

Prophylactic mechanisms of *Cucumis melo* var. *flexuosus* and *Phoenix dactylifera* fruit extracts against diabetic cardiomyopathy in streptozotocin induced diabetic rats

Amna Ali Saddi¹, Azza Mostafa Mohamed^{2,3} and Abdulkader Mohamed Shaikh⁴

¹Biology Department, Faculty of Science, Al Faisaliah, King Abdulaziz University, Jeddah, Saudi Arabia

²Biochemistry Department, Faculty of Science, Al Faisaliah, King Abdulaziz University, Jeddah, Saudi Arabia

³Therapeutic Chemistry Department, Drug Industry Division, National Research Center, Cairo, Egypt

⁴Biology Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract: The aim of this investigation was to study the antidiabetic impact of *Cucumis melo* var. *flexuosus* and/or *Phoenix dactylifera* fruit aqueous extracts and their mechanisms in repressing diabetes induced cardio-myopathy in diabetic rats. Type 2 diabetes was promoted in rats by a single intraperitoneal injection of streptozotocin (30mg/kg body weight). *C. flexuosus* and *P. dactylifera* extracts (200mg/kg body weight, each) were ingested to diabetic rats daily for a month. The results showed that ingestion of either plant extract or their combination to diabetic rats significantly diminished the glucose level and boosted the insulin concentration in the blood. The plant extracts markedly ameliorated the serum inflammatory molecules, tumor necrosis factor (TNF- α) and C-reactive protein (CRP), as well as the alteration in the cardiac malondialdehyde (MDA) and glutathione peroxidase (GPx). The extracts attenuated the increase in cardiac apoptosis enzyme (caspase -3) and the oxidative DNA fragmentation. Treating diabetic rats with plant extracts also scaled down the serum cardiac function enzyme, creatine phosphokinase-MB (CPK-MB). The biochemical results were confirmed by histopathological examination. This study has proven that both the plant extracts particularly their combination have potential hypoglycemic effect and could attenuate cardiomyopathy in diabetic rats.

Keywords: *Phoenix dactylifera*, *Cucumis melo* var. *flexuosus*, antidiabetic, DNA fragmentation.

INTRODUCTION

One of the top ranking causes of mortality worldwide is diabetes mellitus (Wild, *et al.* 2004). One of the primary signs of the disease is loss of homeostatic regulation of blood glucose levels, induced by either an abnormality in the production of insulin, or by the atypical action of insulin on its receptors in the body, which leads to defective metabolism of glucose and other respiratory substrates such as lipids and proteins (Wolpin *et al.* 2013). The continual hyperglycemia can cause glucose toxicity, due to further defectiveness in the ability of β -cells to produce insulin (Wolpin *et al.* 2013). The syndrome is a progressive one and is directly linked with increased risk of atherosclerosis (Wakabayashi & Masuda, 2004), coronary heart disease (Feher, 2004), stroke and peripheral vascular disease (Thomas *et al.*, 2004).

Hyperglycemic cardiovascular damage includes both cardiac muscles (cardiomyopathy) and peripheral blood vessels (Francis, 2001). Diabetic cardiomyopathy (DCM) can lead to loss of the heart's pumping ability, affecting blood circulation throughout the body, a condition called heart failure (Liu *et al.* 2014). Oxidative stress, apoptosis, and inflammation have been considered the key initiating factors of cardiac remodeling and eventually dysfunction (Cesselli *et al.*, 2001, Cai *et al.*, 2002).

Evidence implicates that formation of advanced glycation end-products (AGEs) from the non-enzymatic glycation and oxidation of proteins and lipids (Wang *et al.*, 2006), is one of the pathogenic mechanisms in DCM. AGE production interferes with protein function or stimulates receptor-mediated formation of oxygen reactive species (ORS), disrupting cell integrity (Thornalley, 2002). Excessive production of ORS and a reduction in antioxidant defense can cause a state of oxidative stress that lead to cardiac dysfunction through several mechanisms, including direct damage to proteins and DNA, as well as inducing apoptotic cell death (Cesselli *et al.* 2001). Also, excessive expression of several inflammatory mediators such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and chemokines, during diabetes is another diabetic mechanism that causes heart failure advancement (Drimal *et al.*, 2008).

Targeting oxidative stress, apoptosis and inflammatory mediators signaling through using agents with antioxidant, anti-apoptosis and anti-inflammatory may improve therapeutic options for diabetic cardiomyopathy.

Cucurbitaceous is a family of a large group of plants distributed principally in tropical and subtropical regions of the world (Dhimati *et al.* 2012). Previous reports have revealed that many plants in Cucurbitaceae have potential impacts against diabetes and its complications (Kolawole1 and Ayankunle, 2012, Joseph and Jini, 2013).

*Corresponding author: e-mail: azzamohamed99@yahoo.com

Also, cucurbits possess other medicinal properties, including hepatoprotective, anti-inflammatory, anticancer, antioxidative stress, antibacterial and immunomodulatory, effects (Grover and Yadav, 2004, Bao *et al.*, 2013). The main chemical constituents in Cucurbitaceae family are: steroids, tocopherols, phenolic compounds, flavones, volatile and fixed oils, saponins, amino acids, carotenes and proteins, which have been proved many therapeutic bioactivities, including glucose lowering effect (Han *et al.*, 2008, De Marino *et al.*, 2009).

Cucumis melo var. flexuosus known as snake melon is one of the plants belonging to Cucurbitaceae (Mendi *et al.* 2010). Although the antidiabetic impact of many plants belonging to this family was documented, the effect of this plant against diabetic cardiomyopathy is still unexplored.

