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Original Article

Insulin expression and insulitis degree of diabetic rats after giving sikkam leaves (*Bischofia javanica* Blume)

[Expresión de insulina y grado de insulitis de ratas diabéticas después de administrar hojas de sikkam (*Bischofia javanica* Blume)]

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Abstract

Context: Gallic acid and quercetin in sikkam leaves (*Bischofia javanica)* have scientific potential as antidiabetic agents. Quercetin suppresses hyperglycemia by inhibiting active glucose transport. Meanwhile, gallic acid acts as an antidiabetic is closely related to its antioxidant properties. Antioxidant compounds neutralize cells that experience oxidative stress by donating their hydrogen atoms.

Aims: To analyze the degree of insulitis and insulin expression in the pancreatic cells of rats induced diabetes mellitus (DM) after giving *B. javanica* leaves extract.

Methods: The treatment groups consisted of G0: negative control (-); G1: positive control (alloxan induction + standard feed); G2: alloxan induction + 300 mg/kg BW of *B. javanica* leaves ethanol extract; G3: alloxan induction + 600 mg/kg BW of *B. javanica* leaves ethanol extract; G4: alloxan induction + ethanol extract of *B. javanica* leaves 900 mg/kg BW and G5: alloxan induction + glibenclamide 4.5 mg/kg BW. At day 28, the rats were sacrificed, and the pancreatic tissue dissected. This was analyzed for degree of insulitis and insulin expression by anti-insulin antibodies using immunohistochemistry and hematoxylin-eosin.

Results: There was a significant difference (p = 0.000) in insulin expression and insulitis degree. By the increase of *B. javanica* leaves dose, the insulin expression value also increased, and the degree of insulitis in Langerhans' islets of DM rats was decreased. Islets of Langerhans in insulin production returned to normal after being given *B. javanica* ethanol extract 900 mg/kg BW like glibenclamide.

Conclusions: *Bischofia javanica* ethanol extract increased insulin production and reduced the degree of insulitis in the islets of Langerhans histology. Further research is needed to determine the extent to which this extract protects from the sequelae of diabetes.

Keywords: hypoglycemic agent; hyperglycemia; immunohistochemistry; insulin expression; insulitis; plant extract.

Resumen

Contexto: El ácido gálico y la quercetina en las hojas de sikkam (*Bischofia javanica*) tienen potencial científico como agentes antidiabéticos. La quercetina suprime la hiperglucemia al inhibir el transporte activo de glucosa. Mientras tanto, el ácido gálico actúa como antidiabético y está muy relacionado con sus propiedades antioxidantes. Los compuestos antioxidantes neutralizan las células que experimentan estrés oxidativo al donar sus átomos de hidrógeno.

Objetivos: Analizar el grado de insulitis y expresión de insulina en las células pancreáticas de ratas inducidas por diabetes mellitus (DM) tras la administración de extracto de hojas de *B. javanica*.

Métodos: Los grupos de tratamiento estuvieron constituidos por G0: control negativo (-); G1: control positivo (inducción de aloxano + alimentación estándar); G2: inducción de aloxano + 300 mg/kg de peso corporal de extracto de etanol de hojas de *B. javanica*; G3: inducción de aloxano + 600 mg/kg de peso corporal de extracto de etanol de hojas de *B. javanica*; G4: inducción de aloxano + extracto etanólico de *B. javanica* hojas 900 mg/kg de peso corporal y G5: inducción de aloxano + glibenclamida 4,5 mg/kg de peso corporal. En el día 28, las ratas fueron sacrificadas y el tejido pancreático disecado. Este se analizó para determinar el grado de insulitis y expresión de insulina mediante anticuerpos antiinsulina usando inmunohistoquímica y hematoxilina-eosina.

Resultados: Hubo una diferencia significativa (p = 0,000) en la expresión de insulina y el grado de insulitis. Al aumentar la dosis de hojas de *B. javanica*, aumentó el valor de expresión de insulina y disminuyó el grado de insulitis en el islotes de Langerhans de ratas con DM. Los islotes de Langerhans en la producción de insulina volvieron a la normalidad después de recibir el extracto etanólico de *B. javanica* 900 mg/kg de peso corporal al igual que glibenclamida.

Conclusiones: El extracto de etanol de *Bischofia javanica* aumentó la producción de insulina y redujo el grado de insulitis en la histología de los islotes de Langerhans. Se necesitan más investigaciones para determinar hasta qué punto este extracto protege de las secuelas de la diabetes.

