Sains Malaysiana 53(6)(2024): 1321-1332
http://doi.org/10.17576/jsm-2024-5306-08

Determining the Time Points for the Development of Early and Advanced Stages of Diabetic Cardiomyopathy in Streptozotocin-Induced Type 1 Diabetes Mellitus Rat Model

(Menentukan Titik Masa untuk Pembentukan Tahap Awal dan Lanjutan Kardiomiopati Diabetes pada Model Tikus Diabetes Mellitus Jenis 1 Teraruh Streptozosin)

Fatin Farhana Jubaidi[1], Nur Liyana Mohammed Yusof[1], Satirah Zainalabidin[2], Izatus Shima Taib[1], Zariyantey Abd Hamid[1] & Siti Balkis Budin[1,*]

[1]Center for Diagnostic, Therapeutic and Investigative Studies, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia
[2]Center for Toxicology and Health Risk Studies, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia

Received: 9 February 2024/Accepted: 16 May 2024

ABSTRACT

The characteristics of early and advanced stages of diabetic cardiomyopathy (DCM) are well-understood; however, the time points by which these stages are developed in animal models vary and depend on the hyperglycaemic status and duration of diabetes. This study was aimed to determine the time points for the development of early and advanced stages of DCM from the induction of type 1 diabetes mellitus by identifying the functional and histological changes that occurred. Type 1 diabetes was induced via streptozotocin injection, and rats were divided into 4-week and 8-week diabetic groups. A group of non-diabetic rats served as the normal control. Cardiac functions and structural changes were analysed. Results showed that after four weeks, all diabetic rats displayed early DCM characteristics, including pronounced left ventricular diastolic dysfunction and cardiomyocyte hypertrophy ($P < 0.05$) compared to the normal control. After eight weeks, there was a significant deterioration in both left ventricular systolic and diastolic function compared to the normal control, along with marked cardiomyocyte hypertrophy and myocardial fibrosis ($P < 0.05$), signifying the development of advanced DCM. In summary, this findings revealed the development of early and advanced stages of DCM at four weeks and eight weeks of diabetes respectively in diabetes melitus type 1 rat model.

Keywords: Diastolic dysfunction; fibrosis; hypertrophy; systolic dysfunction; type 1 diabetes mellitus

ABSTRAK

Ciri-ciri peringkat awal dan lanjut kardiomiopati diabetes (DCM) telah dikenalpasti; walau bagaimanapun, titik masa di mana peringkat ini terbentuk di dalam model haiwan adalah berbeza dan bergantung kepada status hiperglisemia dan tempoh diabetes. Kajian ini bertujuan untuk menentukan titik masa pembentukan peringkat awal dan lanjut DCM daripada aruhan diabetes mellitus jenis 1 dengan mengenal pasti perubahan fungsi dan histologi yang berlaku. Diabetes jenis 1 telah diaruh secara suntikan streptozosin dan tikus dibahagikan kepada kumpulan diabetes 4 dan 8 minggu. Sementara itu, tikus bukan diabetes dijadikan kumpulan kawalan. Fungsi dan perubahan struktur jantung dianalisa. Hasil kajian menunjukkan pada empat minggu diabetes, semua tikus diabetes menunjukkan ciri-ciri awal DCM, termasuklah disfungsi diastolik ventrikel kiri dan hipertrofi kardiomiosit yang ketara ($P <0.05$) berbanding kumpulan normal. Selepas lapan minggu diabetes, terdapat kemerosotan yang ketara pada kedua-dua fungsi sistolik dan diastolik ventrikel kiri berbanding dengan kumpulan normal, dengan hipertrofi kardiomiosit dan fibrosis miokardium yang ketara ($P <0.05$), menunjukkan pembentukan DCM peringkat lanjut. Kesimpulannya, DCM peringkat awal berlaku pada empat minggu diabetes manakala DCM peringkat lanjut pula pada lapan minggu diabetes pada model tikus diabetes jenis 1.

Kata kunci: Diabetes melitus jenis 1; disfungsi diastolik; disfungsi sistolik; fibrosis; hipertrofi

Introduction

According to the World Health Organization (WHO), diabetes mellitus has been the direct cause of death to 1.5 million people in 2019 alone (WHO 2021). The prevalence of diabetes mellitus keeps rising at an unnerving rate, whereby it is predicted that 640 million people will be affected with diabetes mellitus by 2040 (IDF 2021). With respect to that, diabetes mellitus is responsible for the increasing risk of developing heart failure, which is not attributable to hypertension and coronary heart disease, known as diabetic cardiomyopathy (DCM) (Paolillo et al. 2019).

DCM is one of the perilous complications of diabetes mellitus that significantly affects the mortality rate of diabetes patients. This cardiac dysfunction condition presents with abnormal cardiac structure and function (Jia, Hill & Sowers 2018). The minimal criteria for a patient to be diagnosed with DCM include left ventricular diastolic dysfunction with/without reduced ejection fraction and the presence of pathological left ventricular hypertrophy and interstitial fibrosis resulting from compensatory and pathological remodelling of the heart and this is known as early stage of DCM (Lorenzo-Almorós et al. 2017). However, due to its silent nature, DCM is often overlooked in diabetic patients; therefore, it may progress into the advanced stage if left untreated. In the advanced stage, extensive hypertrophy and fibrosis may be observed in the cardiac tissue, with prominent dysfunction in both systolic and diastolic function and is associated with high morbidity (Aneja et al. 2008; Huo et al. 2023).

The development of DCM involved left ventricular hypertrophy and fibrosis, which resulted from an adaptive response to increased hemodynamic stress, reduced cardiomyocyte population and neurohormonal activation (Ritchie & Abel 2020). Reduction in the cardiomyocyte population mainly results from diabetes-induced oxidative damage and apoptosis. Consequently, this puts the burden of generating sufficient cardiac contractility on the remaining viable cardiomyocytes to meet the body's demand, thus resulting in the hypertrophy of the viable cells (Nakamura & Sadoshima 2018). The deposition of fibrotic tissue in the extracellular matrix space is to replace the dead cardiomyocytes, and the stiff nature of the collagen tissue causes the heart to lose its contractility and flexibility, thus, resulting in reduced cardiac function (Frangogiannis 2014; Wang et al. 2021).

