Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2016; 8(5); 379-383

ISSN: 0975-5160

Research Article

Protective Effects of Pomegranate Peel Extract on Cardiac Muscle in Streptozotocin-Induced Diabetes in Rats

Ashraf Y Albarakti*

Human Anatomy department, College of Medicine, Taif University, Saudi Arabia

Available Online: 25th October, 2016

ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder in which the carbohydrate and lipid metabolism is improperly regulated by insulin. Diabetes has been recognized to be one of highly risk independent factor for cardiovascular disorders, cardiomyopathy, coronary heart disease, congestive heart failure, peripheral arterial disease and stroke. Pomegranate considers a native fruit of Al-Taif region. Pomegranates contain numerous of antioxidant polyphenolic substances as compared to other fruits and vegetables. Polyphenols have been shown to be cardio protective in different model systems. The present study has been designed to demonstrate the protective effects of pomegranate peel extract against diabetic heart complications in streptozotocin (STZ)-induced diabetic rats. Method: Sixty adult male albino rats weighing 250 – 300 gm, were used in this study and divided into three groups; the first group, normal group; the second was subjected to induction of diabetes; the third group was treated with pomegranate extract orally. At the end of the trial (8 weeks), animals heart specimens were taken after the last injection and processed for histological and ultrastructural studies. Results: Biochemical studies showed increased values of glucose, and cardiac enzymes (Troponin I and CK-MB) and myoglobin in the second group while in the third group there was improvement in values of the examined parameters. Histopathological studies revealed obvious many degenerative changes that were varying from vacuolation to myocytolysis and loss of myofibrils. Ultrastructural examination showed extensive degeneration of the muscle fibers with marked loss and even complete disappearance of myofibrils, with degeneration of many mitochondria. The toxic effects of diabetes on the myocardium were markedly attenuated by pomegranate extract administration in combination with streptozotocin-induced diabetic injections. Conclusion: From this study, it was concluded that, pomegranate peel extract administration markedly attenuated diabetes induced cardiomyopathic changes.

Keywords: pomegranate, diabetes, cardiac muscle, STZ, histopathology

INTRODUCTION

Numerous studies on diabetes have been reported that associated with alterations of normal histology of heart and leading to an increased risk of cardiomyopathy[1]. It is recognized that long-standing diabetes to be an independent risk factor for cardiovascular disease. The negative impact of diabetes extends to all components. It shows the cardiovascular system, including the tiny blood vessels, big heart and arteries, as well as evidence kidneys. A lot of patients who suffer from diabetes type 1 or type 2 diabetes mellitus are prone to several heart disorders and blood vessels including coronary heart disease, stroke and peripheral arterial disease, cardiomyopathy and congestive heart complications failure. Cardiovascular now the main causes of morbidity and mortality caused by diabetes[2]. Pomegranates reach in polyphenols as compared to other fruits and vegetables[3-5]. Supplementation of with pomegranate juice to pregnant mice was recently shown to protect against neuro degeneration in neonatal mice subjected too hypoxic–ischemic brain injury[6]. Pomegranate has a long history of use in medicine[7]. Antibacterial, antifungal, antioxidant, antitumor, antiviral, antimalarial and antimutagenic effects of Pgranatum (PG) have already been supported by different studies[8-10]. Several studies have been reported that the prevention and treatment of some cancer types such as, lung cancer[11] and prostate cancer[12]. In the Middle East there were earlier studies of pomegranate as a fruit native, with type II diabetes prevention and treatment. The effects of fractions pomegranate (peels, flowers, and seeds) and some of the active components on the biochemical changes and metabolism associated with satisfactory marks from type 2 diabetes disease fractures pomegranate affect the case of patients with type 2 diabetes is by reducing oxidative stress and peroxide fat. The fasting glucose levels dropped dramatically in the blood of punicic acid, methylated seed extract and pomegranate peel extract. These findings provide evidence of an anti-diabetic activity of the pomegranate fruit. However, before the pomegranate or any of the abstracts can be recommended for medical management of type 2 diabetes disease, controlled, clinical studies, and there is a need[13].

