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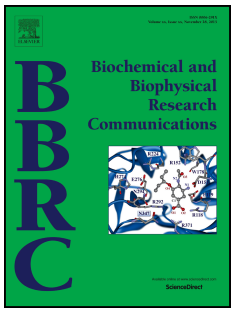

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Cardioprotection by 6-gingerol in diabetic rats

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Abstract:

The current study was conducted to evaluate the effect of 6-gingerol (6G) on cardiac complications in streptozotocin (STZ)-induced diabetic (DM) rats. STZ-induced DM rats (single 50mg/kg i.p. injection, 15 days prior to drug treatment) or time-matched controls were treated with 6G (75 mg/day route orally). After a further 8 weeks, blood was collected for biochemical analysis and 8-isoprostenol was measured in urine. Cardiac hemodynamics and ECG was assessed. 6G significantly attenuated the increased level of blood glucose in diabetic rats and improved cardiac hemodynamics in including RR interval, max dP/dt, min dP/dt and Tau. In addition, 6G alleviated the elevated ST segment, T amplitude and R amplitude with no significant effect on disturbed levels of adiponectin, TGF-β or 8-isoprostenol induced by diabetes. The results showed that treatment with 6G has an ameliorative effect on cardiac dysfunction induced by diabetes. Which may be not related to its potential antioxidant effect.

Key words: diabetes, 6-gingerol, cardioprotection, adiponectin, vascular complications, hemodynamics, electrocardiogram.

1. Introduction

Diabetes mellitus (DM) is a common metabolic disorder and a leading cause of cardiovascular disease worldwide [1]. The increased morbidity and mortality of these patients has been attributed to cardiovascular complications and diabetic cardiomyopathy [2, 3]. Diabetic cardiomyopathy (DCM) is pathologic changes in the heart due to diabetes and characterized by both structural and functional alterations [4]. These alterations include fibrosis, apoptosis, myocyte angiopathy, increased left ventricular (LV) mass and LV hypertrophy and impairment in cardiomyocyte contractility with diastolic and systolic dysfunction [5, 6]. Several factors have

been suggested to be responsible for the pathogenesis of DCM including hyperglycemia, dyslipidemia, insulin resistance, low-grade inflammation, and oxidative stress [7].

Despite great advances in treatment of diabetes, natural products with anti-oxidant effects may be more favorable for reducing diabetes-induced side effects in many diabetic patients. Among plants containing natural anti-oxidants, ginger exhibits antioxidant and anti-inflammatory properties [8]. Phytochemical studies have indicated that the main constituents of ginger extract are the shogaols, gingerols, zingerone and paradol [9]. In addition, 6-gingerol, one of the major elements of ginger, has been found to possess antiangiogenic [10], anti-inflammatory [11], antihyperglycemic [12], and vasorelaxatory effects [13]. Moreover, our previous study revealed that 6-gingerol alleviates exaggerated vasoconstriction in diabetic rat aorta through direct vasodilation and nitric oxide generation [14]. Therefore, the aim of the current study is to investigate the effect of 6-gingerol on alterations in cardiac parameters and vascular complications of diabetic rats.

2. Materials and methods

2.1.Chemicals

Streptozotocin (STZ) and 6-gingerol (6G) were purchased from Sigma-Aldrich (St. Louis, MO, USA), while lipid profile kits were purchased from Randox Laboratories Ltd. (Antrim, UK). 8-Isopropane assay kit was purchased from Cayman Chemical, Ann Harbor, MI, USA. Tumor growth factor beta-1 (TGF-β1) and adiponectin rat enzyme linked immunosorbent assay (ELISA) kits was purchased from R&D Systems, Inc., Minneapolis, MN, USA.

