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Paederia foetida Ameliorates Diabetic Cardiomyopathy in Rats Models by Suppressing Apoptosis

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ABSTRACT

Diabetes mellitus is one of the most prevalent global public health issues associated with a higher risk of cardiovascular diseases, contributing to morbidity and mortality. Research has demonstrated that elevated reactive oxygen species (ROS) generation in diabetes can trigger apoptosis, exacerbating diabetic cardiomyopathy (DCM). This study investigates the cardioprotective effects of *Paederia foetida* in rats' models of type 2 diabetes induced by a high-fat diet (HFD) and streptozotocin (STZ) treatment. The diabetic model was established in Sprague Dawley rats by intraperitoneal injection of streptozotocin (STZ, 40 mg/kg). Sprague Dawley rats were treated with varied concentrations of standardized extract of *P. foetida* (50 mg/kg and 100 mg/kg), administered orally once daily for four weeks. Standardized extract from *P. foetida* has a range of therapeutic potential, including anti-inflammatory, antioxidant, and anti-diabetic properties. The common metabolic disorder indices and myocardial apoptosis were investigated. The findings from this study demonstrated increased expression of Bcl-2 and decreased expression of Bcl-2 Associated X-protein BAX as indicated by IRS scoring in cardiomyocytes, suggesting that *P. foetida* has a significant protective effect on diabetic cardiomyopathy by decreasing apoptosis. Increased Bcl-2 and decreased BAX levels may be related to regulating oxidative stress and mitochondrial pathways involving myocardial apoptosis.

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P. foetida extract could be a potential intervention for attenuating cardiomyopathy in diabetes mellitus.

Keywords: Diabetic cardiomyopathy, hyperglycemia, myocardial apoptosis, *Paederia foetida*, type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus, a metabolic disorder, is one of the most prevalent global public health issues associated with a higher risk of cardiovascular diseases, contributing to morbidity and mortality (Punthakee et al., 2018; Zhao et al., 2018). The leading cause of death globally comprises 17.9 million deaths annually, and 31% of all deaths have been identified as cardiovascular disease (Lu et al., 2021). Diabetes type 1 and type 2 are heterogeneous diseases with a wide range of clinical presentations and disease progression (ElSayed et al., 2023). Insulin resistance and pancreatic β-cell dysfunction are the key factors contributing to developing and progressing type 2 diabetes mellitus (Nishimura et al., 2022). Inflammation in adipose tissues, brought on by immune cell infiltration around hypertrophied adipocytes, such as macrophages, primarily initiates and sustains insulin resistance (Fasshauer & Blüher, 2015). High-fat diet (HFD) consumption modifies the gut microbiota, causing endotoxemia and chronic inflammation in several tissues, including adipose tissue (Unamuno et al., 2018). As a compensation mechanism, obesity-related insulin resistance is frequently accompanied by a transient rise in insulin secretion and the number of pancreatic β-cells. However, diabetes is ultimately brought on by the attenuation of insulin secretion and the decline in β-cell mass that results from this compensation ceasing to be sustained over time (Cani et al., 2007). As a result of excessive production or insufficient clearance of mitochondrial reactive oxygen species (ROS) during persistent hyperglycemia, oxidative stress develops, which is a significant contributing factor to diabetic microangiopathy and a major contributor to diabetic cardiomyopathy (DCM) (Jia et al., 2018). Diabetic cardiomyopathy is a myocardial pathology unique to diabetic patients with persistent hyperglycemia and cardiac failure (Dillmann, 2019). Hence, elevated reactive oxygen species (ROS) and hyperglycemia in diabetic myocardial tissue are risk factors. Type 2 diabetes has been recognized as a substantial risk factor for cardiovascular diseases despite the unclear etiology of cardiovascular morbidity and mortality (Dunlay et al., 2019).

Cardiac failure unrelated to valvular heart disease, hypertension, or artery disease can arise in diabetic cardiomyopathy, a pathophysiological condition (Dillmann, 2019). According to previous research, chronic hyperglycemia affects the energy preferences of cardiomyocytes, increases the production of free radicals, and induces an oxidative stress-like condition (Kukidome et al., 2006). It may remodel cardiac structure, an essential indicator of diabetic cardiomyopathy. Myocardial interstitial, cardiomyocyte hypertrophy, elevated oxidative stress inflammation, perivascular fibrosis and apoptosis, and indicators are

