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INSTANT GRANULES OF *Zingiber officinale* AND *Cajanus cajan* LEAVES IMPROVED LIVER HISTOLOGICAL PROFILE IN DIABETIC MODEL RATS

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ABSTRACT

This study evaluated the effectiveness of instant granules *of Zingiber officinale* and *Cajanus cajan* leaves on liver histological profile in diabetic model rats. A total of 25 male Sprague Dawley rats were assigned into five groups. Group 1 (NC) consisted of healthy rats given distilled water, while Group 2 (PC) included untreated diabetic model rats. The metformin group (MET) received 150 mg/kg metformin, and two treatment groups (T1 and T2) were administered *Zingiber officinale* and *Cajanus cajan* instant granules at 300 mg/kg and 150 mg/kg, respectively, over 28 days**.** The diabetic condition was obtained by single induction of streptozotocin at dose of 35 mg/kg BW. The liver tissue of diabetic model rats was stained using hematoxylin-eosin staining to observe the cytoplasm intensity, central vein diameter, and nucleus intensity. The results showed that administration of the instant granules significantly decreased liver microstructural damage ($P<0.01$). Optimal therapeutic effects were observed with the 150 mg/kg BW (T2) dose, which successfully diminished cytoplasmic intensity, alleviated central vein dilation, and reduced nuclear cell concentration. The research result indicate that instant granules have strong hepatoprotective properties that can repair liver microstructural damage caused by diabetes.

Key words: *Cajanus cajan*; diabetes; instant granule; liver; *Zingiber officinale*

ABSTRAK

Penelitian ini mengevaluasi efektivitas granul instan Zingiber officinale dan daun Cajanus cajan terhadap profil histologi hati pada tikus model diabetes. Sebanyak 25 tikus Sprague Dawley jantan dibagi menjadi lima kelompok. Kelompok 1 (NC) terdiri dari tikus sehat yang diberi air suling, sedangkan Kelompok 2 (PC) terdiri atas tikus model diabetes yang tidak diobati. Kelompok metformin (MET) menerima 150 mg/kg metformin, dan dua kelompok perlakuan (T1 dan T2) diberikan bubuk instan Zingiber officinale dan Cajanus cajan masing-masing pada dosis 300 mg/kg dan 150 mg/kg selama 28 hari. Kondisi diabetes diperoleh dengan induksi tunggal streptozotocin (35 mg/kg BB). Jaringan hati diwarnai menggunakan pewarnaan hematoksilin-eosin untuk mengamati intensitas sitoplasma, diameter vena sentralis, dan intensitas inti sel hati pada tikus model diabetes. Hasil penelitian menunjukkan bahwa pemberian granul instan secara signifikan menurunkan kerusakan mikrostruktur hati ($P<0,01$). Dosis 150 mg/kg BB (T2) menunjukkan efektivitas optimal dengan berhasil menurunkan intensitas sitoplasma, mengurangi dilatasi vena sentralis, dan menurunkan intensitas sel nukleus. Hasil penelitian menunjukkan bahwa granul instan memiliki sifat hepatoprotektif yang kuat yang dapat memperbaiki kerusakan mikrostruktur hati yang disebabkan oleh diabetes.

Kata kunci: Cajanus cajan; diabetes; granul instan; hati; Zingiber officinale

INTRODUCTION

Diabetes mellitus (DM) is a metabolic condition characterized by hyperglycemia (high blood glucose). Diabetes melitus is caused by the body's inability to either produce sufficient insulin or insulin resistance (IDF 2021). The prevalence of diabetes mellitus is continually increasing globally, with a projection of reaching 783 million patients by 2045. Indonesia ranks fifth, with 19.5 million patients in 2021 that suffer from diabetes melitus (IDF 2021). The normal blood glucose range in humans is 70-99 mg/dL, which differs from rats with 50-135 mg/dL (Fiorenza *et al.* 2022).

Chronic hyperglycemia in diabetic condition can induce histological damage in the liver, such as increased cytoplasmic and nuclear intensity (Alfarisi *et al.* 2022) and central vein dilation, which indicates impaired liver function (Salahshoor *et al.* 2019; Khales *et al.* 2024). These findings have prompted the exploration of herbal therapies as an alternative medicine to repair microstructural liver damage.

The hepatoprotective potential of plants such as *Cajanus cajan* (*C. cajan*) and *Zingiber officinale* (*Z. officinale*) is linked to their phytochemical constituents. *Cajanus cajan* leaves extract has been reported by Wresdiyati *et al.* (2024a) to contain a variety of polyphenols, including the flavonoids phloretin, tangeretin, phloridzin, and hesperetin, as well as several phenolic acids like ellagic acid, gallic acid, and rosmarinic acid. The ethanolic extract of *Z. officinale* is also rich in similar compounds, like flavonoids such as phloridzin, naringin, and phenolic acids such as ellagic acid and syringic acid. Active compounds such as ellagic acid, gallic acid, tangeretin, and phloridzin have been proven to repair diabetes-induced liver damage (Ali *et al.* 2020; Bashar *et al.* 2021).

