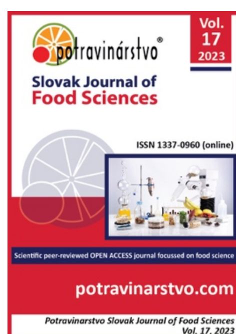

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The effect of Kawa Daun (*Coffea canephora*) decoction on blood glucose levels and pancreatic β-cells regeneration in rats with diabetes

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ABSTRACT

Giving coffee leaves *Kawa Daun (Coffea canephora)*, which contains flavonoids and chlorophyll, which are antioxidants, is one of the therapies that may be used to treat diabetes mellitus, which is expected to affect 783 million people worldwide by 2045. This study, therefore, aims to demonstrate *Kawa Daun* decoction's potency in lowering blood glucose levels and restoring pancreatic β-cells in rats with diabetes mellitus. Wistar rats (2-3 months, 200 g, n = 28) were used in this true experimental study, which applied a pre-post-control group design. Regular feeding + no intervention was for the group (K-); *Kawa Daun* was not provided to (K+) DM (alloxan) rats + regular feeding; (P1) DM rats (alloxan) received 3.6 ml/200 g BW/day of *Kawa Daun* decoction along with regular feeding; *Kawa Daun* decoction 7.2 ml/200 g body weight/day in addition to regular feeding was given to (P2) DM rats (alloxan). For 14 days, the intervention was given orally. A spectrophotometer was utilized to detect blood glucose levels, and histological analysis using H&E staining was employed to determine the state of the pancreatic β-cells. In comparison to the (K+) group, the intervention group significantly decreased blood glucose levels ($p = 0.001$), according to the findings. The P2 group's reduction in blood sugar levels (Δ = 139.33 mg/dl 38.45) was more significant than that of the P1 group (Δ = 109.17 mg/dl 35.32). Compared to the (K+) (27.1% damage) group, the intervention group's pancreatic β-cells revealed improvement according to the histopathological examination results. The group's (P2 = 14.9%) damage area was less than the group's (P1 = 22.4%). This study emphasizes how administering *Kawa Daun* decoction can improve blood glucose levels and reconstruct the pancreatic β-cells damage and its protection. Finally, this kind of leaf could be a substitute compound for diabetic herbal therapy.

Keywords: blood glucose, *Kawa Daun*, pancreatic β-cells, flavonoid, antioxidant

INTRODUCTION

Diabetes mellitus is a disease characterized by chronically elevated blood glucose levels caused by impaired glucose metabolism resulting from pancreatic β-cells damage or insulin resistance **[1]**. The International Diabetic Federation reported an increase in the number of people with diabetes mellitus by 537 million in 2021, with an estimated increase to 643 million in 2030 and 783 million in 2045 **[2]**. Indonesia is ranked seventh in the world with a prevalence of diabetes mellitus incidence of 10.7% **[3]**.

Specifically, non-pharmacological treatment is still one of the most popular treatments in Indonesia, primarily since Indonesia is known as a country with a variety of functional foods. One of them is Robusta coffee leaf (*Coffea canephora*). In West Sumatra, coffee leaves, better known as "*Kawa Daun*," are one of the typical drinks of West Sumatra in great demand by the public. *Kawa Daun (Coffea canephora)* has the potential as an antioxidant, anti-diabetic, antibacterial, and anti-inflammatory since its decoction has several bioactive components. The bioactive components contained in *Kawa Daun* are phenolic components, such as caffeine, chlorogenic acid, flavonoids, chlorophyll, and Mangiferin, which can improve the condition of diabetes mellitus pathogenesis **[4]**.

Flavonoids are one of the most abundant secondary metabolite compounds in *Kawa Daun*, with antioxidant, anti-diabetic, and anti-inflammatory properties. The action of flavonoids in improving the condition of diabetes mellitus is by scavenging free radicals of Reactive Oxygen Species (ROS). When ROS activity can be inhibited, oxidation and inflammation processes in the body can be suppressed. It can allow insulin receptors and pancreatic β-cells to regenerate or repair themselves, decreasing blood glucose levels **[5]**.