Phoenix dactylifera Linn. (also known as date palm, family Arecaceae) fruits are widely consumed in many parts all over the world for their great nutritional value (Al-Shahib and Marshall, 2003). The fruits contain essential minerals (such as calcium, iron, magnesium, phosphorus, potassium, zinc, selenium and manganese), dietary fibers, carbohydrates (such as fructose and glucose), proteins, fatty acids and vitamins (Al-Shahib and Marshall, 2003). *P. dactylifera* fruit aqueous extract has potent antimutagenic, hepatoprotective, anti-inflammatory and antioxidant activities (Abbas *et al.* 2013). The latter being ascribed to the presence of carotenoids and phenolic compounds (Zhang *et al.* 2013). The potential antidiabetic action of *P. dactylifera* fruit extract was also documented (Michael *et al.* 2013), however the exact mode of action of date fruits in controlling diabetes and its progression is still not established.

The present study was undertaken to explore the potential antidiabetic impact of aqueous fruit extracts of *C. flexuosus* and/or *P. dactylifera* and their mechanisms in attenuating cardiomyopathy in diabetic rats.

MATERIALS AND METHODS

All chemicals utilized in this investigation were bought from Sigma and Merck companies. Kits used for evaluating of different measurements were bought from Biogamma, Stanbio, West Germany.

Plants

Cucumis melo var. flexuosus and *Phoenix dactylifera* fruits were bought from the local market.

Preparation of *Cucumis melo var. flexuosus* fruit aqueous extract

The fruits of *C. melo var. flexuosus* were cleaned and cut into small pieces without the seeds. The fruit pieces were

then oven dried at 40°C for two days. The dried fruits were powdered in a grinder. 250g of fruit powder were mixed with 2 liters distilled water. The mixture was then boiled under reflux at 100°C for half a minute and then lyophilized. The lyophilized plant extract was dissolved in distilled water before administration.

Preparation of *Phoenix dactylifera* fruit aqueous extract

Two hundred grams of *P. dactylifera*, fruits were grinding in distilled water (1/10,w/v) using a mortar and pestle . The obtained mixture was left for 24h and then centrifuged for 20min at 4000g. The supernatant was collected, frozen at -0°C, and then dried under freezing. The obtained extract was dissolved in distilled water for supplementation to animals.

Animals

Wistar albino rats -with weights ranging from 180-200g- were utilised for this study. The rats were taken from Experimental Animal Care Center of King Fahad Medical Research Center, King Abdulaziz University. The rats were housed in a thermostatically-controlled room at 21°C with 12h light/12h dark cycle and humidity 60%. Rats were provided with typical food and tap water *ad libitum* for one week for adaptation. Animal handling was carried out according to the guidance provided by the Experimental Animal Laboratory and Animal Care Committee of Faculty of Science, King Abdulaziz University.

Induction of type 2 diabetes

For induction of type 2 diabetes , STZ (Sigma, USA) was dissolved in 50mM citrate buffer (pH 4.5) and administered, as a single dose (30 mg/kg body weight, intraperitoneal injection) (Tian *et al.* 2014), to each rat in diabetic groups . After injection, 5% glucose solution was administered to the animals to prevent hypoglycemia. At 10 days after STZ injection, animals which showed fasting blood glucose level over 220 mg/dl were regarded as diabetic and chosen for the experiment.

Experimental design

The rats were allocated into 5 groups (n=10)

Group 1: Normal control rats.

Group 2: Diabetic rats.

Group 3: Diabetic rats were treated with *C. flexuosus* extract (200mg/Kg body weight, Kolawole *et al.* 2012) daily for a month.

Group 4: Diabetic rats were treated with *P. dactylifera* extract (200mg/Kg body weight, Mard *et al.* 2010) daily for a month.

Group 5: Diabetic rats treated with the combination of *C. flexuosus* and *P. dactylifera* (200 mg/Kg body weight each) daily for a month.

C. flexuosus and *P. dactylifera* fruit extracts were dissolved in distilled water and administered orally to rats commenced 2 weeks after induction of diabetes. At the

end of the experiment (30 days after treatment with the plant extract), the rats were starved for about 14 hours. The blood specimens were gathered and left for clotting for serum isolation and utilized for biochemical analysis. The animals were sacrificed utilizing an anesthetic agent and the cardiac specimens were taken to be washed in phosphate buffer saline (0.1M, pH 7.4), and then utilized for biochemical tissue analysis.

Serum biomarkers analysis

Serum fasting glucose level (biomarker of hyperglycemia) and creatine phosphokinase kinase (CPK)-MB activity (marker of cardiac muscle damage) were estimated utilizing an automatic biochemical analyzer (ci16200, Abbott, USA). Using Merck Millipore's protocol (Millipore, Cooperation, MA, and USA), serum insulin was evaluated by radioimmunoassay (RIA). The concentration of inflammatory cytokine, TNF- α was estimated utilizing ELISA assay kit according to the instructions provided by the manufacturer (DuoSet kits; R&D Systems, Minneapolis, MN, USA). Measurement of CRP was performed with latex-enhanced immunonephelometry on a Behring BN II Nephelometer (Dade Behring).

Cardiac tissue analysis

MDA (index of lipid oxidation) was measured utilizing thiobarbituric acid reactive substances (TBARS) method (Buege and Aust, 1978). This method depends on the reaction between thiobarbituric acid and MDA. MDA level was estimated utilizing the extinction coefficient value (ϵ) of MDA-thiobarbituric acid complex (1.56×10^5 /M/cm). Glutathione peroxidase (GPx) relative activity was measured according to Rotruck *et al.* (1973) utilizing dithio-binitrobenzoic acid method. Caspase 3-like protease was estimated by the assay of Vaculova and Zhivotovsky (2008). DNA fragmentation was estimated by an alkaline Comet assay (Singh *et al.*, 1988). The assay is depending on the unwinding of DNA in an alkaline medium. Tail length, tail DNA % and the tail moment were measured as indices of DNA fragmentation.

Histopathological examination

Small fragments of heart muscles were fixed utilizing 10 % formalin and then incorporated into paraffin. The heart specimens were sectioned (5-6- μ m), stained with hematoxylin-eosin (H& E) and examined by a light microscope.

