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Hibiscus sabdariffa linn. calyx extraction and gallic acid improving cardiac diastolic dysfunction in high fat diet-STZ-induced type 2 diabetic rats

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Abstract

Cardiovascular disease is one of serious complications of chronic diabetes. The objective of this study was to investigate the effects of HS-WE and gallic acid on ventricular function in high fat diet-streptozotocin (HFD-STZ)-induced Type 2 diabetic rats. Male Sprague-Dawley rats were used. The normal control group was fed normal rat chow. Diabetic groups were fed HFD for 4 weeks and followed by intraperitoneal injection of STZ 30 mg/kg. The diabetic rats were divided into 5 groups which were orally administered daily with distilled water, 50% propylene glycol, HS-WE (500 mg/kg), gallic acid (50 mg/kg), and metformin (200 mg/kg) for further 8 weeks. At the end of all treatments, the levels of fasting blood glucose (FBG), serum insulin, Homeostatic Model Assessment-Insulin Resistance (HOMA-IR), plasma and heart malondialdehyde (MDA), heart superoxide production and left ventricular function were determined. Diabetic control rats showed significant increases in levels of FBG, HOMA-IR, MDA and superoxide production. In left ventricular function examination, an increase in diastolic ventricular pressure (DVP) and a decrease in (-) dP/dt were observed. HS-WE and gallic acid treated groups had significant decreases in FBG, HOMA-IR, plasma MDA, heart MDA and heart superoxide production as well as a decrease in DVP and an increase in (-) dP/dt when compared to diabetic control group. These results indicated that HS-WE and gallic acid improve diabetic induced diastolic dysfunction and this effect may be mediated via their antioxidant activities.

Keywords: Roselle, *Hibiscus sabdariffa*, Diabetic cardiomyopathy, Antioxidant

1. Introduction

Diabetes mellitus (DM) is a major chronic disease affecting a large population all over the world. Cardiovascular diseases, including coronary artery disease, stroke, atherosclerosis, hypertension and cardiomyopathy are major complications found in diabetic patients. Diabetic cardiomyopathy is defined as diabetes-associated changes in structure and function of myocardium that occurs independently of coronary artery disease and hypertension in diabetic patients [1]. One of the earliest signs is mild left ventricular diastolic dysfunction with little effect on ventricular filling [1], the later phase is a systolic dysfunction, and it eventually leads to heart failure**.** Left ventricular hypertrophy, extracellular matrix changes, small vessel disease and cardiac autonomic neuropathy have all been reported in animal models [2]. It has been proposed that hyperglycemia, calcium homeostasis changes, advanced glycation end products, protein kinase C, free fatty acids and oxidative stress are involved in the pathophysiology of diabetic cardiomyopathy [3]. Although multiple pathophysiologic mechanisms have been proposed to explain the pathology, hyperglycemia seems to be the central mechanism triggering the processes that lead to the ultimate pathologic changes of myocardium. Hyperglycemia causes an

increase in the levels of free fatty acids and promotes excessive production and release of reactive oxygen species leading to abnormal gene expression, faulty signal transduction and finally, cardiomyocytes apoptosis [2].

Hibiscus sabdariffa Linn. (Malvaceae; common name: ‘‘roselle’’) is widely cultivated in Southeast Asia (Thailand, Malaysia, the Philippines, etc.). It is known as *krajeab daeng* in Thailand, and has been used in Thai folk medicine as a diuretic agent. Several pharmacological activities of roselle calyx extract have been demonstrated such as antihypertensive [4], hepatoprotective [5], antioxidant [6], hypolipidemic [7] and anticancer effects [8]. *Hibiscus sabdariffa* L. calicyx extracts have been reported to contain phenolic acids (such as gallic acid and protocatechuic acid) and several polyphenolic compounds including flavonoids such as anthocyanins [8]. We have previously reported the antidiabetic, antioxidant and vascular response-improving activities of both roselle calyx water extract and gallic acid in chronic STZ-induced type 1 diabetic rats [9 & 10]. As gallic acid is one of essential compounds found in roselle calyx extract, the hypothesis of this study was that roselle calyx water extract and gallic acid may ameliorate cardiomyopathy in chronic high fat diet-STZ-induced diabetic rats.

