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Alleviation of Cardiac Damage by Dietary Fenugreek (*Trigonella foenum-graecum*) Seeds is Potentiated by Onion (*Allium cepa*) in Experimental Diabetic Rats via Blocking Renin–Angiotensin System

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Abstract Hyperglycemia is one of the metabolic and homeostatic abnormalities that increase the cardiovascular mortality in diabetic patients by increased oxidative stress. We have recently reported amelioration of oxidative stress in cardiac tissue by dietary fenugreek (*Trigonella foenum-graecum*) seeds and onion (*Allium cepa*) in streptozotocin-induced diabetic rats. The mechanistic aspects of the cardio-protective influence of dietary fenugreek seeds (10%) and onion (3% powder) both individually and in combination on hyperglycemia-mediated cardiac damage was further investigated in this study on streptozotocin-induced diabetic rats. Cardio-protective influence of these dietary spices was evidenced by their blocking potential on renin–angiotensin system. This might be the consequence of reduced activation of angiotensin-converting enzyme (ACE) and angiotensin type 1 receptor (AT_1) in cardiac tissue. The combination produced an additive effect on ACE and AT_1 protein and mRNA expressions. Increased expression of type IV collagen, fibronectin, Bax, 4-hydroxynonenal, iNOS and metabolites of nitric oxide (nitrate/nitrite) along with disturbed PUFA-to-SFA ratio and activities of cardiac marker enzymes in blood confirmed the myocardial damage. Dietary fenugreek seed, onion and fenugreek + onion were found to ameliorate these pathological changes in the cardiovascular system. The beneficial effect being higher with the combination sometime amounting to additive (iNOS expression) or even a synergistic (cardiac Bax and type IV collagen expression and circulatory marker enzymes) in diabetic rats. Thus, the results of present investigation suggested that the combination of fenugreek seeds and onion offers higher beneficial influence in ameliorating cardiac damage accompanying diabetes.

Keywords Cardio-protection · Oxidative stress · Renin–angiotensin system · Fenugreek seeds · Onion

Introduction

Diabetes mellitus (DM) is the most common chronic metabolic disorders and a leading cause of mortality all over the world. Reactive oxygen species (ROS)-induced oxidative stress is currently suggested to be a mechanism underlying diabetes and its complications. Cardiovascular disease (CVD) is one of the secondary complications of diabetes which include ischemic heart disease, myocardial infarction and cardiomyopathy. CVD accounts for 80% mortality in diabetic patients [1]. These complications are associated with multiple structural and functional abnormalities such as cardiomyocyte hypertrophy, cardiac fibrosis, mitochondrial dysfunction, interstitial accumulation of glycoprotein, systolic and diastolic dysfunction [2]. It is a well-known fact that the hyperactivity of angiotensin-converting enzyme (ACE) and receptor AT_1 of renin–angiotensin system (RAS) is associated with the establishment and progression of CVD and diabetes [3].

Dietary fenugreek (*Trigonella foenum-graecum*) seed is well recognized to possess multiple health beneficial influences which include antidiabetic, hypocholesterolemic, antioxidant, digestive stimulant, gastro-protective and cardio-protective effect as documented in numerous animal studies, as well as limited human trials [4]. Onion (*Allium cepa*), the abundantly used *Allium* spice, has been reported to exert a wide range of health benefits, including antidiabetic,

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anticancer, antithrombotic, hypocholesterolemic effects in both preclinical and clinical studies [5]. Studies have reported that these two spices show a modulatory effect on glucose homeostasis and lipid metabolism in streptozotocin-induced diabetes [6, 7]. Recently, these two spices have also been demonstrated to ameliorate hyperglycemia, hypoinsulinemia and attendant metabolic abnormalities in diabetic rats [8]. We have also recently shown that dietary interventions with fenugreek seeds and onion significantly alleviated the oxidative stress and lipid abnormalities in cardiac tissue of STZ-induced diabetic rats [9].