The results of previous studies explained that giving drinks from Robusta coffee leaves (*Coffea canephora*) can significantly increase insulin levels so that the HOMA-IR index or insulin resistance can be improved. The study was conducted on Wistar rats conditioned to have metabolic syndrome **[6]**. The results of another study also explained that the administration of ethanol extract from Robusta coffee leaves (*Coffea canephora*) could significantly reduce blood glucose levels in Wistar rats fed a high-fat and high-sucrose diet **[7]**.

Based on previous research findings of Anjani (2020) and, the effect of giving *Kawa Daun (Coffea canephora)* decoction on the repair of pancreatic β-cells has not been known. For this reason, this study's significance and objective are to prove the effectiveness of *Kawa Daun* decoction in reducing blood glucose levels and repairing pancreatic β-cells in rats with diabetes mellitus.

Scientific hypothesis

The research hypothesis hinged on the assumption that the *Kawa Daun* decoction *(Coffea canephora)* could positively improve the blood glucose and pancreatic cells' damage of diabetic rats. As an object of the in vivo study in Wistar strain rats, kawa daun (Coffea canephora) in different doses should decrease blood glucose and repair pancreatic disturbance in diabetic rats.

MATERIAL AND METHODOLOGY

This true experimental study used a pre-post-control group design. This research was conducted at the Pharmacy Laboratory and the Laboratory of the Faculty of Health Sciences, Universitas Perintis Indonesia.

Samples

Robusta coffee leaves (*Coffea canephora*) were obtained from the Tanah Datar area of West Sumatra. Sample preparation started with the sample drying process using the drying method in an open container of 27 °C temperature, not exposed to direct sunlight. It aimed to minimize the damage to the bioactive components contained in *Kawa Daun*. The drying process was carried out for two-three days. It was then continued with the manufacture of coarse powder from coffee leaves using a food processor and stored in a dark bottle. The *Kawa Daun* formula was made by boiling 5 g and 10 g of coffee leaves in 200 ml of water until they boiled.

Chemicals

All chemical reagents in the experimental design were of analytical grade quality and were purchased from Sigma-Aldrich, Japan, Leica Biosystem, USA, and Indogen Intertama Supplier, Indonesia.

Experimental Animals

This study used experimental animals obtained from the pharmacy laboratory of Universitas Andalas. The study used white Wistar rats (*Rattus novergicus*) with inclusion criteria of male rats aged 2-3 months weighing 150-200 gr. All groups of rats were given regular feed and drink ad libitum. The given dose was graded, with P1 3.6 ml/200 g BW/day and P2 7.3 ml/200 g BW/day for 14 days. The K- group was a group of healthy mice, only getting regular feed without intervention. Meanwhile, the K+ group was a group of DM rats induced by alloxan, given regular feed, and not given *Kawa Daun*. We involved 28 rats for the experimental groups and separated them in to 4 groups, 7 rats/ group. Before involving these animals, they were acclimatizatized for 7 days with ad-libithum consumption. The Ethics Committee of the Universitas Perintis Indonesia, Padang approved the involvement of these animals following the legislation of the ethical clearance No. 083.1/KEPK.F2/ETIK/2022, KEPK Universitas Perintis Indonesia. We sacrified these animals by dislocating the spinal cord nerve after being anaesthetized with methanol 10%.

Experimental Analysis and Instruments

Proximate and Fiber Analysis: Proximate analysis was conducted on dried *Kawa Daun* powder to determine the protein, fat, carbohydrate, water, and ash content using the 2012 AOAC method **[8]**. Fiber content analysis used the gravimetric method **[9]**.

Chlorophyll Analysis: The chlorophyll content of *Kawa Daun* was obtained through 5 g of powder brewed with 70 °C water for ten minutes, compared to 5 g of powder dissolved in 80% acetone by using Aminot's (2000) standard procedures for chlorophyll determination **[10]**. It was then measured using a 663 nm and 645 nm UV-VIS spectrophotometer of SHIMADZU UV-1280 (Shimadzu Scientific Instruments, Japan). Afterwards, the chlorophyll content was calculated by Arnon's formula **[11]**:

Chlorophyll a = (12.7 × A663) - (2.69 × A645)
Chlorophyll b = (22.9 × A645) - (4.68 × A663)
Total of chlorophyll = (20.2 × A645) + (8.02 × A663)