2. Material and methods

2.1. Preparation of Hibiscus sabdariffa Linn. water extract (HS-WE)

The water extract of *H. sabdariffa* (HS-WE) was kindly provided by Assoc. Prof. Arunporn Itharat, Applied Thai Traditional Medicine Center, Thammasart University, Prathumthani. The dried calyxes of *H. sabdariffa* were blended and boiled in water at 60ºC for 60 minutes, and then filtered. The filtrate was dried using a spray dryer. Using this procedure, the yield was 37.4% of the dried calyx weight. The dried HS-WE was then packed in tight containers and kept at 4-6º C until used. Quantitative determination of the main compounds of HS-WE by HPLC found that it was composed of quercetin 0.50 mg/g, gallic acid 0.15 mg/g and cyanidin-3-glucoside 2.74 mg/g. The gallic acid used in this investigation, was purchased from Sigma Chemical Co Ltd (St Louis, USA).

2.2. Experimental animals and experimental protocol

Male Sprague-Dawley rats weighing 250-280g were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Nakornpathom. Rats were maintained in an air-conditioned room (24±2 ºC) with a 12 h dark-light cycle. All animal experimental protocols were approved by the Animal Ethics Committee of Khon Kaen University, Khon Kean, Thailand (AEKKU 2/2556). The animals were randomly divided into 6 groups with 6-8 rats per group. The normal control group received normal diet and distilled water (DW) throughout the experimental period. The other 5 groups were induced to have Type 2 DM by feeding them a high fat diet (HFD, 40% lard) for 4 weeks, followed by single injection of streptozotocin (STZ) 30 mg/kg [11]. Two weeks after STZ injection, the animals with fasting blood glucose (FBG) over 200 mg/dL were treated with the following agents: DW (diabetic control group), 50% propylene glycol, HS-WE 500 mg/kg/d, gallic acid 50 mg/kg/d (dissolved in 50% propylene glycol) or metformin 200 mg/kg/d. All these treatments were administered daily for further 8 weeks. At the end of treatment period, FBG, serum insulin, plasma and heart malondialdehyde (MDA), heart superoxide production, serum lipid profiles, serum cardiac enzymes and ventricular function were determined after a 12-hour fasting period.

2.3. Blood chemistry analyses

Blood from the lateral tail vein was collected to measure FBG and fasting serum insulin. FBG was measured using a glucometer (Accu-Check Advantage II; Roche Diagnostics, Mannheim, Germany) and insulin using a rat insulin ELISA kit (Millipore MA, USA). Total cholesterol (TC), low density lipoprotein-cholesterol (LDL), high density lipoprotein-cholesterol (HDL), triglyceride (TG), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) were investigated using enzymatic and colorimetric methods (Roche diagnostics, Thailand). HOMA-IR was calculated as follow; HOMA-IR = Fasting glucose (mmol/ml) x Fasting insulin (µIU/L) /22.5.

2.4. Cardiac ventricular function examination

At the end of all treatments, animals were anesthetized using urethane (1.5 g/kg, intraperitoneal injection) and a tracheotomy was performed to facilitate breathing. A micro pressure catheter (Mikro-Tip®, Millar Instruments, USA) was inserted into the right carotid artery and advanced into the left ventricle. Cardiac function parameters, including heart rate, end-systolic pressure (SVP) and end-diastolic pressure (DVP), maximum (+) and minimum (-) dP/dt were recorded using Power Lab® and analyzed using Lab Chart7® (AD Instruments, Australia). At the end of recording, the animal was euthanized and the heart was collected to examine MDA and superoxide production.