2.2.Animals and experimental protocol

Male Wistar rats, 6 weeks age, King Abdulaziz University, Jeddah, Saudi Arabia weighting 240-280 gm were housed (3-4 rats per cage) in clear polypropylene cages and kept under constant environmental conditions with 12 h day and night cycle, well ventilated, temperature 22 ± 2 ^{o}C, and relative humidity of 50–60%. Rats had free access to commercially available rodent pellet diet and purified water. Care was taken to avoid stressful conditions, and all procedures were performed between 8 and 10 a.m. All experimental protocols were approved by the Biomedical and research Ethical committee, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia and were performed in accordance with Saudi Arabia Research Bioethics and Regulations, which are consistent with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Diabetes was induced through single intraperitoneal (i.p.) injection of a fresh solution of STZ dissolved in 0.1 M sodium citrate, at a dose of 50 mg/kg, to overnight fasting rats (14, 15]. Two weeks after STZ administration, DM rats with blood glucose concentration of between 250–350 mg/ dL were selected for drug treatment. Based on the results of our previous studies these animals will go on to develop vascular complications over the subsequent 8 weeks [14, 16].

Age-matched control or the 15-day DM animals were assigned groups with 6-8 animals in each group. Group I (normal controls) received an equivalent volume of the 0.1 M sodium citrate buffer. Group II (DM) diabetic rats received oral single daily dose of 1% CMC-Na, as a vehicle, starting on the 15th day. Group III (DM + 6G) diabetic rats received 6G in a dose of 75 mg/kg orally as a single daily dose, starting on the 15th day. Group IV (control + 6G), normal control rats received 6G in the same dose and duration as in group III. Animals were maintained under standard conditions for a further 8 weeks.

At the end of the experiment, animals were fasted for 8 hours and a tail capillary droplet was used to determine the fasting blood glucose level. To determine cardiac hemodynamics including contractility and conductivity, we followed the same steps previously described in our recent work by Azhar and El-Bassossy [17]. For blood analysis, one ml blood was withdrawn from the vena cava through a small incision in the lower abdomen and 1ml saline injected to prevent hypovolemia. The blood sample was allowed to coagulate for 30 min at 4°C and centrifuged (3000×g, 4°C, 20 min). Serum was divided into aliquots and stored at -20°C for later analysis. Rats were then injected with one ml saline to prevent hypovolemia. At the end of the experiment animals were killed by an overdose of anesthetic.

2.3.Measurement of blood glucose, serum adiponectin and TGF-ß, and urinary 8-isoprostane

For blood glucose determination, blood was collected from a tail vein after overnight fasting, and glucose was measured using a glucometer (ACCU-CHEK®, Roche Diagnostics, Mannheim, Germany). Adiponectin and TGF-β1 were measured in serum using rat ELISA kits (Quantikine®) according to the manufacturer's protocol.

For urine collections, rats were placed in individual metabolic cages for a period of 24-h. The volume of collected urine was determined gravimetrically and urine samples were kept at −80°C until assayed. Urinary 8-isoprostane level was determined by using a sensitive rat enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Harbor, MI, USA).

2.4.Determination of lipid profile

Total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) were determined spectrophotometrically, using commercial kits (ELITech®, ELITech,

Puteaux, France). Low density lipoprotein cholesterol (LDL-C) was calculated by using Friedewald formula [17].

2.5.Cardiac hemodynamic and Electrocardiogram (ECG) recording

Invasive cardiac hemodynamics was recorded as previously described in our recent work [18]. The BP module identified and calculated the left ventricular end ventricular systolic pressure (ESP), left ventricular end-diastolic pressure (EDP), slope of the systolic pressure increment (max dP/dt) and slope of the diastolic pressure decrement (min dP/dt), systolic and diastolic duration, contractility index and the left ventricular diastolic time constant (Tau).

The ECG leads I, II, III, aVR, aVL, aVF were recorded with surface skin electrodes (AD Instruments, Bella Vista, Australia). Corrected QT (QTc) was calculated with mean values and the Bazett's Formula (QTc = QT Interval/√ (RR interval) [19].

2.6.Statistical analysis

Values are expressed as mean ± SD. Statistical analysis was carried out by using one-way analysis of variance (ANOVA) followed by Newman-Keuls' post hoc test using GrapPad InStat statistical software (Prism 5, GraphPad, CA, USA). Significance was measured at $p < 0.05$. The power was greater than 90% with sample sizes of 6-8 rats per group.

3. Results:

3.1.Effect of 6G on body weight, biochemical parameters

A characteristic decrease in body weight and hyperglycemia was observed in the diabetic model in the current study. Treatment with 6G was found to ameliorate significantly this

hyperglycemia with no significant effect on body weight (figure 1A and 1B). There were no significant differences in the lipid profile between different rat groups (table 1).

