Effects of Abscisic Acid on the Diabetic Changes in Rat Myocardium

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Abstract

The present study aims to find out the possible protective effect of abscisic acid (ABA) on the development of diabetic cardiomyopathy (DCM) in type 2 diabetic rats. *Materials and methods:* Fifty male rats divided into: normal control group, diabetic group and three diabetic treated groups with either pioglitazone, or ABA, or both pioglitazone and ABA for 16 weeks. At the end of experiment, ECG was recorded, biochemical measurement of serum glucose, insulin, lipid profile, troponin I, creatine kinase MB (CK-MB), Lactate dehydrogenase (LDH), interleukin-1 beta (IL-1β) and tumor necrosis factor- alpha (TNF-α) was done, caspase 3 and 9 activity in heart, RT- PCR for connexin-43(Cx43) and histopathological examination of cardiac tissue. *Results:* Treatment with ABA exerted positive effects on blood glucose and insulin levels that found to be reflected on heart weight/body weight ratio in diabetic rats. Also, it exerted significant improvement in cardiac markers and pro-inflammatory cytokines. DCM is associated with increased myocyte cell death that indicated by increasing caspase 3 and 9 that improved significantly by ABA. Also, results indicated that myocardial Cx43 mRNA levels were lesser in diabetic versus non-diabetic rats. Cx43 deterioration in diabetics may be behind the prolongation of the QRS and QTc, that improved by ABA. The histopathological findings showed that ABA improved diabetic cardiomyocyte necrosis and fibrosis. *Conclusion:* The diabetic rats benefit from ABA intake due to its hypoglycemic, anti-inflammatory and anti-apoptotic effects. So, intake of ABA in combination with anti-diabetic drugs may be beneficial for the management of type 2 diabetes mellitus.

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INTRODUCTION

Diabetes mellitus (DM) is a global metabolic disease that continues to be a health problem in the developed as well as developing nations. Epidemiological studies predict that it will affect nearly 400 million people by 2030 [1]. Cardiovascular complications are the most common cause of morbidity and mortality in patients with diabetes [2]. Both type1 and type2 diabetes mellitus promoted cardiac dysfunction; a condition described as diabetic cardiomyopathy (DCM), either directly or indirectly. DCM could be induced directly by upregulation of calpain-1 specifically in mitochondria that induce the cleavage of ATP synthase, increase in superoxide generation and apoptosis in cardiomyocytes [3]. In addition, increased formation of myocardial advanced glycation end-products (AGEs) that associated with hyperglycemia has also been reported to promote collagen accumulation and fibrosis in the cardiac tissue [4]. Also, DCM may be promoted indirectly through premature atherosclerosis [5]. The risk incidence of developing heart failure increases in DM regardless of hypertension, obesity, hyperlipidemia or underlying coronary heart disease [6]. In the early stages, diabetic cardiomyopathy may present with diastolic dysfunction and subsequently proceed to systolic dysfunction [7]. The pathogenesis of diabetic cardiomyopathy is incompletely understood, and limited treatment options exist.

2-Cis, 4-trans-abscisic acid (ABA) is a plant hormone that regulates important physiologic functions, such as the response to abiotic stress (water and nutrient availability, UV irradiation) [8]. It is also present in mammalian plasma, and several cell types like human granulocytes, mesenchymal stem cell and pancreatic B cells have been shown to produce it [9]. Bruzzone et al. (2007) found that ABA released from human granulocytes stimulates phagocytosis, cell migration, and production of reactive oxygen species indicating that ABA should be considered as a new pro-inflammatory cytokine in humans [10, 11]. Also, ABA was found to decrease macrophage accumulation in white adipose tissue of obese diabetic mice [12, 13]. Activated platelets or quartz particles stimulate ABA release from monocytes/macrophages which in turn stimulates release of cytokines and prostaglandin E2 in an autocrine manner [14, 15]. IL-8 induces human mesenchymal stem cells to release ABA, which stimulates the release of growth factors from these cells and colony growth from human hemopoietic progenitors [16, 17].

ABA is also suggested to be involved in the regulation of glucose metabolism in humans. Plasma ABA (ABAp) concentration increases in humans after a glucose overload, indicating that it is an endogenous hormone [9]. This glucose overload stimulates β-pancreatic cells release of ABA, which in turn stimulates insulin release [12]. In vitro, ABA stimulates glucose uptake in murine cells, independent of insulin, by increasing the expression and membrane translocation of the glucose transporter (GLUT)-4 in skeletal muscle [2]. Most recently, Ameri et al. [16] noticed that an impairment of the hyperglycemia-induced increase in ABAp was observed in subjects with type 2 diabetes mellitus (T2DM) and with gestational diabetes mellitus (GDM). They also observed an

interesting finding in women with GDM one month after child birth; ABAp either fasting or in response to glucose is restored to normal, along with glucose tolerance. These in vivo results strongly suggest a beneficial effect of elevated ABAp on glycemic control in humans.

Abscisic acid is structurally similar to thiazolidinediones (TZDs) which are PPAR-γ agonists, have been shown to be very effective in improving systemic insulin sensitivity. It is found that ABA could be used as a nutritional supplement to combat T2DM and obesity-related inflammation [17]. No side effects were observed in ABA-fed mice, such as excess weight gain and fluid retention, which are commonly observed with TZDs [18]. Moreover, an in vitro study of Vigliarolo et al. [19] suggested a role for endogenous ABA in the protection against oxygen depletion in cardiomyocytes, by stimulating NO production and glucose uptake. So, we aim in this study to find out the role of abscisic acid in metabolic control of T2DM rats and its possible protective effect on the development of diabetic cardiomyopathy.