STATISTICAL ANALYSIS

The data of the current study were presented as the mean \pm SD. Significance variations between data were calculated utilizing one-way analysis of variance (ANOVA) followed by Bonferroni as a post-ANOVA test. The variations among data were statistically significant at $p \leq 0.05$.

RESULTS

The effects of *C. flexuosus* and/ or *P. dactylifera* on serum glucose and insulin levels in normal and diabetic rat groups are shown in fig. 1. The results showed that induction of diabetes in rats led to marked increase in the blood glucose level and decrease in insulin level (group 2) with respect to control rats (group 1 $p \leq 0.001$). Supplementation of diabetic rats with *C. flexuosus* and/ or *P. dactylifera*, effectively decreased the blood glucose level and increased the serum insulin content with relation to diabetic ones ($p \leq 0.001$).

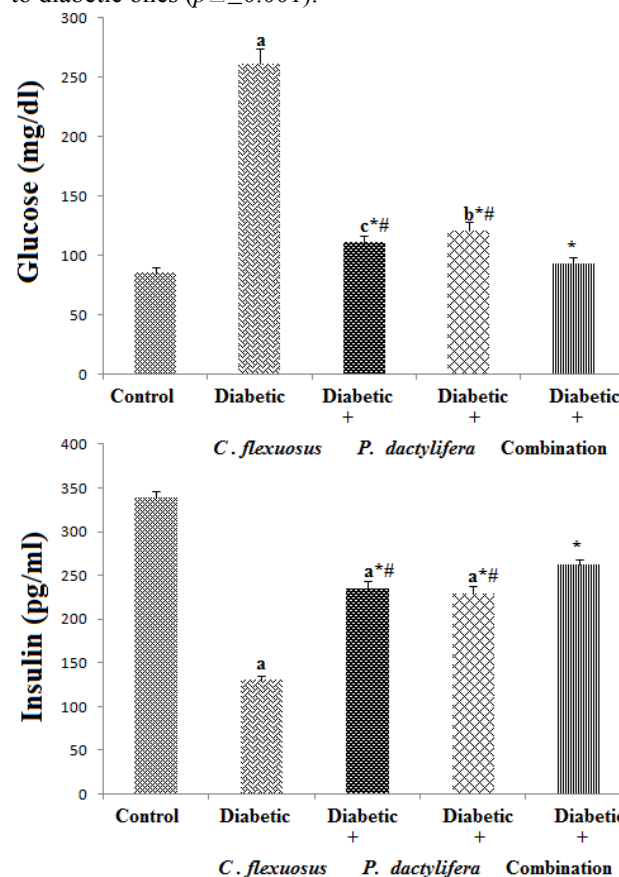


Fig. 1: Serum glucose and insulin levels in control and diabetic rats. Data are presented as mean + SD from 10 rats significant differences at: ^a $p \leq 0.01$, ^b $p \leq 0.01$, ^c $p \leq 0.05$ compared with the control group; ^{*} $p \leq 0.01$ compared with diabetic group; [#] $p \leq 0.05$ compared with the combination (*C. flexuosus* + *P. dactylifera*) group

The concentrations of the serum inflammatory markers (TNF- α and CRP) in different diabetic rat groups are shown in fig. 2. The data revealed that elevation in these indices in serum of diabetic rats versus control ones ($p \leq 0.001$). Oral ingestion of *C. flexuosus* and/ or *P. dactylifera*, significantly reduced the concentrations of these inflammatory proteins compared to diabetic untreated ones ($p \leq 0.001$).

Table 1: Levels of oxidative stress (MDA) and antioxidant (GPx) markers in control and diabetic rats

Parameters	Control	Diabetes	Diabetes + <i>C. flexuosus</i>	Diabetes + <i>P. dactylifera</i>	Diabetes + Combination
MDA	9.8±0.5	26.6±0.85 ^a	13.02±0.76 ^{b*#}	14.05±0.75 ^{b*#}	11.20±0.45 ^{c*}
GPx	164.84±5.02	78.90±8.3 ^a	145.3±5.07 ^{b*#}	140.6 ±3.96 ^{b*#}	155.70±7.5 ^{c*}

Table 2: Levels of cardiac apoptosis (caspase-3) and DNA (tail length, % tail DNA and tail moment) fragmentation markers in control and diabetic rats.

Parameters	Control	Diabetes	Diabetes + <i>C. flexuosus</i>	Diabetes + <i>P. dactylifera</i>	Diabetes + Combination
Caspase-3	3.46±0.45	9.75±0.85 ^a	5.87±0.40 ^{b*#}	6.20±0.90 ^{b*#}	4.40±0.42 ^{c*}
Tail length	1.94±0.08	6.60± 1.08 ^a	3.7±0.20 ^{b*#}	3.4±0.25 ^{b*#}	2.9±0.16 ^{c*}
% Tail DNA	2.4±0.11	6.1±0.68 ^a	3.9±0.27 ^{b*#}	4.1±0.22 ^{b*#}	3.2±0.24 ^{c*}
Tail moment	5.7±0.16	20.7± 1.9 ^a	10.8±1.5 ^{b*#}	11.6±0.70 ^{b*#}	7.5±0.54 ^{c*}

Data are presented as mean±S.D. from 10 rats, ^a*P*≤0.001, ^b*P*≤0.01, ^c*P*≤0.05 compared with the control group, ^{*}*P*≤0.001 compared with diabetic group, [#]*P*≤0.05, ^{SS}*P*≤0.01 compared with combination (*C. flexuosus* + *P. dactylifera*) group.

Table 1 shows the level of cardiac lipid oxidation product marker (MDA) as well as the activity of antioxidant enzyme (GPx) in different diabetic rat groups. The result showed significant increase in the MDA content and reduction in GPx in cardiac of diabetic rats in relation to control ones. Oral intake of *C. flexuosus* and/ or *P. dactylifera* to diabetic rats, effectively modulated the alterations in cardiac MDA and GPX levels versus diabetic untreated ones (*p*≤0.001).