Combined extract of *Z. officinale* and *C. cajan* leaves has been reported to have a hypoglycemic effect

in previous research (Wresdiyati *et al.* 2020; Wresdiyati *et al.* 2024a; Wresdiyati *et al.* 2024b). In addition, the combination extract of *Z. officinale* and *C. cajan* leaves were found to be non-toxic (Wresdiyati *et al.* 2023).

Currently, no research reports the effectiveness of instant granules of *Z. officinale* and *C. cajan* leaves in repairing microstructural liver damage in diabetic model rats. The objective of this research was to evaluate the potential of an instant granule to repair liver microstructural damage in diabetic model rats.

MATERIALS AND METHOD

Tools and Materials

The equipment used in this study included rat enclosures, enclosure covers, aluminum foil, minor surgical instruments, mortars, gastric probe, vortex (VELP ZX3), magnetic stirrers (EYELA MAGNETIC STIRRER RCH3-L), Petri dishes, soft incubators (Eyela SLI-450N), tissue cassettes, rotary microtome (Yamato RV-240), tissue embedding console (Tissue Teck Sakura), water bath (B-ONE), glucometer (Roche), light microscope (Olympus CX24), light microscope equipped with a camera (Olympus CX23-CCD10 USB Camera), water bath (Eyela Waterbath SB-650), hotplate (HP-v2), histological slide box, digital scale (AND HR-250AZ), rat restraint tool.

The materials used in this study were Sprague-Dawley rats, instant granules of *Z. officinale* and *C. cajan* leaves, patented metformin 500 mg (Glucophage Merck), ketamine (Ket-A-100®), xylazine (Interchemie®), antibiotics (Sanprima®), anthelmintic (vermox), vitamins (elkana), streptozotocin (Sigma-Aldrich S0130-1G), xylene (Qrec 184001-1021), picric acid (SAFC 197378-500.00G), formaldehyde 37% (Sigma-Aldrich 1.04003.2500), acetic acid glacial (Merck 1.000063.2500), ethanol (Merck 1.00983.2500), disposable microtome blade (Feather®), entellan (Merck 1.07961.0500), paraffin (Histoplast Thermo Scientific 6774006), hematoxylin, eosin, distilled water, 2 mL microtube, Accu-Check strips (Roche), citric acid (Merck 1.00244.1000), sodium citrate dihydrate (Merck 1.06448.1000), object glass (SAIL BRAND CAT. No. 7105).

Experimental Animal Preparations

All methods used in this study were approved by the Animal Ethics Committee of SKHB IPB (No. 049/KEH/SKE/XI/2022). A total of 25 male Sprague Dawley rats (220-230 g, 8 weeks old) were obtained from BPOM-RI. The rats were acclimatized for 7 days, and administered anthelmintic medication (30 mg/kg BW), antibiotics (30 mg/kg BW), and vitamins (30 mg/kg BW). The rats were kept in cages (50 × 30 × 15 cm) with husks at a temperature of 24° C, a 12-hour light-dark cycle, and a 55-63% humidity.

Instant Granule Preparation from Zingiber officinale and Cajanus cajan leaves

The extraction and production process of instant granules from *Z. officinale* and *C. cajan* leaves was conducted at PT. Insular Multi Natural, Bogor. Extracts from *Z. officinale* and *C. cajan* leaves were made separately using a method of maceration for 30 minutes. The filtrate was concentrated through evaporation process at 70° C for 75 minutes to produce a concentrated extract, which was subsequently dried into a dry extract. The *C. cajan* leaves extract contained flavonoids at 4090.09±338.23 mg/100 g, while the *Z. officinale* extract contained 622.74±203.39 mg/100 g of flavonoids and 701±4.42 mg/100g of 6-gingerol. The instant granules were made using the dried extract of *Z. officinale*, dried extract of *C. cajan* leaves, and additional ingredients such as sodium benzoate (Wuhan Youji Industries), skim milk (Fonterra), melon essence, green food coloring (Apple Green, Indocol), stevia (Soho Nootropic), Povidone (PvP) (Greenco Group), and aerosol (Wacker) (Wresdiyati *et al.* 2024b).