According to Gulsin, Athithan and McCann (2019), the glycemic status and duration of diabetes determine the development rate and progression of DCM towards the advanced stage. The type 1 diabetes mellitus model is among the popular models used to study DCM (Gliozzi et al. 2020; Liang et al. 2021; Liu et al. 2020; Shaher et al. 2020; Wang et al. 2020). However, the time point by which features of early stage and advanced stage of DCM developed after induction of diabetes with streptozotocin (STZ) varies and seems to depend on the dose of STZ used. On top of that, different amounts of STZ used to induce diabetes may impact the severity and development rate of DCM (Luo et al. 2020; Oh et al. 2019; Youssef, Abdelrazek & Moustafa 2021). Commencement of treatment at the early stage of DCM is believed to limit its progression towards the irreversible later stage (Marcinkiewicz, Ostrowski & Drzewoski 2017). Knowing the time points is important because it will assist in determining when treatment for DCM should be started, as it would be less effective when given at the advanced stage. Hence, this study sought to determine the time points for the development of early and advanced stages of DCM from the induction of type 1 diabetes mellitus by identifying the presence of functional and histological characteristics in each DCM stage.

Materials and Methods

Animals

All activities involving the use of animals for research were approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC). Sprague-Dawley male rats were sourced from the Animal Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia. All rats had free access to food and water and were housed at ambient temperature on a 12-hour light/dark cycle. All rats were allowed food and drinks *ad libitum*. All animal designs and protocols were approved by the UKM Ethical Committee (ethical approval number: FSK/2020/SITI BALKIS/25-NOV./ 1120-DEC.-2020-DEC.-2022). All animal care guidelines during the experiment strictly followed the Malaysian Code of Practice for The Care and Use of Animals for Scientific Purposes (Animal Welfare Board 2019), which was set by the Department of Veterinary Services Malaysia (MyCode 2019). The sample size was calculated according to the calculation formula by Wan Nor Arifin and Wan Mohammad Zahiruddin (2017), allocating seven animals per group.

Experimental Design

Male Sprague-Dawley rats weighing 230-250 g received a single intraperitoneal injection STZ (55 mg/kg, in 0.1

mol/l citrate buffer, pH 4.5). After three days of STZ administration, diabetes mellitus was confirmed by measuring the fasting blood glucose using a commercial glucometer (Accu-Chek, Roche). Rats with glucose levels above 15 mmol/l were considered diabetic (Mohammed Yusof et al. 2020). Non-diabetic rats were randomly allocated to citrate buffer vehicles. Two sets of diabetic and non-diabetic groups were set up, one for four weeks and eight weeks of untreated period, to allow the development of DCM. The untreated durations were selected based on our previous findings (Mohammed Yusof et al. 2018, 2020). Each experimental group consisted of 7 rats.

ISOLATED LANGENDORFF-PERFUSED RAT HEARTS

At the end of each study duration, rats were anaesthetised with ketamine/xylazine cocktail (60/12 mg/kg, intraperitoneally) and administered heparin (500 IU, intraperitoneally) to prevent blood clotting (Mohammed Yusof et al. 2018). Upon loss of pedal reflex activity, thoracic surgery was performed, and the heart was rapidly extracted (Lateef, Al-Masri & Alyahya 2015). Euthanasia is achieved by exsanguination during the harvesting of the heart. The isolated heart was then placed in an ice-cold perfusion buffer and immediately cannulated to the Langendorff apparatus (Langendorff isolated heart system, ADInstruments, Australia) via the aorta. The hearts were perfused with Krebs–Henseleit buffer (in mmol/L: NaCl 118.0; KCl 3.2; $MgSO_4$ 1.2; $NaHCO_3$ 25.0; NaH_2PO_4 1.18; $CaCl_2$ 2.5; glucose 11.1) at constant pressure (48 mm Hg) and 37 °C (Lim et al. 2017). A latex balloon was connected to a pressure transducer to obtain intraventricular pressure changes and inserted into the left ventricle through the mitral valve. The balloon volume was filled with water to maintain a left ventricular end-diastolic pressure equivalent to 5–7 mmHg. After 20 min of stabilisation, cardiac function parameters such as left ventricular developed pressure (LVDP), left ventricular maximum rate of pressure development (+dP/dt), left ventricular maximum rate of pressure decrease (-dP/dt), as well as the time constant of isovolumic relaxation (Tau) were assessed using a PowerLab data acquisition system and analysed using chart software (LabChart 7.0, ADInstrument, Australia).

HISTOLOGICAL ANALYSIS

The formalin-preserved cardiac section was embedded in paraffin and cut into tissue slices of 3–5µm thick. The tissue sections were stained with hematoxylin and eosin to analyse cardiomyocyte size and with picrosirius red staining to analyse myocardial fibrosis. Quantitative assessments, including cardiomyocyte size and percentage of fibrotic area, were analysed using the batch mode of the ImageJ macro with 100 randomly chosen high-power fields in each heart (Mohammed Yusof et al. 2018; Tatsuguchi et al. 2007).

STATISTICAL ANALYSIS

Statistical analysis was done using GraphPad Prism software (GraphPad Software 8.0, San Diego, California, USA). Data were presented as arithmetic mean ± SEM. Parametric tests ANOVA (Two-way ANOVA) followed by post hoc Tukey's test were used to confirm the significant difference between groups. A value of $P < 0.05$ was considered statistically significant.

RESULTS

CHARACTERISATION OF DIABETIC PHENOTYPES

An increase in blood glucose and significant weight loss characterises T1DM in patients. In this study, rats administered with STZ were shown to consistently lose weight compared to non-diabetic rats, who had gained weight consistently throughout the experimental period (Table 1). Blood glucose levels were significantly elevated in diabetic rats at weeks 4 and 8, exhibiting the critical characteristic of diabetes mellitus.

TABLE 1. Effects of STZ-induced T1DM on fasting blood glucose and body weight

Group		Week 4		Week 8	
		NDM	DM	NDM	DM
Fasting blood glucose (FBG)		5.767±0.142	23.730±2.067$^{\#}$	6.050±0.200	27.870±2.923$^{\&}$
Body Weight (g)	Week 1	225.70±12.96	228.70±9.07	222.80±14.85	232.2±24.16
	Week 4	244.0±9.17	195.80±6.42$^{\#}$	276.3±16.63	186.0±15.38$^{\&}$
	Week 8			305.30±12.66	177.10±14.97$^{\&}$

Data was analysed using Two-way ANOVA, n=7. $^{\#}P < 0.05$ compared to NDM (Week 4), $^{\&}P < 0.05$ compared to NDM (Week 8)

EFFECTS OF DIABETES ON DIASTOLIC FUNCTION

Cardiac function in experimental rats was analysed using Langendorff's isolated heart perfusion; data is presented in Figure 1. Diastolic function is measured by assessing the left ventricular maximum rate of pressure decrease (-dP/dt), which represents the ability of the ventricular wall to fully relax at the end of the heart's diastolic cycle. Our findings showed that -dp/dt is significantly reduced ($P < 0.05$) in the 4-week untreated diabetes group. In the 8-week diabetes group, the diastolic dysfunction was further aggravated, as shown by the significantly reduced -dP/dt ($P < 0.05$) compared to the 4-week diabetes group. The time taken for the left ventricle to relax fully, presented as the diastolic relaxation time (tau), was also prolonged significantly in both four-week and 8-week diabetes groups.