Palabras Clave: agente hipoglucemiante; expresión de insulina; extracto de planta; hiperglucemia; inmunohistoquímica; insulitis.

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INTRODUCTION

The use of plants as an antidiabetic agent has become a trend in research on diabetes management over the last few decades. The treatment using plant extracts is considered safer, and relatively inexpensive. Several antidiabetic plants have been tested in a laboratory to improve the structure of the pancreas, even the level of β cells insulin factories (Salehi et al., 2019). According to Mai (2017), one of the plants with the potential as an antidiabetic agent based on its chemical content is sikkam (*Bischofia javanica* Blume, *Phyllanthaceae*). This plant is found widely in Vietnam, India, China, Indonesia, and the Philippines (Chi, 2012). *B. javanica* is reported to be rich in flavonoids and phenolic compounds such as quercetin and gallic acid, which have high antioxidant activity (Cambie, 1984; Gupta 1988; Whistler 1992; Ihwan et al., 2018). *B. javanica* has prospective nutraceutical and therapeutic properties. Molecular docking of the compounds identified in this plant demonstrating a strong binding affinity to experimental target receptors that could be potential candidates in the food and pharmaceutical industries (Chowdhury et al., 2020). *B. javanica* leaves of ethanol extract contain many secondary metabolites, and some of them are quercetin and gallic acid, which can use as antidiabetic (Sutharson et al., 2009; Kituyi et al., 2018). *B. javanica* bark has traditionally been used by the Pakpak and Simalungun people in North Sumatra (Indonesia) as an ingredient for the treatment of various types of diseases, including diabetes (Silalahi et al., 2015; 2018). Apart from Indonesia, in India, this plant's bark has been proven in a laboratory to reduce blood sugar levels in the rat (Majeed, 2019).

Diabetes mellitus is a glucose metabolism disorder that occurs because pancreatic β cells do not produce enough insulin or when the body cannot effectively use insulin (Bilous and Donelly, 2014). According to the American Diabetes Association (2014), the number of cases and prevalence of diabetes has continued to increase over the past few decades and will continue if not treated seriously. The condition of hyperglycemic diabetics will be involved in the formation of free radicals. It causes glucose autoxidation, protein glycation, and activation of polyol metabolic pathways, which will further accelerate reactive oxygen formation compounds or reactive oxygen species (ROS) (Fiorentino et al., 2013). This phenomenon occurs in all body cells, including pancreatic β cells as insulin factories (Raciti et al., 2010).

According to Cerf (2013) and Aronson (2008), oxidative stress can be neutralized by antioxidant compounds such as flavonoids and phenolics by donating their hydrogen atoms. Quercetin has shown to prevent damage to pancreatic β cells due to oxidative stress. This flavonol helps improve insulin secretion, increases adiponectin circulation, and inhibits glucosidase activity in the small intestine, and increases GLUT4 transporters in skeletal muscle (Zhang et al., 2011; Arias et al., 2014). Meanwhile, gallic acid provides an antidiabetic effect by increasing glucose for energy and increasing insulin sensitivity (Doan et al., 2015). Based on a literature search, it has been proven that *B. javanica* has an antidiabetic effect in rats tested through parameters of blood sugar levels, body weight, and islets of Langerhans morphometry. Still, no reports have been found about the effect of *B. javanica* leaves extract on the degree of insulitis and insulin expression in β cells. Therefore, this study will examine the effect of the ethanol extract of *B. javanica* leaves on the degree of insulitis and insulin expression in β cells of diabetic rats.

MATERIAL AND METHODS

Preparation of *B. javanica* leaves

B. javanica leaves were collected from Simalungun Regency, Indonesia (2°59′17.5′′N 98°52′ 29.3′′E). The voucher was identified and authenticated by Dr. Nursahara Pasaribu, M.Sc and deposited into the Medanense Botanical Herbarium (Registration number 5395/MEDA/ 2020), at Universitas Sumatera Utara, Medan, Indonesia.

Extraction was carried out using the maceration method with 96% ethanol as solvent. Fresh *B. ja-*

vanica leaves (10 kg) were dried. Then, from 270 g of dry leaves, the ethanol extract of *B. javanica* leaves was obtained. The drying process was based on the *Zanthoxylum acanthopodium* drying procedure by Situmorang et al. (2019a; 2019b; 2020) so that the phytochemical content in this plant was not lost due to heat.