the main pathological characteristics of diabetic cardiomyopathy (Saisho,2014). In addition to accelerating cardiac injury and initiating mitochondrial oxidative damage in diabetes mellitus, oxidative stress under hyperglycemic conditions lowers antioxidant capacity and can cause cell death through necrosis or apoptosis (Sangweni et al., 2021; Sun et al., 2020). Moreover, earlier research has demonstrated that elevated ROS generation in diabetes can trigger apoptosis, exacerbating diabetic cardiomyopathy (Bhatt et al., 2015; Ji et al., 2017). Apoptosis is primarily mediated by a family of cysteine proteases known as caspases. There are intrinsic (mitochondria-driven) and extrinsic caspase-mediated apoptosis pathways (receptor-mediated) (Lee & Pervaiz, 2007). Pro- and anti-apoptotic Bcl-2 proteins in the intrinsic pathway regulate the release of cytochrome C from mitochondria. Notably, the significant generation of superoxide (O_2^-) anion in mitochondria makes the membranes more susceptible to ROS. Consequently, the membrane is broken down by the sustained ROS attack, which leads to permeability, the release of cytochrome C into the cytoplasm, and, ultimately, the induction of apoptotic cell death (Orrenius, 2007; Ott et al., 2007). Cysteine-dependent aspartate-specific proteases (caspases), divided into initiator and effector caspases, are a family of protease enzymes that play a crucial role in apoptosis.

Initially, the effector caspases, including caspase 3, 6, and 7, are activated in a cascade after the initiator caspase, known as caspase 9, forms a compound with cytochrome C (Wen et al., 2022; Zou et al., 2021). Subsequently, chromatin condensation and oligonucleosomal DNA fragmentation are brought on by caspase 3 activating the caspase-activated DNase (CAD) enzyme (Ge et al., 2019). Moreover, the heart is damaged by activating the mitochondria-dependent apoptotic pathway, a direct consequence of oxidative stress and inflammation (Raish,2017). In the extrinsic pathway, the ligand binding to its death receptor engages an adaptor protein that procures and activates procaspase 8. As a result, FasL binds to Fas, resulting in the subsequent activation of the fas-associated death domain (FADD) and caspase-8. Upon ligating the cell surface death receptor(s), the receptor-mediated pathway is activated, initiating a downstream effector mechanism mediated by the caspase family of cysteine proteases (Lee & Pervaiz, 2007). The activation of caspase-3, essential for the induction of apoptotic cell death, is also caused by caspases-8 and caspase-9. Activating effector caspases like caspase 3 is necessary for apoptosis via a mitochondrial or non-mitochondrial pathway (Patar et al., 2018). However, a family of proteins known as B-cell lymphoma-2 (Bcl-2) that control the permeability of the membrane of mitochondria is known to modulate apoptosis. Members of this family include the anti-apoptotic (Bcl-x, Bcl-2, Bcl-XL, and Bcl-w) and pro-apoptotic (Bax, Bak, Bad, and Bim) proteins (Cory & Adams, 2002; Lee et al., 2020). Thus, inhibiting oxidative stress and apoptosis may prevent diabetic cardiomyopathy.

The search for more aggressive cardioprotective treatments remains challenging despite advancements in managing diabetic mellitus (Dunlay et al., 2019; Mordi et al., 2020; Snell-Bergeon & Maahs, 2015). Due to their extensive biological activities, bioactive phytochemicals are currently intensively studied (Lu et al., 2021). Medicinal plants have received considerably more attention as a source of biologically active substances, such as antioxidant, antihyperglycemic, and antihyperlipidemic agents. They play a significant role in identifying new counteractive drugs (Tang & Halliwell, 2010).

Similarly, a previous study has shown that *Paederia foetida* (Rubiaceae) exhibits good anti-diabetic properties and has suggested dl-α-tocopherol, stigmastanol, 2-hexyl-1-decanol, n-hexadecanoic acid and as the bioactive compounds present in the chloroform extract. Others are 2-nonadecanone, stigmast-4-en-3-one, 4,4-dimethyl-, cholest-8(14)-en-3-ol, (3β,5α)-, stigmasterol, 1-ethyl-1-tetradecyloxy-1-silacyclohexane, gamma-sitosterol, stigmast-7-en-3-ol, (3β,5α,24S)-, scopoletin, and α-monostearin. This plant is predominantly found in Asian countries (Tan et al., 2020). Research has extensively documented that *Paederia foetida* (PF) leaf extract exhibited considerable anti-diabetic, antihyperlipidemic, and antioxidant potential in mice models (Kumar et al., 2014). Antioxidant activity of the phenolic content of *Paederia foetida* stems from their role as free radical scavengers, which reduces oxidative stress (Osman et al., 2009). However, the anti-apoptotic effects of the plant on a diabetic heart have not been explained. Therefore, this study investigated the cardioprotective effects of *Peaderia foetida* in rats' models of type 2 diabetes induced by a high-fat diet (HFD) and streptozotocin (STZ) treatment.