Experimental Animal Grouping and In Vivo Test Procedures

Twenty-five male Sprague Dawley rats (8 weeks old, weighing 220-230 g) were used in this investigation and randomly assigned to five treatment groups (n= 5 per group). Group 1 (NC) included healthy rats that were given distilled water, whereas Group 2 (PC) comprised untreated diabetic model rats. The metformin group (MET) consisted of diabetic model rats treated with metformin at a dosage of 150 mg/kg BW. Additionally, two other treatment groups were studied: diabetic model rats receiving *Z. officinale* and *C. cajan* instant granules at 300 mg/kg BW (T1) and 150 mg/kg BW (T2). Diabetes induction was performed on all groups except the NC group via a single intraperitoneal injection of streptozotocin (STZ) at a dose of 35 mg/kg BW. Rats were considered diabetic if blood glucose levels exceeded 300 mg/dL. All interventions were given orally once daily over a 28-day period.

Liver Sampling and Processing

Rats were terminated on day 29 by intraperitoneal (i.p.) injection of anesthesia using ketamine (70 mg/kg BW) and xylazine (10 mg/kg BW). All rats organs were taken and soaked in 0.9% physiological NaCl, then fixated with Bouin's solution. After 4 hours, the liver was sectioned (0.5 cm) to optimize fixation. After 24 hours fixation period, all organs were transferred to 70% alcohol as a stopping point.

Histological preparation followed the method described by Kiernan (2015). The process was initiated with liver tissue trimming, followed by dehydration using graded alcohol and clearing with xylene to remove residual alcohol. The liver tissue was then infiltrated with liquid paraffin and formed into paraffin blocks using a Tissue-Tek (Sakura) device. Tissue sectioning was performed using a rotary microtome at a thickness of 4 micrometers. The sections were stained using hematoxylin-eosin and observed using a light microscope (Olympus CX23) with a USB CCD10 camera. Each liver tissue sample was documented in seven fields of view.

The liver histological analysis included cytoplasmic intensity, central vein diameter, and nuclear cell intensity, which were analyzed using ImageJ 1.45i. Color intensity was evaluated using gray value (gv), which reflects the tissue's ability to absorb hematoxylin-eosin stain; higher gray values indicate lower color intensity, suggesting changes in metabolic activity of the cell (Chan 2014). The central vein diameter was measured using the straight-line selection, and the measure features were analyzed in the SPSS.

Data Analysis

Liver cytoplasm intensity, liver central vein diameter, and liver cell nucleus intensity data were measured using the ImageJ application. All data obtained from this research were analyzed to quantitative analysis through a one-way ANOVA test, using SPSS 25.00 software. A Duncan's multiple range test was performed if the test results showed significant differences ($P<0.05$). If the data did not meet the prerequisites for the ANOVA test, the Mann-Whitney test was performed.

RESULT AND DISCUSSION

Rat Blood Glucose Level

Blood glucose level in diabetic model rats is shown in Figure 1. Blood glucose levels in streptozotocin-induced diabetic rats increased, with the group 2 (PC) at 349±9.53 mg/dL, the MET group at 345.66±4.5 mg/dL, the T1 group at 341.33±5.03 mg/dL, and the T2 group at 341.66±4.72 mg/dL, while the NC group not induced with streptozotocin showed blood glucose levels of 106 ± 3 mg/dL. On the final day of treatment (day 29), the blood glucose levels of the NC group remained stable within the normal range (92.66±2.3 mg/dL), while the blood glucose levels of the PC group increased to 571±50.22 mg/dL. Blood glucose levels in the metformin (MET) group decreased to 137±52.56 mg/dL, while blood glucose levels in the diabetic rat groups administered instant granules at doses of 300 mg/kg BW (T1) and 150 mg/kg BW (T2) also decreased to 149±128. 17 mg/dL and 148.33±93.3 mg/dL, respectively. The administration of low-dose instant granules (T2) showed the optimal effect in lowering blood glucose levels in diabetic model rats compared to other groups.

The effectiveness of metformin and instant granules of *Z. officinale* and *C. cajan* leaves in reducing blood glucose levels is based on different mechanisms of action. Metformin inhibits mitochondrial complex I (Foretz *et al.* 2019; Reczek *et al.* 2024), which triggers AMP-activated protein kinase (AMPK) activation (Agius *et al.* 2020). This activation primarily suppresses hepatic glucose production and modulation of the gut microbiota (Foretz *et al.* 2019). On the other hand, instant granules of *Z. officinale* and *C. cajan* leaves act through multi-target mechanisms with the help of polyphenol content in the instant granules (Wresdiyati *et al.* 2024a). Bioactive compounds such as flavonoids and 6-gingerol synergistically stimulate insulin secretion and increasing the sensitivity of insulin (Iranloye *et al.* 2011; Abdalla *et al.* 2023), improve peripheral glucose uptake through glucose transporter 4 (GLUT4) translocation (Tzeng *et al.* 2011), and enhance cellular protection by targeting the free radicals and inflammation through the nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor kappa B (NF-κB) pathways (Rajappa *et al.* 2019; El-Huneidi *et al.* 2023). This mechanism reduces blood glucose levels in diabetic model rats. Administration of instant granules of *Z. officinale* and *C. cajan* leaves at a dose of 150 mg/kg BW showed optimal results in reducing blood glucose levels in diabetic model rats.