Total Flavonoid Analysis: A total of 5 grams of *Kawa Daun* powder was homogenized with 200 ml of 96% methanol using a magnetic stirrer for 30 minutes. A total of 60 ml of *Kawa Daun* solution was taken and then centrifuged with Cytology Centrifuge Cytospin^{TM} 4 (Thermo Scientific, USA), taking part of the supernatant. A total of 1 ml of the sample solution was mixed with 9 ml of methanol; 2.8 ml of sample solution, 0.4 ml of 5% $AlCl_3$, and 6.8 ml of 5% acetic acid were pipetted and then incubated for 30 minutes. The measurement of total flavonoids used the $AlCl_3$ calorimetry method of ASTM D240 Oxygen Bomb Calorimeter (Changsha Kaiyuan Instruments Co., Ltd, China), with absorbance calculations utilizing UV-VIS spectrophotometry at a wavelength of 428 nm. The calibration curve employed standard quercetin in methanol (0.005 mg/ml), with concentrations of 0 g/ml, 5 g/ml, 10 g/ml, 20 g/ml, 40 g/ml, respectively and 60 g/ml **[12]**.

Antioxidant Activity Analysis: Antioxidant activity was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Sample preparation was carried out by weighing 0.1 mg of *Kawa Daun* powder and then putting it into a microtube containing 1 ml of methanol. The homogenized sample was then centrifuged at 4500 rpm for seven minutes. After that, 200 µl of supernatant was stored in a microtube. The blank used was ethanol as much as 200 µl. In addition, the standard solution was prepared by weighing 0.01 g of ascorbic acid, put into a test tube, and adding 10 ml of distilled water and vortex until homogeneous. The dilution was conducted starting from 1000 ppm to 0 ppm, with the formula V1×N1 = V2×N2.

Moreover, antioxidant activity was performed by pipetting 200µl of each solution into a microtube, and 1000 µl of DPPH was added and then mixed using a vortex and incubated for 30 minutes. Observations were then made utilizing UV-VIS Spectrophotometer at a wavelength of 517 nm. The following formula (1) was used for antioxidant activity calculation (1):

(%IC) = (Blank absorbance - Sample absorbance/Blank absorbance) x 100% (1) **[13]**.

Number of samples analyzed: we analyzed 28 pancreatic organs as a histopathological examination sample from different intervention groups.

Number of repeated analyses: 0

Number of experiment replication: 0

Laboratory Methods

Blood Glucose Level Measurement: Enzymatic measurement of blood glucose levels was done twice: after three days of alloxan induction and 14 days after the intervention. Before blood collection, rats fasted for eight-ten hours. Then, 2 ml of blood was taken through the retroorbital plexus, inserted into a microtube, and centrifuged at 4000 rpm for 15 minutes. Blood glucose levels were measured by adding 10 µl of sample and standard with 1000 µl of o-toluidine reagent. For blanks, they were filled with water and added with reagent as much as 1000 µl. Samples, standards, and blanks were then homogenized using a vortex. Then, samples, standards, and blanks were incubated for ten minutes at a temperature of 20-25 °C or five minutes at a temperature of 37 °C. Reading the absorbance utilized spectrophotometry with a wavelength of 500 nm.

Histopathological Examination: Preparing for histopathological examination began with rats killed by spinal cord dislocation. The tissue was washed with physiological NaCl solution, tissue fixation (36 hours) NBF (Neutral Buffer Formaldehyde) 10%. Dehydration was with graded alcohol for 60 minutes, and clearing was with xylol for two hours (2 times). Soaked in liquid paraffin (three hours), the thinly sliced tissue was immersed in a water bath containing water and gelatine at 40 °C, affixing on a slide. Deparaffination of preparations was carried out with xylol I-III (two minutes). Then, it was immersed in the graded alcohol gradually (two minutes). Staining used hematoxylin for ten minutes and eosin for five minutes. Then, it was dipped in graded alcohol (5 times), immersed for five minutes in xylol I, II, and III, and covered with a cover glass. Afterward, a pancreatic necrosis or regeneration feature was observed with Leica MD2000 LED Optilab Microscope (Leica Microsystem, USA) by measuring the injury area with the ImageJ program (Java, National Institute of Health).