The plant material of *B. javanica* leaves was soaked in a large glass jar, and 1000 mL of 96% ethanol solvent was added. The glass jar was closed tightly, and the top of the pot was covered with aluminum foil and left for three days protected from sunlight, and the soak was stirred every day. After three days, the marinade was filtered and collected in a different bottle. Then, the filtering results were evaporated with an electric heater. After thickened, it was put into a steam cup that wrapped in aluminum foil for one night, then evaporated again until the extract was thick. The leaves of *B. javanica* were transferred to sample bottles for storage.

Furthermore, the plant material waste was macerated again by adding 96% ethanol. The plant material was carried out for three macerations and obtained 100 g of the ethanol extract of *B. javanica* leaves.

Animal handling

Thirty male albino rats of Wistar strain weighing 120-180 g were housed in neat, well-ventilated propylene cages and kept under standard laboratory conditions (light/dark cycle 12 hours, 24°C). The animals were obtained from the Faculty of Pharmacy, University of North Sumatra. They were allowed to adjust for two weeks before the experiment began. Rats were divided into six groups, with five animals in each treatment group. Feeding in the form of milled corn or pellets and giving drinking water *ad libitum*. Alloxan induction was performed by intraperitoneal injection. Before induced, alloxan was dissolved using 0.9% NaCl. Alloxan injection was carried out at a dose of 160 mg/kg BW. Rats induced with DM were used as the treatment group. All experiments and protocols described in the present study were approved by the Animal Research Ethics Committees/AREC with approval number 00703/KEPH-FMIPA/2020. The experimental procedures and animal care were performed by the "Guide for the care and use of laboratory animals" and "Committee for the purpose of control and supervision on experimental animals" (CPCSEA) to minimize pain and discomfort.

Study design

This research was conducted using a non-factorial Completely Randomized Design (CRD). The treatment group consisted of G0: negative control (-), G1: positive control (alloxan induction + standard feed); G2: alloxan induction + 300 mg/kg BW of *B. javanica* leaves ethanol extract; G3: alloxan induction + 600 mg/kg BW of *B. javanica* leaves ethanol extract; G4: induction of alloxan + ethanol extract of *B. javanica* leaves 900 mg/kg BW; and G6: Induction of alloxan + glibenclamide 4.5 mg/kg BW. After the rats experienced hyperglycemia due to alloxan injection, the rats were given ethanol extract of *B. javanica* leaves for 28 days. The dose of *B. Javanica* leaves ethanol extract was given based on the dose conversion results used by Ihwan et al. (2018) in mice. According to their research on the toxicity test for leaves extract of this plant, the safe dosage for mice ranges from 30 mg/30 g BW - 60 mg/30 g BW. This study was modified with an initial dose of 13 mg/30g BW of mice and converted to rat per kg based on the dose conversion table by Laurence and Bacharach (1964). The dose of mice for 30 g BW = 13 mg, so the dose for 20 g BW of mice = (20:30) × 13 mg = 8.6 mg. Convert dose of 20 g mice to rat 200g = 7.0. The dosage for 200 g rat was 8.6 mg × 7.0 = 60.2 mg. Then the dose for mice per kg was = (1000: 200) × 60.2 = 301 mg, to 300 mg. For the subsequent dose treatment had an increase of 300 mg, to obtain a series of induction B. *javanica* leaves ethanol extract treatment doses of 300, 600, 900 mg/kg BW.

Immunohistochemistry of insulin expression

Pieces of pancreatic tissue that were fixed on the slide were heated for 2 h at 60°C by a hot plate Thermo scientific brand. Deparaffination and rehydration of the pancreatic fragments using xylol