Liver Cell Cytoplasm Intensity

Hematoxylin and eosin (HE) staining results of liver tissue in diabetic model rats are presented in Figure 2a,

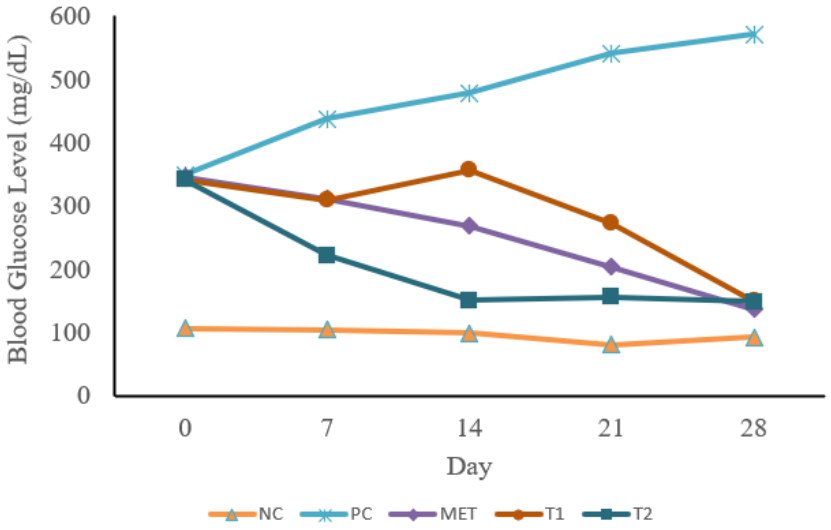

Figure 1. Blood glucose levels of diabetic model rats. Administration of instant granules of *Z. officinale* and *C. cajan* leaves lower blood glucose level in diabetic model rats. NC = Group 1, PC= Group 2, MET= DM + Metformin (150 mg/kg BW), T1= Instant granules of *Z. officinale* and *C. cajan leaves* (300 mg/kg BW), T2= Instant granules of *Z. officinale* and *C. cajan* leaves (150 mg/kg BW)

with the quantitative analysis of cytoplasmic intensity displayed in Figure 2b, while liver cytoplasmic intensity average was shown in Table 1. Administration of instant granules of *Z. officinale* and *C. cajan* leaves to diabetic model rats showed significantly lower cytoplasmic intensity (P<0.01) compared to PC group. The NC group exhibited the lowest (P<0.01) cytoplasmic intensity of liver cells compared to the other groups. Conversely, the PC group showed significantly higher cytoplasmic intensity of liver cells (P<0.01) compared to the other groups. The increased cytoplasmic intensity of liver cells in the PC group indicates damage to the cytoplasm of liver cells due to diabetes, causing them to absorb more eosin dye, thereby increasing cytoplasmic intensity. The MET, T1, and T2 groups showed significantly higher gray values (P<0.01) compared to the PC group. The low-dose instant granule treatment (T2) was identified as the most effective in reducing liver cytoplasmic intensity of diabetic model rats.

Diabetic condition exhibit changes in the basic properties of liver cell cytoplasm, characterized by increased color intensity due to excessive eosin absorption (eosinophilia), as shown in Figure 2a. Eosinophilia is caused by protein denaturation within the cytoplasm of liver cells and the loss of Ribonucleic Acid (RNA) bound to absorb hematoxylin dye (Alfarisi *et al.* 2022), which contributes to excessive eosin absorption. Based on the result, instant granules of *Z. officinale* and *C. cajan* leaves were effective in repairing liver microstructural damage in diabetic model rats.

Oxidative stress is a primary complication of diabetic hyperglycemia, stemming from an imbalance

Table 1. Mean (±SD) cytoplasmic intensity of liver cells (gv) of diabetic model rats

Group	Liver cytoplasm intensity (gv)
NC	222.51±2.46**
PC	219.20±2.53$^{\#\#}$
MET	219.67±2.08*
T1	221.37±1.72**
T2	221.35±1.99**

$^{*, **, \#}$Different superscripts in the analysis results indicate significant differences (P<0.05) between the 2 treatment groups. **P<0.01 Vs. PC; *P<0.05 Vs. PC; $^{\#\#}$P<0.01 Vs. NC in the Mann Whitney test. NC= Group 1, PC= Group 2, MET= DM + Metformin (150 mg/kg BW), T1= DM + Instant granules of *Z. officinale* and *C. cajan* leaves (300 mg/kg BW) and T2= DM + Instant granules of *Z. officinale* and *C. cajan* leaves (150 mg/kg BW)