Statistical Analysis

Statistical analysis was used to see the effect of giving the *Kawa Daun* decoction in decreasing blood glucose levels and repairing pancreatic β-cells in rats with diabetes mellitus. Paired t-test analysis was employed to measure the effect of giving *Kawa Daun* decoction before and after giving the decoction. In addition, paired t-test analysis was also utilized to determine differences in blood glucose levels between groups. The Post Hoc Bonferroni test followed it if the data were normally distributed, and vice versa. The Kruskal-Wallis test analysis was employed if the data were not normally distributed, followed by the Mann-Whitney test. Statistical analysis was performed using SPSS 26 software, with significant differences in ethical *p*-value >0.05 and 95% CI.

RESULTS AND DISCUSSION

Nutrient Content

Analysis of the nutritional content of *Kawa Daun (Coffea canephora)* used the AOAC 2012 method. The analysis results of the nutritional content of *Kawa Daun* are present in Table 1.

Table 1 Nutritional content of *kawa daun (Coffea canephora)*.

Nutritional Content	(%)
Protein	8.75
Fat	5.21
Carbohydrate	83.86
Water	2.07
Ash	0.11
Coarse fiber	8.95

Note: characteristic of kawa daun (*Coffea canephora*).

Bioactive Components and Antioxidant Activity

Analysis of the bioactive components contained in *Kawa Daun (Coffea canephora)* used the $AlCl_3$ calorimetry method for total flavonoids, UV-VIS Spectrophotometer for chlorophyll, and DPPH scavenging for antioxidant activity. The analysis results of bioactive components and antioxidant activity can be seen in Table 2. From the antioxidant activity test results, *Kawa Daun* is known to have intense antioxidant activity (IC50 = 30.99 ppm) **[14]**. Many factors cause *Kawa Daun* to have high antioxidant activity. Analysis of the intact form of *Kawa Daun* suggests the presence of other bioactive substances, which help increase antioxidant activity, including caffeine, chlorogenic acid, mangiferin, and chlorophyll, and are also known to have anti-diabetic, antioxidant, and anti-inflammatory activities. Aside from bioactive components, nutrients, such as carbohydrates and crude fiber, in *Kawa Daun* can also help increase antioxidant activity through the Maillard reaction caused by heating compounds containing carbohydrate derivatives and amino acids **[15]**.

Table 2 Content of bioactive components and antioxidant activity of *kawa daun (Coffea canephora)*.

Sample	Total of Flavonoid (mQE/g)	Chlorophyll (mg/L)	IC50 (ppm)
Kawa Daun	1.3	15.33	30.99

Note: bioactive component of *Kawa Daun* (*Coffea canephora*).

The Kaur et al **[16]** study revealed that the decoction analysis of arabica coffee leaves has a higher flavonoid component than robusta coffee (69.2 mg EQ/g and 47.5 mg EQ/g respectively). However, the *Kawa daun* flavonoid contained in higher enough compound for 1.3 mEQ/g.

The Effect of Giving *Kawa Daun* Decoction on Blood Glucose Levels

Giving *Kawa Daun (Coffea canephora)* decoction was orally carried out for 14 days. Blood glucose levels before and after the intervention can be seen in Table 3. The paired t-test analysis results revealed significant differences in blood glucose levels before and after the intervention in the K+, P1, and P2 groups. A similar statistical test also demonstrated a significant difference in changes in blood glucose levels between the four groups (p <0.001). The Post Hoc Bonferroni test in Table 4 further uncovered a significant difference in changes in blood glucose levels in groups P1 (p = 0.001), and P2 (p = 0.000), compared to group K-, and there was no significant difference in changes in blood glucose levels between treatment groups P1 and P2 (p = 0.119). The intervention results also showed that the decrease in blood glucose levels was the largest in group 2 (139.33 ±38.453). However, if looking at the difference between groups P1 and P2, there was no statistical difference. It indicates that the lowest dose in the intervention could reduce blood glucose levels equivalent to blood glucose levels that received a higher dose intervention.

Table 3 The effect of giving *kawa daun* decoction on blood glucose levels.