Diabetic rats exhibited a significant decrease in serum level of adiponectin when compared with control group, although treatment of diabetic rats with 6G had no significant effect on serum adiponectin levels (figure 1C) while adiponectin levels were reduced by 6G in control normal rats. DM resulted in significantly increased serum TGF-β1 and urinary 8-isoprostane levels and these increased levels were not significant changed by treatment with 6G (figure 1D and 1E).

3.2.Effect of 6G on cardiac hemodynamics

The present study showed a significant attenuation of max dP/dt, min dP/dt, EDP, and ESP in DM rats compared to the nondiabetic control group (figures 2A, B, C and D). Treatment with 6G significantly inhibited max dP/dt and min dP/dt changes with no effect on EDP or ESP. In contrast, DM rats had no changes in the contractility index (figure 2E) but a significantly increased Tau (figure 2F). Despite that, treatment with 6G increased the contractility index significantly when compared with DM rats (figure 3E) and significantly alleviated the prolonged Tau close to the normal nondiabetic value (figure 2F).

3.3.Effect of 6G on cardiac ECG parameters

Diabetic rats study exhibited significant bradycardia with both increased RR and PR interval when compared to control nondiabetic animals (figure 3A, B and C). Treatment with 6G elicited no significant improvement in bradycardia or RR interval when compared with diabetics but did have reduce the PR interval to near normal nondiabetic values (figure 3A, B and C). In addition, DM rats showed increased total cycle duration without changing the diastolic duration when compared to the normal nondiabetic rats. Treatment with 6G produced no significant

differences in total or systolic duration in control or DM rats but increased diastolic duration in DM rats (figures 3D, E, and F).

Moreover, diabetes produced a significant increase in QT, QTc, and JT intervals when compared with control nondiabetic rats, and this changes were not significantly changed by treatment with 6G (figure 4A, B and C). In contrast, diabetes induced changes in ST height, T and R amplitude when compared with control nondiabetic rats (figure 4D, E and F) and these changes were significantly attenuated when diabetic rats were treated with 6G. 6G did not significantly change ST height, T or R amplitude in controls.

4. Discussion

In the present study, DM was induced experimentally in rats by STZ injection which was evident by a marked and significant hyperglycemia and decrease in body weight. STZ causes destruction of β-cells of the pancreas through internalization into the β-cells and liberation of toxic nitrosourea and nitric oxide [20]. Hyperglycemia in DM may directly or indirectly enhance oxidative stress and associated inflammation, leading to impairment of tissues inducing diabetic cardiomyopathy (DCM) and producing abnormal cardiac function [21]. Several previous studies have indicated a possible antihyperglyemic effect of the total ginger extract [22, 23]. 6G is one of the main constituents of ginger, and this antihyperglycemic action may be due to the antioxidant effect of 6G demonstrated in previous studies [24, 25].

Ventricular dysfunction in diabetic rats is evident in the current study by a significant reduction in in Max dP/dt, Min dP/dt, EDP, ESP and Tau confirming the damaging effects of diabetes on cardiac structure and function [26]. Maximum dP/dt is the maximal rate of rise of left ventricular pressure and is used often as an expression of myocardial contractility and systolic function [27]. In addition, minimum dP/dt can be used as a valuable tool in the evaluation of

diastolic function of the left ventricle [28]. Moreover, Weiss et al. [29] found that left ventricular diastolic function can be also assessed by an index called left ventricular diastolic time constant, or Tau. ESP is used as an indicator also for ventricular contractility and EDP is an indicator for preload [30, 31].