Materials and Methods:

Fifty male Sprague-Dawley rats (220–240 g) were procured at our Medical Experimental Research Center of Mansoura University, Egypt and housed in an air-conditioned room at a temperature of 25±2 ℃ and relative humidity of 45% to 55% under 12-h light: 12-h dark cycle. The animals had free access to food pellets except when starvation was required. Water was provided *ad libitum*. The protocol of this research followed the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, National Research Council, Washington, DC and National Academy Press, no. 85-23, revised 1996). Our Local Committee of Animal Care and Use approved this protocol (code number: R/18.03.72). After 1 week of acclimatization, the rats were randomly divided into 5 groups each contains 10 rats: normal control group (C), diabetic group (DM) and three diabetic treated groups with either pioglitazone (DM+PG), or abscisic acid (DM+ABA), or both pioglitazone and abscisic acid (DM+PG+ABA) for 16 weeks. Abscisic acid (1 mg/kg/day) [20] and pioglitazone (10 mg/kg/day) [20] were administered by oral gavage throughout the experimental period. ECG recording and body weight (BW) were measured just before induction of diabetes and at the end of study periods. Overnight fasted rats were anesthetized using urethane (1 g/kg, ip). Urethane was selected as an anesthetic agent as its single dose induces long-term anesthesia and analgesia with minimal cardiovascular and respiratory system depression [21]. Blood was collected immediately from the heart, allowed to clot at room temperature for 10 to 15 min and then centrifuged for 10 min at 3000g; clear serum was obtained and stored at -20°C until measuring serum parameters. Immediately after scarifice, hearts were quickly excised, cleaned, and perfused with ice-cold saline, dried with filter paper to measure the heart weight (HW) to find out HW/BW ratio. Each heart was cut into pieces; One part was fixed in 10% formaldehyde, which was left for H& E, Masson's trichrome staining and the other part was stored at −70 °C for the following

detection of caspases 3,9 and for RT-PCR of connexin-43.

Induction of type 2 diabetes (T2DM)

For induction of T2DM all groups of rats were fed high fat diet (HFD) (58%fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) for the initial period of 8 weeks [22], while normal control rats were fed on normal diet ad libitum (60% CHO/10% fat/30% protein, as a percentage of total kcal).The composition of food given to the groups is presented in table 1 [23]. After 8 weeks of HFD, 40 mg/kg streptozotocin (STZ) (Sigma Chemical Co., St. Louis, MO, USA) dissolved in 10 mM citrate buffer (pH 4.5) was given intraperitoneal in the lower right quadrant of the abdomen to all groups with HFD regimens, while the control negative rats were injected with citrate buffer alone (pH 4.5) in a dose volume of 1ml/ kg. Blood glucose concentration were measured 72 h after STZ injection using the One Touch Blood Glucose Monitoring System (Life Scan, Milpitas, CA) by tail vein puncture blood sampling. The rats with plasma glucose ≥300 mg/ dl were considered diabetic and selected for our study.

Assessment of electrocardiography (ECG)

ECG was recorded twice for each rat, one at the beginning of study to exclude any cardiac abnormality and the second time at the end of the study using BIOPAC System, Inc. (USA). ECG leads were recorded with surface electrodes (AD Instruments), the leads were placed on the right foreleg (negative electrode), left foreleg (positive electrode) and right hind leg (neutral electrode).The mean value for each rat was obtained from four values consisting of four consecutive cardiac cycles using Lab Chart software (AD Instruments). A corrected QT interval (QTc) that takes into account changes in HR is often used as a more objective parameter of depolarization and repolarization of ventricles. QTc were calculated according to the following formula:

$$QTc = \frac{QT}{\sqrt{\frac{RR}{f}}}$$

Where f is the normalization factor according to the basal RR duration in rats, which is 150 ms [24].

Measurement of serum parameters

Serum levels of glucose (GL) concentration (mg/dl) was measured by a glucose oxidase method and fasting insulin (FI) (μU/ml) was estimated using radio immune assay (RIA) kit (Sigma - Aldrich, St Louis, MO, USA).

HOMA-IR was used to assess insulin sensitivity [25].

$$HOMA\text{-}IR = \frac{GL\ (mg/dl)\ x\ FI\ (\mu U/ml)}{405}$$

Serum levels of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) cholesterol were assayed according to the methods of Allain et al [26], Fossati and Prencipe [27] and Burstein et al [28] using diagnostic kits purchased from Analyticon, Biotechnologies AG, (Germany).

Low density lipoprotein cholesterol (LDL) concentration was calculated by using formulae of Friedwald et al. [29]: LDL = [TC] - [HDL+VLDL]

Very Low Density Lipoprotein Cholesterol (VLDL) was calculated using the following equation: VLDL (mg/dl) = TG/5 [29]

Cardiovascular risk indices were calculated according to Ross [30] as follows:

Cardiovascular risk index 1 = Total cholesterol / HDL cholesterol

Cardiovascular risk index 2 = LDL cholesterol / HDL cholesterol

Biological cardiac markers and inflammatory cytokines

Serum levels of troponin I, creatine kinase MB (CK-MB), Lactate dehydrogenase (LDH), interleukin-1 beta (IL-1β) and tumor necrosis factor- alpha (TNF-α) were analyzed with an automatic biochemical analyzer (Abx Diagnostics, Montpellier, France).

Assessment of caspase-3 and caspase9 activity in cardiac tissues

All procedures were performed at 4°C. The heart samples were lysed by 30 strokes using a Konetes homogenizer at a ratio of 100 mg tissue/1 ml lysis buffer [50 mM Tris-HCl (pH 7.4), 2 mM EDTA, 2 mM EGTA, 150 mM NaCl, 1 mM PMSF, 10 μg/ml leupeptin, 1 mM sodium orthovanadate, 1% (v/v) 2-mercaptoethanol, 1% (v/v)

Nonidet P40, and 0.3% sodium deoxycholate]. These homogenates were centrifuged at 100,000 g for 1 h. The supernatant was stored at -70°C for biochemical assays of caspase-3 and 9 activities using colorimetric assay kits; the procedures were performed according to the manufacturer's instructions (Sigma - Aldrich, St Louis, MO, USA). Spectrophotometric measurement of the chromophore p-nitroanilide (pNA) released from DEVD-pNA by caspase-3 and from LEHD-pNA by caspase 9 were performed. The absorbance was measured at 405 nm and results can be calculated as pmol of pNA released per minute per μg protein [31].