MATERIALS AND METHODS

Extraction

Twigs of fresh *Paederia foetida* were air-dried and ground into powder. The powdered twigs of *Paederia foetida* (1.2 kg) were soaked and extracted by organic solvents, namely hexane (Fisher chemical, UK), chloroform (Thermofisher, India), and methanol (Honeywell Research Chemicals), using the cold maceration method. Briefly, the powdered plant material was soaked in solvent hexane in a conical flask for 72 hr. The Whatman filter paper was used to filter the hexane suspension. The filtrate was concentrated under reduced pressure using the rotary vacuum evaporator to get the crude extracts.

Similarly, the hexane solvent was substituted with chloroform and then methanol by applying the similar extraction method mentioned above. These steps were repeated three times for each solvent to optimize the yield. The active crude extract was freeze-dried and kept at -20°C until use.

Animals

Sprague Male Dawley rats ($n = 48$), aged 10–12 weeks and of weight 190–220 g, were used for this study. The animals were acclimatized in a room with a temperature of 25 ± 2°C and a 12-hr day-light cycle for a week. They

were divided randomly into two groups: a normal control group fed a basal diet (n = 8) and an obese group placed on a high-fat diet (n = 40) for four weeks. The obese group was fed HFD to induce obesity. After four weeks, out of 40 obese rats, type 2 diabetes was induced in the 32 obese rats (n = 32) following an overnight fast by intraperitoneal injection of streptozotocin (STZ, 40 mg/kg). In contrast, the normal control rats were injected with normal saline. Rats with more than 170 mg/dL of blood glucose were considered diabetic. After seven days, diabetic rats (n=32) were randomly further divided into four groups, each consisting of 8 treated with either distilled water, metformin, or varied concentrations of standardized extract of *Paederia foetida* (Table 1). The standardized extract and metformin were administered orally once daily for four weeks. All rats were fed *ad libitum* throughout the experiment. The rats used in the experiment were handled following the ethical approval of the Animal Ethics Committee of Universiti Sains Malaysia.

The characteristics of each grouping are as follows:

Group 1: Normal control (NC) (Healthy rats treated with distilled water daily)

Group 2: Obese (O) (High-fat diet rats treated with distilled water daily)

Group 3: Diabetic control (DC) (Diabetic rats treated with distilled water/day)

Group 4: Diabetic + Metformin (D+M) (Diabetic rats treated with 300 mg/kg/day Metformin)

Group 5: Diabetic + 50 mg Standardized extract *Paederia foetida* (D + 50 mgPF) (Diabetic rats treated with 50 mg/kg/day *Paederia foetida*)

Group 6: Diabetic + 100 mg Standardized extract *Paederia foetida* (D + 100 mg PF) (Diabetic rats treated with 100 mg/kg/day *Paederia foetida*)

Immunohistochemistry and Examination of Apoptotic Markers

After 28 days, the rats fasted overnight and were euthanized by exsanguination under ketamine-xylazine [100-300 mg/kg] via intracardiac puncture. The heart was carefully harvested, preserved in 10% formaldehyde, and processed for fixation and paraffin embedding. Fixed tissues were rehydrated and deparaffinized. Immunohistochemistry (IHC) determines the anti-apoptotic markers B-cell lymphoma-2 and Bcl-2 Associated X-protein (Bcl-2 & BAX).

Table 1
Diabetic rats' group

Number of rats (n)	Exposure/Treatment
8	Diabetic control rats treated only with distilled water daily
8	Diabetic rats treated with metformin at 300 mg/kg daily
8	Diabetic rats treated with a standardized extract of *Paederia foetida* at 50 mg/kg daily
8	Diabetic rats treated with a standardized extract of *Paederia foetida* at 100 mg/kg daily

Note. Characteristics of diabetic rats' groups used in the experiment

Assessment of Bcl-2

Positive control tissue for Bcl-2 consists of lymph nodes and tonsils. It was diluted 1:100, and the Leica Bond TM system was used to stain paraffin-embedded human lymph node tissue. Antigen retrieval was carried out by high pressure in a citrate buffer (pH 6) following dewaxing and hydration. The section (5 µm) was occluded for 30 minutes at room temperature with 10% normal goat serum. The primary antibody (1% BSA) had been incubated overnight at 4°C. A biotinylated secondary antibody identified and visualized the primary antibody by an SP system conjugated with HRP.