Previous studies found that hyperglycemia is usually associated with left ventricular hypertrophy in type 2 diabetes [32]. In the present study, the increase in R wave amplitude in the diabetic rats may indicate a change in left ventricular mass. With diabetes, pathological hypertrophy results from myocardial damage and fibrosis [33, 34]. Fibrosis may affect the filling and contractility of the ventricles leading to cardiac dysfunction [35]. The present study also shows that DM produces significant bradycardia with a prolongation of the RR interval. Previous investigations indicate an initial stimulation of the sympathetic nervous system in DM but prolonged exposure to hyperglycemia and elevated catecholamine levels causes a decrease in adrenergic receptors and subsequently bradycardia may occur [36]. This bradycardia was not improved with 6G. In addition, PR intervals are widened in diabetic animals and it has been reported that this is commonly associated with atrial fibrillation [37]. We demonstrate a significant improvement of this prolonged PR interval in diabetic rats treated with 6G. Moreover, there was a prolongation of total cycle duration (especially the systolic duration) indicating an effect on systolic and contractility function in diabetic rats. As QT interval is dependent on the duration of the cardiac cycle, it was also evident in the present study that QT interval was prolonged in diabetic rats. The ventricular repolarization abnormalities in the ECG, including T wave morphology changes and QT interval prolongation, could be markers of ventricular hypertrophy, left ventricular dysfunction or myocardial ischemia [38] and have been associated with an increased risk of sudden death [39]. Acute myocardial ischemia increases the duration of

QT interval [40]. Several mechanisms have been proposed to be involved in the prolongation of the QT interval secondary to acute myocardial ischemia including changes in the myocardial response to catecholamine or cholinergic stimulation, modification of calcium or potassium ion transport, or intracellular hydrogen concentration changes [41]. In addition, The ST-segment elevation is an indicative marker for ischemia [42], which is also evident in the diabetic rats in the current study.

The current results support the concept that the damaging effect of diabetes on the heart may be attributed mainly to the ischemic effect on coronary vessels and blood flow evidenced by ECG findings. In the present study treatment with 6G was found to prolong the diastolic duration, which may improve the coronary filling. During systole, the coronary arteries are compressed by ventricular contraction and greatly increase resistance to coronary blood flow. Therefore, most coronary blood flow in the left ventricle occurs during diastole [43]. We demonstrated improvement in cardiac function (including systolic and diastolic function) in this work. This diabetic model showed a significant decrease in serum adiponectin, and increases in both serum TGF-β1 and urinary 8-isoprostane levels when compared to control non-diabetic animals. Adiponectin is an antidiabetic, antiatherogenic and anti-inflammatory adipokine [44, 45]. In addition, it also has a cardioprotective effect [46]. Previous studies found that significant reductions in adiponectin in rodent models are associated with a higher incidence of diabetes, dyslipidemia, insulin resistance [44, 45], and cardiovascular disease [46]. TGF-β plays an important modulatory role in the immune system and it is usually elevated in DM and diabetic nephropathy [47, 48]. In addition, the 8-isoprostane is an oxidative marker and formed during the free radical-mediated oxidation of arachidonic acid in the cell membrane [49] and is also

elevated in diabetic patients [50]. In the current study, treatment with 6G produced no significant effect on adiponectin, TGF-β or 8-isoprostane levels.

In conclusion, the present study demonstrates that oral administration of the ginger constituent, 6G, not only alleviates the hyperglycemia in diabetic rats but also can ameliorates and improve the cardiac dysfunction induced by diabetes. This ameliorative effect is evident by improving contractility parameters and ischemia evident in ECG through a mechanism which is probably not related to its potential antioxidant effect.

Disclosure

The authors declare that they have no competing interests.

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Table 1: The effect of 6-gingerol (6G) treatment on lipid profiles of diabetes rats (DM) and normal controls including total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), and triglycerides (TG).

	TC	**LDL-C**	**HDL-C**	**TG**
Control	64.38 ± 12.05	50.49 ± 14.78	5.804 ± 2.10	42.20 ± 10.38
DM	77.18 ± 17.65	57.92 ± 14.45	6.472 ± 1.65	45.29 ± 17.33
DM + 6G	75.74 ± 20.98	56.92 ± 16.63	6.914 ± 1.63	36.52 ± 7.18
Control + 6G	69.89 ± 8.64	54.33 ± 7.05	8.744 ± 2.27	37.48 ± 11.61

Values are expressed as the mean ± SD; N=6-8 animals with no significant differences detected by One Way ANOVA.

Figure legends

Figure 1: The effect of 6-gingerol (6G) treatment on body weight (A), blood glucose level (B) serum adiponectin (C), serum TGF-β1 (D), and urinary 8-isoprostane (E) levels in diabetes (DM) and normal control rats

Values are expressed as the mean ± SD; N=6-8 animals. *P<0.05 compared with the corresponding control group values; #P<0.05 compared with the corresponding DM group values. Data were analysed using one-way ANOVA and Newman-Keuls post-hoc test.