I, II and III 70, 80, 90% and absolute ethanol I, II, and III, 90, 80, 70% ethanol were used. These preparations were washed with phosphate buffer solution (PBS), blocking endogenous peroxidase with 3% H_2O_2 solution in distilled water for 30 minutes, then washed with PBS solution three times for 5 minutes each. Next, the ultra-V block was carried out for 5 minutes with 1:20 dilution of normal goat serum in PBS 1× and the rat anti-insulin primary antibody was dropped (1: 300) for one night in the refrigerator, then washed with PBS solution. The secondary antibody used was biotinylated goat anti-polyvalent for 10 minutes, then washed with PBS solution. The streptavidin peroxidase that was diluted to the concentrations 12.5 ng/mL conjugate enzyme was added and rewashed with PBS solution. Substrate and chromogen 3,3'-diaminobenzidine (DAB) 500 nm/mL were added, then incubated for 10 minutes at room temperature in the dark. The final step was washed with distilled water, counterstain with hematoxylin 5% for 10 seconds, washed with running water and distilled water. Then, dehydrated with stratified ethanol I, II, and III, 90% ethanol, 80% ethanol, 70%, clearing with xylol I, II, and III 70, 80, 90% and permanent mounting with Canadian balm (2-4 drops) (Lab Vision, Cat # MS-1378-PO). Cells count pancreatic beta was performed per visual field on 400× magnification with optical microscopy Olympus CX33, Tokyo, Japan. Observation of coloring immunohistochemistry was calculating the average number of pancreatic beta cells by manual cell counting method. Two technicians independently performed manual counts of the obtained images, which were then processed in ImageJ software (Abramoff et al., 2004). Counts were collated with the autocount results blindly. Previous manual counting, images were cropped, scaled to µm, separated by color channels, and artifacts transferred back. An area tool was used to calculate the cells in the area. Cell numbers were expressed as counts/µm^2. The ImageJ cell counter tool recorded mouse clicks on cells that were labeled with green arrows. The results were saved to a spreadsheet, and screenshots were used to record the session.

Hematoxylin-eosin

Deparaffinization of dry pancreatic tissue was carried out in xylol I (70%), II (80%), III (90%) three times (each for 10-15 minutes). This tissue was added to 96% alcohol twice (for 5 minutes each). Then washed with running water until the alcohol was gone. Put it in 5% hematoxylin paint for 7-10 minutes. Washed with running water until it did not flow. Dip it in HCl 0.6% two times for decolorization. Washed again with running water. Soak in water for a while until it turns blue. Put it in the eosin paint for 3-5 minutes. Washed with running water. Put it in the alcohol solution I (washed it off with running water, press the preparation with paper, wipe it with a cotton ball, and then put it in the xylol I (70%), II (80%), III (90%) three times (each for 10-15 minutes). Pressed the trial again with writing, wiped with cotton, then did the mounting. This staining method was needed to identify the insulitis degree classification (inflammatory cell infiltration) in islets of Langerhans and analyzed by optical microscopy Olympus CX33, Tokyo, Japan. The analysis of the islets was carried out according to the criteria established by Signore et al. (1989) and adapted by Ventura-Oliveira et al. (2002), where grade 0 is characterized by absence of inflammatory cell infiltrate, grade 1 (low) was less than 25%, grade 2 (medium) when the islet had a cell infiltration between 25 and 80%, grade 3 represents higher than 80% and less than 100%, while islets, completely overtaken by cellular infiltration, were classified as islets grade 4 (in this research, grade 0 and 4 did not appear). The inflammatory cell was counted by the manual cell counting method. The ImageJ cell counter tool recorded mouse clicks on cells that were labeled with uncolored dots. The results were saved to a spreadsheet, and screenshots were used to record the session.

Statistical analysis

The data were expressed as mean ± SD and analyzed by analysis of variance (ANOVA) followed by the LSD (Least Significance Difference) test in the SPSS 25 program at the 5% significance level.

RESULTS

Insulin expression in islets of Langerhans beta cells after given *B. javanica* leaves

There was a significant difference ($p<0.05$) in the analysis of insulin expression in Langerhans' beta cells after given the extract of *B. javanica* leaves. The DM group that was given standard feed (G1) had the lowest average number of beta cells that express insulin, followed by the DM group given ethanol extract of *B. javanica* leaves at a dose of 300 mg/kg BW (G2). The highest average number of beta cells that express insulin was in the DM group given ethanol extract of *B. javanica* leaves at a dose of 900 mg/kg BW (G4) and a group that given glibenclamide 4.5 mg/kg BW (G5) and the control group (G0) in Table 1.

The Allred score was given by adding up the A score in the form of a proportion score (the number of beta cells that were immunoreactive against antibodies, brown) and a B score in an intensity score (color strength) (Fedchenko and Reifenrath, 2014). The measurement results are presented in Fig. 1. The Allred score's highest score was G4, while the lowest was G2 and G5 (glibenclamide 4.5 mg/kg BW).

The islets of Langerhans microscopic images of Wistar rats showed this region of the pancreas that contain insulin-secreting beta cells displayed with a brown color (Fig. 2). Islets of Langerhans, which have a decreased number of beta cells, were indicated by cavity formation. In the G1 and G2 groups, large cavity formations were found due to the reduced number of beta cells in large numbers. Groups G4 and G5 also showed a lot of cavity formation but followed by the number of beta cells that expressed brown color. Cavity formation can disappear as the beta cells regenerate. The use of stratified doses proved that insulin expression in pancreatic beta cells increased in line with the increasing amount of the extract.