Group	Pre-intervention	Post-intervention	(Δ) Pre-Post	*p*-value
K-	87 ±4.336	87.83 ±3.656	-0.83 ±2.927	0.517
K+	479.50 ±91.352	439.50 ±65.127	40 ±37.084	0.046
P1	230.50 ±29.616	121.33 ±13.736	109.17 ±35.324	0.001
P2	240.00 ±33.478	100.67 ±18.907	139.33 ±38.453	0.001

Note: K- = healthy rat + standard feed; K+ = T2DM + standard feed; P1 = T2DM + *Kawa Daun (Coffea canephora)* decoction 3.6 ml/200 g BW/day; P2 = T2DM + *Kawa Daun (Coffea canephora)* decoction 7.2 ml/200 g BW/day.

Table 4 Post hoc test of changes in blood sugar levels before and after the intervention.

Group	Δ Blood Glucose Level (mg/dl)	Value of *p*			
		K-	K+	P1	P2
K-	-0.83 ±2.927	-	0.039*	0.000*	0.000*
K+	40 ±37.084		-	0.001*	0.000*
P1	109.17 ±35.324			-	0.119
P2	139.33 ±38.453				-

Note: $p < 0.05$ = significant.

Functioned as antioxidants, anti-diabetic, anti-inflammatory, phenols, flavonoids, chlorophyll, Mangiferin, and caffeine has been identified as bioactive components in *Kawa Daun*. Flavonoids are known to have antioxidant activity, which is believed to protect the body against damage caused by reactive oxygen species so that they can inhibit the occurrence of degenerative diseases, such as DM. Flavonoids can also lower blood glucose levels with their ability as antioxidants. Moreover, flavonoids protect against cell damage as insulin producers and can restore insulin receptor sensitivity in cells and even increase insulin sensitivity **[17]**. The mechanism of anti-diabetic activity of flavonoids also occurs in the regulation of carbohydrate digestion, regulation of insulin signalling, and insulin secretion resulting from the recovery time of pancreatic β-cells so that insulin production increases and the increase in glucose uptake into the blood is due to repair of insulin receptor cells **[18]**.

The results of this study align with previous studies, which stated that the administration of steeped Robusta coffee (*Coffea canephora*) could reduce blood glucose levels and increase insulin levels so that the HOMA-IR index could be improved, carried out on Wistar rats with metabolic syndrome **[6]**. Another study also showed that the administration of an ethanolic extract from green coffee beans (*Coffea canephora*) provided intense inhibitory activity against the α-glucosidase enzyme to suppress the conversion rate of carbohydrates into glucose and prevent pancreatic β-cells damage **[6]**, **[19]**.

The Effect of Giving Kawa Daun (*Coffea canephora*) Decoction on Pancreatic β-cells Regeneration

Histological pancreatic β-cells can be determined by assessing the percentage of damage to the cells in the pancreas. The comparison of normal cells and damaged cells can be seen in Figure 1, as the comparison of the percentage of damage in groups P1 and P2. In Figure 1. A (K-), pancreatic Langerhans islet (L) had clear boundaries, no edema in the cells, especially in β-cells morphology, clear connective tissue capsule in Langerhans (yellow arrow), and no cell necrosis. Meanwhile, it is inversely proportional to Figure 1. B (K+), where there were signs of pancreatic β-cells damage due to alloxan induction resulting in cell swelling or edema of the Langerhans islet (yellow arrow) and intralobular duct (O), the degranulation of β-cells in Langerhans, the formation of steatoses (S), the destruction of pancreatic acini cells (Ac), and even tissue necrosis was seen. From the microscopic observation results, it was also known that there was extensive damage in the positive control group by 27.1%.

Figure 1. C depicts the changes in pancreatic β-cells after intervention with *Kawa Daun (Coffea canephora)*. It illustrates a repair process in pancreatic β-cells, and a reduction in cell edema and hydrophobic degeneration could also be seen. Pancreatic tissues showed vacuolation and decreased vasculature with cellular infiltration with cells as lymphocytes and mononuclear cells with focal areas of degeneration and necrosis area (LyM). It indicates the recovery process of tissue regeneration and repair. The microscopic feature reveals that the extent of damage ranged from 22.4%, reduced compared to the positive control group.

On the other hand, in Figure 1.D, the damaged area was reduced to 14.9%. These results were not significantly different from the low-dose intervention group. Furthermore, the histopathological feature indicates improvements feature of the Langerhans islet region, and the tissue shows normal pancreatic acini (NA). It is consistent with the absence of a significant difference in blood glucose levels between the low-dose (P1) and high-dose (P2) treatment groups.