2.5. Determination of lipid peroxidation

The amount of plasma and heart MDA was measured as thiobarbituric acid reactive substances (TBAR) using a spectrophotometric method as has previously been described (12, 13). Approximately 200 mg of the upper part of left ventricle was finely sliced and homogenized in a cold phosphate buffer (pH 7.4). The homogenate was centrifuged at 14000 g, 4 °C for 30 min. The clear supernatant was collected for assay of MDA. 150 µl of samples were reacted with 10% trichloroacetic acid (TCA), 125 µl of 5 mM ethylene diamine tetraacetate (EDTA), 125 µl of 8% sodium dodecyl sulfate (SDS) and 10 µl of 0.5 µl/ml of butylated hydroxytoluene (BHT). The mixture was left for 10 min, and 0.6% thiobarbituric acid (TBA) was subsequently added in an equal volume. The mixture was then heated for 30 min in a boiling water bath. After cooling to the room temperature, the mixture was centrifuged at 10,000 g for 5 minutes at 25 °C. The absorbance of the supernatant was measured at the wavelength of 532 nm by spectrophotometry. The amount of MDA was calculated using a standard curve of 1,1,3,3-tetra-ethoxypropane (0.3-10 µmol/l). The MDA concentration in the tissues was normalized against the protein concentration. Protein concentration was determined using the Bradford dye binding method.

2.6. Determination of superoxide production in heart tissue

Superoxide generation was estimated using lucigenin-enhanced chemiluminescence as has previously been described with some modification [14]. The heart was excised rapidly from animal after euthanasia and placed in ice-cold saline. The upper part of left ventricle was cut into 0.5x1 cm (width x length) strip and incubated with 1 ml oxygenated Krebs-ringer bicarbonate solution at pH 7.4, 37ºC for 30 min. Sixty microliters of 100 µM lucigenin was added to the sample tube and placed in a luminometer. The luminometer integrated the photon count of every 30 sec for 5 min. After that the heart tissue was dried at 45ºC for 24 hours and weighed. Tissue superoxide production was expressed as relative photon count unit per min per dry weight of tissue.

2.7. Statistical analyses

Data are expressed as mean ± S.E.M. The differences among treatment groups were analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls for the post-hoc test. A *p*-value of less than 0.05 was considered statistically significant. Statistical analysis was performed using computer-based software (Stata version 10, College Station, TX).

3. Results

3.1. Effect of HS-WE and gallic acid on cardiac function

The systolic ventricular pressure (SVP) and rate of left ventricle (LV) pressure rise (+dP/dt max) of all diabetic groups were comparable to normal rats (Table 1). Diabetic control rats showed a significant increase in diastolic ventricular pressure (DVP) and a significant decrease in (–)dP/dt as compared to normal control rats, indicating impairment of ventricular dilatation. Interestingly, treatments with HS-WE 500 mg/kg and gallic acid 50 mg/kg caused a significant decrease in DVP and a significant increase in (–)dP/dt as compared to the diabetic control group, which indicated that HS-WE and gallic acid could improve ventricular dilatation (Table 1). The heart rate of the diabetic control group was significantly decreased compared to the normal group. None of the treatments improved the decrease in heart rate in diabetic rats.

The systolic blood pressure (SBP) and diastolic blood pressure (DBP) of diabetic control rats treated with DW were no different from the values of normal control rats (Table 1), and the treatment with HS-WE, gallic or metformin did not affect SBP or DBP. Metformin treatment caused a partial decrease in diastolic ventricular pressure (DVP), but this was not statistically significant when compared to diabetic control group (Table 1). Propylene glycol did not cause any changes in cardiac function of diabetic rats

Table 1 The effect of HS-WE and gallic acid on cardiac function

Cardiac function	Normal control	DM control	DM + 50% Propylene glycol	DM + HS-WE 500 mg/kg	DM + Gallic acid 50 mg/kg	DM + Metformin 200 mg/kg
HR (beats/min)	319 ± 25.11	236 ± 14.87*	237 ± 5.52*	292 ± 29.43	277 ± 20.52	243 ± 15.45
SVP (mmHg)	115.99 ± 4.90	120.65 ± 3.21	123.4 ± 2.65	112.42 ± 3.90	117.27 ± 1.80	117.04 ± 6.86
DVP (mm Hg)	9.41 ± 1.63	25.59 ± 2.77*	21.92 ± 0.98*	6.89 ±1.78$^{\#}$	6.51 ± 1.45$^{\#}$	15.15 ± 2.42
(+)dP/dt (mm Hg/sec)	(+)5757 ± 873	(+)4207 ± 346	(+)4594 ± 159	(+)5514 ± 320	(+)5544 ± 541	(+)4844 ± 476
(-)dP/dt (mm Hg/sec)	(-)5385 ± 893	(-)3350 ± 405*	(-)3629 ± 250*	(-)5163 ± 427$^{\#}$	(-)5129 ± 454$^{\#}$	(-)4420 ±544
SBP in arterial (mmHg)	95.0±3.42	95.50±4.40	93.2 ± 3.29	86.68±6.13	104.70±4.62	88.42±6.87
DBP in arterial (mmHg)	58.74±5.32	46.50±4.34	44.13 ± 1.87	46.42±5.36	48.51±3.98	45.48±4.64