Real-time PCR for connexin-43(Cx43) mRNA assay

RNA isolation and reverse transcription were performed. The obtained single-chain DNA was used for real-time PCR. Amplification was performed in 10 μl of SYBR Green PCR Master Mix containing 30 pmol.l^{-1} of each primer. For amplification of GJP43 gene and beta-actin (the housekeeping gene was not reported to be changed in DM), gene fragments of the following primers were used to determine Cx43-mRNA level: GJP43, sense 5′-TCC TTG GTG TCT CTC GCT TT-3′, antisense 5′-GAG CAG CCA TTG AAG TAG GC-3′; and beta-actin, sense 5′-TCA TCA CTA TCG GCA ATG AGC-3′, antisense 5′-GGC CAG GAT AGA GCC ACC A-3′. Sample volume was adjusted to 20 μl with deionized water. Amplification was performed as previously described [32]. The CT (cycle threshold) was defined as the number of cycles required for the fluorescence signal to exceed the detection threshold. We calculated the expression of the target gene relative to the housekeeping gene as the difference between the CT values of the two genes.

Histopathological studies

The second portion of isolated heart were preserved in 10% formalin for 24 h. Specimens were cut in section of 3–5 μm in thickness to be stained with hematoxyline and eosin (H&E) stain in order to examine the morphological changes. Additionally, heart sections were stained with Masson's trichrome to examine extracellular matrix (ECM) deposition. The grading system used for assessment of parameters was [–: absence of change; +: 0–30% area shows changes (mild); + +: 30–60% area shows changes (moderate); + + +: 60–100% area shows focal changes (severe focal); + + ++: 60–100% area shows diffuse changes (severe diffuse)] [33].

Table (1): Composition of high fat diet

Ingredients	Diet (g/kg)
Powdered NPD	365
Lard	310
Casein	250
Cholesterol	10
Vitamin and mineral mix	60
dl-Methionine	03
Yeast powder	01
Sodium chloride	01

Statistical analysis

Expression of the results were shown as mean ± SD of the rats in each group. The results were statistically analyzed using One-way ANOVA followed by post-hoc test to evaluate the significance between the different investigated groups using SPSS version 16 (Chicago, IL, USA). Values with $P < 0.05$ were statistically considered significant.

RESULTS

The results presented in table (2) revealed a significant reduction in the body weights of diabetic group as compared to control group. As regarding HW/BW ratio in diabetic rats, there was significant increase in this ratio as compared to control rats with significant reduction in all treated groups when compared to the diabetic one. Table (2) also showed significant increase in serum glucose and HOMA-IR with significant reduction in serum insulin levels in diabetic group. However, treated groups showed significant improvement in these measured parameters especially, in ABA and combined treated groups.

Regarding to the lipid profile parameters, figure (1) showed significant elevation in serum levels of TG, TC, LDL, and VLDL with reduction in HDL in diabetic rats as compared to control. These parameters were significantly improved in treated rats especially in the combined treated group. To explore the impact of diabetes-induced hyperlipidemia and protective effect of ABA on the heart, the cardiovascular risk indices were calculated as shown in figure (2). Diabetic rats showed significant increase in both cardiac indices

when compared with the control rats. However, ABA supplemented rats for 16 weeks exhibited marked improvement in these recorded indices.

Table (3) showed significant elevation in serum troponin I, CK-MB, LDH, IL-1β and TNF-α levels of diabetic rats as compared to control ones. While, ABA treated groups showed significant decrease in their serum levels when compared to the diabetic rats. Figure (3) showed that both caspase 3 and 9 activities in cardiac tissues were significantly increased in diabetic group as compared with the control one; while, ABA supplementation gave significant reduction in both caspases activities especially when combined with pioglitazone. Figure (4) showed significant decrease in Cx43 expression in diabetic myocardia; however, supplementation with ABA significantly improved its expression.

Regarding ECG records, the diabetic hearts showed significant changes in QRS amplitude and duration together with QTc and heart rate when compared to the control ones (table 4, figure 5). Treatment with ABA revealed significant improvement of these changes; otherwise, P-R

interval and ST segment did not show any significant changes in all groups.

Histopathological examination reveals that diabetic hearts in figure (7) displayed structural abnormalities, including marked myocardial necrosis associated with nuclear lysis and myophagia, also diabetic hearts shows marked distortion of myocardial fiber associated with marked sarcoplasmic esinophilia (H&E), together with marked fibrous connective tissue proliferation between the atrophied fibers as shown with Masson" stained sections (100 µm),

Treatment with pioglitazone shows marked decrease of myocardial degeneration and myolysis, with focal area of perivascular fibrous connective tissue (figure 8). While, group treated with ABA shows marked decrease of myocardial degeneration and myolysis with mild degree of perivascular fibrous connective tissue (figure 9). Interestingly, in combined treated hearts there is marked decrease of myolysis with only minimal perivascular fibrous connective tissue layer (figure 10).

Table 2: Effect of abscisic acid and pioglitazone on body weight, HW/BW ratio, HOMA-IR, glucose and insulin levels in different studied groups

	C	DM	DM+PG	DM+ABA	DM+PG+ABA
Body weight (g)	403.83±13.27	316.33±6.74[a]	371.17±7.70[ab]	378.33±7.92[ab]	403.00±8.02[bcd]
HW/BW (mg/g)	1.49±0.05	1.97±0.05[a]	1.62±0.04[ab]	1.59±0.05[ab]	1.47±0.03[bcd]
Glucose (mg/dl)	88.83±7.63	349.17±43.22[a]	190.67±10.75[ab]	167.33±7.84[ab]	140.17±16.58[abc]
Insulin (µU/ml)	23.62±3.11	13.68±1.26[a]	16.30±0.90[a]	18.90±0.86[ab]	19.50±0.74[abc]
HOMA-IR	5.19±0.83	11.77±1.65[a]	7.68±0.69[ab]	7.81±0.54[ab]	6.74±0.73[b]

C: control group, DM: diabetic group, DM+PG: diabetic treated with pioglitazone, DM+ABA: diabetic treated with abscisic acid, DM+PG+ABA: diabetic treated with combined pioglitazone & abscisic acid. Data are expressed as mean ± SD, test used: One way ANOVA, followed by post-hoc tukey, P: significance <0.05. a: significance as compared to C group. c: significance as compared to DM+PG group. b:significance as compared to DM group. d:significance as compared to DM+ABA group.