Figure 2: The effect of 6-gingerol (6G) treatment in diabetes rats (DM) and normal control rats on (A) end diastolic pressure (EDP), (B) end systolic pressure, (C) Peak positive pressure (Max dP/dt), (D) Peak negative pressure (Min dP/dt), (E) contractility index and (F) left ventricular diastolic time constant (Tau).

Values are expressed as the mean ± SD; N=6-8 animals. *P<0.05 compared with the corresponding control group values; #P<0.05 compared with the DM group.

Data were analysed using one-way ANOVA and Newman-Keuls post hoc test.

Figure 3: The effect of 6-gingerol (6G) treatment in diabetes rats (DM) and normal control rats on heart rate (A), R-R interval (B), P-R interval (C), cycle duration, (D), systolic duration (E), and diastolic duration (F).

Values are expressed as the mean ± SD; N=6-8 animals. *P<0.05 compared with the corresponding control group values; #P<0.05 compared with the DM group. Data were analysed by using one-way ANOVA and Newman-Keuls post hoc test.

Figure 4: The effect of 6-gingerol (6G) treatment in diabetes rats (DM) and normal control rats on QT interval (A), corrected QT (QTc) interval (B), JT interval (C), ST height (D), T amplitude (E), and R amplitude (F).

Values are expressed as the mean ± SD; N=6-8 animals. *P<0.05 compared with the corresponding control group values; #P<0.05 compared with the DM group. Data were analysed by using one-way ANOVA and Newman-Keuls post hoc test.

