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Histological Profile of Kidney Tissues in Diabetic Model Rats Treated with Instant Granule of *Cajanus cajan* Leaves and *Zingiber officinale*

(PROFIL HISTOLOGI JARINGAN GINJAL TIKUS MODEL DIABETES YANG DIBERI GRANUL INSTAN DAUN UNDIS DAN JAHE)

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels, which can impair kidney function. This study was aimed to analyze the histological profile of kidney tissue in diabetic model rats treated with instant granules of *Cajanus cajan* leaves and *Zingiber officinale*. A total of 25 male *Sprague Dawley* rats were utilized and separated into five groups; negative control (NC), diabetes or positive control (PC), diabetes mellitus (DM) + metformin at 150 mg/kg BW (MET), DM + instant granules at 300 mg/kg BW (G1), and DM + instant granules at 150 mg/kg BW (G2). The condition of DM was obtained by single streptozotocin induction (35 mg/kg BW). The results indicate that the administration of instant granules significantly ameliorate the histological profile of kidneys in diabetic model rats, including an increase in the glomerulus to Bowman's capsule ratio, as well as a decrease in the number of necrotic tubular cells and the tubular dilatation index ($P<0.05$). The administration of instant granules made from *C. cajan* leaves and *Z. officinale* significantly reduced microstructural damage to kidney tissue in diabetic model rats. A dose of 150 mg/kg BW showed the most optimal results in preserving renal microstructure.

Keywords: *Cajanus cajan*; diabetes; kidney; *Zingiber officinale*

ABSTRAK

Diabetes melitus adalah penyakit metabolik kronis yang ditandai dengan peningkatan kadar glukosa darah, yang berdampak buruk pada fungsi ginjal. Penelitian ini bertujuan untuk menganalisis profil histologi jaringan ginjal pada tikus model diabetes yang diberi granul instan dari daun undis (*Cajanus cajan*) dan jahe (*Zingiber officinale*). Sebanyak 25 ekor tikus jantan *Sprague Dawley* digunakan dan dibagi menjadi lima kelompok: kontrol

negatif (KN), kontrol positif atau diabetes (KP), diabetes mellitus (DM) + metformin 150 mg/kg BB (MET), DM + granul instan 300 mg/kg BB (G1), dan DM + granul instan 150 mg/kg BB (G2). Kondisi diabetes melitus diinduksi menggunakan streptozotocin dengan dosis tunggal 35 mg/kg BB. Hasil penelitian menunjukkan bahwa granul instan secara signifikan memperbaiki profil histologi ginjal tikus model diabetes, termasuk peningkatan rasio glomerulus terhadap kapsula Bowman, penurunan jumlah sel nekrosis tubulus dan indeks dilatasi tubulus ($P<0,05$). Pemberian granul instan daun undis dan jahe secara signifikan menurunkan kerusakan mikrostruktur jaringan ginjal pada tikus model diabetes. Dosis 150 mg/kg BB menunjukkan hasil optimal dalam memperbaiki mikrostruktur ginjal.

Kata-kata kunci: undis (*Cajanus cajan)*; diabetes; ginjal; jahe (*Zingiber officinale)*

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by impaired carbohydrate, fat, and protein metabolism, leading to elevated blood glucose levels. It is caused by insufficient insulin production, insulin resistance, or a combination of these factors (ADA, 2020). The global prevalence of diabetes is increasing, with Southeast Asia having the third-highest prevalence at 11.3%. Indonesia ranks fifth in diabetes prevalence. The condition affects 10.5% of the global population aged 20 to 79, affecting approximately 536.6 million individuals (IDF, 2021). Diagnosis is based on hyperglycemia, defined as a random blood glucose level greater than 200 mg/dL or a fasting blood glucose level of 126 mg/dL or higher (ADA, 2020).

Insulin disorders cause insufficient insulin in the bloodstream, preventing glucose from entering and being metabolized within cells. This results in glucose accumulation, leading to elevated blood glucose levels. This decreases glycogenesis, reducing glycogen reserves in the liver and muscles (Kanungo *et al*., 2018). Elevated blood glucose levels promote glucose autooxidation and stimulate metabolic pathways like advanced glycation end products (AGEs), sorbitol pathway, hexosamine pathway, and increased Nicotinamide Adenin Dinukleotide Phosphate (NADPH) oxidase activity. This excess of free radicals overwhelms the body's antioxidant defense mechanisms, leading to oxidative stress (Papachristoforou *et al*., 2020).