Assessment of BAX

Human esophageal malignancy served as the positive control for BAX. A Leica Bond TM system was diluted 1:100 and stained on paraffin-embedded human oesophageal carcinoma tissue. Antigen retrieval was accomplished by high pressure in a citrate buffer (pH 6) following dewaxing and hydration. The section (5 µm) was occluded for 30 min at room temperature with 10% normal goat serum. The primary antibody (1% BSA) had been incubated overnight at 4°C. A biotinylated secondary antibody recognized and visualized the primary antibody by an SP system conjugated with HRP.

Scoring System for Reading IHC Slides

Various methods are used to determine assessment criteria and associated scoring points. These are aligned with the experiment's scientific purpose and the characteristics of the IHC markers used. The reference point involves a percentage of positively stained cells and the observed staining intensity. The scoring system employed for interpreting the IHC results in this study is described below.

Immunoreactive Scoring (IRS) System

The immunoreactive scoring system (IRS) gives a range of 0–12 as a product of multiplication between the positive cells proportion score (0–4) and the staining intensity score (0–3) (Table 2). IRS was utilized for expressing a variety of IHC markers (VEGF, BMP and its receptors, vWF and many others) in bone studies by (Koerdt et al., 2017). The sample under examination stains for IHC marker heterogeneously, then each staining intensity is scored independently, and the results are added. An example of such an approach is given by (Fedchenko & Reifenrath, 2014)when a specimen encompassed 50% of the tumor cells with moderate intensity (2 × 2 = 4), 25% of tumor cells with intense immunostaining (1 × 3 = 3), and 25% of cells with weak intensity (1 × 1 = 1), the score was 4 + 3 + 1 = 8.

Table 2
Interpretation of immunoreactive scoring system (IRS) scoring

IRS scoring	Interpretation
0-1	Negative
2-3	Positive: Weak
4-8	Positive: Moderate
9-12	Positive: Strong

Statistical Analysis

All data were analyzed using the Statistical Package for Social Science (SPSS) for Windows software version 21.0 (SPSS, Chicago, USA). Qualitative data were expressed as percentages. Statistical analysis between groups was made using one-way analysis of variance (ANOVA), and variables were expressed as mean ± standard deviation. P value < 0.05 was considered statistically significant.

RESULTS

Glucose levels in group 1 and group 2 remained at the normal range throughout the experiment. The normal control group increased by 7.03% of blood glucose, while the Obese group increased by 11.01%. There was no significant difference in plasma glucose levels during the obesity induction period. Furthermore, glucose levels increased significantly ($p < 0.05$) in group 3 by 10.29% compared to groups 4, 5, and 6. However, treated diabetic mice showed decreased blood glucose levels than the diabetic control group (group 3). Treating diabetic rats (group 5) with *Paederia foetida* extract (50 mg/kg) significantly decreased blood glucose levels compared to groups 4 and 6. There was a 27.19% reduction in blood glucose levels in group 5, followed by 23.14% in group 4 (Table 3). Thus, the low extract dose seemed to be the most effective antihyperglycemic in this study.

Interpretation of Bcl-2 Scoring

The IRS shown below (Table 4) of all groups predict the increased expression of Bcl-2 in group E and group F as indicated by IRS scores that show strong staining of cells with Bcl-2 that indicates the increased manifestation of Bcl-2 in groups of rats treated with standardized extract (Figure 1). It indicates that the dose given to group E rats has significantly decreased the apoptosis in rats with increased expression of positive cells with Bcl-2 cardiomyocytes, indicating the treatment has a positive impact on decreasing the apoptosis in streptozotocin-induced rats.

Table 3
Plasma glucose levels and changes before and during interventions

Rats' group	Plasma glucose levels (nmol/L)		
	Week 0	Week 4	Changes
Group 1: Normal healthy rats	4.55 ± 0.35^{a}	4.87 ± 0.26^{a}	0.32 ± 0.09^{a} (7.03%)
Group 2 : Obese rats	5.72 ± 0.49^{a}	6.35 ± 1.55^{a}	0.63 ± 1.06^{a} (11.01%)
Group 3: Diabetic control rats	26.64 ± 5.82^{b}	29.38 ± 2.43^{b}	2.74 ± 3.39^{a} (10.29%)
Group 4: Diabetic + Metformin	21.48 ± 1.99^{a}	16.51 ± 11.38^{b}	-4.97 ± 9.39^{c} (-23.14%)
Group 5: Diabetic + 50 mg/kg standardized extract of *Paederia foetida*	13.68 ± 6.70^{c}	9.96 ± 4.15^{a}	-3.72 ± 2.55^{b} (-27.19%)
Group 6: Diabetic + 100 mg/kg standardized extract of *Paederia foetida*	16.14 ± 4.07^{d}	13.43 ± 9.99^{a}	-2.71 ± 5.29^{c} (-16.79%)