MATERIALS AND METHODS

Experimental Animals

*Author for Correspondence: a.albrakati@tu.edu.sa

60 adult male Wistar rats (250- 300 gm), were obtained from King Abdul Aziz University, Jeddah, Saudi Arabia. They were housed and maintained at an air-conditioned room with a 12-h light/12-h dark cycle and allowed free access to water and food.

Chemicals and Reagents

All the chemicals and streptozotocin (STZ) were purchased from Sigma Chemical Co., St. Louis, MO, USA). Glucose was estimated by a spectrophotometric assay using kit purchased from biodiagnostic, Egypt. Troponin I, CK-MB and myoglobin were assayed by ELISA technique using assay kit purchased from abcam, Cambridge, UK according to the instructions provided.

Induction of Diabetes

A single intraperitoneal injection dose (60 mg/kg/b.w) dissolved in 0.05 ml/l sodium citrate buffer, pH 4.5 was used for induction the diabetes. Control animals group were fasted and received normal saline. Hyperglycemia was confirmed 3 days after injection by measuring blood glucose level using an Accu-Check Sensor as a glucose meter. The animals with fasting blood glucose levels ≥250 mg/dl were considered diabetic.

Preparation of Pomegranate Peels Extract

The mature granatum fruits were obtained from the local market. Peels were dried in the ground for extraction at room temperature. Subsequently, granatums were extracted using absolute methanol in Soxhlet apparatus for 24 h at room temperature. Thereafter, the extract was followed by filtration and centrifuged at 8000 rpm for 15 min, the clear supernatant were collected, and then the methanol were evaporated at 45 °C. Crude extract (23.5%, w/w) was kept at 20 °C. Pomegranate peel extract (500 mg/kg/b.w) were administered orally in aqueous solution once per day[14].

Experimental Design

60 rats were divided randomly into three groups (n=20). In the group I, rats were left as a control and they were given normal saline solution, group II and group III, rats were subjected to induction of diabetes. Whereas, group II, untreated diabetic rats were given normal saline solution. In the group III, diabetic rats were given pomegranate peel extract 500 mg/kg/b.w oral administrations for 8 weeks. At the end of the experimental period, the animals are killed under light anesthesia and the heart were sampled and fixed for histological and ultrastructural examination.

Biomedical Analysis

The blood samples (2 ml) were collected from retro-orbital vein to assay levels of serum glucose, troponin I, CK-MB and myoglobin using Spectrophotometer[15].

Statistical Analysis

The data obtained from the biochemical analysis of different groups were presented as a mean ±SE and statistical significance was assessed by one-way ANOVA using SPSS statistical version 16 software package (SPSS® 4 Inc., USA) to assess significant differences among treatment groups. The statistical significance was set at $P < 0.05$.

RESULTS

The biochemical results showed that the values of blood glucose, troponin I, myoglobin and the activity of CK-MB have been elevated significantly ($P<0.05$) in the STZ treated-animals as compared to the control group, meanwhile, the diabetic animals treated with pomegranate peel extract caused significantly decrease in all tested parameters to be near the control levels (Table 1).

Histopathological Examinations of the Cardiac Muscle

Histological examination of the cardiac muscle of control group, revealed normal structure of myocardium, branching and anastomosis cardiac muscle fibers, acidophilic sarcoplasm and central elongated vesicular nuclei (Fig. 1). On the other hand, cardiac tissues of diabetic group, showed loss of the cardiac architecture with degeneration of the myocardial fibers, and congested coronary blood vessels (Fig. 2). By contrast, cardiac sections of the pomegranate treated rats, showed the preserved appearance of myocardium, branching and anastomosis cardiac muscle fibers with acidophilic sarcoplasm and central elongated vesicular nuclei (Fig. 3).

Ultrastructural Examination of the Cardiac Muscle

Examination of the control cardiac sections, showed cardiac muscle myofibrils, with alternating dark and light bands, they were separated by regular rows of mitochondria (Fig. 4). Group II showed damaged and widely separated myofibrils with some of myofibrils were loosed of striations with some fat droplet in between. Mitochondria were small in number, abnormal in shape and disturbed distribution (Fig. 5). However, cardiac sections in the rats of the group III showed preserved myofibrils with alternating dark and light bands, cardiac myofibrils were separated by regular rows of mitochondria and have been fat droplets in-between (Fig. 6).