Oxidative stress, caused by free radicals, can lead to structural and functional impairments in biomacromolecules like proteins, lipids and carbohydrates (Juan *et al*., 2021). This damage, characterized by elevated levels of *malondialdehyde* (MDA), can cause apoptosis and accelerate diabetes related complications (Alfarhan *et al*., 2020). Structural and functional abnormalities in organs can also contribute to microvascular and macrovascular complications, including nephropathy, retinopathy, stroke, neuropathy, peripheral vascular diseases and coronary artery disease (Rawshani *et al*., 2017).

Diabetes related microvascular complications, like nephropathy, can lead to Diabetic Kidney Disease (DKD), a progresssive decline in kidney function (Zhang *et al*., 2024). The number of individuals affected by DKD is projected to rise from 463 million in 2019 to 700 million by 2045, with the disease potentially progressing to Chronic Kidney Disease (CKD) and End-Stage Renal Disease (ESRD), increasing mortality and morbidity rates among diabetes patients (Scilletta *et al*., 2023).

The kidneys play a crucial role in maintaining body physiology, filtering waste products, regulating blood volume, secreting hormones, maintaining acid base balance and metabolizing vitamin D (Imenez Silva and Mohebbi, 2022). However, early histological alterations, such as thickening of the glomerular basement membrane, glomerulosclerosis and matrix accumulation, contribute to kidney function

deterioration (Adeva-Andany *et al*., 2023). Treatment for nephropathy typically involves drugs like metformin and herbal medicines to lower blood glucose levels and protect the kidneys, such as synthetic drugs like metformin and herbal medicines like adipose tissue (Aroda and Ratner, 2018).

Herbal medicines are natural, affordable and have fewer side effects (Verma *et al*., 2018). *Cajanus cajan* leaves and *Zingiber officinale* are potential medicinal plants with antihyperglycemic, anti-inflammatory and antioxidant benefits (Wresdiyati *et al*., 2024a). Both contain flavonoids and gingerols with antidiabetic and hypoglycemic activities. Combining both shows a more pronounced hypoglycemic effect than using either individually (Wresdiyati *et al*., 2020a). Single *Z. officinale* extract can improve kidney histology in diabetic rat models. Both plants have potential medicinal applications (Al Hroob *et al*., 2018; Almatroodi *et al*., 2021).

Previous studies have shown that single *Z. officinale* extract can improve kidney tissue structure in diabetic rat models, while a combination of *C. cajan* leaves extract and *Z. officinale* has hypoglycemic properties. However, no research has been conducted on using instant granules containing both extracts to improve kidney tissue histology in diabetic rats. This study was aimed to analyze the histological profile of kidney tissue in diabetic rats treated with instant granules of *C. cajan* leaves and *Z. officinale*.

RESEARCH METHODS

Materials

This study used *C. cajan* leaves from Lombok, West Nusa Tenggara, and *Z. officinale* from Solo, Central Java, Indonesia. Both plant species were identified at the National Research and Innovation Agency. Instant granules of *C. cajan* leaves and *Z. officinale* were manufactured by PT. Insular Multi Natural in Bogor, West Java, Indonesia. Eight-week-old male *Sprague Dawley* rats were used in the study. Other materials used included streptozotocin (Sigma-Aldrich S0130-1G), metformin (Glucophage® 500 mg), ketamine (Ket-A-100®), xylazine (Interchemie®), saturated picric acid, formalin (Sigma-Aldrich 1.04003.2500), glacial acetic acid (Merck 1.00063.2500), ethanol (Merck 1.00983.2500), xylol (QrëC®), paraffin (Histoplast Thermo Scientific), distilled water, entelan, hematoxylin and eosin.