Results are expressed as mean ± S.E.M., *: $P<0.05$; significant difference as compared to normal control rats using ANOVA followed by Newman-Keuls test, $^{\#}$: $P<0.05$; significant difference as compared to diabetic control rat receiving DW. DM: diabetic rats.

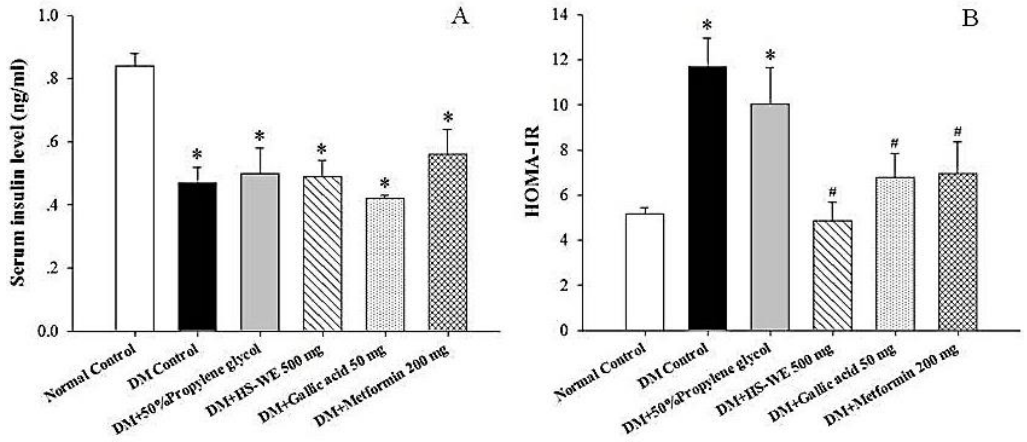

Figure 1 The Effects HS-WE and gallic acid on insulin levels (A) and HOMA-IR (B). Results are expressed as mean ± S.E.M. (*: $p<0.05$: significant difference as compared to normal control, #: $p<0.05$: significant difference as compared to DM control group)

3.2. Effect of HS-WE and gallic acid on heart weight of diabetic rats

The body weights of diabetic control group were significantly decreased compared to normal control rats. HS-WE, gallic acid and metformin treated groups had the significant increases in the body weights compared to the diabetic control group. The whole heart weight (HW) and left ventricular weight (LVW) of diabetic rats were not significantly different compared to normal rats (Table 2). However, the relative weight of the left ventricle to body weight (LVW/BW) of the diabetic control rats was significantly increased compared to normal control rats, indicating that the left ventricle of diabetic rat hearts were hypertrophic. Administration of HS-WE and gallic acid caused a significant decrease of LVW/BW values when compared to the diabetic control rats (Table 2), indicating a beneficial effect of HS-WE and gallic acid in reducing left-ventricular hypertrophy. Metformin had no effect on relative weight of the left ventricle. Propylene glycol did not cause any changes in HW, LVW or LVW/BW of diabetic rats compared to the diabetic control group.