While it may be speculated to have cardio-protective influence by virtue of modulating cholesterol homeostasis, thrombosis or antiplatelet aggregatory activity, it remains to be examined whether these spices can offer cardio-protection in conditions of diabetes. Since there is a possibility of additive effect/or synergy among these spices in their hypoglycemic and insulin sparing action, this needs to be evaluated with their specific combination since they exert their action by different mechanisms. Hence, this animal study was designed to investigate the cardio-protective influence of fenugreek seeds, as a provider of dietary fiber, and onion, a well-established insulinotropic and antioxidant agent, both individually and in combination using streptozotocin-diabetic model. The present study has particularly focused on the level of specific oxidative stress markers and to unravel its mechanism in mitigating cardiac damage under diabetic condition.

Materials and Methods

Materials

All the chemicals were procured from Sigma-Aldrich Chemical Co., (St. Louis, MO, USA) or Sisco Research Laboratories (Mumbai, India) and were of analytical grade and highest purity. Fenugreek seeds (*Trigonella foenum-graecum* L.) were purchased from the local market and powdered and stored at 4 °C. Onion (*Allium cepa* L.) which was procured from the local market was chopped, freeze-dried and powdered and stored at 4 °C. Casein (food grade) was procured from Nimesh Corporation (Mumbai, India). Maize starch, cane sugar powder and refined groundnut oil were purchased from the local market.

Animals and Experimental Design

The animal study was carried out with due approval from the Institutional Animal Ethics Committee (CSIR-CFTRI, Mysore, India). Male Wistar rats (140–150 g body mass, $n = 80$) raised in the Experimental Animal Production Facility Unit of this institute were housed in individual cages under standard laboratory conditions with a 12-/12-h light–dark cycle. All the animals had ad libitum access to food and water. Experimental diabetes was induced by a single *i.p.* injection of 45 mg/kg STZ (Sigma-Aldrich, St Louis, MO, USA) dissolved in freshly prepared citrate buffer (pH 4.5). On the third day, blood was drawn from the retro-orbital plexus; fasting blood glucose was determined by the method of Huggett and Nixon [10]. Animals with a fasting blood glucose level > 250 mg/dL were recruited as diabetic animals. Rats were divided into eight groups, out of which four groups were diabetic (12 rats in each group), and the other four groups were non-diabetic (eight rats in each group). One group of diabetic animals ($n = 12$ per group) and a group of normal animals ($n = 8$ per group) were maintained on a semisynthetic basal diet. The basal diet consisted of (%): casein, 21; cane sugar, 10; corn starch, 54; NRC vitaminized starch, 1; Bernhardt–Tommarelli modified NRC salt mixture, 4; fat-soluble vitamins at the recommended levels; and refined peanut oil, 10. The animals were maintained on various experimental semisynthetic diets and water ad libitum for 6 weeks. The three experimental diets consisted of 10% fenugreek seed powder, 3% onion powder and a combination of 10% fenugreek seed and 3% onion powder, respectively, replacing an equivalent amount of starch in the basal semisynthetic diet. At the end of 6th week, the animals were killed under euthanasia. Blood was collected in heparinized tubes by heart puncture, and plasma was separated by centrifugation. The heart tissue was quickly excised, weighed and processed for various analyses.

Marker enzymes of Cardiac Damage

Activities of LDH [11], aspartate aminotransferase and alanine aminotransferase [12], alkaline and acid phosphatase [13] and angiotensin-converting enzyme activity [14] were measured in the plasma and homogenate of the heart tissue. Creatine phosphokinase-MB (CK-MB) was assayed by using a diagnostic kit (Agappe Diagnostic Pvt Ltd, Thane, India).

Measurement of Troponin-T and Nitric Oxide

Cardiac marker Troponin-T was estimated by commercially available ELISA kit (Catalog # CSB-E16443r, CUSABIO Biotech Co., Wuhan, China). Nitric oxide (NO) level was estimated using a commercially available kit (Catalog # K262, Biovision, Mountain View, CA, USA).