Note. All values were expressed as mean ± SD of 6 groups. Data with different superscripts (a, b, c, and d) in the same column were considered significantly ($p < 0.05$) different

Table 4
Immunoreactive scoring system (IRS) scoring for B-cell lymphoma 2 (Bcl-2)

Groups		% Positive cells	Staining intensity	IRS scoring
Bcl2 - Control		3	4	12
Group 1	Normal Control	3	2	6
Group 2	Obese	2	2	4
Group 3	Diabetic control	2	1	2
Group 4	Diabetes + metformin	3	3	9
Group 5	Diabetes + 50 mg *Paederia foetida*	3	2	6
Group 6	Diabetes + 100 mg *Paederia foetida*	2	2	4

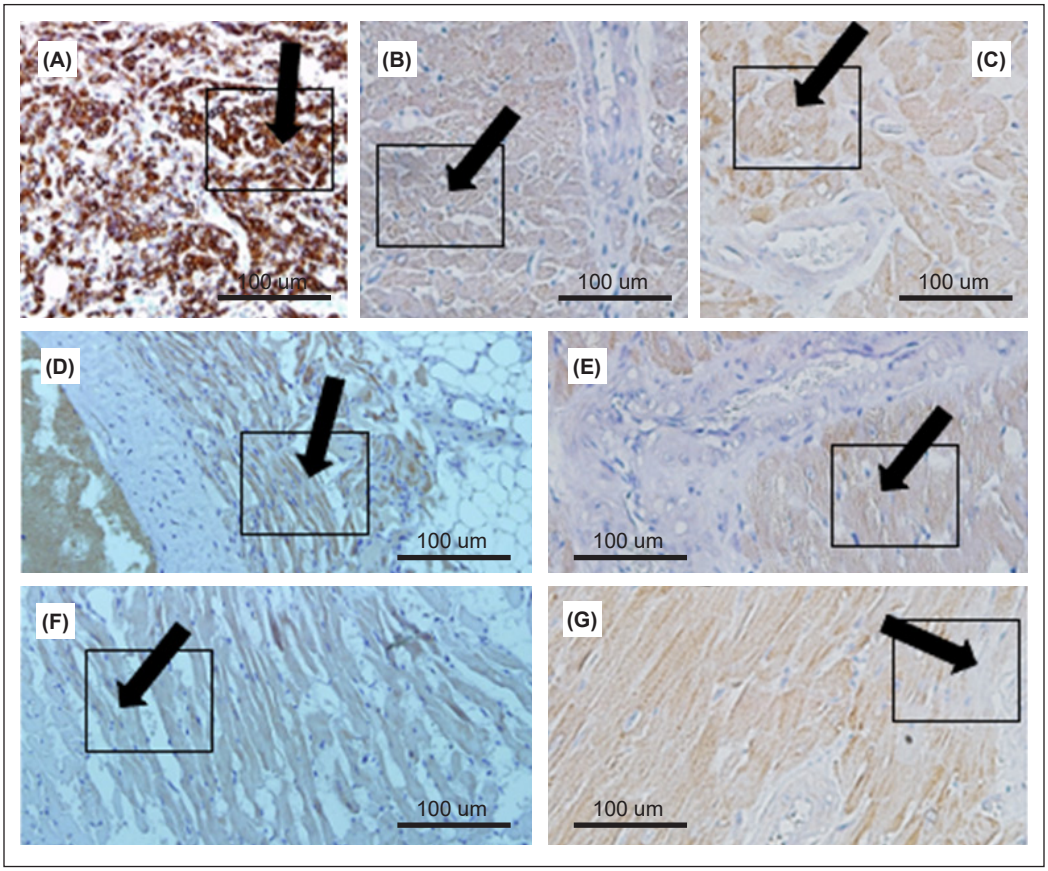

Figure 1. Representative photomicrograph of rats' cardiomyocytes with B-cell lymphoma 2 (Bcl-2)
Note. (A) Bcl-C = Cells of small figure (strong staining); (B) NC = Cells of small figure (weak staining); (C) O = Cells in small figure (strong staining); (D) DC = Cells in small figure (moderate staining); (E) D + M = Cells in small figure (moderate staining); (F) D + 50 mg PF = Cells of small figure (weak staining); (G) D + 100 mg PF = Cells of small figure (weak staining); Normal Control (NC); Obese (O); Diabetic control (DC); Diabetes + metformin (D + M); Diabetes + 50 mg *Paederia foetida* (D + 50 mg/kg PF); Diabetes + 100 mg *Paederia foetida* (D + 100 mg/kg PF)

Moreover, as identified by the immunoreactivity score (IRS), the increased expression of Bcl-2 in group 5 and group 6 indicated more staining of Bcl-2 cells as compared to the control, particularly in group 5, treated with a low dose (50 mg/kg) of standardized *Paederia foetida* extract.