The cardiac apoptosis index, caspase 3, was markedly elevated in diabetic rats (table 2) versus control healthy animals. Intake of *C. flexuosus* and/ or *P. dactylifera* to diabetic rats, obviously reduced the increase in caspase-3 versus diabetic untreated rats (*p*≤0.001). The level of DNA damage are illustrated in table 2 and fig. 3. Marked increases in DNA fragmentation markers (Tail DNA length, % tail DNA and tail moment) were seen in the heart tissue of diabetic rats. Ingestion of *C. flexuosus* and/ or *P. dactylifera* to diabetic rats, significantly reduced the increases in these markers versus diabetic untreated rats (*p*≤0.001).

Histopathological examination

The histological examination of cardiac pictures from the control and the diabetic animal groups are shown in fig. 5. The cardiac section from control rat showed regular arrangement of the myocyte fibers, with central round or oval nuclei. The cardiac picture of diabetic rat demonstrated disorganization of the myocardial arrays, discontinuous myofibrils and degeneration of myocytes. Cardiac histological pictures of diabetic rats treated with *C. flexuosus* and/ or *P. dactylifera* showed more or less normal cardiomyocyte features.

DISCUSSION

Extensive evidence revealed that diabetic hyperglycemia is one of the major pathogenic mechanisms in DCM (Liu

et al. 2014). Limitations of the currently available drugs to manage diabetic hyperglycemia and its complications, made it necessary to develop new drugs which can behave as complementary and/or alternative remedies. The fewer side effects and the multidimensional mode of action of natural plant products for controlling diabetes, have made them a center of interest (Wadkar *et al.* 2008).

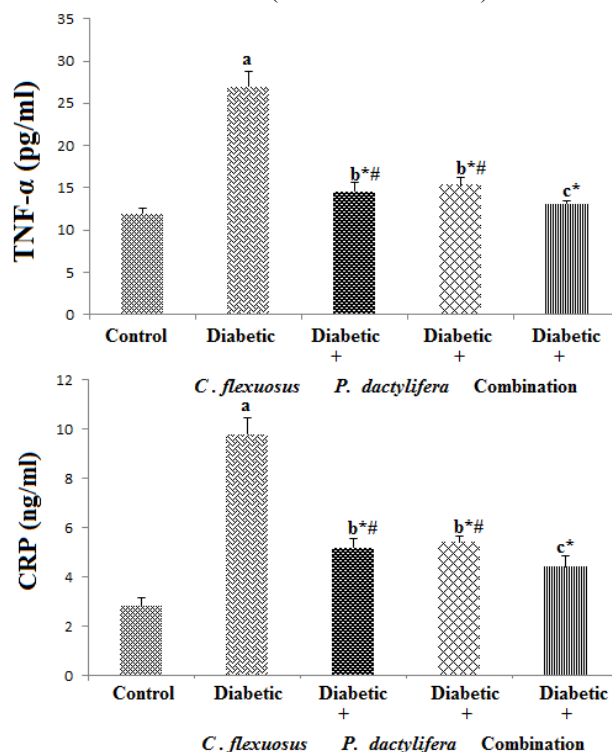


Fig. 2: Levels of serum inflammatory indices (TNF-a and CRP) in control and diabetic rats. Data are presented as mean + SD from 10 rats. Significant difference at: ^a*p*≤0.001, ^b*p*≤0.001, ^c*p*≤0.005 compared with the control group; ^{*}*p*≤0.001 compared with diabetic group; [#]*p*≤0.05 compared with the combination (*C. flexuosus* + *P. dactylifera*) group.

The current study demonstrated the beneficial protective mechanisms of aqueous extracts of *C. flexuosus* and/or *P. dactylifera* against diabetic cardiomyopathy in diabetic rats. To the extent of our knowledge, this study is the first one to illustrate the protective impact of *C. flexuosus* and/or *P. dactylifera* against diabetes cardiomyopathy.

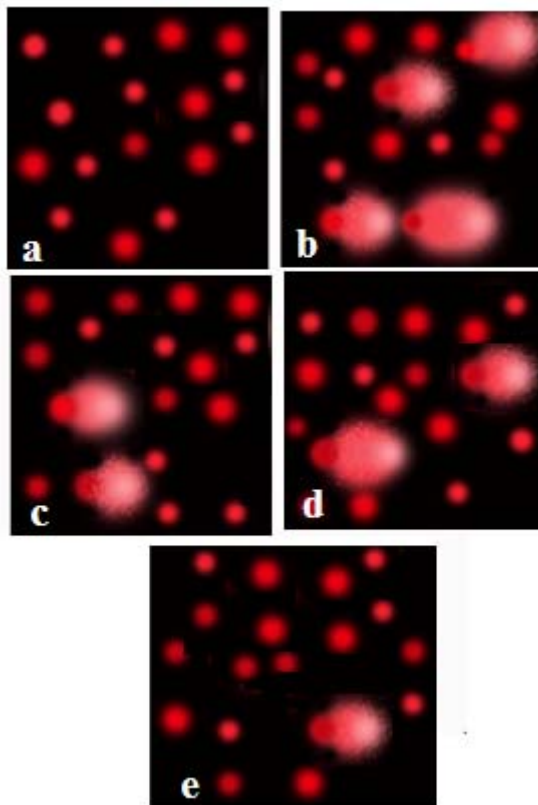


Fig. 3: Comet assay showing the effect of *C. flexuosus* and/or *p. dactylifera* on the degree of DNA fragmentation in the cardiac of diabetic rats. (a) control group, (b) Diabetic group, (c) Diabetic rats treated with *C. flexuosus*, (d) Diabetic rats treated with *P. dactylifera*, (e) Diabetic rats treated with the combination (*C. flexuosus* and *P. dactylifera*).