Table 3: Effect of abscisic acid and pioglitazone supplementation on cardiac markers and inflammatory cytokines in all experimental groups

	C	**DM**	**DM+PG**	**DM+ABA**	**DM+PG+ABA**
Troponin I (ng/ ml)	119.17±5.56	211.17±8.26[a]	170.00±6.60[ab]	157.17±6.62[abc]	139.33±6.25[abcd]
Creatine kinase (U/L)	95.33±6.65	193.67±9.42[a]	155.00±5.97[ab]	140.50±5.99[abc]	112.67±5.92[abcd]
LDH (U/L)	70.50±7.56	198.33±9.85[a]	158.17±6.34[ab]	137.67±6.83[abc]	112.83±6.62[abcd]
TNF-α (ng/L)	147.00±5.40	298.33±12.91[a]	227.33±10.03[ab]	184.50±4.51[abc]	165.17±3.43[abcd]
IL-1β (ng/L)	13.20±0.55	21.35±1.57[a]	17.65±0.47[ab]	15.83±0.65[abc]	14.43±0.42[bc]

C: control group, DM: diabetic group, DM+PG: diabetic treated with pioglitazone, DM+ABA: diabetic treated with abscisic acid, DM+PG+ABA: diabetic treated with combined pioglitazone & abscisic acid. Data are expressed as mean ± SD, test used: One way ANOVA, followed by post-hoc tukey, P: significance <0.05. a: significance as compared to C group. c: significance as compared to DM+PG group. b:significance as compared to DM group. d:significance as compared to DM+ABA group.
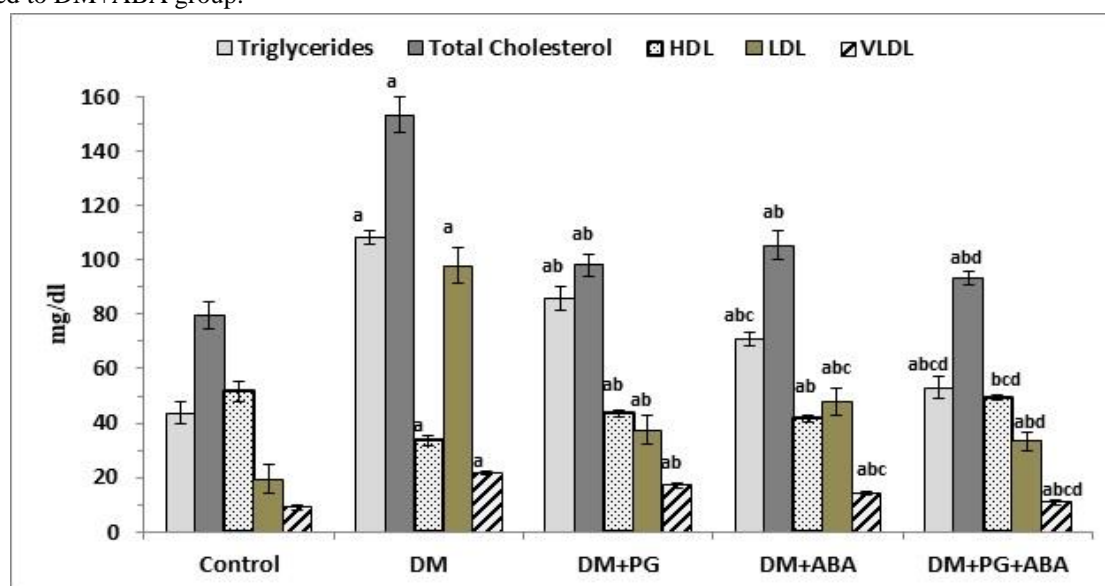
Figure 1: *Shows the lipid profile in the experimental groups. Data are expressed as mean ± SD, test used: One way ANOVA, followed by post-hoc tukey, P: significance <0.05. a: significance as compared to C group. c: significance as compared to DM+PG group. b:significance as compared to DM group. d:significance as compared to DM+ABA group.*

Table (4): ECG parameters in different experimental groups

	C	**DM**	**DM+PG**	**DM+ABA**	**DM+PG+ABA**
P-R (sec)	0.053±0.006	0.056±0.008	0.049±0.007	0.049±0.006	0.046±0.005
QRS duration (sec)	0.025±0.003	0.032±0.003[a]	0.024±0.003[b]	0.027±0.003[b]	0.026±0.003[b]
QRS amplitude (mV)	0.242±0.008	0.176±0.025[a]	0.234±0.007[b]	0.233±0.005[b]	0.234±0.011[b]
QTc	40.560±4.274	57.535±4.257[a]	41.928±2.124[b]	40.380±3.932[b]	40.027±4.113[b]
S-T segment	0.034±0.003	0.032±0.003	0.033±0.005	0.030±0.004	0.034±0.003
HR (beat/min)	278.667±5.465	222.167±7.026[a]	270.667±7.118[b]	266.667±4.719[ab]	276.333±2.733[bd]

