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Original Article

Apoptosis via cytochrome c in aortic tissue of diabetes mellitus after giving sikkam leaves (*Bischofia javanica* Blume)

[Apoptosis vía citocromo c en tejido aórtico de diabetes mellitus después de dar hojas de sikkim (*Bischofia javanica* Blume)]

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Abstract

Context: Diabetes mellitus (DM) is a metabolic hyperglycemia disease caused by abnormalities in insulin secretion and the highest increase in diabetes sufferers that occurred in Southeast Asian countries, including Indonesia. The sikkam leaf (*Bischofia javanica*) ethanolic extract contains many secondary metabolites, and some of them are quercetin and gallic acid, which have the potential to be used as antidiabetic.

Aims: To analyze apoptosis via cytochrome c in aortic tissue of DM after giving *B. javanica* leaves.

Methods: 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 G5: Induction of alloxan + glibenclamide 0.5 mg/kg BW. Fourteen days later, the rats were dissected, and the aortic tissue was analyzed for apoptosis by tunnel assay and cytochrome c by immunohistochemistry.

Results: There was a statistically significant difference ($p<0.01$) in the apoptotic value and cytochrome c. Increasing the dose of *B. javanica* leaves, the cytochrome c expression, and apoptotic value decreases in the aorta of DM rats. The cell shape returned to normal after being given *B. javanica* ethanol extract 900 mg/kg BW than glibenclamide.

Conclusions: *Bischofia javanica* ethanolic extract reduced apoptosis via cytochrome c in aortic histology, and this plant can be developed into a diabetic drug candidate.

Keywords: apoptosis; cytochrome c; hyperglycemia; hypoglycemic agents; immunohistochemistry; plant extracts.

Resumen

Contexto: La diabetes mellitus (DM) es una enfermedad de hiperglucemia metabólica causada por anomalías en la secreción de insulina y el mayor aumento de pacientes con diabetes que se produjo en los países del sudeste asiático, incluida Indonesia. El extracto etanólico de la hoja de *Bischofia javanica* contiene muchos metabolitos secundarios, y algunos de ellos son la quercetina y el ácido gálico, que tienen el potencial de usarse como antidiabéticos.

Objetivos: Analizar la apoptosis por citocromo c en tejido aórtico de DM tras la administración de hojas de *B. javanica*.

Métodos: El grupo de tratamiento consistió en 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 etanólico de hojas de *B. javanica*, G4: Inducción de aloxano + extracto etanólico de hojas de *B. javanica* 900 mg/kg de peso corporal y G5: Inducción de aloxano + glibenclamida 0,5 mg/kg de peso corporal. Catorce días más tarde, las ratas fueron disecadas y el tejido aórtico fue analizado para apoptosis por ensayo de túnel y citocromo c por inmunohistoquímica.

Resultados: Hubo una diferencia estadísticamente significativa ($p<0.01$) en el valor apoptótico y el citocromo c. Al aumentar la dosis de hojas de *B. javanica*, la expresión del citocromo c y el valor apoptótico disminuyen en la aorta de ratas DM. La forma de la célula volvió a la normalidad después de recibir extracto de etanol de *B. javanica* 900 mg/kg de peso corporal que glibenclamida.

Conclusiones: El extracto etanólico de *Bischofia javanica* redujo la apoptosis a través del citocromo c en histología aórtica y esta planta puede convertirse en un candidato a fármaco diabético.

Palabras Clave: agentes hipoglucemiantes; apoptosis; citocromo c; extractos de plantas; hiperglucemia; inmunohistoquímica.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic hyperglycemia disease caused by abnormalities in insulin secretion and action. DM is a problem in society and even the world because the number increases from year to year. The prevalence of diabetes in the world related to age increased from 5.9-7.1% in the 20-79 years old. In 2012, the highest increase in people with diabetes occurred in Southeast Asian countries, including Indonesia (Idris et al., 2017). The Indonesian population diagnosed with DM reached 9.1 million in 2014, and Indonesia ranked 5th globally (Idris et al., 2017).