Insulitis degree in islets of Langerhans beta cells after given *B. javanica* leaves

There was a significant difference ($p<0.05$) in the analysis of insulitis degree of islets of Langerhans after given *B. javanica* leaves (Table 2). The degree of insulitis 0 (average) was not found in any group. Even in the control group (G0), there were mononuclear inflammatory cells (lymphocytes), although the number was below 15 and grade 1 (low). Apart from the negative control group, stage 1 was also found in the G4 that given ethanol extract of *B. javanica* leaves at a dose of 900 mg/kg BW. The highest degree of insulitis 2 (medium) was in the G3 that gave ethanol extract of *B. javanica* leaves at a 600 mg/kg BW dose. The highest degree of insulitis 3 (high) was in G1. End-stage level islet (degree 4) was not found in any group in this experiment (Fig. 3). Degree of insulitis in hematoxylin-eosin stain in the form of mononuclear inflammatory cells (lymphocytes) around and in the islets of Langerhans pancreas. Microscopic image of the insulitis degree presented in Fig. 4.

DISCUSSION

The differences in insulin expression and inflammation in islets of Langerhans between the healthy and the DM groups were due to alloxan induction. The DM group had few beta cells expressing insulin and a high number of infiltrated lymphocytes. An increase in the number of beta cells expressing insulin and a decrease in the number of infiltrating lymphocytes in the group given the ethanol extract of *B. javanica* leaves at a dose of 900 mg/kg BW proved that this plant has an antihyperglycemic effect.

B. javanica leaves ethanol extract contains gallic acid and quercetin (Gupta, 1988; An 2009; Mai, 2017), which are part of phenols and flavonoids. Gallic acid and quercetin are thought to synergize in supporting antioxidant activity by increasing cellular antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (Martinez et al., 2007). The antioxidant plays a role in preventing DNA damage to pancreatic beta cells caused by DNA alkylation by alloxan (Herra and Mulja, 2005). This extract's antioxidant effect also prevents damage to pancreatic β cells by inhibiting oxidative stress through the radical scavenging mechanism or radical scavenging.

Table 1. Statistical analysis of insulin expression data in beta cells.

Group	n	Mean rank ± SD	ANOVA	LSD
G0	6	33 ± 6.29[a]		a
G1	6	12 ± 5.02[a]		a
G2	6	13 ± 4.69[b]	0.000	b
G3	6	40 ± 9.00[b]		b
G4	6	67 ± 12.64[bc]		bc
G5	6	58 ± 15.45[bc]		bc

G0: Control; G1: DM + standard feed; G2: DM + 300 mg/kg BW of *B. javanica* ethanol extract; G3: DM + 600 mg/kg BW of *B. javanica* ethanol extract; G4: DM + *B. javanica* ethanol extract 900 mg/kg BW; and G5: DM + glibenclamide 4.5 mg/kg BW (a,b,bc = significance different notation $p<0.05$). G4 was significantly different from G1 as a positive control, and G0 as a negative control. It was mean that G4 had an improvisation in expression of beta cells.

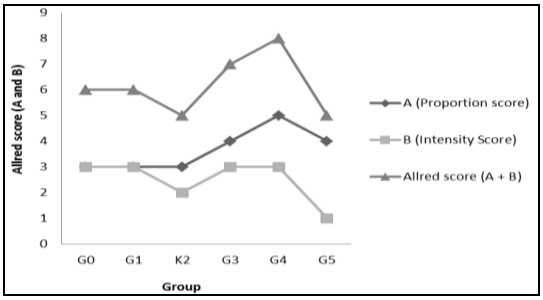

Figure 1. Allred score measurement results.
Data represent mean ± SD (n = 6). G0: Control; G1: DM + standard feed; G2: DM + 300 mg/kg BW of *B. javanica* ethanol extract; G3: DM + 600 mg/kg BW of *B. javanica* ethanol extract; G4: DM + *B. javanica* ethanol extract 900 mg/kg BW; and G6: DM + glibenclamide 4.5 mg/kg BW.

Table 2. Statistical analysis on insulitis degree in islets of Langerhans beta cells.