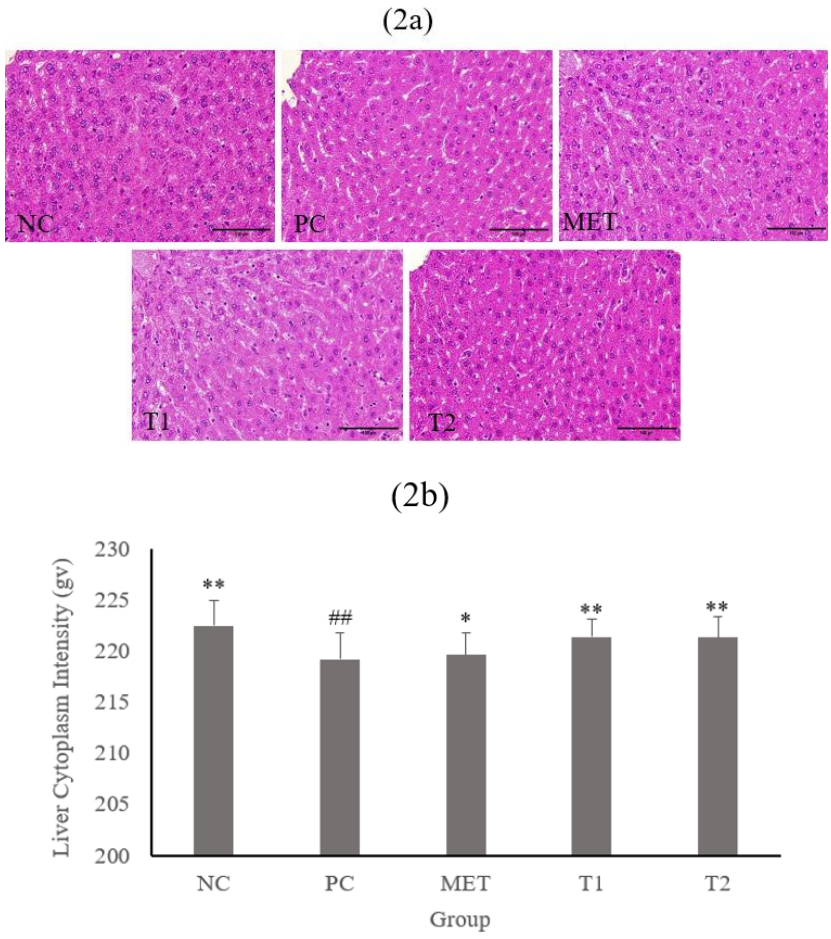

Figure 2. Photomicrograph (2a) and graph (2b) of the liver cytoplasm intensity in diabetic model rats. Administration of instant granules of *Z. officinale* and *C. cajan* leaves lower the cytoplasm intensity in diabetic rats (P<0.01). NC= Group 1, PC= Group 2, MET= DM + Metformin (150 mg/kg BW), T1= Instant granules of *Z. officinale* and *C. cajan* leaves (300 mg/kg BW), T2 = Instant granules of *Z. officinale* and *C. cajan* leaves (150 mg/kg BW), scale= 100 µm

between the overproduction of Reactive Oxygen Species (ROS) and the body's antioxidant production. This overproduction is driven by multiple increases in metabolic pathways, including an increase in polyol and hexosamine pathways, increase in activation of Protein Kinase C (PKC), and the increase in formation of Advanced Glycated End Products (AGEs) (González *et al.* 2023). The liver, due to its vital role in glucose metabolism, is particularly vulnerable to the damage inflicted by ROS on cellular lipids, proteins, and DNA. This pathological condition is further exacerbated by the dysfunction of mitochondrial complex I, which also elevates ROS levels and leads to structural damage within the cytoplasm of hepatocytes (Yaduvanshi *et al.* 2012; Dal *et al.* 2016; Hernández-Esparza *et al.* 2020).