EFFECTS OF DIABETES ON SYSTOLIC FUNCTION

Results showed that left ventricular systolic function in the four weeks diabetes group was not affected by diabetes condition as presented by the no significant difference in left ventricular maximum rate of pressure development (+dP/dt) compared to the normal group

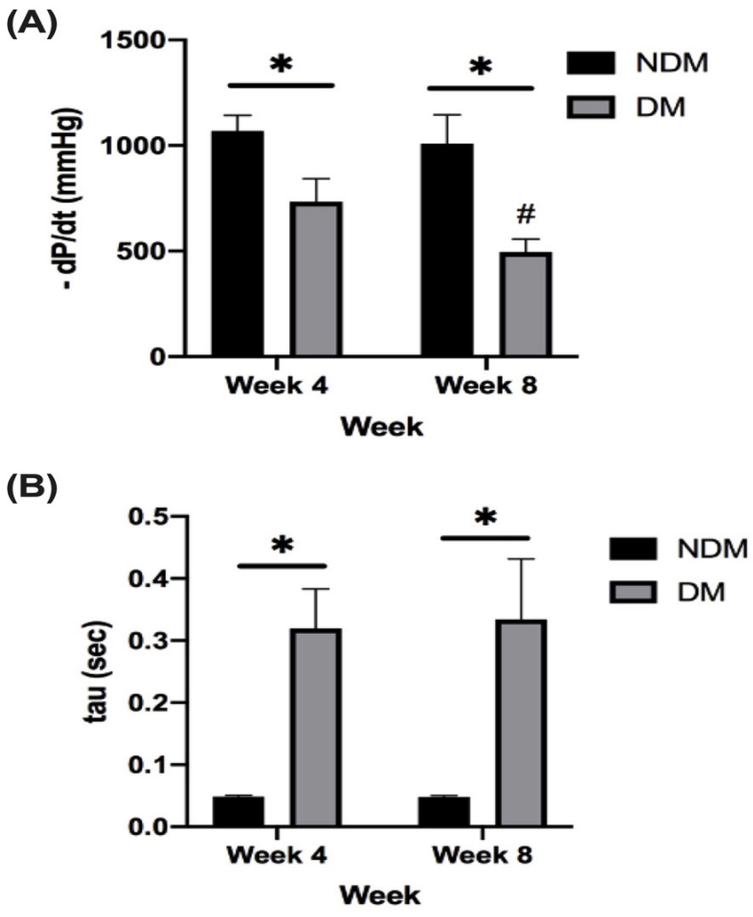

FIGURE 1. Effects of STZ-induced T1DM on (A) lleft ventricular maximum rate of pressure decrease (-dP/dt), (B) diastolic relaxation time (tau). Data analysed using Two-way ANOVA, n=7.

*$P < 0.05$, #$P < 0.05$ compared to week 4 DM

(Figure 2). In contrast, eight weeks of diabetes duration further exacerbated the systolic function, as shown by a significant reduction in the +dP/dt ($P < 0.05$), compared to normal control and four weeks diabetes groups. Meanwhile, left ventricular developed pressure (LVDP) was significantly reduced ($P < 0.05$) at 4 weeks and 8 weeks diabetes groups as compared to respective normal controls. The LVDP was also further decreased as the untreated diabetes period was prolonged to 8 weeks compared to the 4-week diabetic group.

CHARACTERISATION OF CARDIAC STRUCTURAL CHANGES

Cardiomyocyte hypertrophy and increased fibrosis are the typical structural hallmarks of diabetic hearts. In this study, we found a significant increase in cardiomyocyte size in both 4 and 8 weeks diabetes groups compared to their respective normal controls ($P < 0.05$) (Figure 3). In contrast, a marked increase in collagen deposition was observed in the 4-week diabetes group (Figure 4). However, in the 8-week diabetes group, collagen deposition was markedly increased ($P < 0.05$), which showed a significant cardiac remodelling process.

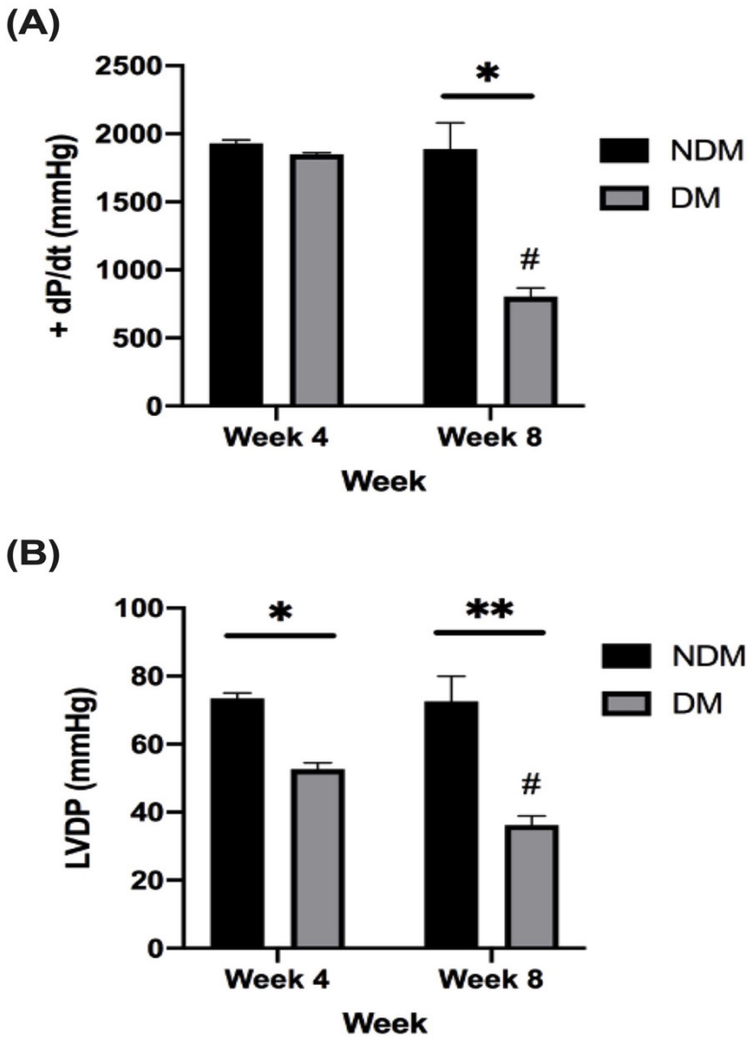

FIGURE 2. Effects of STZ-induced T1DM on (A) left ventricular maximum rate of pressure development (+dP/dt), (B) left ventricular developed pressure (LVDP). Data was analysed using Two-way ANOVA, n=7