In the present study diabetic rats showed a clear increase in the serum glucose level accompany with a deficiency in the serum insulin level compared with control rats, implying development of diabetic state in rats. Oral supplementation of *C. flexuosus* and/or *P. dactylifera* extracts to diabetic rats, noticeably depleted the blood glucose level and boosted the insulin concentration compared with diabetic untreated ones. The data of our result indicated that the combination of the two plant extracts was the successful one in regulating the blood glucose and the insulin contents, suggesting that the fruit extracts of both plants synergistically improved the capability of diabetic rats to utilize the excess blood glucose by promoting the pancreatic beta-cells to produce more insulin, which stimulates glucose uptake and utilization by the tissues through glycolysis. Similar

hypoglycemic effect of *P. dactylifera* fruit extract has previously documented (Michael *et al.* 2013). The hypoglycemic impact of *C. flexuosus* fruit extract has not been previously studied, however the hypoglycemic effects of leaf extract of *C. flexuosus* as well as fruit extracts of other Curcubitaceae plants have been documented (Kolawole and Ayankunle, 2012, Ibrahim, 2017).

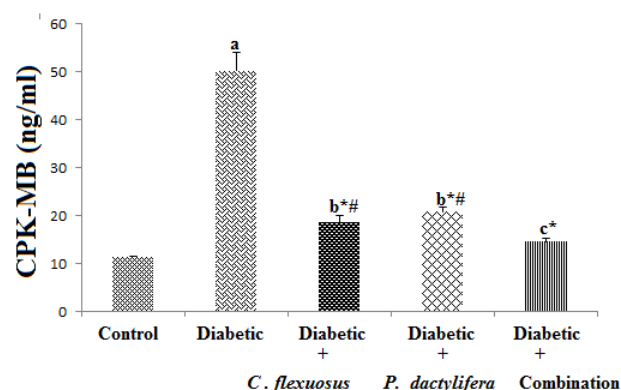


Fig. 4: Level of serum cardiac damage index (CPK-MB) in control and diabetic rats. Data are presented as mean + SD from 10 rats. Significant differences at: ^a $P \leq 0.001$, ^b $P \leq 0.01$, ^c $P \leq 0.05$ compared with the control group; * $p \leq 0.001$ compared with diabetic group; # $P \leq 0.05$ compared with the combination (*C. flexuosus* + *P. dactylifera*) group.

It is worth mentioning that investigating the effect of *C. flexuosus* and/or *P. dactylifera* extracts on inflammatory biomarkers of diabetic rats was the first study of its kind. In this study, induction of diabetes caused up-regulation of serum inflammatory markers (TNF- α , and CRP) in diabetic rats with respect to non-diabetic ones. The overproduction of reactive radicals in response to hyperglycemia can induce myocardial inflammation, which is characterized by the production of inflammatory cytokines (Roslan *et al.* 2017). Some authors has reported that over-production of such inflammatory mediators during diabetes is one of the diabetic mechanism that lead to the progression of heart failure (Drimal *et al.*, 2008). TNF- α stimulates the generation of reactive free radicals, which cause DNA damage in cultured cardiomyocytes (Suematsu *et al.* 2003). The increase in expression of TNF- α promotes the generation of IL-6, the main stimulator of CRP production, causing inflammatory cardiac damage (De Ferranti and Rifai 2007). Some investigations showed that high serum CRP accelerates arterial atherosclerosis and thrombosis (Danenberg *et al.* 2003, Paul *et al.* 2004) and exacerbates the vascular injury (Teoh *et al.* 2008). Thus, a prophylactic strategy that ameliorates the expression of inflammatory proteins could attenuate organ dysfunction.

Treatment of diabetic rats with *C. flexuosus* and/or *P. dactylifera* fruit extracts, markedly reduced the serum TNF- α and CRP contents versus diabetic untreated animals. The combination of the two extracts was more effective in lowering the levels of these markers. The possible mechanism by which the two plant extracts elicit anti-inflammatory effect in diabetic rats might be through inhibiting the infiltration of inflammatory cells and/or repressing NF- κ B activation. The suppressing effect of *P. dactylifera* fruit extract on the expression of inflammatory cytokines has been demonstrated in an experimental animal model (Al-Rasheed *et al.* 2015). Also some dietary cucurbits show suppressing impact on the inflammatory cytokines, including interleukin (IL)-1 β and TNF- α in sera of lipopolysaccharide (LPS)-inflamed mice (Hamad *et al.* 2015). In addition, A recent study has reported that *C. flexuosus* leaf extract could reduce TNF- α and interleukin levels in brains of diabetic rats (Ibrahim, 2017).