Group	n	Mean rank ± SD	ANOVA	LSD
G0	6	12 ± 6.29[a]		a
G1	6	69 ± 12.64[a]		a
G2	6	59 ± 15.45[b]	0.000	b
G3	6	43 ± 9.00[b]		b
G4	6	11 ± 5.02[bc]		bc
G5	6	37 ± 6.49[bc]		bc

G0: Control; G1: DM + standard feed; G2: DM + 300 mg/kg BW of *B. javanica* ethanol extract; G3: DM + 600 mg/kg BW of *B. javanica* ethanol extract; G4: DM + *B. javanica* ethanol extract 900 mg/kg BW; and G5: DM + glibenclamide 4.5 mg/kg BW (a,b,bc = significance different notation $p<0.05$). G4 was significantly different from G1 as a positive control, and G0 as a negative control. It was mean that G4 had an improvisation on insulitis degree.

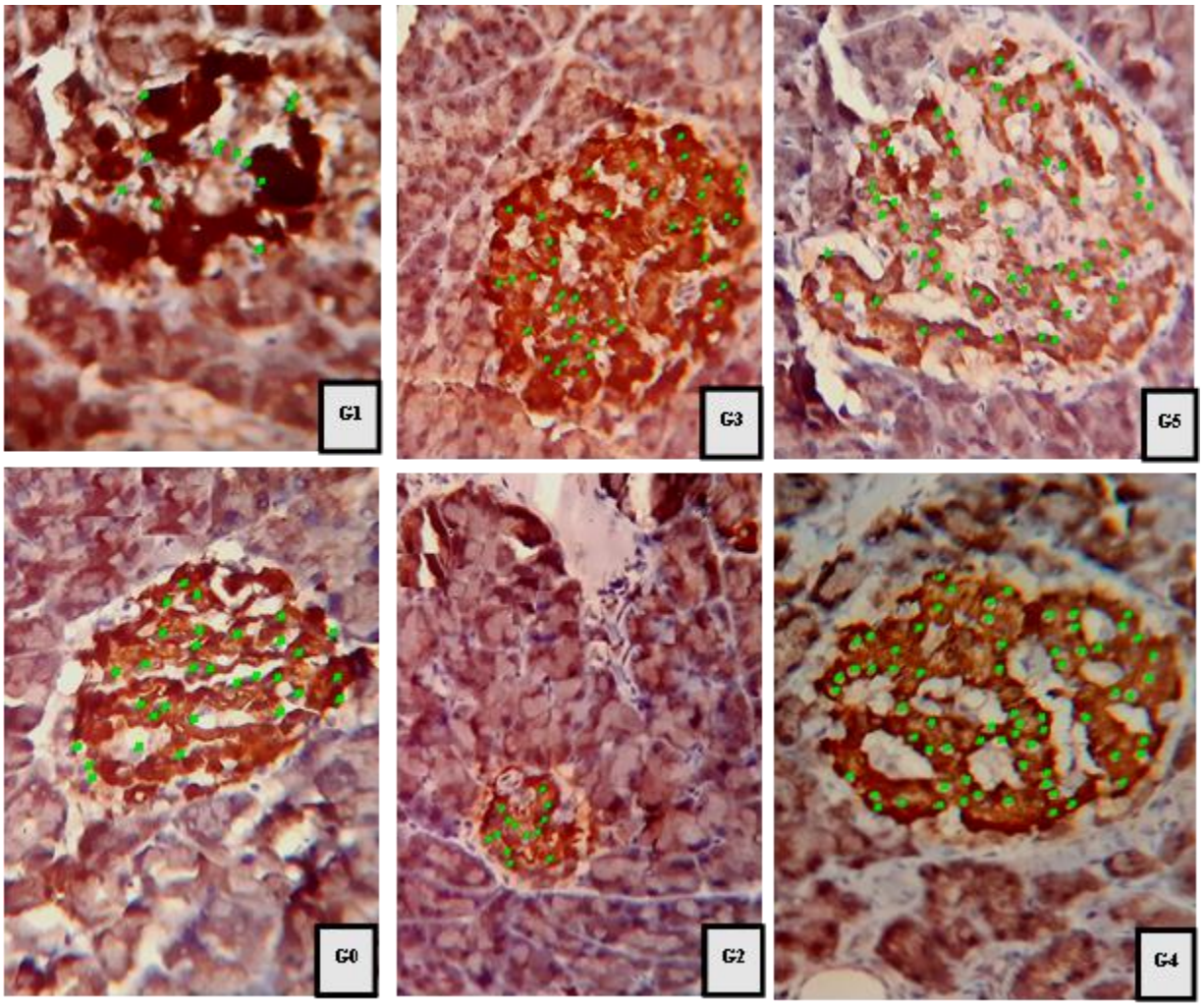

Figure 2. Insulin expression in islets of Langerhans beta cells in each group.