DISCUSSION

Streptozotocin is an N-methyl nitrosocarbamil-glucosamine-structured substance synthesised by *Streptomyces achromogenes*[16]. It is known that it destroys the DNA of the related cell by increasing pancreatic β-cell poly adenine diphosphate ribose synthetase activity, and also causes degenerative lesions by decreasing NAD levels with these effects, it blocks pro-insulin synthesis and leads to type I diabetes characterised by insulin insufficiency[17]. The biochemical results observed that there was a significant increase in the plasma glucose levels in STZ-treated group when compared to control rats. This may support the findings of Lenzen[18] who stated that the treatment with STZ induced increase in blood glucose level response, accompanied by corresponding inverse changes in the plasma insulin concentration. These findings have been associated with vascular metabolic derangements associated with hyperglycemia[19]. Regarding the effect of pomegranate peel extract, animals treated with both STZ and pomegranate peel extract revealed an improvement in biochemical alterations when compared with animals received STZ alone. These results were agreement with Saad *et al.*[20], who reported that administration of Punica granatum reduced the concentration of glucose, triglycerides, cholesterol, in the blood of diabetic rats treated with alloxan. On the other

Table 1: Effects of pomegranate peel extract (500 mg/kg/b.w) on glucose, Troponin I, CK-MB and myoglobin levels in STZ treated-animals.

Parameters	Group I (Control)	Group II (STZ treated group)	Group III (STZ + pomegranate)
Glucose (mg/dl)	125 ±5	285 ±3	88 ±3
Troponin I (ng/ml)	0.004±0.011	0.69±0,012 [a]	0.007±0.003 [b]
CK-MB (ng/ml)	1.87±0.41	27.1±1.83 [a]	1.29±0.531 [b]
Myoglobin (pg/ml)	118.59±0.60	301.74±1.38 [a]	132.11±.0.231 [ab]

Data are represented as mean ± SE (n= 20 in each group).
a: Significant at $p < 0.05$ with respect to control group.
b: Significant at $p < 0.05$ with respect to STZ treated group.

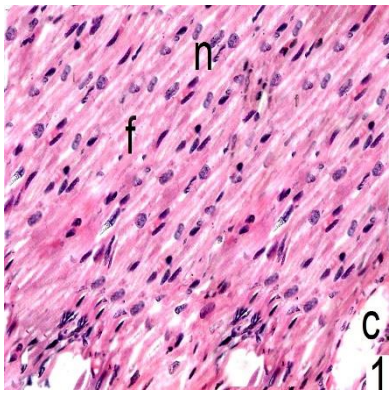

Figure 1: A photomicrograph of section in the heart tissues of group I, shows branching and anastomosis cardiac muscle fibers (f) with acidophilic sarcoplasm and central elongated vesicular nuclei (n) with normal coronary blood vessels, (H and E, X400).

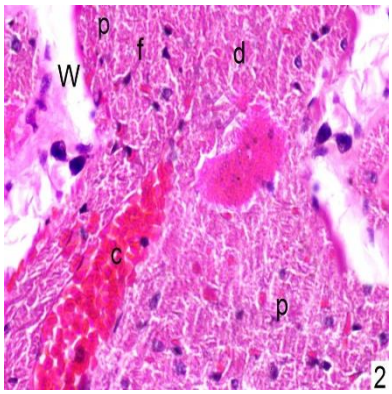

Figure 2: A photomicrograph of transverse section in heart tissues of the group II, shows loss of normal cardiac architecture, fragmentation and degeneration of the myocardial fibers (f), wide separation of muscle fibers (w) with pycknotic nuclei (p), degenerated area (d) and congested coronary blood vessels (c), (H and E, X400).

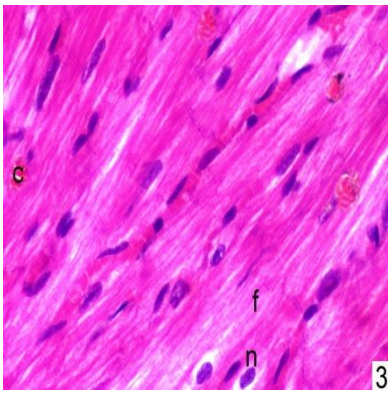

Figure 3: A photomicrograph of transverse section of heart tissues of group III, shows normal branching and anastomosis cardiac muscle fibers (f) with acidophilic sarcoplasm and central elongated vesicular nuclei (n) and congested coronary blood vessels (c), (H and E, X400).