3.3. Effect of HS-WE and gallic acid on FBG, serum insulin, HOMA-IR and lipid profiles

All diabetic rats had the significantly high FBG compared to normal rats. Treatments with HS-WE or gallic acid significantly reduced FBG ($p<0.05$) as compared with diabetic control rats (Table 3). Diabetic rats treated with HS-WE and gallic acid had the percent reductions of FBG of 52% and 37%, respectively. Interestingly, treatments with HS-WE or gallic acid did not significantly affect fasting serum insulin compared to diabetic control rats. However, these treatments caused a decrease in HOMA-IR values (Fig. 1A and 1B). The results indicated that HS-WE or Gallic acid can improve insulin sensitivity. Metformin, a standard oral hypoglycemic drug, significantly decreased FBG and HOMA-IR. Propylene glycol treatment had no effect on FBG and HOMA-IR compared to diabetic control rats.

In diabetic control rats, levels of TG were significantly higher, and levels of HDL were significant lower than those of normal rats (Fig. 2), whereas levels of TC and LDL were comparable to normal rats. HS-WE and gallic acid significantly decreased TG and TC levels in diabetic rats with no effect on LDL and HDL (Fig. 2) while metformin reduced only TG levels compared to diabetic control rats.

Table 2 Effect of HS-WE and gallic acid on heart weight of diabetic rats

Groups	BW (g)	HW (g)	LVW (g)	LVW/BW (mg/g)
Normal control	557.67 ± 5.82	1.56 ± 0.10	1.23 ± 0.09	2.21 ± 0.07
DM control	395.13 ± 13.10*	1.58 ± 0.08	1.23 ± 0.08	3.10 ± 0.16*
DM + 50% Propylene glycol	391.4 ± 3.78*	1.78 ± 0.07	1.29 ± 0.07	3.20 ± 0.19*
DM + HS-WE 500 mg	444.75 ± 19.38#	1.52 ± 0.04	1.09 ± 0.03†	2.63 ± 0.14#
DM + Gallic acid 50 mg	433.5 ± 18.25#	1.42 ± 0.05	1.10 ± 0.04†	2.55 ± 0.12#
DM + Metformin 200 mg	457.63 ± 8.30#	1.67 ± 0.06	1.26 ± 0.04	3.21 ± 0.14

Results are expressed as means ± S.E.M., *: $P<0.05$; significant difference as compared to normal control group, #: $P<0.05$; significant difference as compared to the diabetic control group. BW: body weight, HW: heart weight, LVW: left ventricular weight

Table 3 The effect of HS-WE and gallic acid on fasting blood glucose (FBG) in diabetic rats

Groups	Fasting Blood Glucose		
	Before treatment (mg/dL)	After 8 weeks of treatments	
		(mg/dL)	(% Change)
Normal control	96 ± 1.24	98.83 ± 1.72	2.95
DM control	348.25 ± 39.88*	404.63 ± 13.11*	16.19
DM+50% Propylene glycol	302.80 ± 13.92*	353.00 ± 14.49*	16.57
DM+HS-WE 500 mg	338 ± 37.06*	161.25 ± 20.15#	-52.29
DM+Gallic acid 50 mg	359 ± 34.10*	225.88 ± 22.02#	-37.08
DM+Metformin 200 mg	362.75 ± 45.34*	192.25 ± 22.33#	-47

Results are expressed as mean ± S.E.M., *: $P<0.05$; significant difference as compared to normal control group, #: $P<0.05$; significant difference as compared to the diabetic control group receiving DW

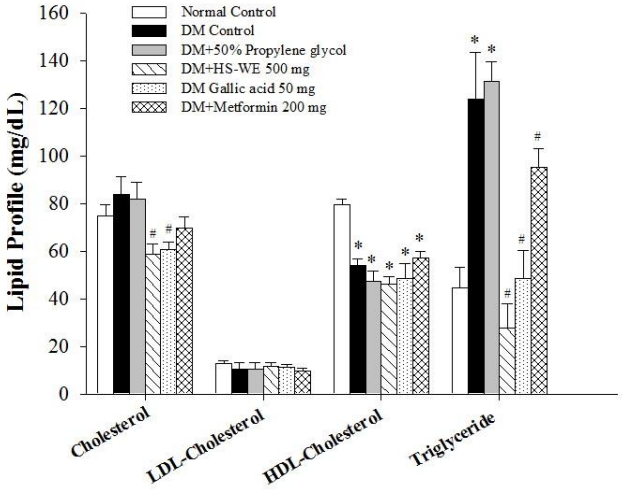

Figure 2 Effect of HS-WE and gallic acid on lipid profiles in diabetic rats. Results are expressed as mean ± S.E.M. (*: p<0.05: significant difference as compared to normal control, #: p<0.05: significant difference as compared to DM control group)