C: control group, DM: diabetic group, DM+PG: diabetic treated with pioglitazone, DM+ABA: diabetic treated with abscisic acid, DM+PG+ABA: diabetic treated with combined pioglitazone & abscisic acid. Data are expressed as mean ± SD, test used: One way ANOVA, followed by post-hoc tukey, P: significance <0.05. a: significance as compared to C group. c: significance as compared to DM+PG group. b:significance as compared to DM group. d:significance as compared to DM+ABA group.
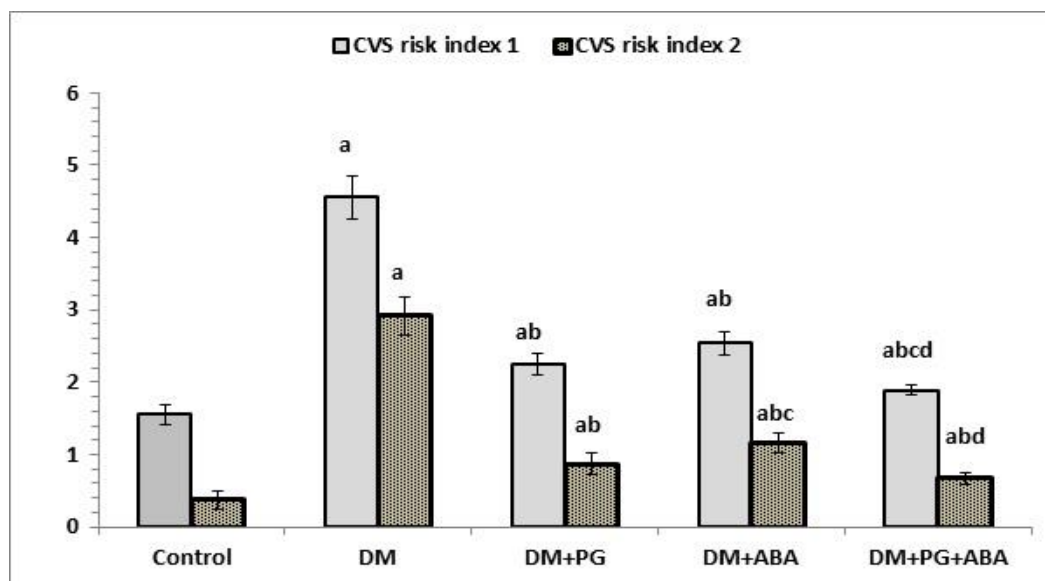
Figure 2: cardiovascular risk indices in the experimental groups. Data are expressed as mean ± SD, test used: One way ANOVA, followed by post-hoc tukey, P: significance <0.05. a: significance as compared to C group. c: significance as compared to DM+PG group. b: significance as compared to DM group. d: significance as compared to DM+ABA group.

Table (5): Semi quantitative scoring of myocardial lesions within different studied groups.

	Control	DM	DM+PG	DM+ABA	DM+PG+ABA
Normal fibers histology	++++	+	++	+++	+++
Myocardial degeneration	-	+++	++	+	+
Myolysis	-	+++	+	+	-
Fibrosis	-	++	+	+	+
Myocarditis	-	+++	+	+	-

(-) indicates no detectable lesions; (+) indicates mild lesions; (++) indicates moderate lesions; (+++) indicates severe focal lesions; (++++) indicates severe diffuse lesions.
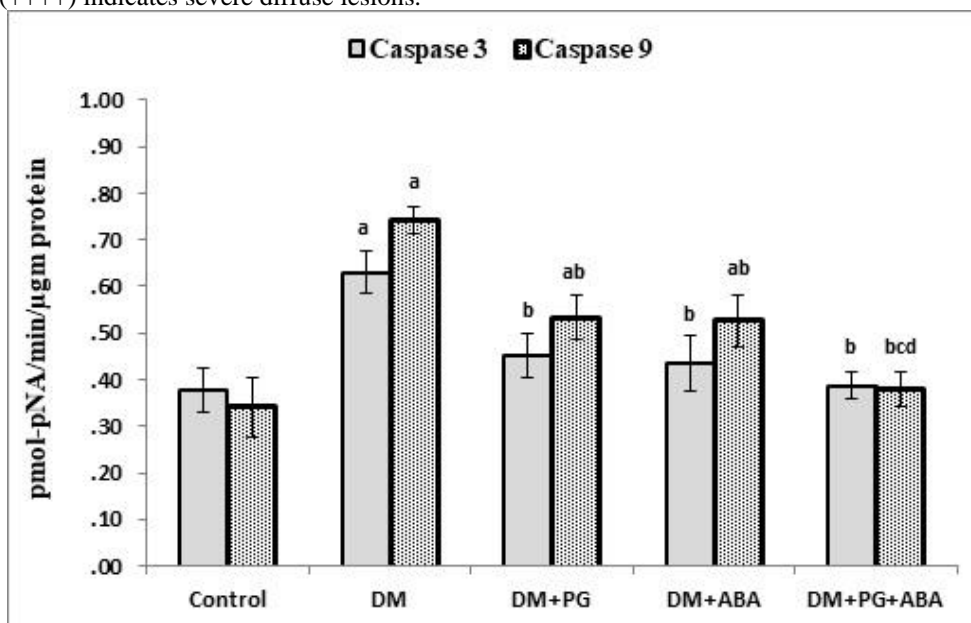
Figure 3: shows caspase3 and 9 detections in cardiac tissues of all experimental groups. Data are expressed as mean ± SD, test used: One-way ANOVA, followed by post-hoc Tukey, P: significance <0.05. a: significance as compared to C group. c: significance as compared to DM+PG group. b: significance as compared to DM group. d: significance as compared to DM+ABA group.

Discussion:

In the present study, a low dose of STZ injection (40 mg/kg body weight) after 8-week feeding of the high-energy diet was used to obtain type 2 diabetes animal model, since it simulates the real course of human type 2 diabetes mellitus [34]. Results proved the production of diabetes by marked decrease in serum insulin together with significant elevation of serum glucose and HOMA-IR which were more similar to those of type2 diabetes than those of type1 diabetes. Consistent with Radosinska et al. [35], using this model of diabetes, our results indicate that the body weights of T2DM rats were significantly lower than those of non-diabetic rats. This body weight loss could be explained by Yang et al. [36] who found that insufficient insulin in diabetics results in prevention of the body from getting glucose as an energy source, so, the body starts burning fat and muscle for getting energy, reducing the overall body weight.

While, HW/BW ratio in diabetics showed significant increase than those of control ones, which could be a sign for myocardial hypertrophy in consistent with Fang et al. [37] results. Myocardial hypertrophy could be explained by increasing the fatty acid oxidation in diabetics, leading to lipid accumulation in the myocardium. Treatment with abscisic acid exerted positive effects on blood glucose and HOMA-IR that found to be reflected on HW/BW ratio and the greatly alleviated BW in diabetic rats. This beneficial effect of ABA could be directly attributable to the lowered serum total cholesterol, triglycerides, LDL and VLDL cholesterol together with increased HDL cholesterol levels in ABA treated groups that improved the diabetic lipid profile. In addition, the recorded values of cardiac risk indices showed the bad impact of diabetes-induced dyslipidemia on the cardiovascular system that were improved with ABA supplementation which could strongly support the notion that ABA may reduce the risk of developing heart diseases.