DM causes disorders of carbohydrate, fat, and protein metabolism (Nurliyani and Sunarti, 2015). DM also causes chronic complications such as blindness, kidney failure, nerves, increased blood cholesterol levels, aortic disorder, increased uric acid levels, and blood vessels (Landon et al., 2020). The 72% of DM patients experienced lipid profile abnormalities, namely increased triglycerides and total blood cholesterol levels and decreased levels of high-density lipoprotein HDL by 21.7% (Juren et al., 2013; Muhammad, 2017). 4.2% of the population over 15 years old has DM (Pramono et al., 2010). The high prevalence of DM requires serious attention from all stakeholders such as doctors, epidemiologists, and the Ministry of Health of the Republic of Indonesia. 13% of people with diabetes mellitus in Indonesia where age, gender, place of residence, employment status, obesity, hypertension, and dyslipidemia are factors that cause DM (Idris et al., 2017).

In the molecular analysis, the aortic tissue by apoptosis plays an important role in the development of insulin deficiency. Lack of insulin can be a loss from uninterrupted insulin secretion of β cells or owing to total β cells. Apoptosis of blood vessel cells in the mitochondrial transmembrane and is inhibited by overexpression of Bcl-2 can interfere with body metabolism and molecular signal transduction. The apoptotic pathway can be used as a molecular strategy to block apoptosis-causing proteins (Lee and Pervaiz, 2007).

As a mega biodiversity country, Indonesia has many medicinal plant species that can be used as a source of basic ingredients in the medical world. More than 42 species of phytotherapy plants on the glucose profile in the body, regeneration, degeneration of pancreatic β cells, and the diameter of Langerhans (Adhikari et al., 2007). The reasons for plants as medicine are environmentally friendly, non-addictive, easy and cheap to use, and have no harmful side effects.

Bischofia javanica Blume (family *Phyllanthaceae*), known as sikkam, is a wild plant that 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). The *B. javanica* leaves of ethanol extract contain many secondary metabolites, and some of them are quercetin and gallic acid, which have the potential to be used as antidiabetic (Sutharson et al., 2009; Kituyi et al., 2018).

This study aimed to determine apoptosis in aorta tissue via cytochrome c after given *B. javanica*, because the aorta functions as a place for blood to flow from the heart to the body. Aortic disorders can cause bleeding, atherosclerosis, obesity, and disturbances in DM sufferers. This research is expected to produce these plants that can be developed into candidates for diabetes drugs in the future.

MATERIAL AND METHODS

Chemicals

The important reagents and chemicals used in this study consisted of dimethyl sulfoxide (Germany, Catalog Number: 1.02952.2500). Fetal bovine serum (Bethesda, MD, USA). Trypsin (0.25%), 3,3′-diaminobenzidine (Scytek Laboratories, USA). The antibody used was polyclonal cytochrome c antibody, catalog number: PAA594Mi01 (buffer: 0.01M PBS, pH7.4, containing 0.05% Proclin-300, 50% glycerol) Cloudclone brand originating from

W. Fernhurst, TX 77494, USA. Detection was also performed using the ABC Elite Kit (Vector Laboratories, Burlingame, CA, USA) with either a biotinylated goat anti-rabbit secondary antibody or a biotinylated goat anti-mouse secondary antibody (Jackson ImmunoResearch, Pennsylvania, USA.) TUNEL detection kit for apoptosis used Promega, Cat # G7130 (USA).

Vegetal material

Bischofia javanica, locally named as cingkam, was from Karo Regency in North Sumatra. The plants were collected around Semangat Gunung, Merdeka, Cinta Rayat, Doulu, and Lingga villages (02°47.168'N, 098°30.310'E). Generally, they have access to forest areas near Mt. Sibayak. Geographically, these villages are situated about 1162–1453 m above sea level (Purba et al., 2018).

Preparation of sikkam leaves (*Bischofia javanica*)

B. javanica leaves (*Bischofia javanica*) were collected from Simalungun Regency. Extraction was carried out using the maceration method with 96% ethanol as solvent. Fresh *B. javanica* leaves (10 000 g) were dried, then the dried leaves were obtained 270 g of ethanol extract of *B. javanica* leaves. The drying process was based on the drying of *Zanthoxylum acanthopodium* by (Situmorang et al., 2019a;b; 2020) so that the phytochemical content in this herb was not lost due to heat.