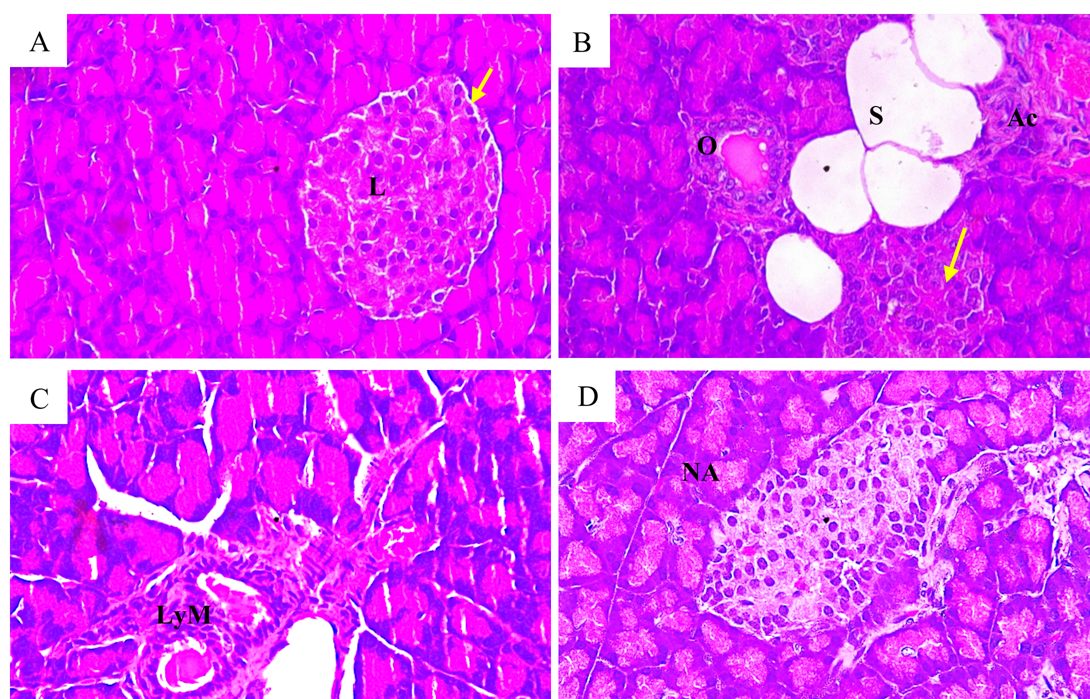

Figure 1 Illustration of pancreatic tissue preparations in x40 magnification: (A) negative control of DM, (B) positive control of DM with alloxan induction, (C) intervention dosage of 3.6 ml/200 g BW/day, (D) intervention dosage of 7.2 ml/200 g BW/day.

Several research results have demonstrated tissue repair after the administration of *Coffea canephora* in some tissues that have undergone degeneration and necrosis. Anti-inflammatory and analgesic properties in *Coffea canephora* reduce the occurrence of edema in tissues previously induced by formalin and modulate pain reduction **[5]**. The results of Handayani **[20]** studies also disclosed an improvement in liver and kidney tissue through an increase in the mean value of ALT (alanine transaminase) and AST (aspartate transaminase) enzymes in liver tissue and an increase in plasma urea levels, thus triggering the kidneys to excrete urea more quickly after *Coffea canephora* intervention, and we believe this effect positively related to the renewal of pancreatic islet cells. Another mechanism thought to improve the condition of pancreatic β-cells is the mechanism of activity of the enzyme α- glucosidase, which is deemed a molecular target in diabetes therapy. This enzyme extracted from *Coffea canephora* has solid inhibitory and high antioxidant activities that suppress insulin resistance, pancreatic cell damage, and impaired tissue glucose utilization. This enzymatic reaction also allows for an improvement in the function of the pancreas of Langerhans, which has been diagnosed with diabetes mellitus **[19]**.