Cajanus cajan Leaves and *Z. offi-cinale* Extraction and Instant Granule Production

The extracts and instan granules production of *C. cajan* leaves and *Z. officinale* were carried out at PT. Insular Multi Natural in Bogor, West Java, Indonesia. The extraction process of *C. cajan* leaves and *Z. officinale* was carried out separately using the maceration method with 70% ethanol for 30 minutes. The filtrate obtained was evaporated at a temperature of 70°C for 75 minutes to produce a concentrated extract, which was then made into dry extracts of *C. cajan* leaves and dry extracts of *Z. officinale* with the addition of starch and aerosol (Wresdiyati *et al*., 2024a). Dry extracts of *C. cajan* leaves and *Z. officinale* are the main ingredients for making instant granules, which are combined with fillers, such as sodium benzoate (Wuhan Youji Industries), skim milk (Fonterra), melon essence, green dye (Apple Green, Indocol), stevia (Soho Nootropic), Povidone (PVP) (Greenco Group) and aerosil (Wacker).

Preparation and Treatment of Rats Model

This study has obtained approval from the Animal Ethics Commission of SKHB IPB No. 049/KEH/SKE/XI/2022. The study involved 25 male *Sprague Dawley* rats (220–230 g, 8 weeks old), acclimatized for a week, and administered antihelminthics, antibiotics and vitamins. They were kept in a temperature controlled environment (a temperature of 22-24°C, a 12-hour light-dark cycle, humidity of 50-63%) and fed a standard diet.

The rats were divided into two groups, namely the control and treatment groups. The control group consisted of:

negative control (NC) group and positive control (PC), while the treatment group included: DM + metformin (MET) group, DM + instant granules 300 mg/kg BW (G1), and DM + instant granules 150 mg/kg BW (G2). Rats in the PC, MET, G1 and G2 groups were induced using streptozotocin (STZ) at a dose of 35 mg/kg BW. STZ was dissolved in a 0.1 M citrate buffer solution pH 4.5. The study involved diabetic model rats with blood glucose levels 250-350 mg/dL, given instant granules daily for 28 days. Fasting blood glucose levels were measured using the Accu-Chek® device every seen days.

Kidney Sampling and Processing

The kidney organ sampling began with anesthetizing the rats using a combination of 70 mg/kg BW ketamine and 10 mg/kg BW xylazine intraperitoneally. The rat kidneys were then taken and fixed with Bouin's fixative solution for 24 hours. The kidney tissue was then dehydrated with graded ethanol, cleared using xylol and infiltrated with paraffin. Tissue samples were embedded method, then cut with a thickness of 4 μm using a microtome. The tissue sections were then stained with hematoxylin-eosin (Kiernan, 2015).

Histomorphological Evaluation of Kidney Tissues

Kidney tissue was analyzed using parameters, include glomerular width to Bowman's capsule ratio, number of tubular necrosis cells and tubular dilation index (TDI) (Alfarisi *et al*., 2023). Then, the number of grid points covering the tubular lumen was recorded and expressed as a percentage of the total sampling points (Fattah *et al*., 2019). The tubular dilation index was assessed by creating a grid consisting of 117 sampling points (13x9) on an image. The number of tubular necrotic cells was assessed using the following criteria: 0 for normal conditions; 1 mild necrosis (<10%); 2 moderate necrosis (10-25%); 3 moderate to severe necrosis (25-50%); 4 severe necrosis (50-75%); and 5 very severe necrosis (>75%) (Bussmann *et al*., 2014).

Data Analysis

The data of renal corpuscular ratio (Bow-man's capsule and glomerulus), number of tubular necrosis cells and tubular dilation index were measured using the imageJ application. The data obtained were analyzed using the One-Way Analysis of Variance test through the SPSS version 25.0 program. If the test results show a significant difference ($P<0.05$) or very significant ($P<0.01$), then continue with the Duncan multiple range test.