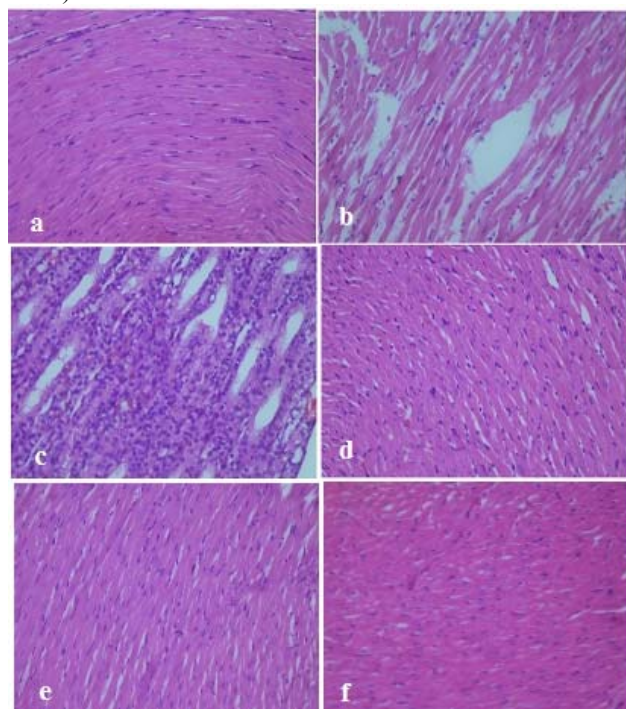


Fig. 5: Cardiac histomorphologic pictures of control and diabetic rats. (a) Cardiac section of control rat showing regular arrangement of the cardiac muscle fibers, with central nuclei (b& c). Cardiac sections of diabetic rats, (b) showing disorganization array of the muscle fiber structure, discontinuous of myofibrils and degeneration of myocytes (arrow); (c) showing infiltration of inflammatory immune cells (arrow). (d, e & f) Cardiac sections of diabetic rat treated with *C. flexuosus*, *P. dactylifera* and their combination respectively, showing more or less normal cardiac architecture (H&E X 400).

A number of investigations have revealed that an increase in lipid peroxidation and decreases in antioxidants are involved in the pathogenesis of diabetic cardiac injury

(El-Missiry *et al.* 2015). Parallel with a former investigation, the present data showed that significant increase in the level of oxidative stress index, MDA (an index of lipid oxidation) with a concomitant reduction in the antioxidant enzyme, GPx, in cardiac of diabetic rats with relation to control ones (Aksakal *et al.*, 2011). The production of MDA may attribute to that diabetic hyperglycemia promotes lipid auto-oxidation and protein glycation, leading to generation of reactive radicals which act against polyunsaturated fatty acids in cell membranes, causing the production of lipid peroxidation products, including MDA (Pryor, 1990). MDA is known to interact with DNA, leading to cytotoxicity, genotoxicity and carcinogenicity (Esterbauer, 1991). The drop in the relative activity of antioxidant enzyme, GPx, may ascribe to deactivation caused by over generation of reactive species. Treatment of diabetic rats with *C. flexuosus* and/or *P. dactylifera* fruit extracts, pronouncedly decreased the cardiac MDA level and ameliorated the decrease in GPx, implying their antioxidant abilities. Similarly, Ibrahim (2017) has found a reduction in MDA in brain of diabetic rats treated with *C. flexuosus* leaf extract. Our study has documented the antioxidant effect of *C. flexuosus* fruit extract for the first time. However, the antioxidant beneficial impact of *P. dactylifera* fruit extract has been previously confirmed (Abbas *et al.*, 2013).

The current study revealed that induction of diabetes in rats induced apoptosis of cardiomyocytes as shown by an elevation in the cardiac apoptotic enzyme, caspase 3, in diabetic rats in comparison to control animals. Some authors reported that increase in oxidative stress level and inflammation in cardiomyocytes can promote apoptosis (Tsai *et al.* 2013, Pan *et al.* 2014). Apoptosis has been considered as the key initiating hazard factor in prompting cardiomyocyte hypertrophy and fibroblast proliferation, both leading to cardiac remodeling and eventually cardiac dysfunction (Cai L and Kang, 2003). Administration of *C. flexuosus* and/or *P. dactylifera* fruit extracts to diabetic rats, significantly reduced the diabetic increase in cardiac caspase 3 compared with diabetic untreated rats. The reduction in apoptosis in cardiac of diabetic rats by these extracts may ascribe to their ability to suppress diabetic oxidative stress and inflammation and /or attenuate the apoptosis pathways via modulating mitochondrial and death receptor apoptotic pathways.

The current investigation also showed that diabetes caused apoptotic cardiomyopathy was documented by cardiac DNA damage. A significant relationship between high blood glucose level and DNA damage was clinically confirmed (Song *et al.* 2007). Administration of *C. flexuosus* and/or *P. dactylifera* fruit extracts to rats with diabetes markedly protected the heart tissue from diabetic DNA damage, as shown by the suppression of the increased Comet markers, including tail length, tail DNA

%, and tail moment compared to diabetic untreated rats. The precise mechanism is unknown, however, this may be due to the beneficial antioxidant capacity of both extracts. The combination of the two extracts was the successful one in ameliorating the heart DNA damage.

In addition, this investigation showed that an elevation in the serum marker of cardio-myocyte injury (CK-MB) in diabetic animals compared with control ones. This result was confirmed by histopathological observation of cardiac muscles. Cardiac section of diabetic rat showed disorganization of cardiac architecture as observed by discontinuous of myofibrils, degeneration of myocytes and infiltration of inflammatory immune cells. Treatment with *C. flexuosus* and/or *P. dactylifera* fruit extracts, effectively ameliorated the increase in CK-MB as well as the cardiac histomorphologic pictures in diabetic rats with respect to diabetic untreated ones. The beneficial impact of both extracts against diabetes induced cardiac muscle damage may be due to their abilities to mitigate inflammation, lipid peroxidation, apoptosis and DNA damage along with restoration of antioxidant.

Conclusion: The current investigation proved that both *C. flexuosus* and/or *P. dactylifera* fruit extracts have pleiotropic protective mechanisms against diabetic cardiomyopathy, including anti-diabetic, anti-inflammatory, antioxidant and antiapoptosis activities. The combination of the two extracts was synergistically the successful one in attenuating diabetic cardiac tissue damage. This supports the utilization of this combination as a promising novel therapy in treatment of diabetic cardiomyopathy.

ACKNOWLEDGMENTS

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, Saudi Arabia, under grant no. (G/55/363/38). The authors, therefore, acknowledge with thanks 228 DSR technical and financial support.