FIGURES

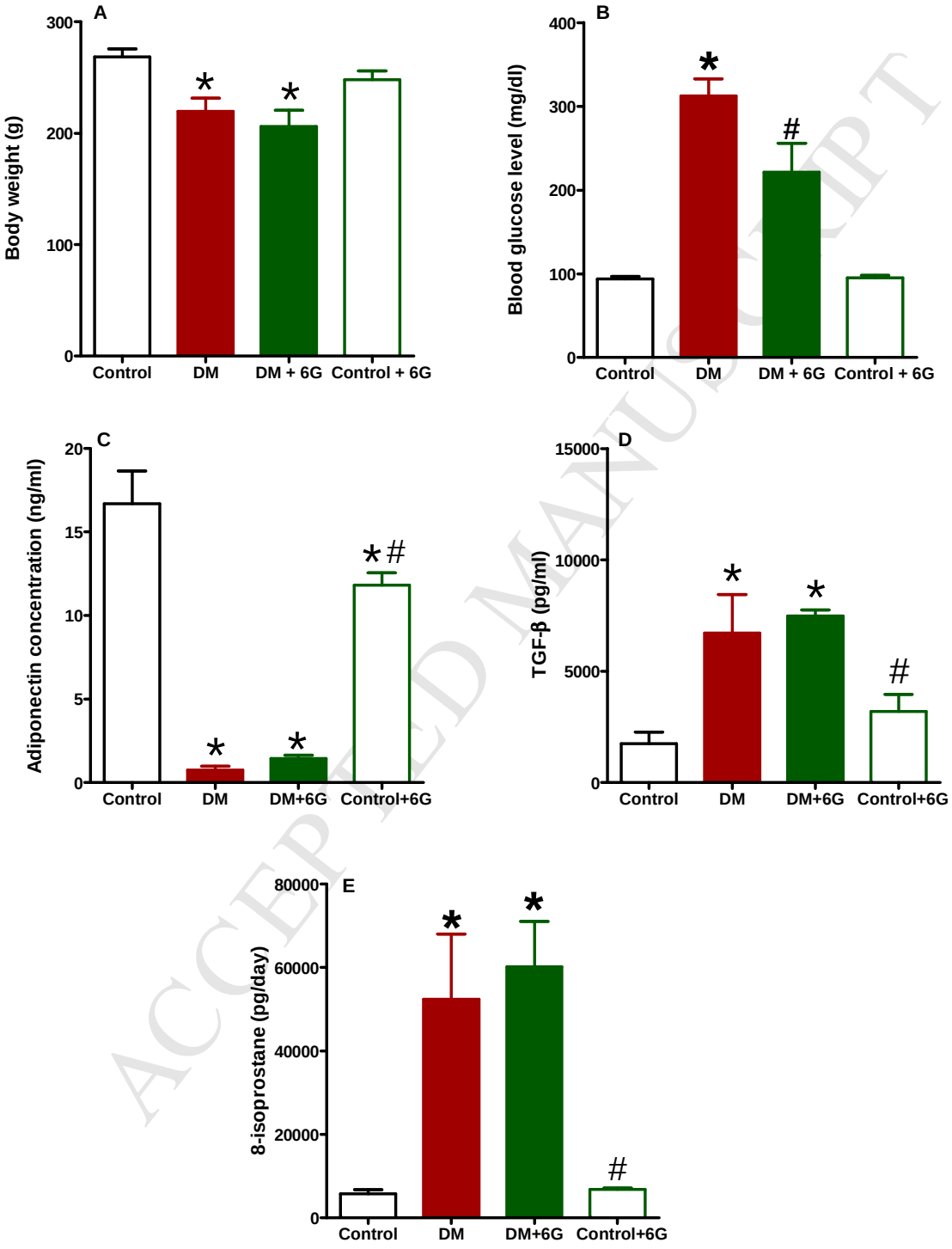

Figure 1

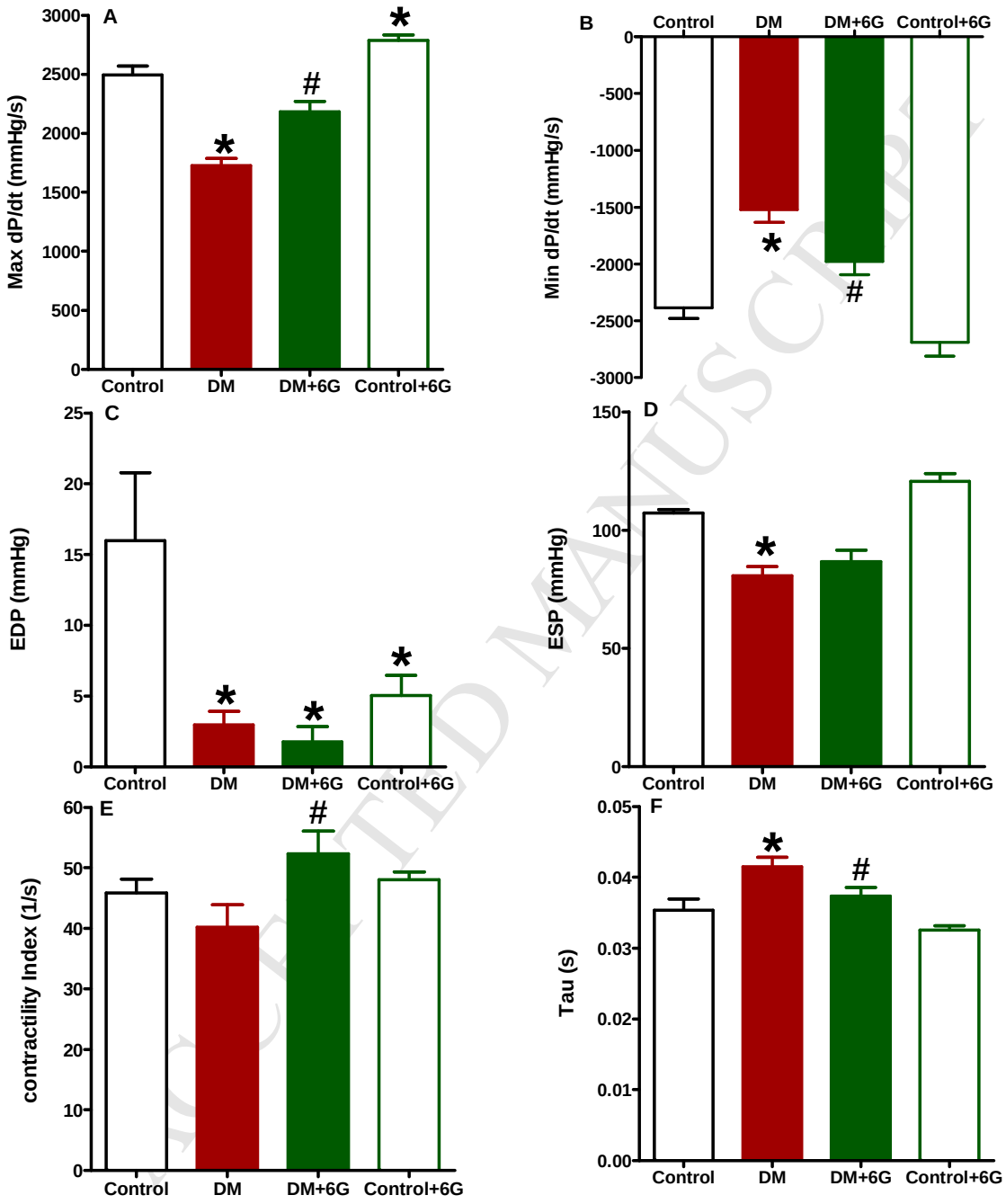
A
Max dP/dt (mmHg/s)
3000
2500
2000
1500
1000
500
0
Control
DM
DM+6G
Control+6G
B
Min dP/dt (mmHg/s)
0
-500
-1000
-1500
-2000
-2500
-3000
C
EDP (mmHg)
25
20
15
10
5
D
ESP (mmHg)
150
100
50
E
contractility Index (1/s)
60
50
40
30
20
10
F
Tau (s)
0.05
0.04
0.03
0.02
0.01
0.00