RESULTS AND DISCUSSION

Fasting blood glucose levels, after streptozotocin induction, in treated rats on day 0 showed higher results in the positive control group (PC) (303.00 ± 36.01 mg/dL) compared to the negative control group (NC) (103.66 ± 2.33 mg/dL). The MET group had blood glucose levels of (299.00 ± 33.72 mg/dL), the G1 group was (330.00 ± 8.08 mg/dL), and the G2 group was (339.00 ± 8.73 mg/dL). At the end of the treatment period (day 28), the blood glucose levels of the NC group remained stable at (100.00 ± 3.21 mg/dL), while the PC group increased to (391.00 ± 19.00 mg/dL). The diabetic group given metformin (MET) indicates a decrease in blood glucose levels to (132.66 ± 34.35 mg/dL), the diabetic group given 300 mg/kg BW instant granules (G1) to (158.33 ± 66.69 mg/dL), and the diabetic group given 150 mg/kg BW instant granules (G2) showed a more significant decrease in blood glucose levels to (105.33 ± 5.04 mg/dL), compared to the MET and G1 groups. Administration of *C. cajan* leaves and *Z. officinale* instant granules can reduce fasting blood glucose levels in diabetic model rats.

Hematoxylin and eosin staining of kidney tissue of treated rats is shown in Figure 1A. The ratio of glomerulus/Bowman's capsule (G/KB) of kidney tissue is shown in Figure 1B. Administration of instant granules of *C. cajan* leaves and *Z. officinale* to diabetic rats showed a significant increase in the ratio of

glomerulus/Bowman's capsule (P<0.05). The negative control group (NC) showed the highest ratio significantly compared to the other groups. Conversely, the positive control group (PC) indicates a significant decrease in the ratio of G/KB compared to the other groups. The decrease in the ratio of G/KB in PC group indicates microstructural damage to the renal corpuscle of the kidneys of diabetic rats. The MET, G1 and G2 groups showed a significantly higher ratio of G/KB (P<0.05) compared to the PC group, but there was no significant difference between the three groups (P>0.05). In addition, the G2 group showed a ratio of G/KB that was not significantly different from the NC group. This shows that administration of instant granules at a low dose (G2) is more effective in increasing the glomerulus/Bowman's capsule ratio in the kidney tissue of diabetic rat models.

The number of tubular necrosis cells in kidney tissue is shown in Figure 2. The negative control group showed a significantly lower number of tubular necrosis cells compared to the other groups (P<0.05). Conversely, the positive control group (PC) indicates a significant increase in the number of tubular necrosis cells compared to the other groups (P<0.05), indicating damage to the number of tubular cells in diabetic rats. The MET, G1 and G2 groups showed a significantly lower number of tubular necrosis cells compared to PC group P<0.05), but the MET group was significantly different from G1 and G2 (P<0.05). The number of tubular necrosis cells in G1 and G2 did not differ significantly (P>0.05). Administration of instant granules of *C. cajan* leaves and *Z. officinale* was effective in reducing the number of tubular necrosis cells in the kidney tissue of diabetic rat models.

The image capture point on the tubular dilation index with a box containing 117 (13x9) (Alfarisi *et al*., 2023) is shown in Figure 3A. The tubular dilation index is shown in Figure 3B. The administration of instant granules of *C. cajan* leaves and *Z. officinale* can reduce the tubular dilation index in the kidney tissue of diabetic rat models (P<0.05). The negative control group (NC) showed a lower and significantly different tubular dilation index compared to the positive control group (PC) (P<0.05). Conversely, the MET, G1 and G2 groups showed no significant difference from the negative control group (NC) (P>0.05). The positive control group (PC) showed a significant increase in the tubular dilation index compared to the MET, G1 and G2 groups (P<0.05). Free radicals in oxidative stress conditions can cause structural cha-nges and functional abnormalities in biomacromolecules, thereby accelerating complications of diabetes mellitus and decreased kidney function.

Hyperglycemia can lead to the accumulation of reactive oxygen species (ROS) through various metabolic pathways, including increased glucose flux, activation of protein kinase C, increased formation of AGEs, overactivity of the hexosamine pathway, and decreased antioxidant defense (González *et al*., 2023). These conditions can damage the kidneys structure and function, trigger inflammatory pathways and worsen chronic inflammation. These interactions form a pathological circle, reinforcing each other, leading to the progression of kidney damage in diabetes. The combination of hyperglycemia, oxidative stress and inflammation can exacerbate kidney damage and lead to hemodynamic changes (Hesp *et al*., 2022).