The diabetic cardiomyopathy was confirmed by the elevated levels of CK-MB, LDH and troponin I, which represent sensitive and powerful predictors of increased cardiac complications. Furthermore, studies have demonstrated the relation between CK-MB and troponin I elevation and the onset of myofibrillar disintegration and increased permeability caused by inflammation [38]. These markers were significantly improved after supplementation with ABA.

As a consequence of oxidative stress in DM, cardiac inflammation and apoptosis have been recognized. Therefore, the state of chronic low-grade inflammation, a feature of obese T2DM, seems to play a major role in DCM progression [39]. In the present study, the proinflammatory cytokines IL-1β and TNF-α showed a significant increase in the diabetic rats. Results revealed a significant improvement in the levels of these cytokines with ABA supplementation which indicates a beneficial effect of ABA on DCM. In this context, Guri et al. [40] pointed to an anti-inflammatory effect exerted by ABA (100 mg/kg of a racemic ABA mixture for 84 days) on atherosclerosis by suppressing macrophage and CD4+ T cell recruitment into the aortic wall of obese mice. Also, Bassaganya-Riera et al. [41] reported that ABA can regulate inflammation via activation of PAPAR -γ.
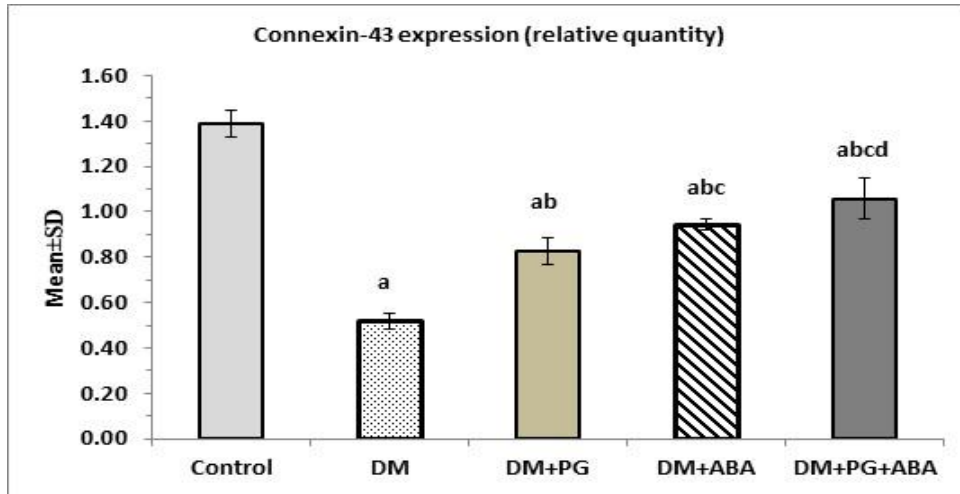
Figure 4: *Expression of connexin 43 in the myocardia of all studied groups.* *Data are expressed as mean ± SD, test used: One way ANOVA, followed by post-hoc tukey, P: significance <0.05. a: significance as compared to C group. c: significance as compared to DM+PG group. b:significance as compared to DM group. d:significance as compared to DM+ABA group.*
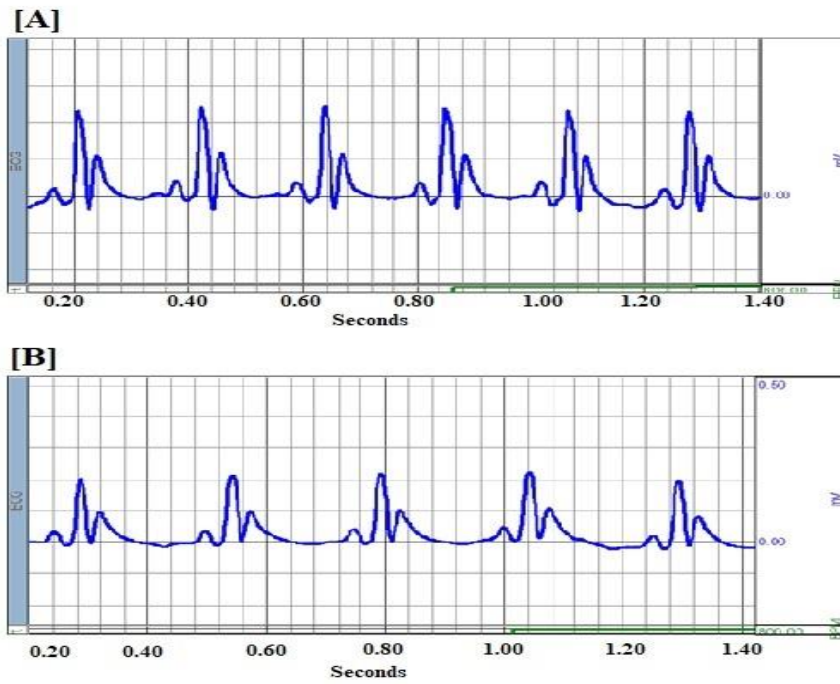

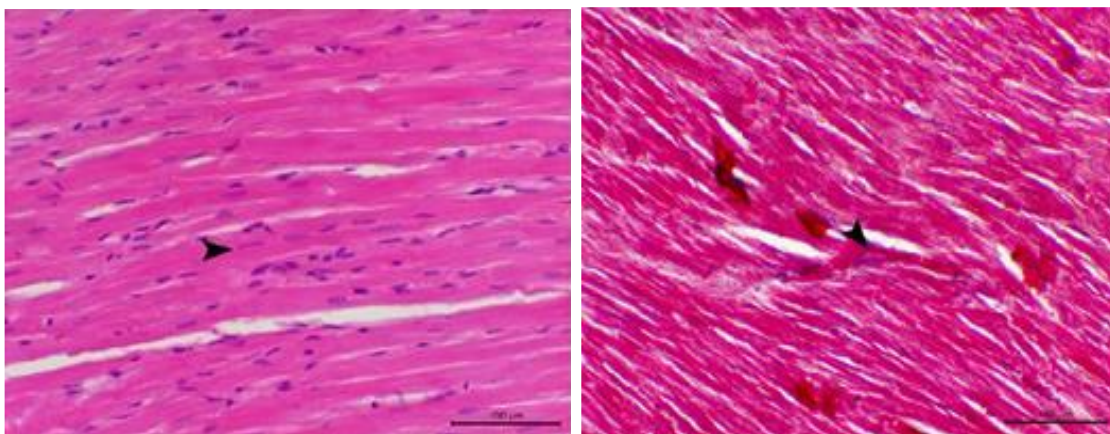**Figure 5:** *ECG records in normal [A] and diabetic [B] rats*