*$P < 0.05$, **$P < 0.01$ compared to NDM; #$P < 0.05$ compared to week 4 DM

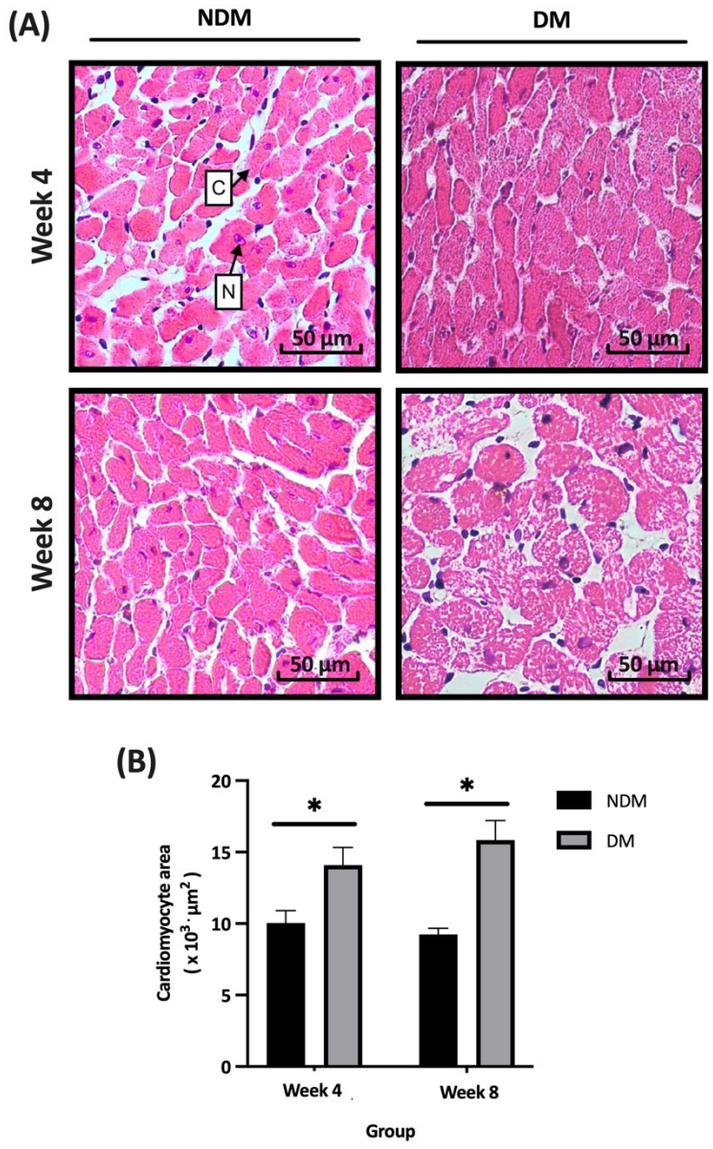

FIGURE 3. Effects of STZ-induced T1DM on cardiomyocyte size. (A) histological image of cardiomyocytes in hematoxylin and eosin stain, magnification ×400; and (B) cardiomyocyte area. Data was analysed using Two-way ANOVA, n=7

*P <0.05 compared to NDM, #P < 0.05 compared to Week 4 DM. C: Cardiomyocyte; N: Nucleus

DISCUSSION

DCM is no doubt a silent killer among diabetic patients as its progression is notoriously asymptomatic and only detectable when it already reached the irreversible advanced stage (Paolillo et al. 2019). Therefore, without proper treatment and management, DCM could rapidly regress towards heart failure. It has been suggested that intervening in the development of DCM at its early stage may limit its progression towards the advanced stage and subsequent heart failure (Marcinkiewicz,

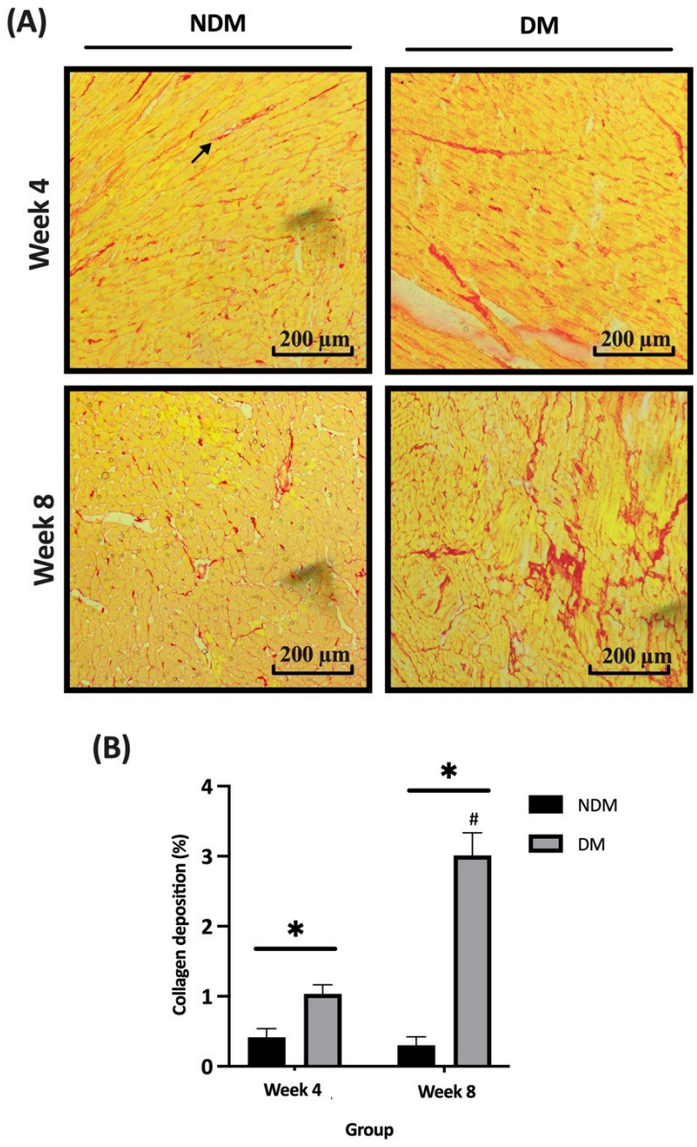

FIGURE 4. Effects of STZ-induced on collagen deposition (A) histological image using picrosirius red staining, magnification ×100. Data was analysed using Two-way ANOVA, n=7.