The powder of *B. javanica* leaves was soaked in a large glass jar, and 1000 mL of 96% ethanol solvent is added. The glass jar was closed tightly, and the top of the jar was covered with aluminum foil and left for 3 days protected from sunlight, and the soak was stirred every day. After 3 days, the marinade was filtered and collected in a different bottle. Then the filtering results were evaporated with an electric heater. Once thick, it was put into a steam cup wrapped in aluminum foil for one night, after which it was evaporated again until the extract is thick. *B. javanica* leaves were then moved into sample bottles for extract. Furthermore, the powder waste was macerated again by adding 96% ethanol. This process was done up to three macerations and obtained 100 g of *B. javanica* leaf ethanol extract.

Animal handling

The animals used were 30 male Wistar rats acclimatized for 7 days. Male rats were grouped into 6 groups were in 1 treatment group contained 5 male rats. The rats were given food in the form of milled corn or pellets and given drinking water. Alloxan induction was performed by intraperitoneal injection. Before being induced, alloxan was dissolved first using 0.9% NaCl. Alloxan injection was carried out at a dose of 160 mg/kg BW. Rats that have been induced with DM be used as the treatment group. The rats were placed in a plastic cage measuring 40 × 30 cm. The research was approved by the Health Research Ethics Committee of USU Medan (Ethical Clearance: No. 010/KEPH-FMIPA/2020).

Study design

This research was conducted using a non-factorial Completely Randomized Design (CRD) and used SPSS 22 program for statistical analysis. The research was conducted in 6 groups of treatment in the study (Fig. 1). 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* leaf ethanol extract, G3: Alloxan induction + 600 mg/kg BW of *B. javanica* leaf ethanol extract, G4: Induction of alloxan + ethanol extract of *B. javanica* leaves 900 mg/kg BW and G5: Induction of alloxan + glibenclamide 0.5 mg/kg BW. After the rats experienced hyperglycemia due to alloxan injection, the rats were given ethanol extract of *B. javanica* leaves for 14 days. The rats were anesthetized by being given a ketamine hydrochloride injection of 100 mg/kg BW in the femur, and then the animal was sacrificed to take the aorta.

Preparation of the aortic tissue in paraffin and hematoxylin-eosin staining

Aortic samples obtained from rats were fixed with a solution of formalin neutral buffer. Then dehydrated with graded alcohol and purified with xylol, impregnated, and implanted in a paraffin

block. So, cutting can be done using a microtome. Hematoxylin-eosin staining started from the deparaffinization process using xylol I, II, and III solutions for 3-5 min each, then followed by a rehydration process using graded alcohol for 3-5 min each. The preparations were stained with hematoxylin for 30-45 s then rinsed with distilled water. Furthermore, the preparations were restained with eosin for 30-45 s, then followed by a dehydration process using graded alcohol and absolute alcohol for 5 times each. The preparations were purified with xylol solution for 3 min (Situmorang et al., 2018). The aortic tissue was observed using the Axiocamp ERc 5s microscope (Germany, 2015) with 40× magnification, and the tunica thickness measured by Optilab Advance software with high-quality Progressive 5 MP CMOS Color Image Sensor (PT. Miconos, Yogyakarta, Indonesia, 2016).

Immunohistochemistry of cytochrome c

The aortic tissue has implanted in paraffin was cut using a microtome with a thickness of 4 µm. For pre-treatment, the tissue was heated in citrate buffer at pH 6.0 and 350 W. After washing with PBS, the tissue was incubated with cytochrome c antibodies, respectively, at 37°C then washed again with PBS before applying avidin-biotin-peroxidase. 3,3-Diaminobenzidine (DAB) hydrochloride was used for chromogenic visualization reaction and then stained with hematoxylin Mayer. The aortic tissue on the slide was stained with hematoxylin.