Figure 6: *Heart of normal control animal. [A] H&E stain shows normal myocardial fibers (arrowhead), [B] Masson's trichrome stain shows a thin layer of fibrous connective tissue mainly perivascular (arrowhead) [x100 μm].*
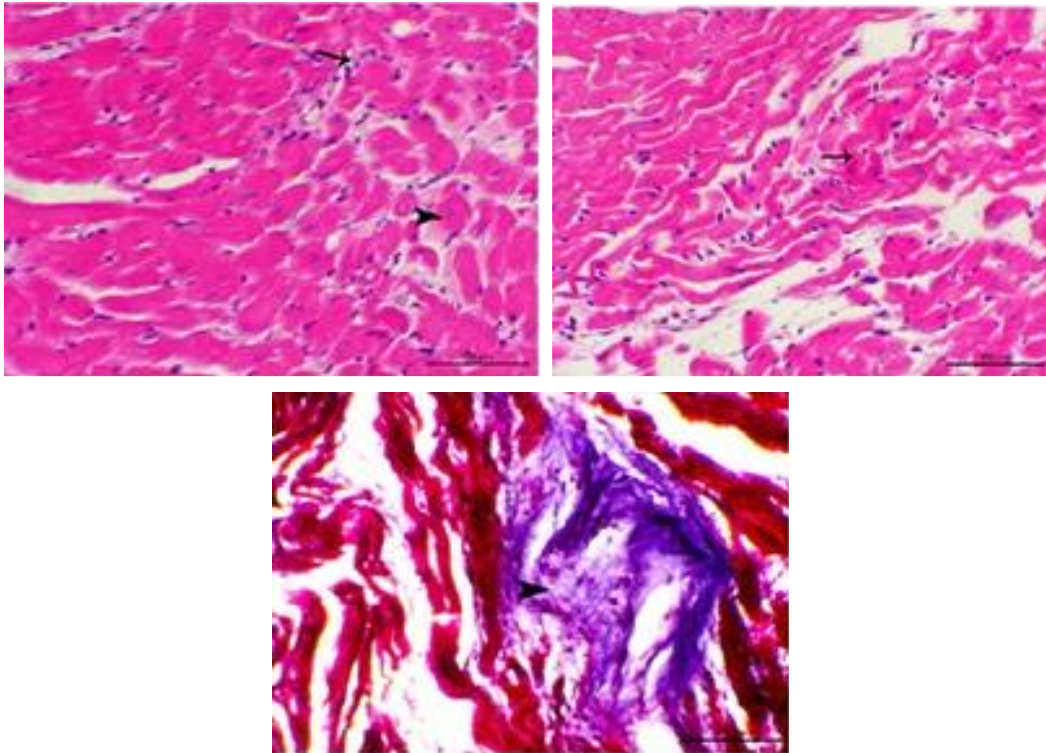
Figure 7: *Heart of diabetic animal. [A] H&E stain shows marked myocardial necrosis associated with nuclear lysis (arrowhead) and myophagia (arrow), [B] H&E stain shows marked distortion of myocardial fiber associated with marked sarcoplasmic eosinophilia (arrow), [C] Masson's trichrome stain shows marked fibrous connective tissue proliferation between the atrophied fibers (arrowhead). [x100 μm].*
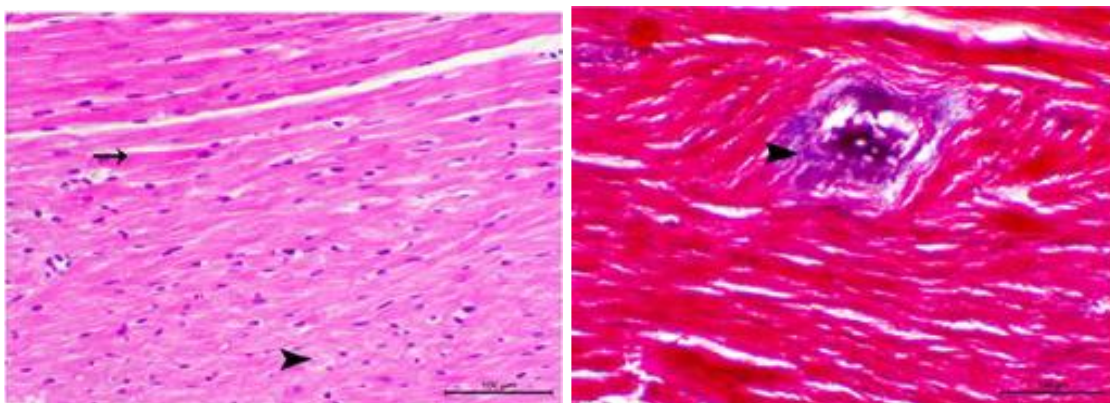