*P <0.05, #P < 0.05 compared to Week 4 DM. Arrow shows collagen deposition stained in red

Ostrowski & Drzewoski 2017). As hyperglycemia plays an essential role in the pathogenesis of this condition, glycaemic management hence plays a significant role in controlling DCM progression. Indeed, findings by Tate, Grieve and Ritchie (2017) supported this notion, whereby hyperglycaemic control by insulin replacement therapy given to type 1 DM rats that featured the early stage of DCM was able to limit the progression of DCM from worsening towards the advanced stage. This finding serves as an important foundation for future intervention

studies aiming to treat and limit the progression of DCM. Past studies have utilised various experimental models to study the development and mechanisms underlying the pathogenesis of DCM. STZ-induced type 1 DM is among the most common DM models for DCM studies (Soetikno et al. 2012; Tate et al. 2017). The similarities in diabetes signs and symptoms STZ-induced rats share with human make them one of the best animal strains to be utilised in studying diabetes-induced complications such as DCM (Wei et al. 2003). In addition, Sprague-Dawley rats are docile and easy to handle, which facilitates experimental procedures and reduces stress-related confounders. Their adaptability to laboratory environments also minimises variability in experimental outcomes. It is essential to design a type 1 DCM rat model that distinguishes the time elapsed from the induction of DM with STZ to the time the rats start to exhibit characteristics of DCM. In this study, we investigated whether the features of early and advanced stages of DCM can be identified after 4 and 8 weeks post-induction with STZ, respectively, based on the structural and functional features of each stages as seen in clinical settings (Huynh et al. 2014; Paolillo et al. 2019).

Evidence shows that different doses of STZ may influence hyperglycaemic status and the development and progression rate of DCM in rat models. Administration of higher doses of STZ (higher than 40 mg/kg, intraperitoneally) seems to induce more severe pancreatic β cell damage and, therefore, cause severe hyperglycaemic status that is long and stable without fluctuation in blood glucose level (Mostafavinia et al. 2016). On the other hand, administration of STZ at lower doses (30-40 mg/kg, intraperitoneally) induces temporary hyperglycaemia that may revert the diabetic condition to normal. The severity of hyperglycaemia indeed influences the experimental model's mortality rate and diabetic complications development (Mostafavinia et al. 2016). There is a consistent pattern whereby studies that utilised lower doses of STZ may span more than 16 weeks, and studies using higher doses typically ran for 12 weeks at most (Gliozzi et al. 2020; Shaher et al. 2020; Wang et al. 2020). Hyperglycaemia is known to play a vital role in the pathogenesis of DCM, whereby it, directly and indirectly, causes damage to the cardiomyocytes, fibroblasts, and endothelial cells via the accumulation of reactive oxygen species and over-activation of various pathogenetic pathways (Ansley & Wang 2013). Hence, it is important to note that the development of DCM is very much dependent on the glycaemic status and duration of diabetes (Gulsin, Athithan & McCann 2019).

In this study, we induced type 1 DM by administering 55 mg/kg of STZ intraperitoneally as previously performed (Mohammed Yusof et al. 2018). We found that after 4 weeks of untreated diabetes, the diabetic hearts exhibited prominent diastolic dysfunction as evidenced by reduced by reduced -dP/dt and LVDP. In addition, increased in tau, the time relaxation constant, showed that the diabetic hearts took longer time for the heart to relax thus exhibiting a feature of diastolic dysfunction fully (Chen et al. 2021). Hoit et al. (1999) also reported that diastolic dysfunction was detected after 4 weeks of diabetes induction with STZ at a slightly higher dose (65 mg/kg intraperitoneally). However, a study using a lower dose of STZ (45 mg/kg, intraperitoneally) reported that diastolic dysfunction only appears after 12 weeks of diabetes (Akula 2003). On the other hand, studies that used a higher STZ dose (70 mg/kg, intraperitoneally) reported significant alteration in both diastolic and systolic functions after 4 weeks of diabetes induction (Luo et al. 2020; Oh et al. 2019). Becher et al. (2013) also reported that 2 weeks after induction of diabetes with STZ at 70 mg/kg intraperitoneally, both diastolic and systolic function were significantly reduced. This further confirms that differences in STZ doses may impact the development rate of DCM, as shown in the different time points at which the diastolic dysfunction appeared.

This finding is supported by the histological observation by which cardiac remodelling induced by diabetes mellitus was assessed. As evidenced, prominent left ventricular hypertrophy and moderate interstitial collagen deposition were observed in the diabetic hearts after 4 weeks. In DCM, left ventricular hypertrophy developed as an adaptive response to increase the hemodynamic pressure loss from the reduced diastolic function, as well as to compensate for the reduced viable cardiomyocyte population (Mohan et al. 2021). Extensive cardiomyocyte death results from oxidative damage and apoptosis induced by chronic hyperglycaemia, leaving the burden to work on the viable cardiac cells and causing them to undergo hypertrophy. In addition, chronic hyperglycaemia itself was able to activate several mechanistic pathways that directly induce the enlargement of the cardiomyocytes (Salvatore et al. 2021; Wang et al. 2019). Activation of PKC/MAPK and the downstream pathways is suggested to enhance cardiac hypertrophy, whereby activation of p38-MAPK and c-Jun N-terminal kinase (JNK) pathways dull the cell survival effect of ERK1/2 by increasing the Bax/Bcl-2 apoptotic ratio, thus further aggravates cardiac hypertrophy (Liu

et al. 2021; Wang et al. 2016; Xia et al. 2007; Xu et al. 2016).

Interestingly, after 4 weeks of untreated diabetes, myocardial fibrosis was not significantly observed compared to normal rats. This finding was supported by Marchini et al. (2020), whereby they reported a similar observation at 3 weeks of untreated diabetes. This shows that the DCM condition has not yet progressed into the advanced stage by week 4 post induction of diabetes with STZ at 55 mg/kg intraperitoneally. However, it important to note that a previous study that used a higher dose of STZ (70 mg/kg) reported severe myocardial fibrosis along with cardiac dysfunction and hypertrophy by week 4 (Luo et al. 2020; Moral-Sanz et al. 2012; Oh et al. 2019). This indicates that dose is indeed an important factor that should be taken into account when inducing an early stage of DCM, aside from the duration of diabetes.