Lipids and Fatty Acid Profile

Heart lipids were extracted by the procedure of Folch et al. [15]. The fatty acid methyl esters were analyzed by gas chromatography (PerkinElmer) using Elite-Wax (30 m × 9.25 mm) fused silica capillary column, a flame

ionization detector (FID). The analysis was carried out in an isothermal condition. The operating conditions were as follows: column temperature 240 °C, injection temperature 250 °C and detector temperature 260 °C. Nitrogen was used as the carrier gas. Individual fatty acids were identified by comparing with the retention times of reference fatty acids. The results are expressed as relative percentage peak area of individual fatty acids.

Western Blot Analysis

The cardiac tissues were homogenized in ice-cold lysis buffer (pH 7.5). Equal protein concentration (μg) of each sample was electrophoresed on 10% SDS-PAGE gel and electrotransferred to PVDF membrane. The protein blots were blocked with 5% skim milk solution for 2 h and incubated with primary antibodies for angiotensin-converting enzyme 1 (Cat.# ab134709), iNOS (Cat.# ab3523), 4-hydroxynonenal (4-HNE) (Cat.# ab46545), β-actin (Cat.# ab25894; Abcam, Cambridge, MA, USA) and AT_1 (Cat.# sc-1173-G, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) all of which were used as per the instructions given by the manufacturer. Immunoreactive bands were then detected by incubating with horseradish peroxidase (HRP)-conjugated secondary antibody and visualized using enhanced chemiluminescence reagents (Sigma Chemicals Co., St. Louis, MO, USA). Band intensity was quantified as the ratio to β-actin using Gene Tools v4.01 analysis software (Syngene).

Real-Time PCR Analysis

Total RNA was extracted from cardiac tissues using TRI reagent as per instructions given by the manufacturer which were previously stored in RNA later. The RNA samples were quantified using Biospectrophotometer. Complementary DNA strand was synthesized by using Verso cDNA synthesis kit (Thermo Fisher Scientific Inc., MA, USA) as per instructions given by the manufacturer. Primers for the PCR amplifications used were synthesized using supplies from Sigma-Aldrich (St. Louis, MO, USA) as listed in Table 1. The RNA expression was quantified using Bio-Rad CFX96 Touch RT-PCR and SYBR Green PCR reagents. The relative quantification was done considering Ct value as per the manufacturer's instructions. The fold changes were calculated by considering Ct of β-actin amplified with samples in order to assure normalized quantification.

Statistical Analysis

Results are expressed as the mean ± SEM for eight animals in each group. Comparisons between different groups were made by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad Instat statistical software (GraphPad Software, Inc., La Jolla, CA, USA). The values with $p < 0.05$ were considered statistically significant.

Results

Influence on Cardiac Renin–Angiotensin System

Influences of dietary fenugreek seeds and onion on circulatory and cardiac ACE activity are presented in Fig. 1. Diabetic rats exhibited a significant ($p < 0.05$) increase in circulatory (66%) and cardiac (95%) ACE activity compared to that of the healthy control group. Dietary treatment to diabetic rats with fenugreek seeds, onion and fenugreek + onion significantly countered this with the maximum effect being produced by the latter ($p < 0.05$). In addition, this study verified the protein and mRNA expression of ACE and AT_1 levels in cardiac tissue (Fig. 2). Diabetic rats encountered up-regulated protein and mRNA expression of ACE and AT_1 compared to normal control. These up-regulated expressions of ACE and AT_1 in cardiac tissue were partially down-regulated by fenugreek seeds (protein by 34 and 27%; mRNA by 12 and 18%, respectively) or onion (protein by 40 and 17%; mRNA by 10 and 13%, respectively), as compared to diabetic control. The combination of fenugreek and onion showed maximum beneficial effect by significantly ($p < 0.05$) down-regulating both protein and mRNA