Figure 8: *Heart of diabetic rat treated with pioglitazone. [A] H&E stain shows marked decrease of myocardial degeneration (arrow) and myolysis (arrowhead), [B] Masson's trichrome stain shows focal area of perivascular fibrous tissue (arrowhead). [x100 μm].*
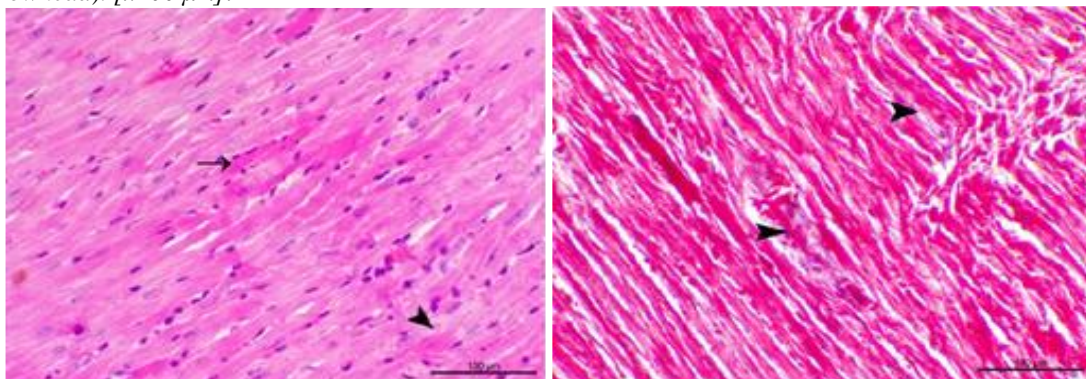

Figure 9: *Heart of diabetic rat treated with abscisic acid. [A] H&E stain shows marked decrease of myocardial degeneration (arrow) and myolysis (arrowhead), [B] Masson's trichrome stain shows mild degree of perivascular fibrous connective tissue. [x100 μm].*

DCM is associated with increased myocyte cell death and apoptosis. Hyperglycemia and increased formation of ROS are the main causes of accelerated necrosis and apoptosis [42]. In synergy with this hypothesis, our investigations revealed significant increase in both caspase 3 and 9 activities in diabetic cardiac tissues. In ABA treated groups, the previous cardiac caspases activities showed significant reduction than the diabetic ones which points to a novel anti-apoptotic effect of ABA in diabetes. According to the present study, ABA anti-apoptotic effect may be attributed to its glucose reducing and anti-inflammatory mechanisms.

Intercellular Cx43 channels are essential for direct communication between cardiomyocytes, ensuring action potential and molecular signal propagation resulting in synchronized heart function [35]. The present results indicated that myocardial Cx43 mRNA levels were lesser in diabetic versus non-diabetic rats and were enhanced by ABA supplementation. This deterioration of Cx43 levels affects the intercellular communication which may be behind the prolongation of the QRS, QTc and slowing of heart rate, thereby affecting heart function in DM. Previous data suggested that type 1 and 2 diabetes mellitus lead to the deterioration of myocardial intercellular communication mediated by connexin-43 (Cx43) channels [35, 43].

The histological findings showed marked myocardial necrosis associated with nuclear lysis and marked sarcoplasmic esinophilia, together with large patches of strong fibrous tissue reactivity between the atrophied fibers in the heart sections of diabetic rats. These alterations could be highly attributed to the hyperglycemia-induced oxidative stress and inflammation that leading to DNA damage and apoptosis [44]. Cardiac fibrosis occurs as a consequence of inflammation and cell injury as confirmed by various studies [45, 46] however, supplementation with ABA has been shown to improve DCM, cardiac architecture, and reduce cardiac fibrosis. Here, abscisic acid was able to protect the heart against diabetes-induced cellular injury and fibrosis probably through its ability to reduce hyperglycemic induced inflammation and apoptosis together with improving connexin-43 expression.

Conclusion: DCM involves the damage of the myocardium through several mechanisms, namely inflammatory induced hypertrophy, fibrosis, apoptosis and necrosis of cardiomyocytes. Abscisic acid (ABA), a phytohormone, is recently shown to possess insulin sensitizer activity through PPAR-γ activation. The diabetic rats benefit from ABA intake due to its hypoglycemic, anti-inflammatory, antiapoptotic effects, upregulation of myocardial Cx43, and preservation of cardiovascular ultrastructure. Our findings indicate that intake of abscisic acid for 16 weeks in combination with antidiabetic drugs may be beneficial for the management of type 2 diabetes mellitus.

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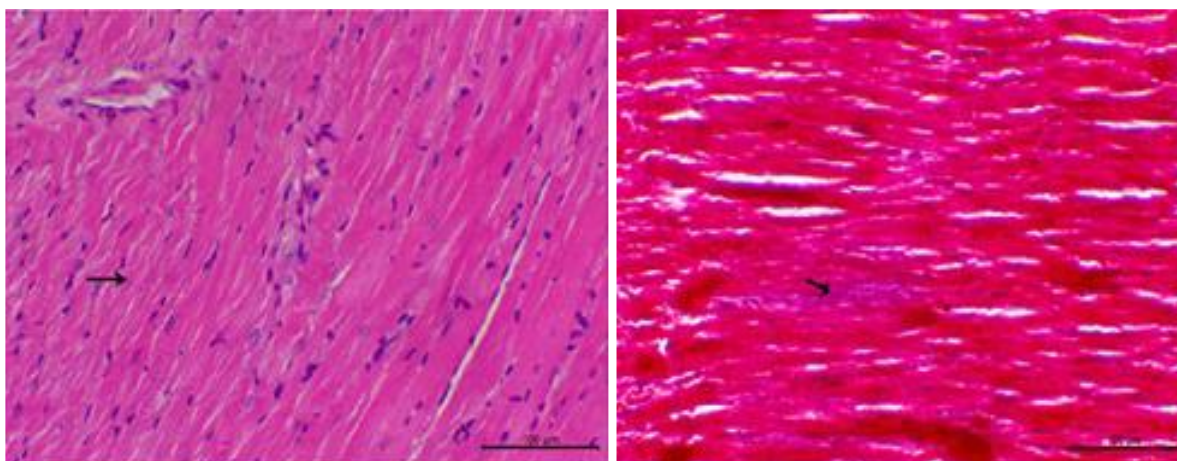
Figure 10: *Heart of diabetic rat treated with pioglitazone and abscisic acid. [A] H&E stain shows marked decrease of myolysis (arrow), [B] Masson's trichrome stain shows minimal perivascular fibrous connective tissue layer (arrow). [x100 μm].*

Mahdi analyzed the data and participated in writing the research

Conflict of interest: The authors declare that there is no conflict of interest.

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