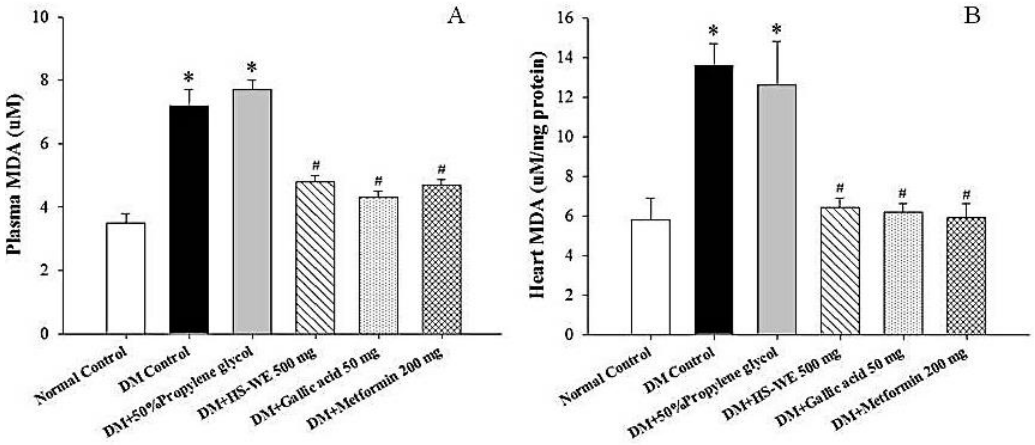

Figure 3 The effect of HS-WE and gallic acid on MDA level in plasma (A) and heart tissue (B). Results are expressed as mean ± S.E.M. (*: p<0.05, significant difference as compared to normal control, #: p<0.05, significant difference as compared to DM control group

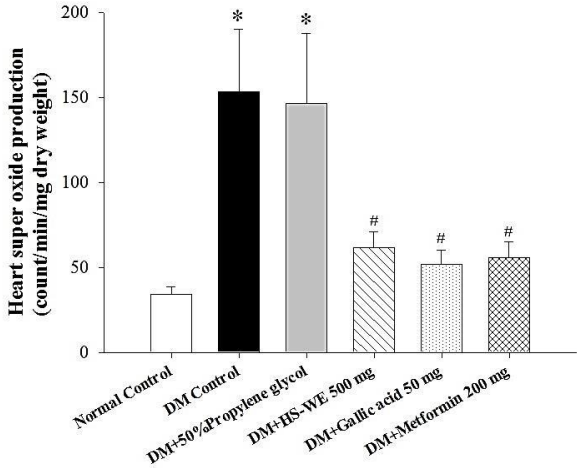

Figure 4 The effect of HS-WE and gallic acid on superoxide production in the heart tissue. Results are expressed as mean ± S.E.M. (*: P<0.05; significant difference as compared to normal rats, #: P<0.05; significant difference as compared to diabetic control rats receiving DW)

3.4. Effect of HS-WE and gallic acid on serum and heart MDA formations

Plasma and heart tissue MDA formations were significantly increased in diabetic control rats (Fig. 3A and 3B). HS-WE, gallic acid and metformin significantly reduced the formation of MDA both in plasma and heart tissue (Fig. 3A and 3B). Propylene glycol did not affect MDA formation.

3.5. Effect of HS-WE and gallic acid on heart superoxide production

The heart tissue superoxide production in diabetic rats receiving distilled water and 50% propylene glycol (153.28 ± 36.98 and 146.28 ± 41.40 count/min/mg dry weight) was significantly higher than that of normal control rats (34.26 ± 4.20 count/min/mg dry weight) ($P<0.05$). Treatment with HS-WE or gallic acid significantly decreased the superoxide production in heart tissue (61.50 ± 9.34 and 51.62 ± 8.38 count/min/mg dry weight) as compared to diabetic control rats receiving DW ($P<0.05$) (Figure 4). The superoxide production in the heart tissue of the metformin-treated group was also significantly decreased (55.48 ± 9.45 count/min/mg dry weight).