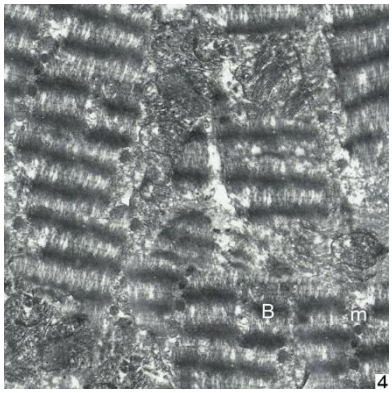

Figure 4: Transmission electron micrograph of rat cardiac muscle in the group I, shows myofibrils with alternating dark and light bands (B), they were separated with rows of mitochondria (m), (TEM, X6000).

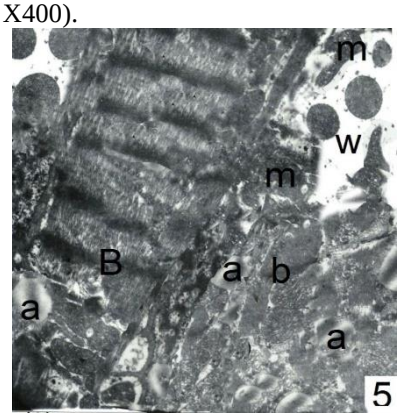

Figure 5: A photomicrograph of section in the cardiac muscle electron microscopic picture of group II, shows wide separation(w) of myofibrils (B) with alternating dark and light bands (B), they were separated with abnormal shape of mitochondria (m), some damaged myofibrils were seen (b) with distribution of fat droplets (a), (TEM, X6000).

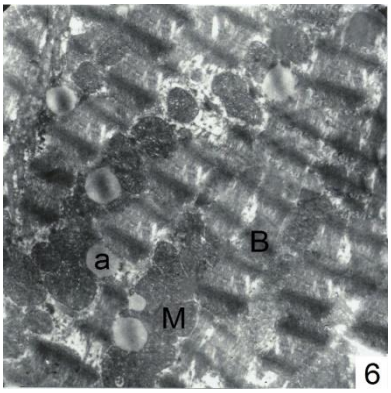

Figure 6: A photomicrograph of section in the cardiac muscle electron microscopic picture of group III, shows relatively improvement of myofibrils with alternating dark and light bands (B), they were separated with rows of mitochondria (M) with distribution of fat droplets (a) in between myofibrils, (TEM, X6000).