It shows that the plant extract administered to these groups as an intervention has affected the expression of Bcl-2 in cardiomyocytes, indicating a positive impact on decreasing apoptosis in streptozotocin-induced diabetic rats, as shown by the IRS scoring (Figure 2).

Interpretation of BAX Scoring

The IRS scores shown in Table 5 predicted the decreased expression of BAX in the group given 50 mg/kg *Paederia foetida* and the group given 100 mg/kg *Paederia foetida* as indicated by weak IRS scores that showed weak staining of cells with Bax as shown in Figure 3. It indicated the decreased expression of BAX in groups of rats treated with PF extract. It indicated that the 50 mg/kg PF extract given to groups of rats decreased the apoptosis in rats' cardiomyocytes as compared to 100 mg/kg PF as well as the diabetic control

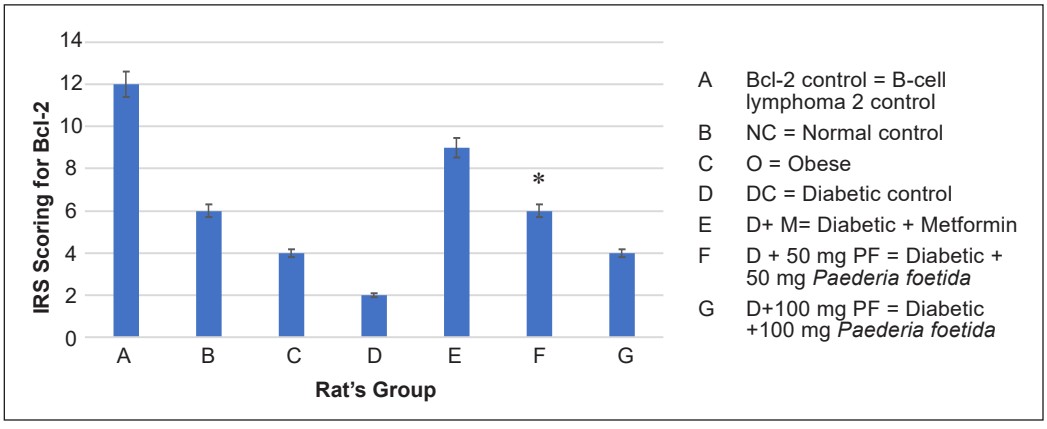

Figure 2. Immunoreactive scoring system (IRS) scoring for B-cell lymphoma 2 (Bcl-2) in rats' groups
Note. Group with * statistically significant ($p < 0.05$)

Table 5
Immunoreactive scoring system (IRS) scoring for B-cell lymphoma 2 (Bcl-2)-associated X protein BAX in different groups of rats

Groups		% Positive cells	Staining intensity	IRS scoring
BAX - Control		3	3	9
Group 1	Normal control	3	2	6
Group 2	Obese	3	3	9
Group 3	Diabetic control	3	4	12
Group 4	Diabetes + Metformin	2	3	6
Group 5	Diabetes + 50 mg PF	3	1	3
Group 6	Diabetes + 100 mg PF	2	2	4

Note: BAX control = Bcl-2 Associated X protein control

group, indicating that the treatment has a positive effect on decreasing the apoptosis in streptozotocin-induced diabetic rats (Figure 4).