3.6. Effect of HS-WE and gallic acid on cardiac enzymes

In treated or non-treated with HS-WE, gallic acid or metformin diabetic rats, serum AST levels were not significantly different among experimental groups (Table 4). LDH levels in diabetic rats receiving DW or 50% propylene glycol were significantly higher than that of the normal control rats. The treatment with HS-WE, gallic acid and metformin significantly lowered the LDH level (Table 4) indicating that these substances could protect the diabetic rats against cardiac injury.

4. Discussion

In the present study, the effects of HS-WE and gallic acid on diabetic cardiomyopathy were investigated using a high-fat diet and a low-dose streptozotocin-induced Type 2 diabetic rat model. Our study demonstrated that chronic HFD-STZ-induced diabetes can cause oxidative stress and impairment of ventricular dilatation and we found that HS-WE and gallic acid can improve the dilatation of the ventricle as shown by the reduction in EDP and increase in (-) dP/dt which is presumably due to their antioxidant activity as shown by the reduction in heart superoxide and MDA levels.

Experimental diabetes produced by a low dose of STZ combined with HFD is regarded as an effective strategy to obtain Type 2 diabetes animal models (15). The HFD-induced insulin resistance, followed by a low dose of STZ causes partial dysfunction of beta cells in insulin-secretion suppression, which imitates the low insulin levels present in Type 2 diabetes. In our study, the HFD-plus-low-dose-STZ-induced diabetes model presented hyperglycemia, dyslipidemia, insulin resistance and decreased fasting serum insulin. Importantly, this HFD-low dose STZ model could induce diabetic cardiomyopathy as indicated by the decrease in heart rate and ventricular dilatation dysfunction (increase in DVP and decrease in (-) dp/dt.

Diabetic cardiomyopathy, defined as ventricular dysfunction with increased risk of heart failure in the absence of hypertension, coronary artery and valvular heart diseases, has been reported in both diabetic humans and animals [3]. In the present study, the diabetic rats that were left untreated for 14 weeks were characterized by a decline in diastolic (increase in DVP and decrease in (-) dp/dt, but not systolic-myocardial performance with increased relative left ventricular weight. LV diastolic stiffness and relaxation disturbances are recognized as the earliest manifestation of DM-induced LV dysfunction [2]. Abnormal diastolic function has been noted in 27–70% of asymptomatic diabetic patients, which may be in part due to increased LV mass and fibrosis [2]. Our experimental results showed the increased relative left ventricular weight in control diabetic rats. This may reflect increased fibrosis formation. Unfortunately, we haven't had the opportunity to perform histological examination of the ventricle to confirm. Interestingly, HS-WE and gallic acid caused a decrease in the relative ventricular weight of diabetic rats. This may imply that HS-WE and gallic acid cause the decrease in myocardium fibrosis formation. However, this would also require a histological examination to confirm.

The pathogenesis of diabetic cardiomyopathy is a combination of many causes. Several hypotheses have been proposed, including autonomic dysfunction, metabolic derangements, abnormalities in Ca^{2+} homeostasis, alteration in structural proteins, and interstitial fibrosis [2].

Hyperglycemia leads to an increase in oxidative stress by intensifying glucose oxidation and mitochondrial generation of reactive oxygen species (ROS), which induce DNA damage and contribute to accelerate apoptosis [16]. In addition, hyperglycemia activates the local renin–angiotensin–aldosterone system (RAAS), contributing to myocyte necrosis and fibrosis [17]. In the case of hyperglycemia, ROS are also generated through the formation of advanced glycation end products (AGEs), altered polyol pathway activity and activation of NADPH oxidase

Table 4 The effect of HS-WE and gallic acid on cardiac enzymes.