hand, increase some cardiac enzymes such as cardiac troponin I, creatine kinase and its isoenzyme MB (CK-MB) were used as a gold standard for detection of acute myocardial infarction in people and small animals[21,22]. In this study, our results also showed that the troponin I, CK-MB and myoglobin of animals treated with STZ were significantly increased in the plasma. These results were in parallel with Hayat *et al.*[23], who reported that diabetes leads to toxic myocardial damage in form degeneration inducing myocardial infarction due to hyperaemia. The enhancement effects of pomegranate peel extract on the cardiac enzymes, animals received both STZ and pomegranate peel extract revealed an improvement in troponin I, CK-MB and myoglobin when compared with animals received STZ alone. These results were in parallel with Chidambara *et al.*[24], who reported that the Punica grantum aqueous peel extract possesses strong antioxidant property can act as a free radical scavenger also it may increase insulin receptor. After eight weeks of treatment with STZ, histopathological results of group II, revealed a disturbance in the structural organization of cardiac tissues, fragmentation and degeneration of the myocardial fibers, wide separation of muscle fibers with pycknotic nuclei, degenerated area and congested coronary blood vessels (Fig 5). The degenerative changes of the nuclei, it may explain form of pyknosis cells of cardiac tissue. These results were in agreement with Dowling *et al.*[25], who reported that fetal hearts from diabetic pregnancy experience has hypertrophy, hyperplasia of the cardiomyocytes, fibroblasts, which marked vacuolar degeneration of the cardiomyocytes. Moreover, take *et al.*[1], also pointed out that there were significant degenerative changes in the myocardium after STZ injection. Also, Bahçeci *et al.*[26] have been added that the myocardium was also affected minimally in the acute phase of diabetes; and heart-related disorders started in this phase. The histopathological findings in this study showed a marked vascular congestion, hemorrhage with endothelial lining in the myocardium, after application of STZ. These results were in coincidence with Manjarrez *et al.*[27]. In addition, results of the present study showed that damaged and widely separated myofibrils with some of myofibrils had loss of striations and mitochondrial degeneration. These results were agreement with the results of Take *et al.*[1], who reported that the electron microscopic analyses of the left ventricle wall revealed prominent degenerative alterations in the myocardial cells of diabetic rats, swelling and crystolysis in the mitochondria. Also in our study, it was observed that the diabetic rats had lipid deposition in the cytoplasm these results were in agreement with Take *et al.*[1], who reported that lipid deposition in the cytoplasm, and widespread of myofilaments were detected in the myocardial cells of diabetic rats. In this study, TEM examination evaluated the cytoplasmic vacuoles. These results were in agreement with Take *et al.*[1], who reported that degeneration process and vacuolar changes in the myocardial cells of diabetic rats. TEM examination results in the present study showed relatively improvement of myofibrils at the ultrastructural changes with alternating dark and light bands, also it showed separate with rows of mitochondria. These results were in agreement with the results of Zafar *et al.*[28], who reported that the antihyperglycaemic effect of the peel extract of pomegranate peel led to inhibitory intestinal absorption of glucose in the diabetic rats. To gather, all these results may show clearly that treatment with STZ had induced cardio-toxicity and then leads to myocardial injury Histological results of the cardiac muscles proved the protective effect of pomegranates peel by reducing the fragmentation and degeneration of the myocardial fibers, wide separation of muscle fibers with pycknotic nuclei, degenerated area. However, antioxidant is present in the several of the natural products, could be one of the possible methods to reduce the incidence of these diseases[29]. Pomegranates, a natural product and it is rich with polyphenolic components as one of the important antioxidant activity compound[30]. The present results showed that pomegranates decreased the cardiac toxicity, the peroxidation marker, and decreased the cardiac enzymes.

CONCLUSION

In conclusion, the present results indicated the myocardial-protective role of pomegranate peel extract which may be attributed to its antioxidant effects.

REFERENCES

1. Gülnur Take1, G.K., A. Canan Yazici, Deniz Erdoğan, J Uludag Univ Med Fac, 2004. 30(3): p. 199-204.
2. Grundy SM, B.I., Burke GL, Chait A, Eckel RH, Howard BV,Mitch W, Smith SC, Sowers JR Circulation, 1999. 100(10): p. 1132-3.
3. Kelawala, N.S. and L. Ananthanarayan, International Journal of Food Sciences and Nutrition, 2004. 55(6): p. 511-516.
4. Ali S I., Mohamed A A., Sameeh M Y., Darwesh O M., and Abd El-Razik T M. Res. J. Pharm., Biol. Chem. Sci., 2016; 7(1): 524-532.
5. Darwesh OM, Hassan M, Barakat OS and Abd El-Rahim WM. Res. J. Pharm., Biol. Chem. Sci., 2015; 6(1): 1202-1211.
6. Loren, D.J., et al., *Maternal dietary supplementation with pomegranate juice is neuroprotective in an animal model of neonatal hypoxic-ischemic brain injury.* Pediatr Res, 2005. 57(6): p. 858-64.
7. Abdollahzadeh, S., et al., *Antibacterial and Antifungal Activities of Punica Granatum Peel Extracts Against Oral Pathogens.* Journal of Dentistry (Tehran, Iran), 2011. 8(1): p. 1-6.
8. Ahmad, I. and A.Z. Beg, *Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens.* J Ethnopharmacol, 2001. 74(2): p. 113-23.
9. Prashanth, D., M.K. Asha, and A. Amit, *Antibacterial activity of Punica granatum.* Fitoterapia, 2001. 72(2): p. 171-3.
10. El-Baz F K., Mahmoud K, El-Senousy W M., Darwesh O M. and El Gohary A E. Int J Pharm Sci Rev Res, 2015; 31(1): 262-268.