Reduced function of β-cells in diabetes leads to two types of pathogenic conditions caused by immune-mediated diabetes condition (T1DM) and metabolic mechanisms diabetes disorder (T2DM). Some studies analyzed the Langerhans islet morphology and the β-cell gene expression in both T1DM and T2DM, which revealed the genes at the β-cell level and the endoplasmic reticulum stress signal contributes to β-cell failure in T1DM (mostly IRE1 driven) and T2DM (mostly PERK–eIF2α dependent) **[21]**. Another study approved that not only affects pancreatic islet damage but contributes to other vital organ degeneration, such as kidney and hepatic lobes. Primal and Ahriyasna's (2022) findings had approved that the diabetes condition contributed to renal failure and necrosis problems. The study improved the quality of renal nephrons after the high-antioxidant compound from Indonesian traditional leaves extraction. Its high flavonoid level contributes to the regeneration of the nephrotic structure by decreasing the inflammatory effect on the tissue **[22]**. We believe that the internal stress of the β-cell organelle could positively contribute to the cell's normal regulation as an insulin producer. The glomerular infiltration and hypertrophy increase significantly in the diabetic disorder, which persistently generates nephrotic site functional failure. This glomerular disturbance will lead to the expansion of the mesangium and accumulation of the extracellular matrix. Furthermore, it can lead to a loss of podocytes, disruption of the

mesangium (mesangiolysis), and glomerular fibrosis. This renal failure condition proceeded by chronic cardiac hypertension accelerated from the arterial atherosclerosis of the coronary artery **[23]**.

Deliberating the antioxidant composition in *Coffea canephora*, it has been approved that flavonoid compound has a parallel effect on the repairing process of the pancreatic islet. Accordingly, β-cells mass and function are important structures to be primarily affected by diabetes. Growing argumentation evidence supports the efficacy of flavonoids for preventing and attenuating diabetes consequences. In T2DM, the metabolic disruption leads to sustained chronic hyperglycemia, hyperlipidemia, and inflammatory cytokines elevation. These trigger the metabolic pancreatic gland to experience endoplasmic reticulum (ER) stress, lysosomal destabilization, and oxidative stress. The cell injury in the Langerhans islet structure immediately stimulates cell death through apoptosis, autophagy, or necroptosis activities. The disruption of pancreatic islet should be prevented to preserve this organ's physiological function, which could be protected from the flavonoid's antioxidant and anti-imflammatory effects **[21]**, **[24]**. Based on the diabetic rats observed, pancreatic steatosis may be one of the phenotypes of metabolic syndrome, which is characterized by obesity with visceral fat accumulation, diabetic conditions, hyperlipidemia, and hypertension **[25]**. It can be stated that steatosis is induced by exhibiting severe abnormalities in the disposal of hepatic triglycerides (hepatocellular lipids, HCLs) and impaired insulin action in the pancreas.

The pancreatic Langerhans cells death stimuli, such as TNF-α (TNFR-1 receptor) and Fas ligand (Fas-L) in the cell receptors superficies, trigger a series of intracellular signals which activate caspases-3 and -7. These caspases cleavage the poly (ADP-ribose) polymerase, lamin, and XK-related protein 8 substrates, contributing to the apoptotic process in the β-cells system as nuclear condensation, membrane blebbing, even the DNA-fragmentation. TNFR-1 expression will start the necroptosis from the lysosomal membrane permeation and the ROS pathways by promoting the necrosome formation that leads to autophagy. Moreover, the apoptotic pathways of pancreatic β-cells could be reinforced over the ER stress and stimulate the inflammatory and immunity mediators such as nitric oxide (NO), and human leukocyte antigen. In this circumstances, flavonoid's antioxidant function could reduce the pancreatic islet cells' apoptotic and autophagy process. Another research report revealed the protective effects of flavonoid compounds in some tropic plant extraction against diabetes β-cells degeneration. The primary molecular mechanisms by which flavonoids protect β-cells survival are the suppression of oxidative stress and subsequent inhibition of the caspase cascade and DNA damage **[24].**

These β-cells protection pathways, especially from the flavonoid effect, increase the cells' antioxidant capacity, inhibiting the cells' ROS accumulation and lipid peroxidation and protecting the cells' death. Flavonoids are believed to preserve the survival of β-cells by inhibiting pro-apoptotic expressions and downregulating the anti-apoptotic genes. Similarly, oxidative stress also results in insulin resistance in the pancreas, liver, and muscles by increasing Diasyl Glycerol (DAG) and Protein Kinase-C (PKC). It leads to the disruption of phosphorylation in the Insulin Receptor Substrate (IRS-1 and IRS-2), indicating those organs' insulin resistance. Moreover, an increasing amount of fatty acids circulating in vessels is generated due to oxidative stress, which positively will produce large amounts of Nitric Oxide (NO). This overproduction of NO presence may induce endoplasmic reticulum (ER) stress by reducing the level of Ca^{2+} in ER; later on, it is toxic to pancreatic β-cells and finally results in these cells' death **[24]**, **[26]**. Eventually, the flavonoid may interfere with the inflammation signalling cascades and positively prevent NO overproduction and its deleterious consequences in shock and ischemia-reperfusion of tissue injury **[27]**.