Table 1 Sequences of the primers used in this study

Gene	Sequence 5′–3′ (forward)	Sequence 5′–3′ (reverse)
ACE	CACCGGCAAGGTCTGCTT	CAACAAGACTGCCACCTGCTGCTCC
AT_1	AATTTTTTCCCCAGAAAGCC	GCCAAAGTCACCTGCATCAT
Type IV collagen	GCCCTACGTTAGCAGATGTACC	TATAAATGGACTGGCTCGGAAT
Fibronectin	GTGATCTACGAGGGACAGC	GCTGGTGGTGAAGTCAAAG
Bcl-2	GGGATGCCTTTGTGGAACTA	CTCACTTGTGGCCCAGGTAT
Bax	CGAGCTGATCAGAACCATCA	CTCAGCCCATCTTCTTCCAG
iNOS	AACCCAAGGTCTACGTTCAAG	AAAGTGGTAGCCACATCCCG
β-Actin	AGGCCCCTCTGAACCCTAAG	CCAGAGGCATACAGGGACAA

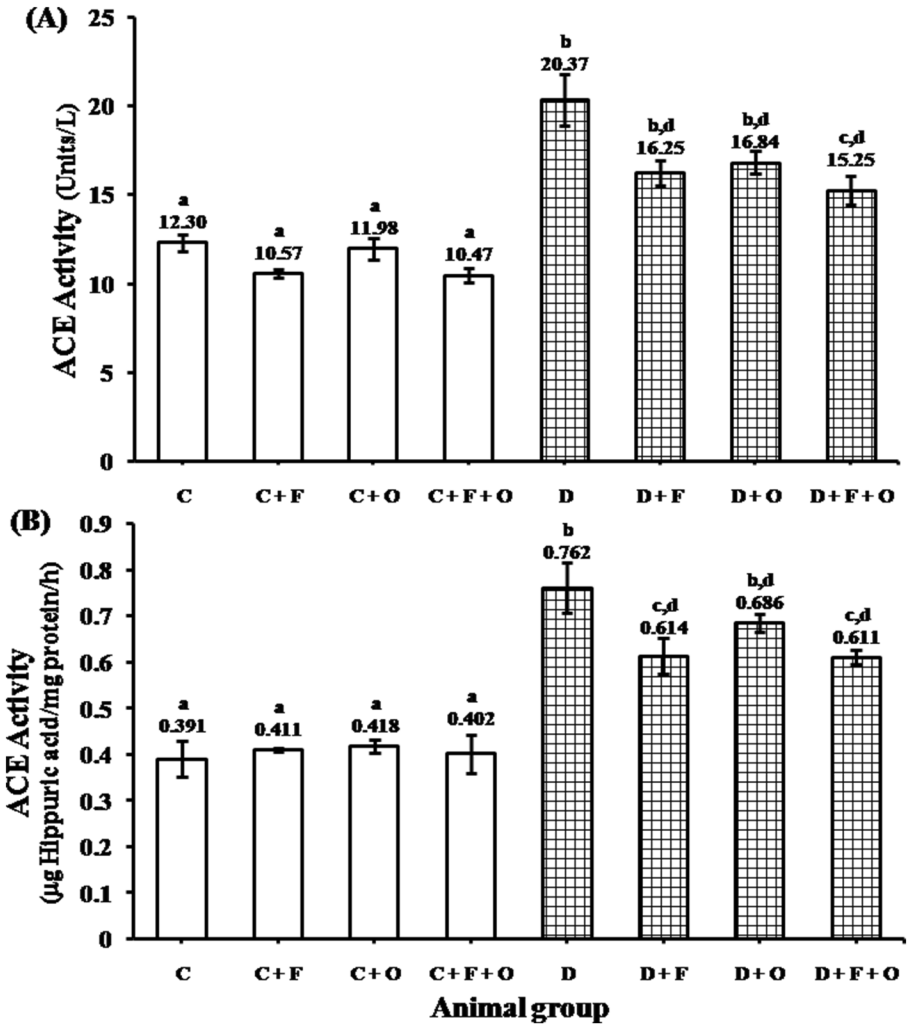

Fig. 1 Influence of dietary fenugreek seeds and onion on activity of angiotensin-converting enzyme in **a**. Plasma and **b** cardiac tissue in diabetic rats. Values are mean ± SEM of eight animals in each group. Each bar carrying different letters (a, b, c, d) is significantly different ($p < 0.05$). C, normal control; D, diabetic control; F, fenugreek; O, onion

expression of ACE (mRNA by 28%) and AT_1 (60 and 27%, respectively), amounting to an additive effect.

Influence on Metabolites of Nitric Oxide and iNOS Expression in Cardiac Tissue

The beneficial influence of fenugreek seeds and onion on nitric oxide metabolites in plasma and cardiac tissue is presented in Fig. 3. In our study, NO metabolites (nitrite/nitrate) were significantly ($p < 0.05$) higher (threefold) in the plasma of diabetic rats compared to normal control. Dietary intervention to these diabetic rats significantly countered the NO level by 