G0: Control; G1: DM + standard feed; G2: DM + 300 mg/kg BW of *B. javanica* ethanol extract; G3: DM + 600 mg/kg BW of *B. javanica* ethanol extract; G4: DM + *B. javanica* ethanol extract 900 mg/kg BW; and G6: DM + glibenclamide 0.5 mg/kg BW. Green arrow = beta cells that express insulin.

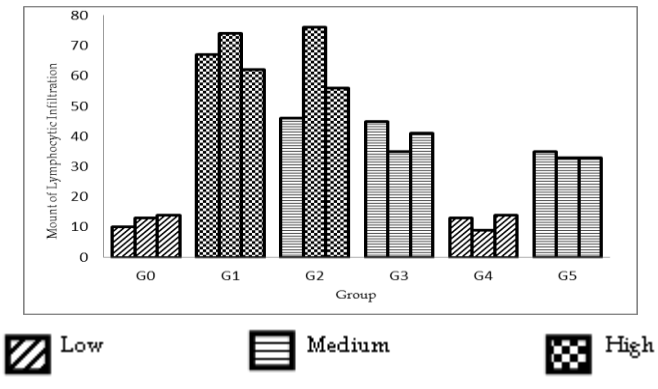

Figure 3. Degree of insulitis per group.

G0: Control; G1: DM + standard feed; G2: DM + 300 mg/kg BW of *B. javanica* ethanol extract; G3: DM + 600 mg/kg BW of *B. javanica* ethanol extract; G4: DM + *B. javanica* ethanol extract 900 mg/kg BW; and G6: DM + glibenclamide 4.5 mg/kg BW. The three bars are representative of each group to show the category "degree of insulitis" (low, medium, high).

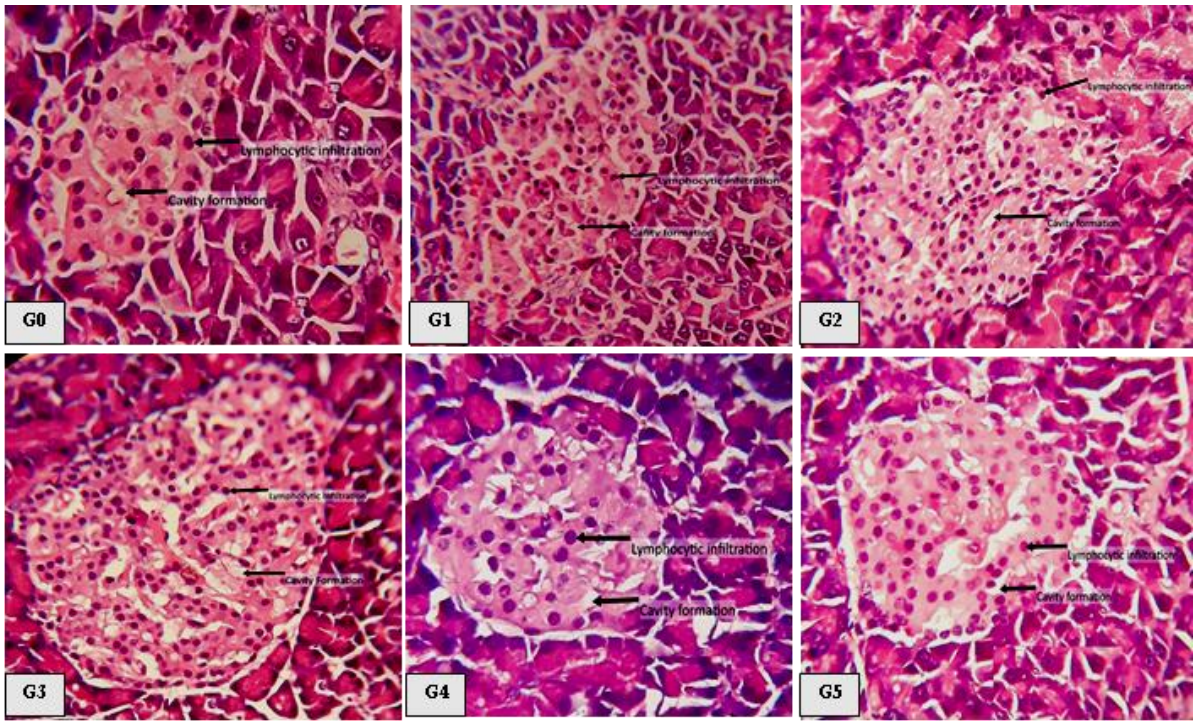

Figure 3. Microscopic view of insulitis degree in islets of Langerhans beta cells in each group.