Figure 2

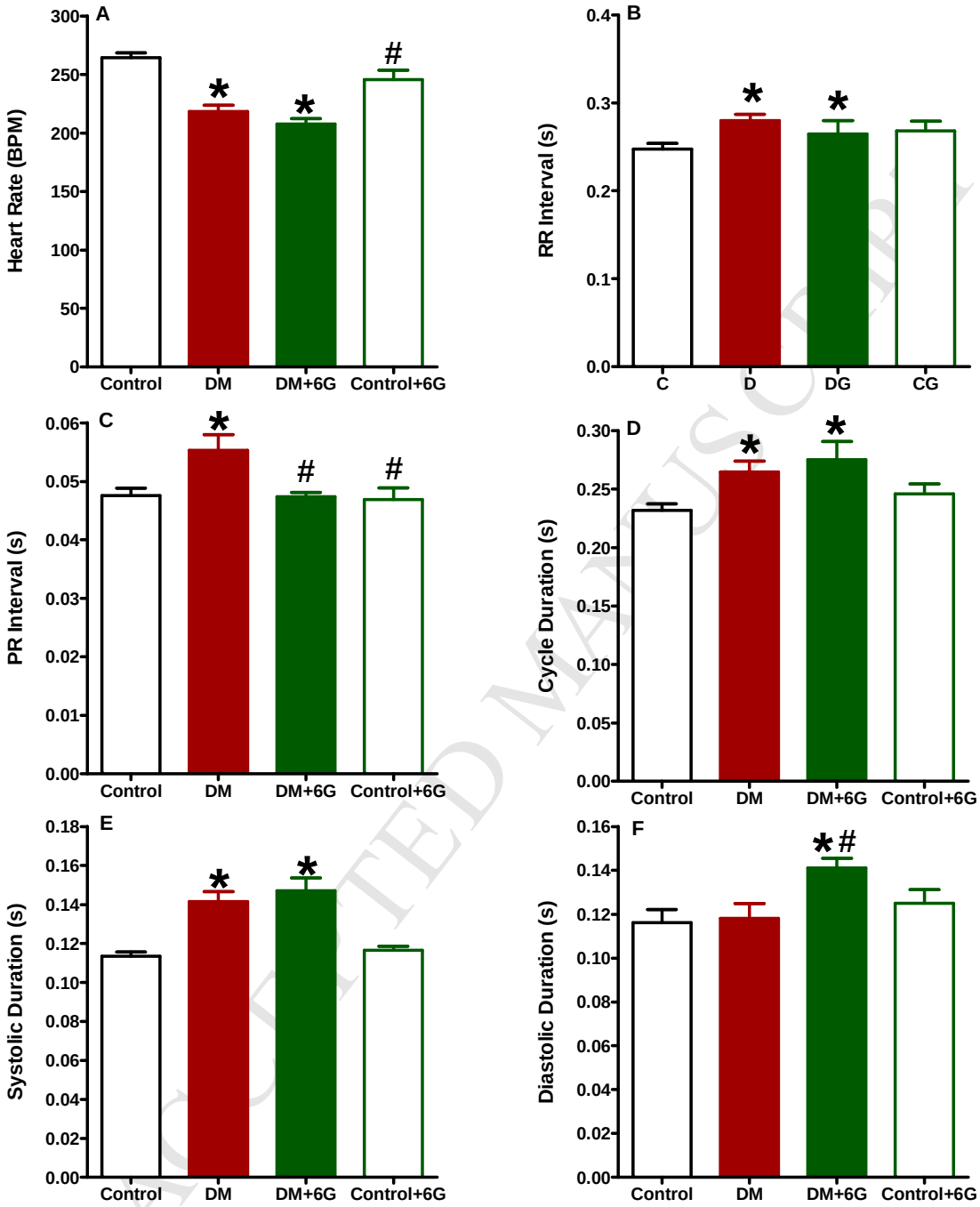
A
Heart Rate (BPM)
300
250
200
150
100
50
0
Control
DM
DM+6G
Control+6G
B
RR Interval (s)
0.4
0.3
0.2
0.1
0.0
C
D
DG
CG
C
PR Interval (s)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
D
Cycle Duration (s)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
E
Systolic Duration (s)
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
F
Diastolic Duration (s)
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00