40% (fenugreek), 45% (onion) and 41% (fenugreek + onion), respectively. Cardiac NO level was significantly decreased under diabetic condition (85%) compared to normoglycemic animals. Treatment with dietary fenugreek seeds, onion and fenugreek + onion significantly restored the level by 4.3-, 6.0- and 4.8-fold, in the respective groups. On the contrary, cardiac iNOS protein (2.8-fold) and mRNA (2.1-fold) expression were significantly up-regulated in diabetes. Dietary intervention to diabetic rats significantly down-regulated the iNOS expression 26 and 14% by fenugreek seeds, 9 and 17% by onion and 31 and 34% by fenugreek + onion, respectively, the combination thus producing an additive effect, when compared to the diabetic control.

Influence on Cardiac Oxidative Stress Markers

Favorable effect by dietary fenugreek seeds and onion was also evidenced by the countering effect on diabetes-induced fibrosis, apoptosis and auto-oxidation of PUFA at the organ level as illustrated in Figs. 4 and 5. mRNA transcription of fibrosis (type IV collagen and fibronectin) and pro-apoptotic (Bax) genes in the cardiac tissue were augmented with down-regulated antiapoptotic (Bcl-2) gene under diabetic condition (2.07-, 2.29-, 1.53- and 0.82-fold, respectively), compared to that of normal control. Altered expressions of these genes were significantly countered by dietary fenugreek (12, 32, 13% and 3.0-fold, respectively), onion (39, 36, 21% and 3.2-fold, respectively) and their combination