REFERENCES

- Ahmed OM, Gabar MA and Ali TM (2012). Impacts of the coexistence of diabetes and hypothyroidism on body weight gain, leptin and various metabolic aspects in albino rats. *J. Diabetes Complications*, **26**(6): 491-500.
- Abbas MH, Elkheir TM, Mustafa HA and Abd Almahmoud IA (2013). Effect of aqueous fruit extract of Phoenix dactylifera, L. (date palm) on improvement of liver disorders induced by traditional Sudanese liquor (Aragi), *Sudan. J. Public Health*, **8**: 52-59.
- Aksakal E, Akaras N, Kurt M, Tanboga IH, Halici Z, Odabasoglu F, Bakirci EM and Unal B (2011). The role of oxidative stress in diabetic cardiomyopathy: An experimental study. *Eur. Rev. Med. Pharmacol. Sci.*, **15**(11): 1241-1246.
- Al-Rasheed NM, Attia HA, Mohamad RA, Al-Rasheed NM, Al-Amin MA and Al-Onazi A (2015). Aqueous date flesh or pits extract attenuates liver fibrosis via suppression of hepatic stellate cell activation and reduction of inflammatory cytokines, transforming growth factor- β 1 and angiogenic markers in carbon tetrachloride-intoxicated rats, *Evid. Based. Complement Alternat. Med.* 2015: 247357.
- Al-Shahib W and Marshall RJ (2003). The fruit of the date palm: its possible use as the best food for the future?" *Int. J. Food Sci. Nutr.*, **54**: 247-259.
- Bao B, Chen YG, Zhang L, Na Xu YL, Wang X, Liu J and Qu W (2013). Momordica charantia (Bitter Melon) reduces obesity-associated macrophage and mast cell infiltration as well as inflammatory cytokine expression in adipose tissues. *PLoS One*, **8**(12): e84075.
- Buege JA and Aust SD (1978). Microsomal lipid peroxidation. *Methods Enzymol.*, **52**: 302-315.
- Cai L and Kang YJ (2003). Cell death and diabetic cardiomyopathy. *Cardiovasc. Toxicol.*, **3**: 219-228.
- Cai L, Li W, Wang G, Guo L, Jiang Y and Kang YJ (2002). Hyperglycemia-induced apoptosis in mouse myocardium: Mitochondrial cytochrome C-mediated caspase-3 activation pathway. *Diabetes*, **51**: 1938-1948.
- Cesselli D, Jakoniuk I, Barlucchi L, Beltrami AP, Hintze TH, Nadal-Ginard B, Kajstura J, Leri A and Anversa P (2001). Oxidative stress mediated cardiac cell death is a major determinant of ventricular dysfunction and failure in dog dilated cardiomyopathy. *Circ. Res.*, **89**: 279-286.
- Danenberg HD, Szalai AJ, Swaminathan RV, Peng L, Chen Z, Seifert P, Fay WP, Simon DI and Edelman ER (2003). Increased thrombosis after arterial injury in human C-reactive protein-transgenic mice. *Circulation*, **108**: 512-515.
- De Ferranti SD and Rifai N (2007). C-reactive protein: A nontraditional serum marker of cardiovascular risk. *Cardiovasc. Pathol.*, **16**: 14-21.
- De Marino S, Festa C, Zollo F and Iorizzi M (2009). Phenolic glycosides from Cucumis melo var. inodorus seed. *Phytochem. Lett.*, **2**: 130-133.
- Dhimati K, Gupta A, Sharma DK, Gill NS and Goyal A (2012). A review on the medicinally important plant of the family cucurbitaceae. *Asian J. Clin. Nutr.*, **4**: 16-26.
- Drimal J, Knezl V, Navarova J, Nedelceva J, Paulovicova E, Sotnikova R, Snirc V and Drimal D (2008). Role of inflammatory cytokines and chemo-attractants in the rat model of streptozotocin-induced diabetic heart failure. *Endocr. Regul.*, **42**(4): 129-35.
- El-Missiry MA, Amer MA, Hemieda FAE, Othman AI, Sakr DA and Abdulhadi HL (2015). Cardio-ameliorative effect of punicalagin against streptozotocin-induced apoptosis, redox imbalance,