This study showed the metformin group (MET) and instant granules of *Z. officinale* and *C. cajan* leaves significantly lower hepatocyte cytoplasmic intensity in diabetic model rats, indicating improved liver microstructure. Metformin, the first-line therapy for type 2 diabetes mellitus, effectively lowers fasting glucose and improves insulin sensitivity (Lin *et al.* 2018; Susilawati *et al.* 2023). Furthermore, 96% ethanol extracts of *Z. officinale* and *C. cajan* leaves are rich in flavonoids and phenolic acids, which have antioxidant properties and play a role in lowering blood glucose levels (Wresdiyati *et al.* 2024b). Active compounds such as phloretin, tangeretin, and phloridzin can prevent hyperglycemia complications and improve glucose metabolism (Wang *et al.* 2016; Ying *et al.* 2018; Guo *et al.* 2020). Furthermore, hesperetin, naringenin, and eriodictyol protect pancreatic β cells from oxidative stress (Zhang *et al.* 2012; Rajappa *et al.* 2019; Abdou *et al.* 2022), while daidzin and rhoifolin enhance endothelial function and insulin sensitivity (Tzeng *et al.* 2011). Other flavonoids such as naringin, genistin, apigenin, glycitein, and daidzein also contribute to blood glucose control through various molecular mechanisms (Esmaeili dan Sadeghi 2009; Cheong *et al.* 2014; Mao *et al.* 2015; Subramanian *et al.* 2019; Ahmed *et al.* 2020; Siriviriyakul *et al.* 2022). The results confirm the effect of instant granules of *Z. officinale* and *C. cajan* leaves in lowering blood glucose and repairing liver damage in diabetic model rats, primarily through the antioxidant activity and hypoglycemic effects of flavonoids and 6-gingerol (Wresdiyati *et al.* 2024b).

Liver Central Vein Diameter

Photomicrograph analysis showed that administration of instant granules of *Z. officinale* and *C. cajan* leaves significantly reduced the diameter of the central vein ($P<0.01$) in diabetic model rats (Figures 3a-b), with the average diameter of the central vein shown in Table 2. The PC group exhibited the most severe dilation of the central vein diameter compared to all other groups, indicating tissue damage. Shruthi *et al.* (2024) reported that diabetic conditions can trigger inflammation, congestion, or fibrosis that alters the central vein diameter. The NC group exhibited the lowest central vein diameter. The T2 group (150 mg/kg BW dose) and the metformin group (MET) showed a significantly higher reduction in liver central vein diameter ($P<0.01$) compared to the PC group. The diabetic group given instant granules of *Z. officinale* and *C. cajan* leaves, especially at a dose of 150 mg/kg BW (T2) showed a significantly lower central vein diameter compared to the PC group ($P<0.01$). Meanwhile, administration of instant granules at a dose of 300 mg/kg in diabetic model rats showed a higher central vein diameter but did not show a significant difference compared to the PC group. Thus, the administration of instant granules of *Z. officinale* and *C. cajan* leaves in the T2 group was more effective in repairing liver damage and reducing liver central vein dilation in diabetic model rats compared to the T1 group.

The administration of instant granules of *Z. officinale* and *C. cajan* leaves led to a significant reduction in the dilation of the central vein within the livers of diabetic model rats (Figure 3a). This finding is relevant because chronic hyperglycemia in diabetes mellitus triggers microvascular damage, including hepatic vascular dysfunction and central vein dilation,

Table 2. Mean (±SD) diameter of central vein of liver tissue of diabetic model rats

Group	Liver central vein diameter (μm)
NC	55.04 ± 23.12^{a}
PC	72.76 ± 22.23^{d}
MET	59.60 ± 20.82^{ab}
T1	67.09 ± 22.93^{cd}
T2	62.23 ± 22.87^{bc}

[a, b, c, d] Different superscripts in the analysis results indicate significant differences ($P<0.01$). NC= Group 1, PC= Group 2, MET= DM + Metformin 150 mg/kg BW, T1= Instant granules of *Z. officinale* and *C. cajan* leaves (300 mg/kg BW) and T2= Instant granules of *Z. officinale* and *C. cajan* leaves (150 mg/kg BW)

Table 3. Mean (±SD) intensity of liver cell nuclei of diabetic model rats

Group	Liver cell Nuclear intensity (gv)
NC	$139.94\pm8.1^{**}$
PC	$128.12.50\pm6.73^{\#\#}$
MET	$132.25\pm8.12^{**}$
T1	$136.32\pm7.10^{**}$
T2	$134.95\pm7.47^{**}$

[**, ##]Different superscripts ([*, #]) in the analysis results indicate significant differences ($P<0.05$) between the 2 treatment groups. **$P<0.01$ Vs. NC; ##$P<0.01$ Vs. PC in the Mann Whitney test. NC= Group 1, PC= Group 2, MET= DM + Metformin (150 mg/kg BW), T1= DM + Instant granules of *Z. officinale* and *C. cajan* leaves (300 mg/kg BW) and T2= DM + Instant granules of *Z. officinale* and *C. cajan* leaves (150 mg/kg BW)

as Khales *et al.* (2024) reported. The decrease in liver central vein diameter of diabetic model rats is associated with the active compounds in the granules.

The extract of *Z. officinale* and *C. cajan* leaves has been shown to have hepatoprotective effects and to lower blood glucose levels (Wresdiyati *et al.* 2020; 2024b; Ogbu *et al.* 2022). The hepatoprotective and hypoglycemic effects are supported by active compounds such as flavonoids, saponins, and 6-gingerol. Flavonoids contribute to lowering hyperglycemia by inhibiting alpha-glucosidase and alpha-amylase enzymes (Manzo dan Vitor 2017), while 6-gingerol increases the expression and translocation of GLUT4 to improve glucose metabolism (Lee *et al.* 2015). Thus, improving glucose metabolism and antioxidant activity to combat free radicals in diabetic conditions from these compounds cumulatively reduces stress on the hepatic vascular system, thereby improving the diameter of the central vein that had previously dilated due to diabetes.