TUNEL assay

Paraffin aortic tissue was cut using a microtome with a thickness of 4 µm. The aortic tissue in the slide was immersed in fresh xylene for 5 min. The slides were rehydrated with multilevel ethanol and washed with NaCl 0.85% and PBS for 5 min. After rehydration, incubation was conducted for 15 min at room temperature with proteinase K (20 µg/mL). The final labeling reaction was carried out by adding the reaction mixture of rTdT to the slides in a humid room (37°C for 1 h). The reaction of rTdT enzyme was ended by immersing the slide in a buffer at room temperature. The slides were washed for 5 min with PBS. The endogenous peroxidase was blocked by 0.3% hydrogen peroxide to PBS. A streptavidin-HRP solution was added to the tissue and incubated at room temperature. A chromogenic substrate DAB was added to the slide. All slides were dehydrated with graded ethanol and cleaned in 100% xylene for 5 min every three times.

Statistical analysis

The data were expressed as mean ± SD and analyzed by ANOVA test and non-parametric data from Kruskal Wallis test in SPSS 22 program. Asterisks indicate the level of statistical significance.

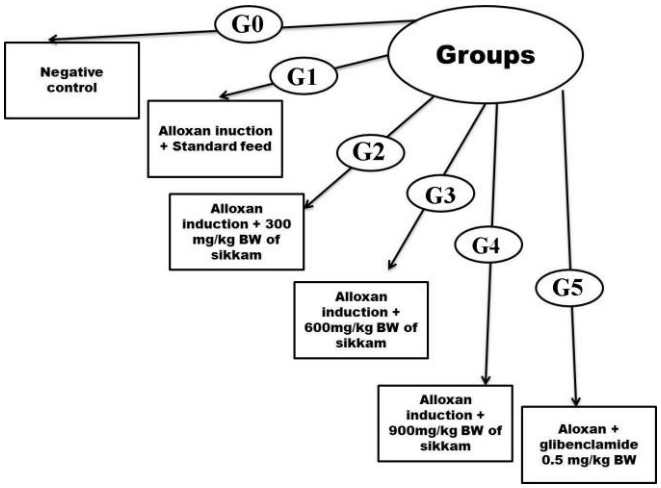

Figure 1. Study design of apoptosis via cytochrome c in aortic tissue of diabetes mellitus after giving *B. javanica* leaves.

RESULTS

Histology of aortic tissue after given an ethanolic extract of *B. javanica* leaves

There was a significant difference ($p<0.05$) in the analysis of tunica thickness in the aortic tissue. The DM group that was given standard feed (G1) had the highest average thickness of tunica, followed by the DM group who was given ethanol extract of *B. javanica* leaves at a dose of 300 mg/kg BW (G2). The lowest tunica thickness was in the DM group given glibenclamide 0.5 mg/kg BW (G5) and the control group (G0) in Fig. 2.

The microscopic image of the aorta of Wistar rats shows the aorta layer, which consists of three layers, namely the tunica intima, tunica media, and tunica adventitia (Fig. 3). In the G1 and G2 groups, the foam cells in the tunica intima were clearly visible. In DM rats, there are foam cells and intracellular smooth muscle lipids but no extracellular lipids, lipid nuclei, calcification, fibrateroma, surface defects, hematoma, and thrombus. Meanwhile, groups G0 and G5 showed a normal condition of the aortic vessels.

Analysis of cytochrome c expression in aortic tissue

There was a significant difference ($p<0.01$) in each DM group. The highest mean cytochrome c expression was found in the DM group given standard feed (G1), and the lowest cytochrome c expression was in the control group (G0) and the DM group after being given ethanol extract 900 mg/kg BW (Table 1 and Fig. 4). Based on the Mann-Whitney test analysis, the value of cytochrome c expression in each treatment group indicated, increasingly the dose of *B. javanica* leaves, the cytochrome c expression decreases in the aorta of DM rats. Cytochrome c was strongly expressed in the G1 and G2 groups (red arrows), which were marked by brown cells (Fig. 5). The expression gets weaker when the dose of *B. javanica* leaves is increased (purple and yellow arrows).