As described by Paolillo et al. (2019), the early stage of DCM is characterised by impaired diastolic function, and the appearance of systolic function signifies its entrance into the advanced stage. Indeed, DCM progresses and worsens with time. In this study, in addition to observing the early DCM features in 4 weeks of untreated diabetic rats, we also studied whether a longer untreated diabetes period to a total of 8 weeks would regress the DCM into the advanced stage. The advanced, late stage of DCM is characterised by cardiac fibrosis, further impairment of diastolic function and the appearance of systolic dysfunction (Paolillo et al. 2019). With the progression of DCM, diastolic dysfunction and reduced cardiac compliance typically coexist with systolic dysfunction, leading to reduced ejection fraction, prolonged pre-ejection performance, shortened ejection period and increased resistance to filling with increased filling pressure (Jia, Hill & Sowers 2018). In this study, we observed that after 8 weeks of untreated diabetes, the diabetic hearts exhibit not only prominent diastolic dysfunction but also accompanied by systolic dysfunction evidenced by significantly reduced contractility rate of the heart with reduced left ventricular developed pressure. Hoit et al. (1999), which used a higher dose of STZ as compared to the present study, reported that systolic dysfunction was manifested after 6 weeks of diabetes induction with STZ at 65 mg/kg intraperitoneally. In contrast, our finding exhibited that systolic dysfunction was developed after 8 weeks of diabetes induction. The difference in development rate is probably due to the difference in hyperglycemia severity considering the difference in the STZ dose used. Tate et al. (2017) and Wang et al. (2020) both used STZ dose at 55 mg/kg, also reported that diastolic and systolic dysfunctions can be observed after 8 weeks of untreated diabetes.

Cardiac remodelling was even more extensively observed in 8 weeks of untreated diabetic rats. This is manifested by prominent cardiomyocyte hypertrophy and myocardial fibrosis. According to Jia, Hill and Sowers (2018), alterations in the myocardial structure directly affect cardiac function, which is why cardiac contractility and relaxation were both significantly affected, as seen in the present study. Our findings are also in line with previous studies reporting that cardiac fibrosis and hypertrophy were observable after 8 weeks of untreated diabetes (Alomar et al. 2020; Wang et al. 2020). However, by using a higher STZ dose (70 mg/kg), Becher et al. (2013) showed that cardiac hypertrophy and fibrosis can be seen only after 2 weeks of diabetes induction. Development of the DCM model by using high doses of STZ may certainly take a shorter time for the cardiac complications to develop. However, it leaves very little therapeutic window for the introduction of treatment or intervention as the DCM progresses very rapidly towards the irreversible advanced stage.

Diastolic dysfunction was exhibited by the reduction in -dP/dt and is related to the changes in LVDP. Reduced LVDP at 4 weeks of untreated diabetes unveiled reduced wall compliance, whereby the heart was unable to fully relax due to reduced cardiac relaxation function as exhibited by the reduced -dP/dt (Silbiger 2019). The reduced ability of the heart to fully relax causes a reduction in both chamber size and pressure at the end-diastolic phase, which may affect the passive flow of blood from the left atrium. It was also demonstrated in this study that 8 weeks of untreated diabetes showed a further reduction in LVDP. The more extensive cardiac remodelling activities at this time point, as shown by the increase in cardiac hypertrophy and fibrosis, lead to the inefficiency of left ventricular relaxation and contraction function. This shows that the left ventricular wall has become stiffer as it loses its ability to contract and relax fully, thus reducing LVDP and may affect the cardiac output as a consequence (Luo et al. 2020).

Based on the results, we found that the early stage of DCM was fully established after 4 weeks of untreated diabetes condition, while the advanced stage of DCM developed after 8 weeks. Correspondingly, any treatment of interest aiming to limit the progression or treating DCM

condition is to be commenced optimally before the DCM condition progresses into the later irreversible advanced stage in order to guarantee the treatment is given within the effective window (Marcinkiewicz, Ostrowski & Drzewoski 2017). The development of DCM should be prevented as early as possible. Using our proposed DCM model, treatment should be given before the condition enters the advanced stage, which is by the eighth-week post-induction of diabetes. Induction of DCM at higher doses not only increases the mortality rate, but the DCM progresses very rapidly to exert an effective treatment. Meanwhile, the development rate of DCM using a low STZ dose is rather slow and may unnecessarily lengthen the study period aside from the risk of reverting back to normal conditions.

Conclusion

It is important to note that the dose of STZ chosen to induce DCM is an important factor in the development rate of DCM, mainly due to the severity of hyperglycaemia depending on the dosage used. Therefore, it is important to choose the most appropriate dose of STZ to induce type 1 DM cardiomyopathy model accordingly. The dose used in this study allows the early stage and the advanced stage of DCM to be developed at 4 weeks and 8 weeks after diabetes induction, respectively. Therefore, any treatment to limit the progression of DCM should be given before the development of the advanced stage takes place.

ACKNOWLEDGEMENTS

This research was fully funded by the Kementerian Pengajian Tinggi (KPT), Malaysia under Fundamental Research Grant Scheme (FRGS/1/ 2019/STG03/ UKM/01/3).

REFERENCES

Akula, A. 2003. Biochemical, histological and echocardiographic changes during experimental cardiomyopathy in STZ-induced diabetic rats. *Pharmacological Research* 48(5): 429-435.

Alomar, F.A., Al-Rubaish, A., Al-Muhanna, F., Al-Ali, A.K., McMillan, J., Singh, J. & Bidasee, K.R. 2020. Adeno-associated viral transfer of glyoxalase-1 blunts carbonyl and oxidative stresses in hearts of type 1 diabetic rats. *Antioxidants* 9(7): 592.

Aneja, A., Tang, W.H.W., Bansilal, S., Garcia, M.J. & Farkouh, M.E. 2008. Diabetic cardiomyopathy: Insights into pathogenesis, diagnostic challenges, and therapeutic options. *The American Journal of Medicine* 121(9): 748-757.

Animal Welfare Board. 2019. *Animal Welfare Board Mycode for the Care and Use of Animals for Scientific Purposes Malaysian Code of Practice for the Care and Use of Animals for Scientific Purposes* (MyCode). Edisi ke-2. Putrajaya: Department of Veterinary Services Malaysia.

Ansley, D.M. & Wang, B. 2013. Oxidative stress and myocardial injury in the diabetic heart. *The Journal of Pathology* 229(2): 232-241.

Becher, P.M., Lindner, D., Frölich, M., Savvatis, K., Westermann, D. & Tschöpe, C. 2013. Assessment of cardiac inflammation and remodeling during the development of streptozotocin-induced diabetic cardiomyopathy *in vivo*: A time course analysis. *International Journal of Molecular Medicine* 32(1): 158-164.