Liver Cell Nuclear Intensity

The HE staining results of liver tissue in diabetic model rats are shown in Figure 4a, while the liver cell nucleus staining intensity graph is presented in Figure 4b. The average liver cell nucleus intensity is shown in Table 3. Administration of the instant granules of *Z. officinale* and *C. cajan* leaves to diabetic model rats resulted in significantly lower ($P<0.01$) liver cell nucleus intensity compared to the PC group. The NC group exhibited the lowest liver cell nucleus intensity compared to all other groups, indicating normal morphological conditions of the cell nucleus. In contrast, the PC group showed significantly higher liver cell nucleus intensity ($P<0.01$) compared to the other groups. This increase in intensity of liver cell nucleus reflects the damage to the cell nucleus, such as DNA damage caused by hyperglycemia, which leads to increased eosin absorption. The MET, T1, and T2 groups showed significantly higher gray values ($P<0.01$) than the PC group. These results indicate that treatment with instant granules of *Z. officinale* and *C. cajan* leaves, particularly at low doses (T2), is more effective in increasing gray value, indicating a reduction in liver cell nucleus intensity. This may be attributed to bioactive compounds antioxidant and hepatoprotective properties in instant granules of *Z. officinale* and *C. cajan* leaves against liver cell nucleus damage caused by oxidative stress induced by hyperglycemia in diabetes.

These findings support previous reports stating that diabetes can trigger genotoxic stress and chromosomal instability. Manifestations of this damage include various nuclear abnormalities such as micronuclei, binucleation, nuclear buds, karyolysis, and paler nuclei (Atmadja *et al.* 2023; Aafreen and Kumar 2024).

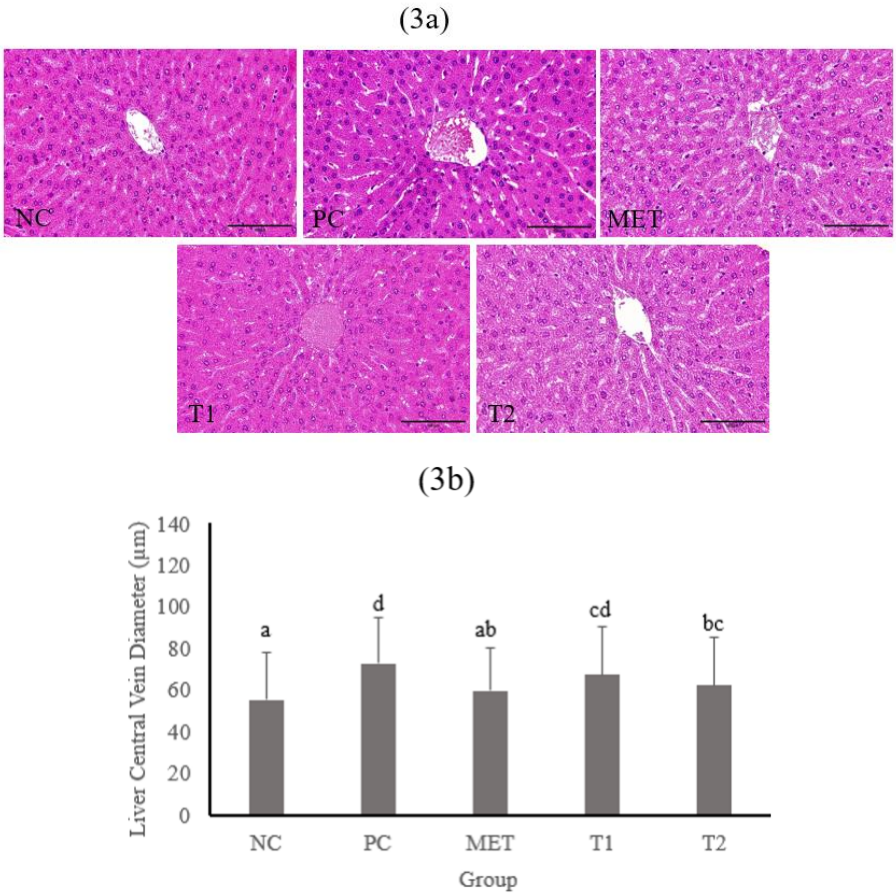

Figure 3. Photomicrograph (3a) and graph (3b) of the diameter of the central vein of the liver tissue of diabetic model rats. Administration of instant granules of *Z. officinale* and *C. cajan* leaves reduce the size of the diameter of the central vein in diabetic rats. NC= Group 1, PC= Group 2, MET= DM + Metformin (150 mg/kg BW), T1= DM + Instant granules of *Z. officinale* and *C. cajan* leaves (300 mg/kg BW), T2= DM + Instant granules of *Z. officinale* and *C. cajan* leaves (150 mg/kg BW), scale= 100 µm