Analysis of apoptotic value in aortic tissue

There was a significant difference ($p<0.01$) in the apoptotic value of each DM group. The highest apoptosis average was in the DM group that was given standard feed (G1), and the lowest apoptosis was in the control group (G0) and the DM group after being given ethanol extract 900 mg/kg BW (Table 2 and Fig. 6). The *B. javanica* ethanol extract 900 mg/kg BW was better at reducing apoptosis than glibenclamide 0.5 mg/kg BW. Based on the Mann-Whitney analysis, the test of cytochrome c expression values in each treatment group showed by increasing the dose of *B. javanica* leaves, and the apoptosis was reduced in the aorta of DM patients. Apoptosis through the TUNEL assay was strongly expressed in the G1 group (red arrow), which was marked by brown cells (Fig. 7). The expression weakened further when the dose of *B. javanica* leaves was increased (purple and yellow arrows). The cells form returned to normal after being given *B. javanica* ethanol extract 900 mg/kg BW.

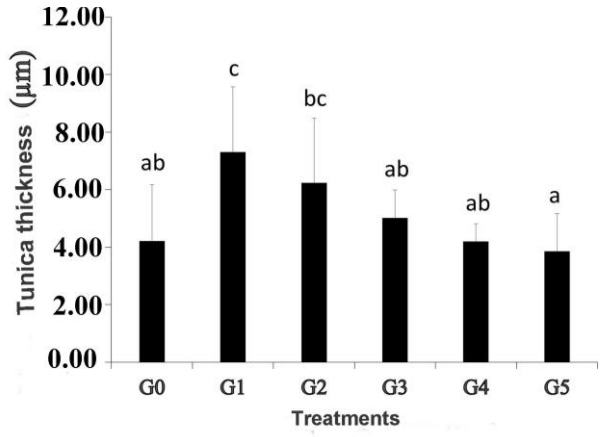

Figure 2. The graph error bar on the thickness of the heart aortic tunica intima of diabetic rats, after giving several doses of *B. javanica* extract.

Data are represented as mean ± standard deviation (n = 6). Different letters indicate statistically significant differences between bars ($p<0.05$) using the Kruskal Wallis test. 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.

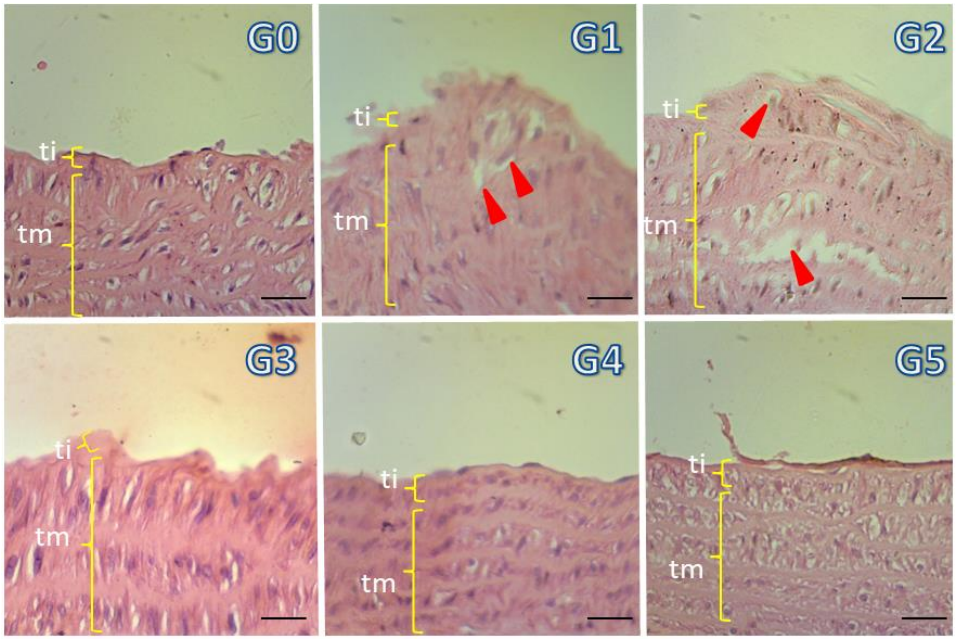

Figure 3. Histology of cardiac aortic tissue in diabetic rats, after several doses of *B. javanica* extract.