Diabetes negatively impacts kidney function and can lead to nephropathy complications. Studies show that diabetic rats and adenine-induced diabetic rats with Chronic Kidney Disease (CKD) exhibit dilation of Bowman's capsule space, necrosis of tubules and tubul dilation in both diabetic and CKD induced rats (Esposito *et al*., 2023).

The diabetic rat model showed significant renal tissue pathological changes, including damage to tubular epithelial cells, mild proliferation of glomerular endothelial and mesangial cells and expansion of Bowman's capsule space (Hu *et al*., 2024)

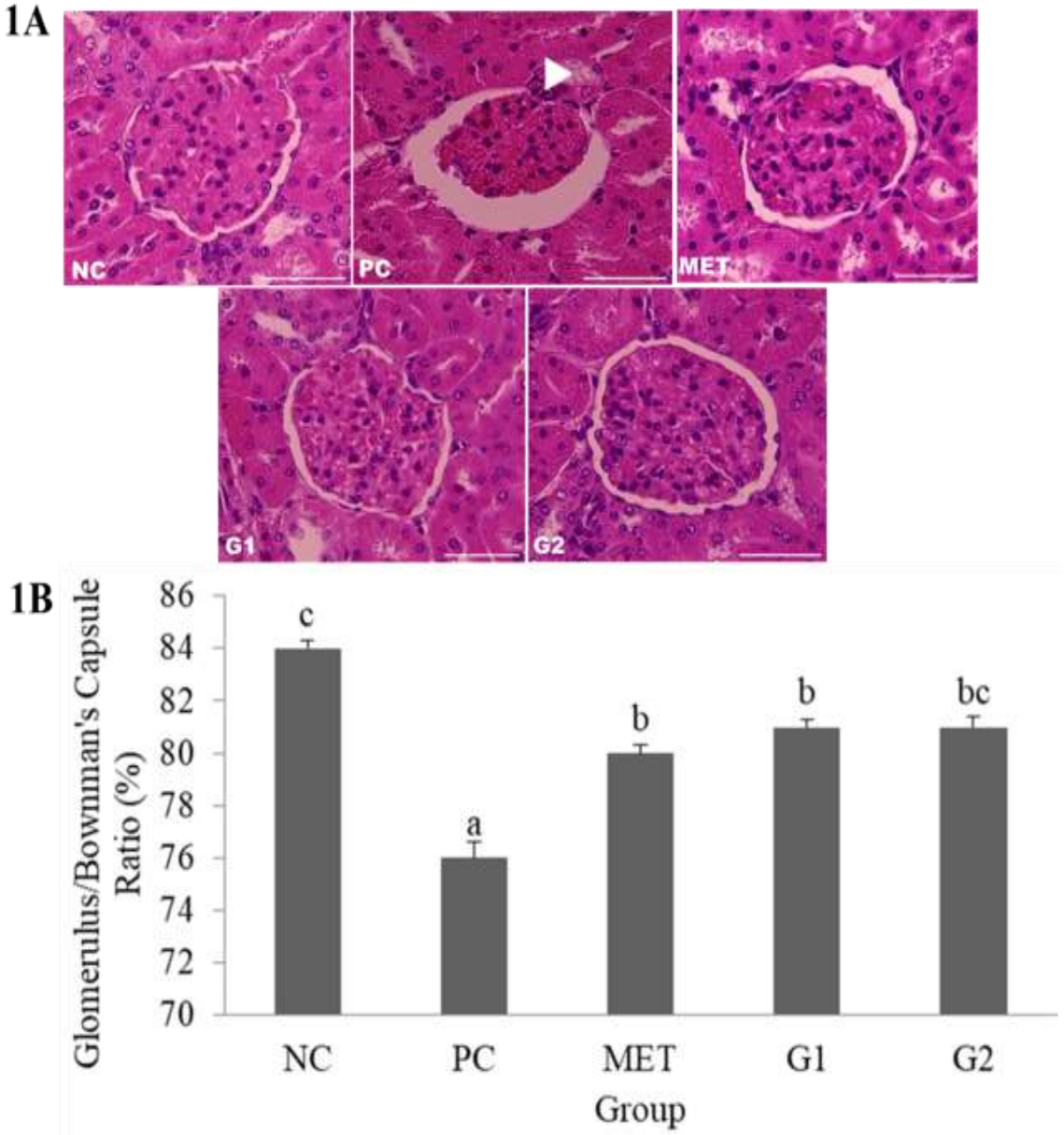

Figure 1. Administration of instant granules of *Cajanus cajan* leaves and *Zingiber officinale* to diabetic rats increasing the ratio of glomerulus to Bowman's capsule ($P<0.05$). 