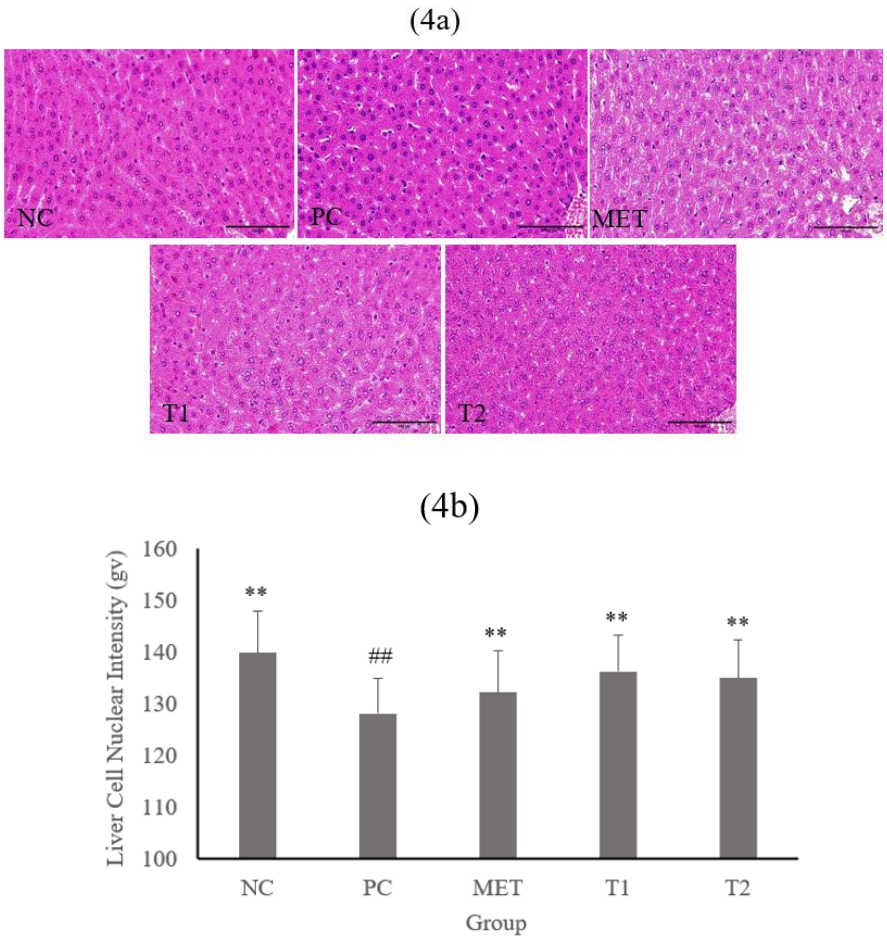

Figure 4. Photomicrograph (4a) and graph (4b) of the intensity of the cell nucleus of the liver tissue of diabetic model rats. Administration instant granules of *Z. officinale* and *C. cajan* leaves lower the intensity of the cell nucleus in diabetic rats. NC = Group 1, PC= Group 2, MET= DM + Metformin (150 mg/kg BW), T1= DM + Instant granules of *Z. officinale* and *C. cajan* leaves (300 mg/kg BW), T2= DM + Instant granules of *Z. officinale* and *C. cajan* leaves (150 mg/kg BW), scale= 100 µm

The repair of liver nuclear damage observed in this study is likely due to the synergistic action of active compounds in the granules, such as flavonoids and 6-gingerol. These compounds are known to have strong antioxidant and antihyperglycemic activities by lowering blood glucose levels and counteracting oxidative stress. The active ingredients in instant granules protect DNA in liver cell nuclei from damage. As a result, the integrity of the cell nucleus structure improves, allowing hematoxylin dye absorption to return to normal and cell nucleus intensity to become normal.

CONCLUSION

Instant granules of *Z. officinale* and *C. cajan* leaves can repair microstructural damage in the liver tissue of diabetic model rats by reducing blood glucose level, cytoplasmic intensity, central vein dilation, and the intensity of liver cell nuclei. A dose of 150 mg/kg BW showed the optimal effectiveness in lowering blood glucose level and restoring liver histological conditions in diabetic model rats. These findings confirm that instant granule of *Z. officinale* and *C. cajan* leaves has the potential to be developed as a phytotherapy in treating complications of liver damage due to diabetes.

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