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. "ti" = tunica intima, "tm" = tunica medica, red arrow = greater vacuolization, black line = 200 µm. Hematoxylin eosin (HE) staining and 400× magnification.

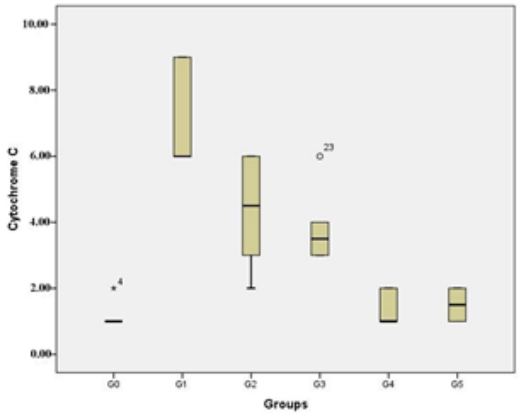

Figure 4. Box plot of cytochrome c expression data in cardiac aortic tissue of diabetic rats.

Data are represented as mean ± standard deviation (n = 6). *p<0.05 indicates statistically significant differences between G1 compared with G2 until G5. 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.

Table 1. Kruskal-Wallis and Mann-Whitney analysis of cytochrome c expression in aortic tissue.

Groups	n	Mean rank	Kruskal-Wallis	Mann-Whitney					
				G0	G1	G2	G3	G4	G5
G0	6	8.08	0.000	-	0.002**	0.003**	0.003**	0.523	0.241
G1	6	32.17		-	-	0.031*	0.016*	0.003**	0.003**
G2	6	25.25		-	-	-	0.799	0.005**	0.006**
G3	6	24.58		-	-	-	-	0.003**	0.003**
G4	6	9.67		-	-	-	-	-	0.575
G5	6	11.25		-	-	-	-	-	-

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 0.5 mg/kg BW. *p<0.05, **p<0.01 indicate statistically differences respect G1 compared with G2 until G5 (n = 6).

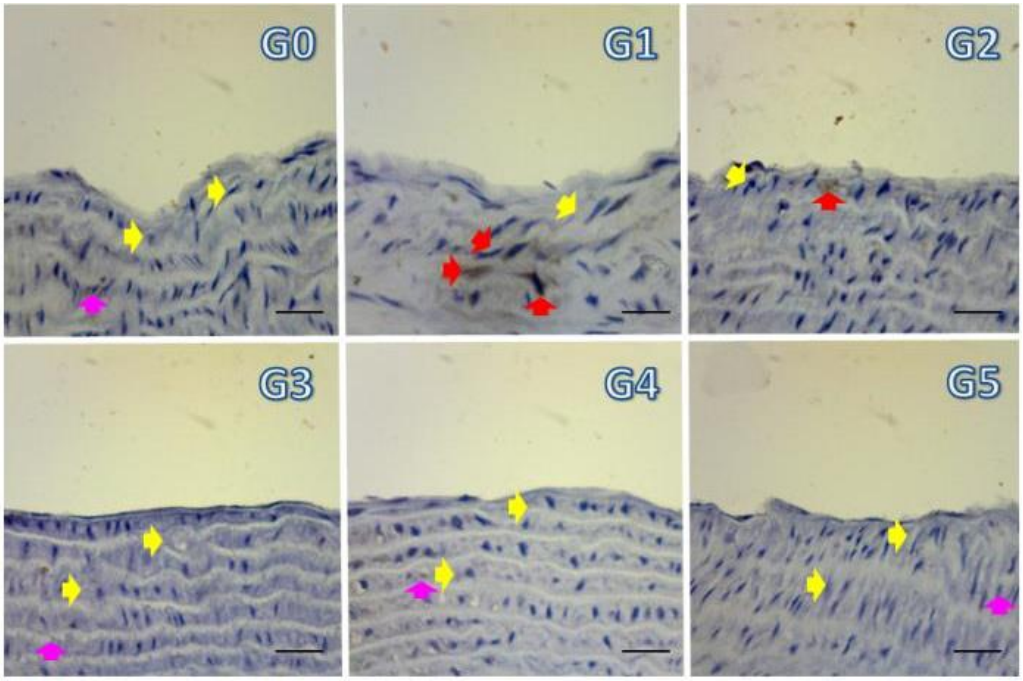

Figure 5. Expression of cytochrome c in the cardiac aortic tissue of diabetic rats.