1A:Photomicrograph of kidney tissue of treated rats; 1B: glomerulus/Bowman's capsule ratio (G/KB). Negative control (NC), diabetic control (PC), DM+metformin 150 mg/kg BW (MET), DM+instant granules of *Cajanus cajan* leaves and *Zingiber officinale* 300 mg/kg BW (G1), DM+instant granules of *Cajanus cajan* leaves and *Zingiber officinale* 150 mg/kg BW (G2). Bar=50 µm.

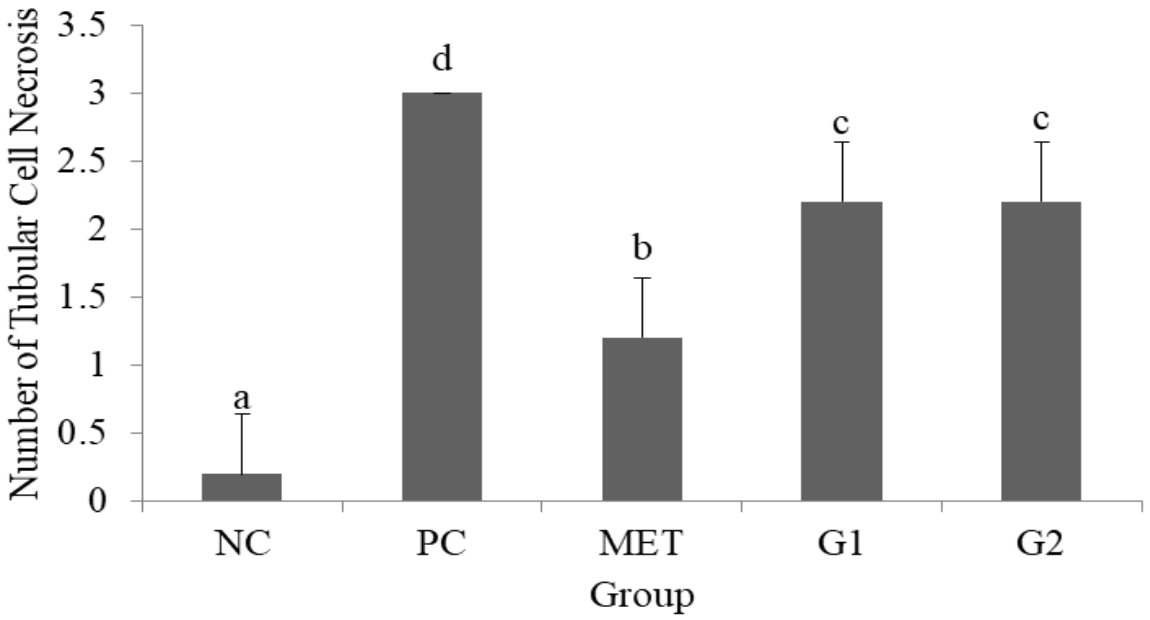

Figure 2. Number of renal tubular necrosis cells. Administration of instant granules of *Cajanus cajan* leaves and *Zingiber officinale* to diabetic rats has been proven effective in reducing the number of renal tubular necrosis cells (P<0.05). Negative control (NC), diabetic control (PC), DM+metformin 150 mg/kg BW (MET), DM+instant granules of *Cajanus cajan* leaves and *Zingiber officinale* 300 mg/kg BW (G1), DM+instant granules of *Cajanus cajan* leaves and *Zingiber officinale* 150 mg/kg BW (G2).