Chen, X., Ashraf, S., Ashraf, N. & Harmancey, R. 2021. UCP3 (Uncoupling Protein 3) insufficiency exacerbates left ventricular diastolic dysfunction during angiotensin II-induced hypertension. *Journal of the American Heart Association* 10(18): e022556.

Frangogiannis, N.G. 2014. The inflammatory response in myocardial injury, repair, and remodelling. *Nature Reviews Cardiology* 11(5): 255-265.

Gliozzi, M., Scarano, F., Musolino, V., Carresi, C., Scicchitano, M., Ruga, S., Zito, M.C., Nucera, S., Bosco, F., Maiuolo, J., Macrì, R., Guarnieri, L., Mollace, R., Coppoletta, A.R., Nicita, C., Tavernese, A., Palma, E., Muscoli, C. & Mollace, V. 2020. Role of TSPO/VDAC1 upregulation and matrix metalloproteinase-2 localization in the dysfunctional myocardium of hyperglycaemic rats. *International Journal of Molecular Sciences* 21(20): 7432.

Gulsin, G.S., Athithan, L. & McCann, G.P. 2019. Diabetic cardiomyopathy: Prevalence, determinants and potential treatments. *Therapeutic Advances in Endocrinology and Metabolism* 10: 204201881983486

Hoit, B.D., Castro, C., Bultron, G., Knight, S. & Matlib, M.A. 1999. Noninvasive evaluation of cardiac dysfunction by echocardiography in streptozotocin-induced diabetic rats. *Journal of Cardiac Failure* 5(4): 324-333.

Huo, J-L., Feng, Q., Pan, S., Fu, W-J., Liu, Z. & Liu, Z. 2023. Diabetic cardiomyopathy: Early diagnostic biomarkers, pathogenetic mechanisms, and therapeutic interventions. *Cell Death Discovery* 9(1): 256.

Huynh, K., Bernardo, B.C., McMullen, J.R. & Ritchie, R.H. 2014. Diabetic cardiomyopathy: Mechanisms and new treatment strategies targeting antioxidant signaling pathways. *Pharmacology and Therapeutics* 142(3): 375-415.

International Diabetes Federation (IDF). 2021. *IDF Diabetes Atlas*. 10th ed. Brussel, Belgium: International Diabetes Federation. www.diabetesatlas.org.

Jia, G., Hill, M.A. & Sowers, J.R. 2018. Diabetic cardiomyopathy: An update of mechanisms contributing to this clinical entity. *Circulation Research* 122(4): 624-638.

Lateef, R., Al-Masri, A. & Alyahya, A. 2015. Langendorff's isolated perfused rat heart technique: A review. *International Journal of Basic and Clinical Pharmacology* 4(6): 1314-1322.

Liang, R., Zhao, Y., Shi, M., Zhang, G., Zhao, Y., Zhang, B. & Liang, R. 2021. Skimmin protects diabetic cardiomyopathy in streptozotocin-induced diabetic rats. *The Kaohsiung Journal of Medical Sciences* 37(2): 136-144.

Lim, Y.C., Budin, S.B., Othman, F., Latip, J. & Zainalabidin, S. 2017. Roselle polyphenols exert potent negative inotropic effects via modulation of intracellular calcium regulatory channels in isolated rat heart. *Cardiovascular Toxicology* 17(3): 251-259.

Liu, X., Guo, B., Zhang, W., Ma, B. & Li, Y. 2021. MiR-20a-5p overexpression prevented diabetic cardiomyopathy via inhibition of cardiomyocyte apoptosis, hypertrophy, fibrosis and JNK/NF-κB signalling pathway. *The Journal of Biochemistry* 170(3): 349-362.

Liu, X., Song, F., Liu, C. & Zhang, Y. 2020. 25OHPPD inhibits hypertrophy on diabetic cardiomyopathy via the PI3k/Akt/GSK3β signaling pathway. *Experimental and Therapeutic Medicine* 20(3): 2141-2147.

Lorenzo-Almorós, A., Tuñón, J., Orejas, M., Cortés, M., Egido, J. & Lorenzo, Ó. 2017. Diagnostic approaches for diabetic cardiomyopathy. *Cardiovascular Diabetology* 16: 28.

Luo, J., Yan, D., Li, S., Liu, S., Zeng, F., Cheung, C.W., Liu, H., Irwin, M.G., Huang, H. & Xia, Z. 2020. Allopurinol reduces oxidative stress and activates Nrf2/p62 to attenuate diabetic cardiomyopathy in rats. *Journal of Cellular and Molecular Medicine* 24(2): 1760-1773.

Marchini, G.S., Cestari, I.N., Salemi, V.M.C., Irigoyen, M.C., Arnold, A., Kakoi, A., Rocon, C., Aiello, V.D. & Cestari, I.A. 2020. Early changes in myocyte contractility and cardiac function in streptozotocin-induced type 1 diabetes in rats. *PLoS ONE* 15(8): e0237305.

Marcinkiewicz, A., Ostrowski, S. & Drzewoski, J. 2017. Can the onset of heart failure be delayed by treating diabetic cardiomyopathy? *Diabetology & Metabolic Syndrome* 9(1): 21.

Mohammed Yusof, N.L., Tengku Affendi, T.N.T., Jubaidi, F.F., Zainalabidin, S. & Budin, S.B. 2020. *Hibiscus sabdariffa* Linn. (Roselle) polyphenols-rich extract prevents hyperglycemia-induced cardiac oxidative stress and mitochondrial damage in diabetic rats. *Sains Malaysiana* 49(10): 2499-2506.

Mohammed Yusof, N.L., Zainalabidin, S., Mohd Fauzi, N. & Budin, S.B. 2018. *Hibiscus sabdariffa* (Roselle) polyphenol-rich extract averts cardiac functional and structural abnormalities in type 1 diabetic rats. *Applied Physiology, Nutrition and Metabolism* 43(12): 1224-1232.

Mohan, M., Dihoum, A., Mordi, I.R., Choy, A.M., Rena, G. & Lang, C.C. 2021. Left ventricular hypertrophy in diabetic cardiomyopathy: A target for intervention. *Frontiers in Cardiovascular Medicine* 8: 746382.

Moral-Sanz, J., Lopez-Lopez, J.G., Menendez, C., Moreno, E., Barreira, B., Morales-Cano, D., Escolano, L., Fernandez-Segoviano, P., Villamor, E., Cogolludo, A., Perez-Vizcaino, F. & Moreno, L. 2012. Different patterns of pulmonary vascular disease induced by type 1 diabetes and moderate hypoxia in rats. *Experimental Physiology* 97(5): 676-686.