In recent reviews, flavonoids stated to have the capacity to enhance glucose-stimulated insulin release and counteract the cytokine-induced dysfunction of pancreatic β-cells. Cytokines are released by the inflammatory cells around β-cells and generate the inducible nitric oxide synthase (iNOS) expression and overproduction of NO, which are leading causes of cell damage **[24]**. We highly approve that the improvement of pancreatic islet repair based on the histopathological features is affected by the previous pathophysiological explanation we stated. The presence of lymphatic and macrophage cells indicates the expression of leukocyte antigen, and the regeneration characteristics indicate the protection of the pancreatic Langerhans islet cells colony. Decreasing neutrophil numbers and preceding autoreactive T-cell accumulation in blood streams are in pre-symptomatic stages of type-1 diabetes and are associated with worsening the pancreatic β-cells function **[27]**, **[28]**.

The histopathological study on Tamarix articulata (FRETA) extract with flavonoid-rich compounds has a potential effect on antidiabetic and antihyperlipidemic circumstances. It has been tested that the experimentally induced diabetes animal had improved the blood lipid profile and histopathological changes in both the liver and pancreas and rectified the Oral Glucose Tolerance Test (OGTT) **[29]**. We acknowldge that the high percentage of antioxidants in flavonoids contributes to the protection of the pancreatic Langerhans islet. It simultaneously repairs the β-cells injury in the induced-diabetic pancreatic organs. By enhancing the expression of some anti-inflammatory proteins and stimulating the presence of leukocyte cells as tissue protection, the antioxidant chains can produce more free radicals, which immediately inhibit excess oxidation leading to tissue damage. Mirmalek

et al. **[30]** indicated that antioxidant contained proteins ameliorate the pancreatic repair of the islet injury. This indicates that the antioxidant as an anti-inflammatory compound has the potential in therapeutic role in pancreatic regeneration resulting in the decreasing percentage of lower intestinal edema, lowering the infiltration of inflammatory cells, and alleviating acinar cell necrosis.

CONCLUSION

Giving *Kawa Daun (Coffea canephora)* decoction could improve diabetes mellitus by lowering blood glucose levels by repairing pancreatic β-cells for 14 days. In addition, there was a significantly improved the blood glucose level in the intervention group with 3.6 mL/200g/day and 7.2 mL/200g/day dose in the positive diabetic group (K+). The histopathological feature reveals that the extent of damaged cells has an effective improvement in both experimental groups, respectively reduced compared to the positive control group. Moreover, the qualitative analysis indicates the improvements in features of the Langerhans islet region and the tissue of the pancreatic acini. Anti-inflammatory and analgesic properties in *Coffea canephora* proved to reduce the occurrence of edema in injured tissues of the pancreatic organ, even for other organs such as hepatic lobes, renal, peripheral innervation, and lymphatic function. Furthermore, flavonoids' high composition in Coffea canephora protects and ameliorates β-cells survival by suppressing oxidative stress and subsequently inhibiting the caspase cascade and DNA damage in diabetic rats. It has a positive consistency without significantly changing blood glucose levels between the low-dose (P1) and high-dose (P2) treatment groups. It suggests that low doses of using *Kawa Daun* have improved the condition of diabetes mellitus equivalent to high doses and can be used for further biochemical analysis, pharmacological developments, and optional nutritional substitution in food and medico-herbal therapy.

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Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

The use of animals in this research was approved by the Ethics Committee of the Universitas Perintis Indonesia, Padang following the legislation of the ethicla clearence No. 083.1/KEPK.F2/ETIK/2022, KEPK Universitas Perintis Indonesia, Indonesia.

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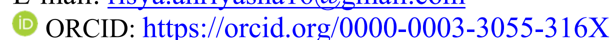
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