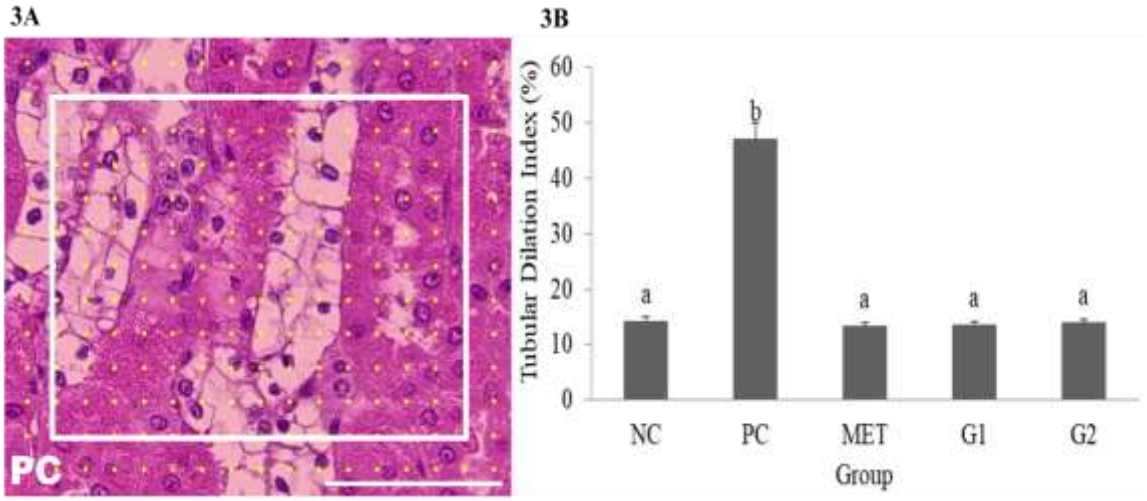

Figure 3. Administration of instant granules of *Cajanus cajan* leaves and *Zingiber officinale* to diabetic rats can reduce the tubular dilation index of kidney tissue (P<0.05). 3A: Box containing 117 (13x9) image capture points; 3B: tubular dilation index. Negative control (NC), diabetic control (PC), DM+metformin 150 mg/kg BW (MET), DM+instant granules of *Cajanus cajan* leaves and *Zingiber officinale* 300 mg/kg BW (G1), DM+instant granules of *Cajanus cajan* leaves and *Zingiber officinale* 150 mg/kg BW (G2). Bar=50 μm.

Changes in the ratio of glomerulus to Bowman's capsule indicate narrowing or widening of Bowman's space (Kanbay *et al*., 2024). Widening of Bowman's space can be caused by glomerular hypertrophy and atrophy, obstructed blood flow or tissue hypoxia and glomerular hyperfiltration, resulting in increased hydrostatic pressure in Bowman's capsule (Chagnac *et al*., 2019).

High hydrostatic pressure can damage the glomerular structure, widening Bowman's space and increasing renal glomerular diameter (Löwen *et al*., 2021). This is due to dilation of capillaries, extracellular matrix deposition and cell proliferation. Hypertrophied proximal tubules also show increased diameter and length. Infiltration of the inner renal tissue by myofibroblasts, deposition of interstitial collagen and tubulointerstitial fibrosis can result in narrowing of the tubular lumen and impaired renal function (Shadmanov *et al*., 2022).

Tubular necrosis in diabetic kidneys results in renal microstructural damage, affecting reabsorption and excretion processes. This damage leads to apoptosis and necrosis of tubular epithelial cells, affecting normal renal function (Maremonti *et al*., 2022). The kidneys microstructural changes cause tubular lumen dilatation, causing the lumen to become more expansive. Degeneration of microvilli on the apical surface reduces the tubular cells ability to reabsorb nutrients and water from urine. Damage to the tubular epithelial cells also alters the basement membrane, potentially leading to the formation of fibrotic tissue in the renal interstitium (Liu *et al*., 2018).

The proximal and distal tubule, located in the renal cortex, are affected by damage, leading to tubular dilation (Zhuo and Li, 2012). This occurs when necrotic tubular epithelial cells are detached from the basement membrane, causing normal tubular epithelial cells to widen and cover the exposed membrane. Dilated cells become flatter, causing the lumen to become more expansive (Priante *et al*., 2019).