G0: Control; G1: DM + standard feed; G2: DM + 300 mg/kg BW of *B. javanica* ethanol extract; G3: DM + 600 mg/kg BW of *B. javanica* ethanol extract; G4: DM + *B. javanica* ethanol extract 900 mg/kg BW; and G6: DM + glibenclamide 0.5 mg/kg BW.

This mechanism consists of donating one electron to an unpaired electron in free radicals to reduce the number of free radicals (Fiorentino, 2013). *In vitro*, flavonoids are potent inhibitors of lipid peroxidation, as traps for reactive oxygen or nitrogen species, and can also inhibit the activity of lipoxygenase and cyclooxygenase enzymes (Herra and Mulja, 2005). Flavonoids are also able to stimulate an increase in insulin release from pancreatic beta cells. This action occurs by regulating peroxisome proliferator-activated receptors (PPAR-α and PPAR-γ) (Gerritson et al., 1995). The beneficial action of flavonoids in diabetes is their ability to avoid glucose absorption or improve glucose tolerance. Furthermore, flavonoids stimulate glucose uptake in peripheral tissues, regulate the activity and expression of enzymes involved in carbohydrate metabolism pathways and act like insulin (insulin-mimetic) by influencing the insulin signaling mechanism (Manject and Ghosh, 1999).

In the DM group, namely the G1 group, there was a relatively high insulin secretion increase (Fig. 2). This increase is because insulin has properties to protect damaged beta cells. The protective effect of insulin on alloxan-induced mouse pancreatic β cells is likely due to reduced demand for NAD, identical to the effects of nicotinamide and aminobenzamide, a poly (ADP-ribose) synthetase that maintains intracellular NAD+ balance, accompanied by inhibition of cellular uptake against alloxan (Saraf et al., 2007; Wadsworth and Koop, 1999). This situation allows insulin-mimetic agents, including flavonoids, to play a role in preventing damage to pancreatic β cells caused by DNA alkylation by alloxan (Herra and Mulja, 2005).

Also, flavonoid compounds are known to have potential anti-inflammatory effects (Silalahi et al., 2015). Quercetin compounds have been shown to inhibit the production of TNF-α and nitric oxide by lipopolysaccharides from activated macrophages (Wadsworth and Koop, 1999). TNF-α suppression was thought to became via inhibition of NF-κB activation. TNF-α inhibition occurs post transcription, while inhibition of inducible nitric oxide synthase occurs in the transcription phase

(Gerritson et al., 1995). It is possible that *Bischofia javanica* extract directly inhibits the production of AGEs. Inhibition of NF-κB activation will weaken the autoimmune response and inflammatory response, which in this study inhibited the inflammatory process in islets of Langerhans (insulitis) (Gerritson et al., 1995; Manject and Ghosh, 1999; Wadsworth and Koop, 1999; Saraf et al., 2007).

The results of this study prove that *B. javanica* leaves extract ethanol has antidiabetic properties and is corroborated by statements from previous studies regarding the content in *B. javanica* leaves extract, namely gallic acid and quercetin, which are flavonoids that act as potent antioxidants (Cambie 1984; Gupta 1988; Whistler 1992; Ihwan et al., 2018). Giving *B. javanica* leaves extract ethanol reduces insulitis degree and improves insulin expression in islets of Langerhans beta cells better than glibenclamide. This herb can be further developed in diabetic treatment.

CONCLUSIONS

In this study, it was demonstrated that the administration of *Bischofia javanica* leaves ethanolic extract protects the islets of Langerhans from the alloxan-induced damage in rats. This was manifested in the improvement in the insulin expression in beta cells and the decrease in the insulitis degree. *Bischofia javanica* leaves ethanol extract was better than glibenclamide displayed in the histologic pancreatic study of rats. Further research is needed to determine the extent to which this plant leaves extract protects from the sequelae of diabetes.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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AUTHOR CONTRIBUTION:

Contribution	Hutahaean S	Ilyas S	Rumahorbo CGP
Concepts or ideas	x	x	
Design	x	x	
Definition of intellectual content	x	x	x
Literature search			x
Experimental studies			x
Data acquisition	x	x	x
Data analysis	x	x	x
Statistical analysis	x	x	x
Manuscript preparation			x
Manuscript editing	x	x	
Manuscript review	x	x	x

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