- metabolic changes and inflammation. *EJBAS.*, **2**: 247-260.
- Esterbauer H, Schaur RJ and Zollner H (1991). Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. *Free Radic. Biol. Med.*, **11**(1): 81-128.
- Feher MD (2004). Diabetes: preventing coronary heart disease in a high risk group. *Heart*, **90**: 18-21.
- Francis GS (2001). Diabetic cardiomyopathy: Fact or fiction? *Heart*, **85**: 247-248.
- Grover JK and Yadav SP (2004). Pharmacological actions and potential uses of *Momordica charantia*: A review. *J. Ethnopharmacol.*, **93**: 123-132.
- Hamad I, Abdelgawad H, Al Jaouni S, Zinta G, Asard H, Hassan S, Hegab M, Hagagy N and Selim S (2015). Metabolic analysis of various date palm fruit (*Phoenix dactylifera* L) cultivars from Saudi Arabia to assess their nutritional quality. *Molecules*, **20**: 13620-13641.
- Han C, Hui Q and Wang Y (2008). Hypoglycemic activity of saponin fraction extracted from *Momordica charantia* in PEG/salt aqueous two-phase systems. *Nat. Prod. Res.*, **22**: 1112-1119.
- Ibrahim DS (2017). Neuroprotective effect of *Cucumis melo var. flexuosus* leaf extract on the brains of rats with streptozotocin-induced diabetes. *Metab Brain Dis.*, **32**(1): 69-75.
- Joseph B and Jini D (2013). Antidiabetic effects of *Momordica charantia* (bitter melon) and its medicinal potency. *Asian Pac. J. Trop. Dis.*, **3**(2): 93-102.
- Kolawole OT and Ayankunle AA (2012). Seasonal variation in the anti-diabetic and hypolipidemic effects of *Momordica charantia* fruit extract in rats. *Eur. J. Med. Plants*, **2**: 177-185.
- Liu Q, Wang S and Cai L (2014). Diabetic cardiomyopathy and its mechanisms: Role of oxidative stress and damage. *Diabetes Invest.*, **5**: 623-634.
- Mard SA, Jalalvand K, Jafarinejad M, Balochi H and Naseri MK (2010). Evaluation of the antidiabetic and antilipaemic activities of the hydroalcoholic extract of Phoenix Dactylifera palm leaves and its fractions in alloxan-induced diabetic rats, *Malaysian J. Med. Sci.*, **17**: 4-13.
- Mendi YY, Eldoğan S, Gutakev R, İpek M, Çürük P and Cetiner S (2010). Regeneration and histological analysis of snake melon (*Cucumis melo var. flexuosus* L. Naudin) by direct organogenesis, *Turk. J. Agric. For.*, **34**: 309-317.
- Michael HN, Salib JY and Eskander EF (2013). Bioactivity of diosmetin glycosides isolated from the epicarp of date fruits, Phoenix dactylifera, on the biochemical profile of alloxan diabetic male rats, *Phytother. Res.*, **27**(5): 699-704.
- Pan Y, Wang Y, Zhao Y, Peng K, Li W, Wang Y, Zhang J, Zhou S, Liu Q, Li X, Cai L and Liang G (2014). Inhibition of JNK phosphorylation by a novel curcumin analog prevents high glucose-induced inflammation and apoptosis in cardiomyocytes and the development of diabetic cardiomyopathy *Diabetes*, **63**(10): 3497-511.
- Paul A, Ko KW, Li L, Yechoor V, McCrory MA, Szalai AJ and Chan L (2004). C-reactive protein accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. *Circulation*, **109**: 647-655.
- Pryor WA and Porter NA (1990). Suggested Mechanisms for the Production of 4-Hydroxy-2-Nonenal from the Autoxidation of Polyunsaturated Fatty Acids. *Free Radic. Biol. Med.*, **8**(6): 541-543.
- Roslan J, Giribabu N, Karim K and Salleh N (2017). Quercetin ameliorates oxidative stress, inflammation and apoptosis in the heart of streptozotocin-nicotinamide-induced adult male diabetic rats. *Biomed. Pharmacother.*, **86**: 570-582.
- Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG and Hoekstra WG (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science*, **179**: 588-590.
- Singh NP, McCoy MT, Tice RR and Schneider EL (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell. Res.*, **175**: 184-191.
- Song F, Jia W, Yao Y, Hu Y, Lei L, Lin J, Sun X and Liu L (2007). Oxidative stress, antioxidant status and DNA damage in patients with impaired glucose regulation and newly diagnosed Type 2 diabetes. *Clin. Sci.*, **112**: 599-606.
- Suematsu N, Tsutsui H, Wen JMD, Kang D, Ikeuchi M, Ide T, Hayashidani S, Shiomi T, Kubota T, Hamasaki N and Takeshita A (2003). Oxidative stress mediates tumor necrosis factor- α -induced mitochondrial DNA damage and dysfunction in cardiac myocytes. *Circulation*, **108**: 1418-1423.
- Tian F, Zhang YJ, Li Y and Xie Y (2014). Celecoxib ameliorates non-alcoholic steatohepatitis in type 2 diabetic rats via suppression of the non-canonical Wnt signaling pathway expression, *PLoS One*, **9**: e83819.
- Tsai CY, Wang CC, Lai TY, Tsu HN, Wang CH, Liang HY and Kuo WW (2013). Antioxidant effects of diallyltrisulfide on high glucose-induced apoptosis are mediated by the PI3K/Akt-dependent activation of Nrf2 in cardiomyocytes. *Int. J. Cardiol.*, **168**(2): 1286-97.
- Teoh H, Quan A, Lovren F, Wang G, Targari S, Szmitko PE, Szalai AJ, Ward ME and Verma S (2008). Impaired endothelial function in C-reactive protein overexpressing mice. *Atherosclerosis*, **201**(2): 318-325.
- Thomas GN, Ho SY, Lam KS, Janus ED, Hedley AJ and Lam TH (2004). Impact of obesity and body fat distribution on cardiovascular risk factors in Hong Kong Chinese. *Obes Res.*, **12**(11): 1805-1813.
- Thornalley PJ (2002). Glycation in diabetic neuropathy: Characteristics, consequences, causes and therapeutic options. *Int. Rev. Neurobiol.*, **50**: 37-57.

- Vaculova A and Zhivotovsky B (2008). Caspases: determination of their activities in apoptotic cells. *Methods Enzymol.*, **442**: 157-181.
- Wadkar K, Magdum C, Patil S and Naikwade N (2008). Anti-diabetic potential and Indian medicinal plants. *J. Herb. Med. Toxicol.*, **2**(1): 45-50.
- Wakabayashi I, Masuda H (2004). Relationship between obesity and atherosclerotic risk in elderly type 2 diabetic patients. *Nihon Ronen Igakkai Zasshi.*, **41**(2): 223-7.
- Wang J, Song Y, Wang Q, Kralik PM and Epstein PN (2006). Causes and characteristics of diabetic cardiomyopathy. *Rev. Diabet. Stud.*, **3**: 108-117.
- Wild S, Roglic G, Green A, Sicree R and King H (2004). Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care*, **27**: 1047-1053.
- Wolpin BM, Bao Y, Qian ZR, Wu C, Kraft P, Ogino S, Stampfer MJ, Sato K, Ma J, Buring JE, Sesso HD, Lee IM, Gaziano JM, McTiernan A, Phillips LS, Cochrane BB, Pollak MN, Manson JE, Giovannucci EL and Fuchs CS (2013). Hyperglycemia, insulin resistance, impaired pancreatic beta-cell function, and risk of pancreatic cancer. *J. Natl. Cancer Inst.*, **105**: 1027-1035.
- Zhang CR, Aldosari SA, Vidyasagar PS, Nair KM and Nair MG (2013). Antioxidant and anti-inflammatory assays confirm bioactive compounds in *Ajwa* Date fruit. *J. Agric. Food Chem.*, **61**: 5834-5840.