Metformin and instant granules from *C. cajan* leaves and *Z. officinale* have been found to preserve the kidney microstructure in diabetic rats. Metformin is a crucial first line treatment for type 2 diabetes (Susilawati *et al*., 2023). *Zingiber officinale* extracts, including flavonoids, saponins and quinones, have been found to have hypoglycemic and antidiabetic effects in diabetic rats. *Cajanus cajan* leaves extract, with its high antioxidant properties, has been shown to effectively reduce blood glucose levels in diabetic mice, rats and humans (Wresdiyati *et al*., 2020b). The combination of these extracts from *C. cajan* leaves and *Z. officinale* produces a stronger hypoglycemic effect than when used individually. Both extracts have been shown to have hypoglycemic effects in diabetic rats (Suhaema and Widiada, 2023; Wresdiyati *et al*., 2020a).

Cajanus cajan leaves and *Z. officinale* contain flavonoids and tannins, which work together to reduce blood glucose levels and maintain normal range. Flavonoids protect pancreatic beta cells, stimulate insulin signaling, promote insulin secretion and inhibit glycogenolysis and gluconeogenesis. They also block digestive enzymes, preventing carbohydrates from being converted into blood glucose (Xiao, 2022; Sun and Miao, 2020). Tannins lower glucose absorption by inhibiting digestive enzymes, extending carbohydrate metabolism time and preventing hyperglycemia (Türkan *et al*., 2019).
Tannins help reduce glucose absorption in the digestive system by inhibiting enzymes like alpha glucosidase and alpha amylase, prolonging carbohydrate metabolism and preventing hyperglycemia (Kaur *et al*., 2021). They reduce oxidative stress in diabetes and lower blood glucose by enhancing glucose absorption through Glucose Transporters (GLUT) 4 activation and translocation, as well as insulin signaling pathways like p38 Mitogen-Activated Protein Kinase (MAPK) and Phosphoinositide 3-kinase (PI3K) (Syafril *et al*., 2019). Additionally, tannins lower blood glucose levels and act as free radical scavengers (Omar *et al*., 2022).
Saponins, which stimulate insulin secretion from pancreatic beta cells and enhance glucose utilization in peripheral tissues, can lower blood glucose levels in diabetic individuals. They also contribute to pan-

creatic beta cell regeneration and inhibit gluconeogenesis in the liver (Yu *et al*., 2022). A study found a hypoglycemic effect in type 2 diabetes model mice, involving increased glucose uptake activity (He *et al*., 2023).

Zingiber officinale extract, rich in bioactive compounds like 10-gingerol, 8-gingerol, 6-gingerol, tannins, polyphenolic compounds, flavonoids, triterpenoids, shogaol, and paradol, has hypoglycemic effects by maintaining cell functions related to receptors and membrane transport (Ma *et al*., 2021; Momoh *et al*., 2022). 6-gingerol stimulates increased glucose uptake and translocation of glucose transporter 4 (GLUT4) to the cell membrane, reducing kidney tissue damage and fibrosis (Lee *et al*., 2015). Gingerol also plays a role in lowering blood glucose levels, reducing malondialdehyde levels, improving kidney function and decreasing kidney hypertrophy (Aboismaiel *et al*., 2024). Research by Almatroodi *et al*. (2021) shows that 6-gingerol in *Z. officinale* extract helps repair kidney tissue damage in diabetic rats. Both *C. cajan* and *Z. officinale* extracts also reduce blood glucose levels, lower hyperglycemia, reduce oxidetive stress and increase exogenous antioxidant content in diabetic rat models.

The results of this study indicate that administration of instant granules of *C. cajan* leaves and *Z. officinale* can effectively reduce blood glucose levels and repair kidney microstructural damage in diabetic model rats. This is likely due to the effectiveness of the flavonoid and 6-gingerol antioxidant content contained in the instant granules (Wresdiyati *et al*., 2024b).

CONCLUSION

Instant granules of *C. cajan* leaves and *Z. officinale* can repair microstructural damage to kidney tissue in diabetic rat models by increasing the ratio of glomerulus to Bowman's capsule, decreasing tubular necrosis scores and dilation index. Administration of instant granules of *C. cajan* leaves and *Z. officinale* at a dose of 150 mg/kg BW provides more optimal results in repairing the kidney microstructure of diabetic rat models.

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