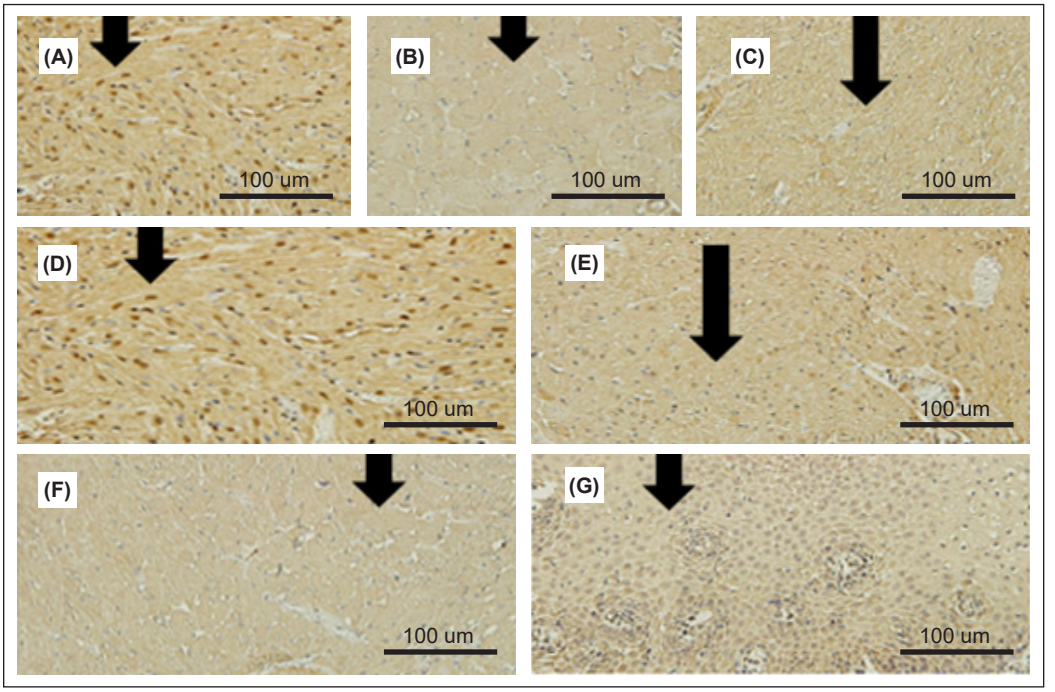

Figure 3. Representative photomicrograph of rat's cardiomyocytes with B-cell lymphoma 2 (Bcl-2)-associated X protein (BAX)
Note: (A) BAX-C = Cells of small figure (strong staining); (B) NC = Cells of small figure (weak staining); (C) O = Cells in small figure (strong staining); (D) DC = Cells in small figure (moderate staining); (E) D + M = Cells in small figure (moderate staining); (F) D + 50 mg PF = Cells of small figure (weak staining); (G) D + 100 mg PF = Cells of small figure (weak staining); Normal Control (NC); Obese (O); Diabetic control (DC); Diabetes + metformin (D + M); Diabetes + 50 mg *Paederia foetida* (D + 50 mg/kg PF); Diabetes + 100 mg *Paederia foetida* (D + 100 mg/kg PF)

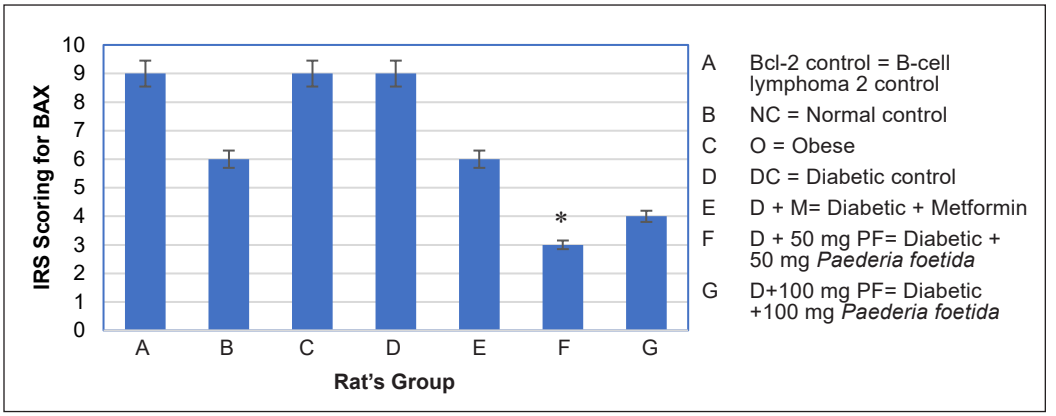

Figure 4. Immunoreactive scoring system (IRS) scoring for BAX in rats' groups
Note. Group with * considered significant ($p < 0.05$)

DISCUSSION

This research observed a slight rise in the level of blood sugar in normal control rats. In obese rats, the level of blood glucose was mildly raised. The metabolic dysfunction induced by a high-fat diet (HFD) in rats is a valuable model for studying the pathogenesis of type 2 diabetes (Heydemann, 2016). Obesity develops due to increased calorie intake (Pang et al., 2013). Obesity causes white adipose tissue to become resistant to the antilipolytic effect of insulin and the amount of non-esterified fatty acids (NEFA) to increase. As a result, the ability of fat cells to bind fat decreases, and NEFA increases insulin resistance further in liver, muscle, and pancreatic beta-cells, along with impaired insulin secretion (Bays et al., 2004). Most chronic sequelae, such as cardiac tissue in interstitial metabolism disorders patients, impaired cellular function, fibrosis in vascular smooth muscle, and diminished contractile activity, are first caused by hyperglycemia after the onset of the disease (Shokoohi et al., 2019). However, the mice model treated with metformin and varied concentrations of *Paederia foetida* extract in this study showed a marked decrease in the level of blood glucose. Similarly, previous research on the extract from *Paederia foetida* demonstrated that the plant has a range of therapeutic potential, including anti-inflammatory, antioxidant, and anti-diabetic properties (Kumar et al., 2014).