Figure 3

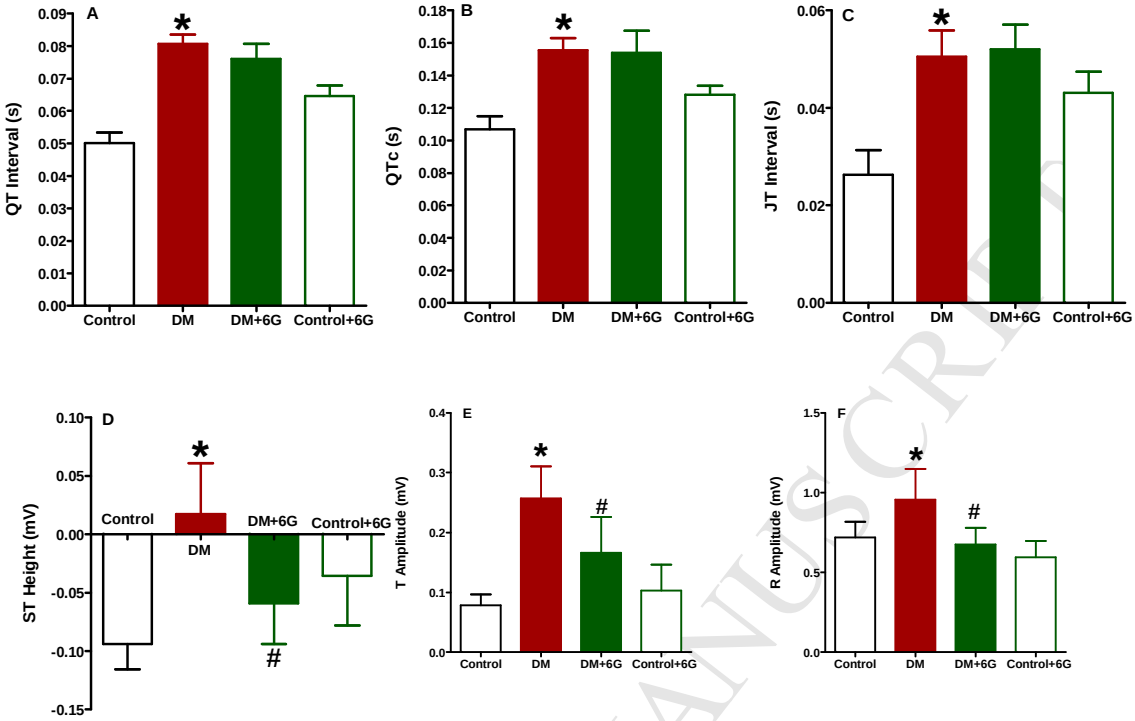

Figure 4