et al.* (2019), the

molecule cinnamaldehyde, which was extracted from cinnamon oil, has an IC50 value of 27.96 ppm against the α-glucosidase enzyme, indicating that it has great potential as an antidiabetic compound that inhibits the activity of the α-glucosidase enzyme. The presence of cinnamaldehyde molecules in cinnamon essential oil has a preventive effect on damage to rat kidney cells. This study was supported by research conducted by Hayward *et al.* (2019). In the histopathological description of the kidneys, the structure of the kidney cells at P2 and P3 appears normal.

Antioxidant therapy inhibits the production of intracellular free radicals and increases the ability of defense enzymes to prevent the emergence of oxidative stress and vascular complications related to diabetes. Cinnamon essential oil can treat kidney damage caused by oxidative stress and free radical buildup in the kidneys. The antioxidants in cinnamon essential oil work by donating their electrons to unstable cells.

Conclusion

The results of the study indicate that the histological images of the kidneys of white rats (*R. norvegicus*) generated by STZ are altered by the administration of cinnamon essential oil (*C. burmannii*). *Cinnamomum burmannii* essential oil has an effective dosage of 400 mg/kg BW. The current study has some limitations that only focused on the histopathology of rat kidneys. Therefore, further research on the pancreatic organ as a producer of insulin is needed.

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Conflict of interest

The authors declare no conflict of interest.

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Author's contributions

BB, SMY, and HP: Conceptualization and design. NLNT and KHPR: Data acquisition. MHE, RZA, and IBM: Formal analysis and interpretation of data. ARK, AP, and MA: Writing-original draft preparation. ENU, BPP, and WW: Writing-review and editing. All authors have read and agreed to the publication of the final version of the manuscript.

Data availability

All data are available in the manuscript.

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