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 0.5 mg/kg BW. Red arrow: strong positive, purple arrow: weak positive, and yellow arrows: negative.

Table 2. Kruskal-Wallis and Mann-Whitney analysis of apoptotic values in aortic tissue.

Groups	n	Mean rank	Kruskal-Wallis	Mann-Whitney					
				G0	G1	G2	G3	G4	G5
G0	6	7.00	0.000	-	0.002**	0.002**	0.002**	0.317	0.019*
G1	6	30.83		-	-	0.309	0.016*	0.002**	0.003**
G2	6	27.00		-	-	-	0.356	0.010*	0.003**
G3	6	23.67		-	-	-	-	0.015*	0.003**
G4	6	9.83		-	-	-	-	-	0.201
G5	6	12.67		-	-	-	-	-	-

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. *p<0.05, **p<0.01 indicate statistically differences respect G1 compared with G2 until G5 (n = 6).

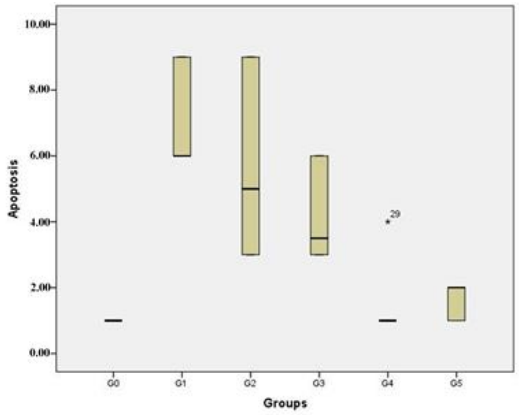

Figure 6. Box plot of apoptotic expression data in cardiac aortic tissue of diabetic rats.

Data are represented as mean ± standard deviation (n = 6). *p<0.05 indicates statistically significant differences between G1 compared with G2 until G5. 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.

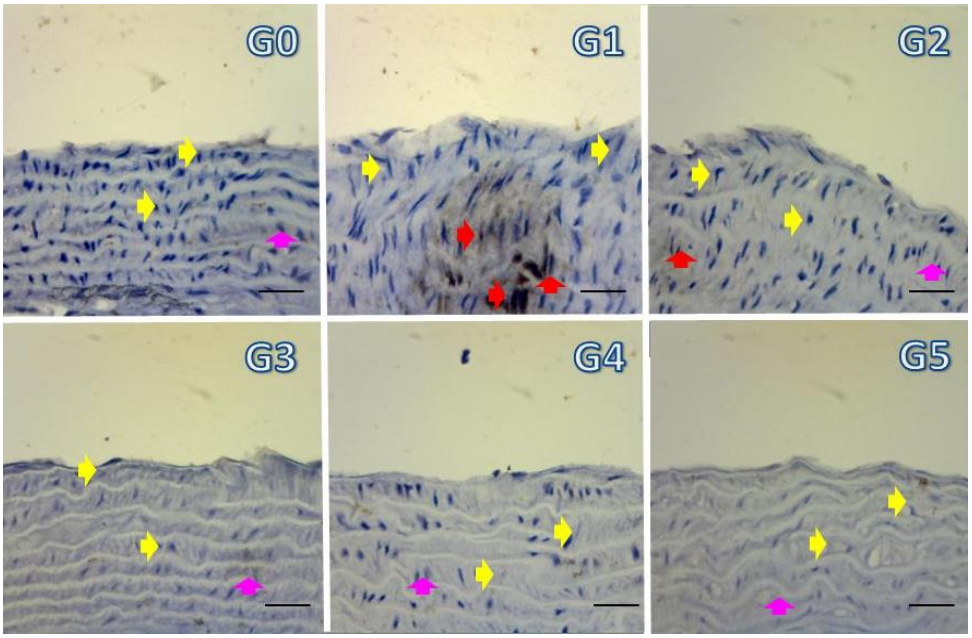

Figure 7. Apoptotic expression of cells in the aortic tissue of the rat heart.