11. Khan, N. and H. Mukhtar, *Pomegranate fruit as a lung cancer chemopreventive agent.* Drugs of the Future, 2007. 32(6): p. 549-554.
12. Malik, A. and H. Mukhtar, *Prostate cancer prevention through pomegranate fruit.* Cell Cycle, 2006. 5(4): p. 371-373.
13. Banihani, S., S. Swedan, and Z. Alguraan, *Pomegranate and type 2 diabetes.* Nutr Res, 2013. 33(5): p. 341-8.
14. Lapornik, B., M. Prosek, and A.G. Wondra, *Comparison of extracts prepared from plant by-products using different solvents and extraction time.* Journal of Food Engineering, 2005. 71(2): p. 214-222.
15. Enderlein, G., *Daniel, Wayne W.: Biostatistics — A Foundations for Analysis in the Health Sciences. Wiley & Sons, New York—Chichester—Brisbane—Toronto—Singapore, 6th ed. 1995, 780 S., £58.—, ISBN 0–471–58852-0 (cloth).* Biometrical Journal, 1995. 37(6): p. 744-744.
16. Hinz, M., et al., *Significance of streptozotocin induced nicotinamide-adenine-dinucleotide (NAD) degradation in mouse pancreatic islets.* FEBS Letters, 1973. 30(2): p. 225-228.
17. Yamamoto, H., Y. Uchigata, and H. Okamoto, *Streptozotocin and alloxan induce DNA strand breaks and poly(ADP-ribose) synthetase in pancreatic islets.* Nature, 1981. 294(5838): p. 284-286.
18. Lenzen, S., *The mechanisms of alloxan- and streptozotocin-induced diabetes.* Diabetologia, 2008. 51(2): p. 216-26.
19. Akgun-Dar, K., et al., *Vanadyl sulfate protects against streptozotocin-induced morphological and biochemical changes in rat aorta.* Cell Biochem Funct, 2007. 25(6): p. 603-9.
20. Saad, e.a., et al., *antidiabetic, hypolipidemic and antioxidant activities and protective effects of punica granatum peels powder against pancreatic and hepatic tissues injuries in streptozotocin induced iddm in rats.* 2015, 2015: p. 6.
21. Jeremias, A. and C.M. Gibson, *Narrative review: alternative causes for elevated cardiac troponin levels when acute coronary syndromes are excluded.* Ann Intern Med, 2005. 142(9): p. 786-91.
22. O'Brien, P.J., *Cardiac troponin is the most effective translational safety biomarker for myocardial injury in cardiotoxicity.* Toxicology, 2008. 245(3): p. 206-18.
23. Hayat, S.A., et al., *Diabetic cardiomyopathy: mechanisms, diagnosis and treatment.* Clinical Science, 2004. 107(6): p. 539-557.
24. Chidambara Murthy, K.N., G.K. Jayaprakasha, and R.P. Singh, *Studies on antioxidant activity of pomegranate (Punica granatum) peel extract using in vivo models.* J Agric Food Chem, 2002. 50(17): p. 4791-5.
25. Dowling, D., et al., *Cardiomyopathy in Offspring of Pregestational Diabetic Mouse Pregnancy.* Journal of Diabetes Research, 2014. 2014: p. 6.
26. Bahçeci S., C.N., Nergiz Y., Söker S., Gökalp D.,Akbalık M.E., Tutşi Y. , *Light microscopic evaluation of cardio-vasculare system in alloxan-induced diabetic rats in acute period.* Dicle Tıp Dergisi, 2007. 34: p. 111-115.
27. Manjarrez-Gutierrez, G., et al., *[Anatomopathological findings during development of diabetic cardiomyopathy in rats].* Cir Cir, 2014. 82(1): p. 11-9.
28. Zafar, R.a.S., J.,, *Antidiabetic activity of Punica granatum Linn.* Sci.Culture (1990). 7(3): p. .
29. Saber A. Sakr1* , A.Y.A., *Effect of cinnamon on cypermethrin-induced nephrotoxicity in albino rats.* International Journal of Advanced Research, 2014 2(7): p. 578-586.
30. Barakat K M, Mattar M Z, Sabae S Z, Darwesh O M, and Hassan S H. Res. J. Pharm., Biol. Chem. Sci., 2015; 6(5): 933-943