In the present study, the diabetic model was established by Sprague Dawley mice injected with streptozotocin. Standardized *Peaderia foetida* extract treatment ameliorated pathological features of diabetic cardiomyopathy, including cardiomyocyte damage caused by oxidative stress and myocardial apoptosis. In addition, the main positive effect of intervention with *Peaderia foetida* in the diabetic model is likely through increased expression of Bcl-2 by cardiomyocytes and subsequent regulation of the anti-apoptotic pathway. In addition, the phenolic component of *Paederia foetida* has antioxidant activity and functions as a scavenger of free radicals, which decreases oxidative stress in diabetic conditions. Modulating oxidation-related pathways could protect vital organs such as the heart from diabetes-induced pathology. The histological hallmark of diabetic cardiomyopathy is increased myocardial apoptosis, associated with increased heart weight, decreased ventricular compliance, and heart failure. The current study observed that *Paederia foetida* treatment significantly prevents myocardial apoptosis in streptozotocin-induced type 2 diabetes in Sprague Dawley rats.

Furthermore, the damage to the myocardium in diabetes mellitus is broadly accepted to be caused by oxidative stress (Cai et al., 2002). Consequentially, hyperglycemia increases oxidative stress via mitochondrial ROS generation, accelerating cardiomyocyte apoptosis and cellular DNA damage and decreasing cardiac contractility, ultimately resulting in myocardial fibrosis(Aragno et al., 2006). Apoptosis is a primary mechanism of cell death characterized by a set of processes

that initiate a chain of molecular events culminating in cell death (Zhou et al., 2018). Persistent hyperglycemia has been linked to increased levels of reactive oxygen species (ROS) and diminished antioxidant defense efficiency, both of which can trigger signaling cascades leading to cell death (Wang et al., 2014; Zhang et al., 2016). The Bcl-2 protein family significantly controls the mitochondrial pathway of apoptosis. Antioxidant pathways in the endoplasmic reticulum, extracellular membrane, nucleus membrane, and inner membrane of the mitochondria can be listed as sites wherein Bcl-2 is found (Aslan et al., 2023).

Paederia foetida treatment in this study ameliorates Bcl-2 levels in cardiomyocytes, which mitigates apoptosis associated with diabetic cardiomyopathy. In response to apoptotic stimuli, Bcl-2 promotes survival by preventing the release of mitochondrial cytochrome C (Lv et al., 2012). After apoptotic stimuli, the ratio of BAX to Bcl-2 determines cell survival or demise (Emamaullee et al., 2006; Evans et al., 2002). It has been shown that the elevation of BAX expression and downregulation of Bcl-2 promotes cell death (Aravani et al., 2020). The higher levels of BAX and caspase-8 and a reduction of Bcl-2 in diabetic rats were demonstrated by their significant *P* value ($p < 0.05$) and IRS score (Fullstone et al., 2020).In the present research, the IRS scores of groups treated with *Paederia foetida* extract predicted the reduced expression of BAX and caspase-8 in both groups, as indicated by feeble staining of cells in both groups with low IRS scores more with 50 mg/kg PF dose as compared to 100 mg/kg PF possibly by reducing the endothelial dysfunction and reducing the β-cell apoptosis in diabetic rats. It indicated that the 50 mg/kg PF extract administered to rats decreased cardiomyocyte apoptosis, indicating that the treatment had a positive effect on reducing apoptosis in STZ-induced rats. This corresponds to a study examining the effect of the methanolic extract of *Paederia foetida* on prostate cancer cells (Pavlou & Kirmizis, 2016).

CONCLUSION

This study suggests that *Paederia foetida* treatment ameliorates diabetes-associated-cardiomyopathy, as evidenced by its improvement of mitochondrial function via increasing Bcl-2 levels and decreasing BAX levels, which could subsequently inhibit myocardial apoptosis. This research demonstrated that standardized *Paederia foetida* extract could be a potential intervention for attenuating cardiomyopathy in diabetes mellitus.

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