Mostafavinia, A., Amini, A., Ghorishi, S.K., Pouriran, R. & Bayat, M. 2016. The effects of dosage and the routes of administrations of streptozotocin and alloxan on induction rate of type1 diabetes mellitus and mortality rate in rats. *Laboratory Animal Research* 32(3): 160.

Nakamura, M. & Sadoshima, J. 2018. Mechanisms of physiological and pathological cardiac hypertrophy. *Nature Reviews Cardiology* 15(7): 387-407.

Oh, J.E., Jun, J.H., Hwang, H.J., Shin, E.J., Oh, Y.J. & Choi, Y.S. 2019. Dexmedetomidine restores autophagy and cardiac dysfunction in rats with streptozotocin-induced diabetes mellitus. *Acta Diabetologica* 56(1): 105-114.

Paolillo, S., Marsico, F., Prastaro, M., Renga, F., Esposito, L., De Martino, F., Di Napoli, P., Esposito, I., Ambrosio, A., Ianniruberto, M., Mennella, R., Paolillo, R. & Gargiulo, P. 2019. Diabetic cardiomyopathy: Definition, diagnosis, and therapeutic implications. Heart Failure Clinics 15(3): 341-347.

Ritchie, R.H. & Abel, E.D. 2020. Basic mechanisms of diabetic heart disease. *Circulation Research* 126(11): 1501-1525.

Salvatore, T., Pafundi, P.C., Galiero, R., Albanese, G., Di Martino, A., Caturano, A., Vetrano, E., Rinaldi, L. & Sasso, F.C. 2021. The diabetic cardiomyopathy: The contributing pathophysiological mechanisms. *Frontiers in Medicine* 8: 695792.

Shaher, F., Wang, S., Qiu, H., Hu, Y., Zhang, Y., Wang, W., AL-Ward, H., Abdulghani, M.A.M., Baldi, S. & Zhou, S. 2020. Effect and mechanism of *Ganoderma lucidum* spores on alleviation of diabetic cardiomyopathy in a pilo**t** *in vivo* **study.** *Diabetes, Metabolic Syndrome and Obesity* 13: 4809-4822.

Silbiger, J.J. 2019. Pathophysiology and echocardiographic diagnosis of left ventricular diastolic dysfunction. *Journal of the American Society of Echocardiography* 32(2): 216-232.e2.

Soetikno, V., Sari, F.R., Sukumaran, V., Lakshmanan, A.P., Mito, S., Harima, M., Thandavarayan, R.A. Suzuki, K., Nagata, M., Takagi, R. & Watanabe, K. 2012. Curcumin prevents diabetic cardiomyopathy in streptozotocin-induced diabetic rats: Possible involvement of PKC–MAPK signaling pathway. *European Journal of Pharmaceutical Sciences* 47(3): 604-614.

Tate, M., Deo, M., Cao, A.H., Hood, S.G., Huynh, K., Kiriazis, H., Du, X.J., Julius, T.L., Figtree, G.A., Dusting, G.J., Kaye, D.M. & Ritchie, R.H. 2017. Insulin replacement limits progression of diabetic cardiomyopathy in the low-dose streptozotocin-induced diabetic rat. *Diabetes and Vascular Disease Research* 14(5): 423-433.

Tate, M., Grieve, D.J. & Ritchie, R.H. 2017. Are targeted therapies for diabetic cardiomyopathy on the horizon? *Clinical Science* 131(10): 897-915.

Tatsuguchi, M., Seok, H.Y., Callis, T.E., Thomson, J.M., Chen, J-F., Newman, M., Rojas, M., Hammond, S.M. & Wang, D-Z. 2007. Expression of microRNAs is dynamically regulated during cardiomyocyte hypertrophy. *Journal of Molecular and Cellular Cardiology* 42(6): 1137-1141.

Wan Nor Arifin & Wan Mohammad Zahiruddin 2017. Sample size calculation in animal studies using resource equation approach. *Malaysian Journal of Medical Sciences* 24(5): 101-105.

Wang, H., Huang, S., Xu, M., Yang, J., Yang, J., Liu, M., Wan, C., Liao, H., Fan, D. & Tang, Q. 2019. Galangin ameliorates cardiac remodeling via the MEK1/2–ERK1/2 and PI3K–AKT pathways. *Journal of Cellular Physiology* 234(9): 15654-15667.

Wang, L., Wu, H., Deng, Y., Zhang, S., Wei, Q., Yang, Q., Piao, S., Bei, W., Rong, X. & Guo, J. 2021. FTZ ameliorates diabetic cardiomyopathy by inhibiting inflammation and cardiac fibrosis in the streptozotocin-induced model. *Evidence-based Complementary and Alternative Medicine* 2021: 5582567.

Wang, S., Ding, L., Ji, H., Xu, Z., Liu, Q. & Zheng, Y. 2016. The role of p38 MAPK in the development of diabetic cardiomyopathy. *International Journal of Molecular Sciences* 17(7): 1037.

Wang, Y., Sun, H., Zhang, J., Xia, Z. & Chen, W. 2020. Streptozotocin-induced diabetic cardiomyopathy in rats: Ameliorative effect of PIPERINE via Bcl2, Bax/Bcl2, and caspase-3 pathways. *Bioscience, Biotechnology, and Biochemistry* 84(12): 2533-2544.

Wei, M., Ong, L., Smith, M.T., Ross, F.B., Schmid, K., Hoey, A.J., Burstow, D. & Brown, L. 2003. The streptozotocin-diabetic rat as a model of the chronic complications of human diabetes. *Heart, Lung and Circulation* 12(1): 44-50.

Xia, Z., Kuo, K-H., Nagareddy, P.R., Wang, F., Guo, Z., Guo, T., Jiang, J. & McNeill, J.H. 2007. N-acetylcysteine attenuates PKCbeta2 overexpression and myocardial hypertrophy in streptozotocin-induced diabetic rats. *Cardiovascular Research* 73(4): 770-782.

Xu, Z., Sun, J., Tong, Q., Lin, Q., Qian, L., Park, Y. & Zheng, Y. 2016. The Role of ERK1/2 in the development of diabetic cardiomyopathy. *International Journal of Molecular Sciences* 17(12): 2001.

Youssef, M.E., Abdelrazek, H.M. & Moustafa, Y.M. 2021. Cardioprotective role of GTS-21 by attenuating the TLR4/NF-κB pathway in streptozotocin-induced diabetic cardiomyopathy in rats. *Naunyn-Schmiedeberg's Archives of Pharmacology* 394(1): 11-31.

*Corresponding author; email: balkis@ukm.edu.my