Treatment group	8 weeks of treatment	
	AST (U/L)	LDH (U/L)
Normal control	173.33 ± 17	1986.50 ± 381
DM control	217.60 ± 32	3549.20 ± 503*
DM + 50% Propylene glycol	251.00 ± 45	3193.00 ± 355*
DM + HS-WE 500 mg	304.38 ± 44	2812.13 ± 210#
DM + Gallic acid 50 mg	240.25 ± 23	2479.75 ± 180#
DM + Metformin 200 mg	218.00 ± 50	2149.33 ± 197#

Results are expressed as mean ± S.E.M., *: $P<0.05$; significant difference as compared to normal control group, #: $P<0.05$; significant difference as compared to the diabetic control group receiving DW.

via protein kinase C [3]. ROS are thought to be a cause of myocardial cell death, hypertrophy, fibrosis, abnormalities of calcium homeostasis, and endothelial dysfunction [18] & [19]. Elevated free fatty acid (FFA) levels are believed to be another contributing factor to diabetic cardiomyopathy. Elevation of circulating FFAs is caused by enhanced adipose tissue lipolysis, and high heart tissue FFAs are caused by the hydrolysis of myocardial triglyceride stores. High circulating and cellular levels of FFAs may result in abnormally high oxygen requirements during FFA metabolism and the intracellular accumulation of potentially toxic intermediates of FFA, all of which lead to impaired myocardial performance and severe myocardial changes [20].

HS-WE and gallic acid produce improved glycemic control and antioxidant activity. Thus, these two significant effects are most likely important mechanisms in alleviating cardiomyopathy in diabetic rats. HS-WE and gallic acid also diminished serum triglyceride, which may be at least one of their actions in alleviating cardiomyopathy.

LDH and AST are released during tissue damage and they are also markers of common injuries and diseases such as hemolysis, heart failure and myocardium injury. In the present study, only the level of LDH was significantly elevated in diabetic group. HS-WE and gallic acid were able to protect diabetic rats against cardiac injury, which is evidenced by the decreased LDH levels in HS-WE and gallic acid-treated groups. The AST level in diabetic rats was slightly increased and not statistically different, which may be due to the pathological change of diabetic heart tissue being in an early state.

Several pharmacological activities of *Hibiscus sabdariffa* calyx extract have been reported. These include antibacterial, antipyretic, antinociceptic, anti-inflammatory, antioxidant, antidiabetic, anti-hypertensive, diuretic, hepatoprotective, antidyslipemic and anti-obesity activities [21]. Micucci and coworkers (2015) have demonstrated the cardiovascular effect of *Hibiscus sabdariffa* calyx water extract such as the antioxidant and cytoprotective effects in vascular endothelial cells and negative inotropic and vasorelaxant effects without any chronotropic effects in isolated guinea-pig atria and aorta [22]. Our findings correspond to this report in that we found that HS-WE has antioxidant properties in heart tissue and improves ventricular dilatation without an effect on heart rate in diabetic rats.

It is clear that all the effects of 50% propylene glycol were the same as those of diabetic rats receiving distilled water. Thus we conclude that the solvent of gallic acid has no interferent effect on gallic acid activity. Several mechanisms of metformin (a standard oral antidiabetic drug) are proposed including suppressing hepatic glucose production, increasing insulin sensitivity, and enhancing peripheral glucose uptake. However, metformin has been contraindicated in patients with heart failure because of the risk of lactic acidosis. In this study, even though metformin showed hypoglycemic, hypolipidemic and antioxidant activities, it caused only slightly decreased DVP in diabetic animals and did not improve (-)dP/dt. This may be due to metformin did not alleviate the ventricular fibrosis or structural changes (no change in the relative ventricular weight). These results also suggest that metformin may not be a proper drug for alleviating diabetic cardiomyopathy.

In conclusion, our study demonstrated that chronic HFD-STZ-induced Type2 diabetes causes the oxidative stress and impairment of ventricular dilatation as evidenced by increases in heart superoxide and MDA levels, and end diastolic pressure. Interestingly, HS-WE and gallic acid can (i) improve insulin sensitivity as shown by a reduction in HOMA-IR and (ii) attenuate dysfunction of ventricular dilatation as shown by the reduction in EDP and increase in (-)dP/dt, most likely by decreasing the oxidative stress as shown by the reduction in heart superoxide and MDA levels.

5. Acknowledgments

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