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. Yellow arrow = negative cytochrome expression, red arrow = strong positive cytochrome expression, purple arrow = weak positive expression. 400× magnification. Black line = 200 µm.

DISCUSSION

The microscopic view of the aorta of the Wistar rats shows different tunica cells from the normal aorta and DM (Figs. 2 and 3). This arterial intima consists of flattened endothelium and epithelium, separated from the internal elastic by loose connective tissue with few fibroblasts, sometimes smooth muscle cells, and fine collagen fibers (Milutinović et al., 2020). The endothelium is a smooth barrier layer for vessels and a partial selective diffusion barrier between the blood and the outer tunica of the vessel wall (Milutinović et al., 2020). Cardiovascular disease is characterized by the thickening of the carotid intima-media tunica (TIM) in DM patients. Type 2 DM is characterized by an increase in lipoproteins containing lots of triacylglycerols (chylomicrons and LDL particles), which can increase oxidative stress and interfere with endothelial function directly indirectly by increasing the production of small-dense LDL particles and reducing HDL (Scuruchi et al.,2019). This event also enhanced intima thickness as a marker of atherosclerosis (Saarikoski et al., 2017). Atherosclerosis causes an increase in the thickness of the intima-media layer of the carotid arteries associated with intima-media hypertrophy (Saarikoski et al., 2017). The *B. javanica* leaves can repair the aortic tissue (Fig. 3) because the *B. javanica* leaf of ethanol extract contains many secondary me-

tabolites, and some of them are quercetin and gallic acid, which has the potential to be used as antidiabetic (Sutharson et al., 2009; Kituyi et al., 2018).

Chronic hyperglycemia selectively impairs endothelial muscle function but not smooth vascular (Nieves-Cintrón et al., 2020). The antidiabetic plants can reverse endothelial dysfunction by normalizing NO-mediated relaxation of acetylcholine and the contribution of vasoconstrictor prostanoids. This vasoprotective action promotes NO-mediated relaxation of the aorta (Nieves-Cintrón et al., 2020). Endothelial dysfunction is a major trigger in the context of inflammation, apoptosis, reactive oxygen species, and phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathways during chronic diabetes. Stimulating apoptotic signaling pathways and regenerating natural antioxidants, such as glutathione and vitamins C and E, can also repair apoptotic aorta (Nieves-Cintrón et al., 2020). Increasingly, the dose of *B. javanica* leaves, the expression of cytochrome c in the aorta of DM sufferers is reduced. Apoptosis through TUNEL assay is strongly expressed in DM. The expression is getting weaker when the dose of *B. javanica* leaves is increased. The cell shape returned to normal after being given *B. javanica* ethanol extract 900 mg/kg BW than glibenclamide. Glibenclamide tends to cause hypoglycemia, hyperinsulinemia, and weight gain. Side effects of glibenclamide have also been reported to disturb the teeth of children who are often chewing these tablets (Kumaraguru et al., 2009). Herbal medicine is environmentally friendly, non-addictive, easy, and cheap to use, and have no harmful side effects.

Overexpression of cytochrome c on the tissue can cause this protein to leave the mitochondria after changes in electrochemical potentiation in the membrane (Redza-Dutordoir and Averill-Bates, 2016). A response to deadly stimuli such as hypoxia, oxidative stress, and DNA damage can activate this pathway (Lanneau et al., 2008). This pathway involves mitochondria because it contains pro-apoptotic factors such as cytochrome c and apoptosis-inducing factors. Giving *B. javanica* leaves extract ethanol reduces apoptosis via cytochrome c better than glibenclamide so that this herb can be further developed in diabetic treatment or repairing aortic tissue.

CONCLUSIONS

In this study was demonstrated that the injection of alloxan and given *Bischofia javanica* extract ethanol affects apoptosis via cytochrome c in aortic histology. *Bischofia javanica* extract ethanol was better than glibenclamide in the histologic aorta of rats. This plant can be developed into a diabetic drug candidate.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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AUTHOR CONTRIBUTION:

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

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