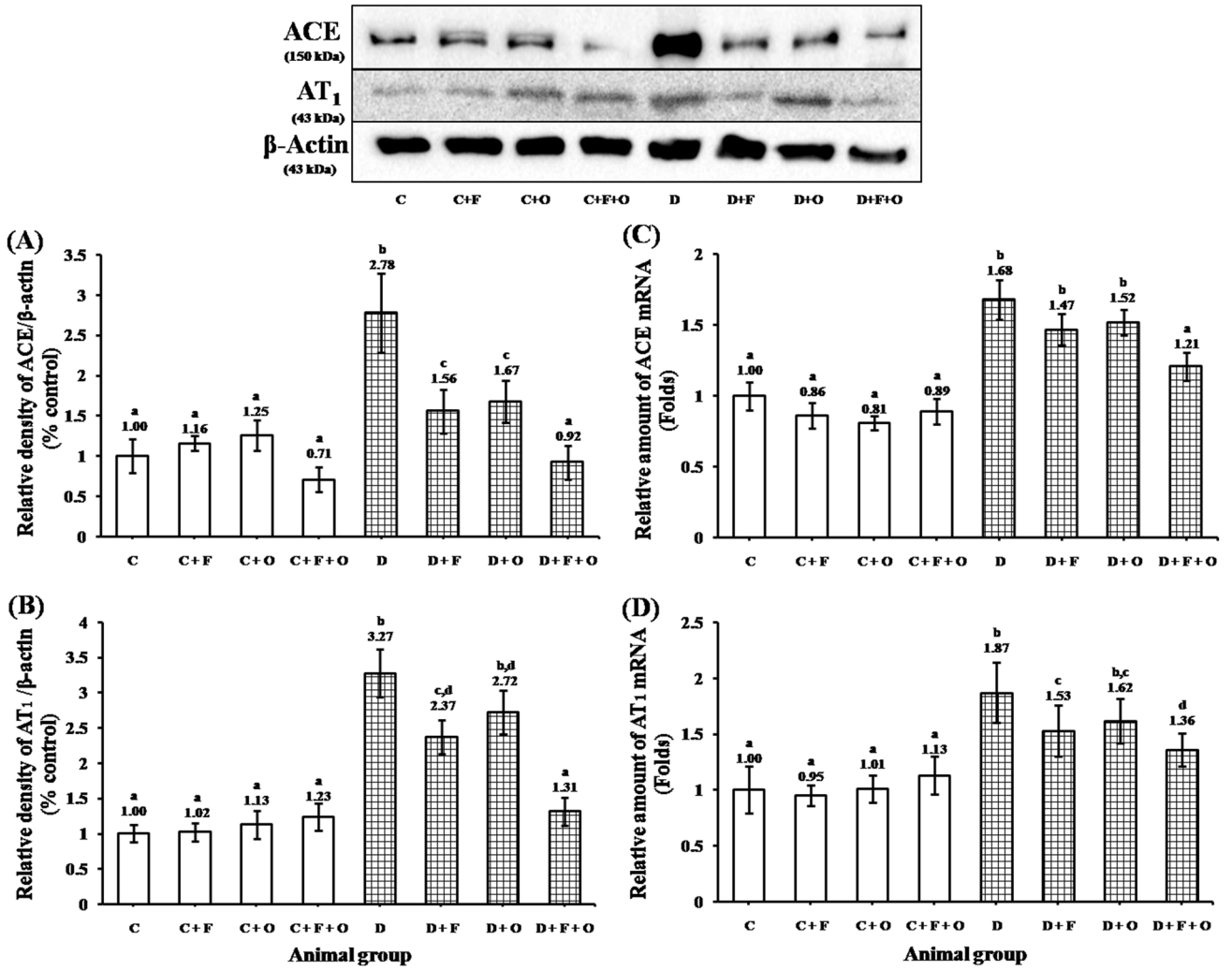

Fig. 2 Influence of dietary fenugreek seeds and onion on cardiac ACE and AT_1 (**a**, **b**). Protein and **c**, **d** mRNA expression in diabetic rats. Values are mean ± SEM of eight animals in each group. Each bar carrying different letters (a, b, c, d) is significantly different ($p < 0.05$). C, normal control; D, diabetic control; F, fenugreek; O, onion

(60, 48, 43% and 3.5-fold, respectively) in the respective mRNA expressions. Increased expression of cardiac lipid peroxidative by-product 4-HNE protein in diabetic condition (2.1-fold, compared to normal control) was significantly countered by dietary fenugreek seeds (15%), onion (20%) and fenugreek + onion (27%) treatment, respectively.

Influence on Cardiac Fatty Acid Profile

Fatty acid composition in the heart tissue revealed that diabetic rats exhibited a prominent decrease ($p < 0.05$) in PUFA–arachidonic acid (20:4) (by 34%), compared to normal control (Table 2). The reduction in arachidonic acid in diabetic rats was also accompanied by a significant increase in PUFA–linoleic acid (by 12%), MUFA–oleic acid (by 33%) and SFA-palmitic acid (by 46%), respectively. The PUFA/SFA ratio was thus significantly decreased under diabetic condition (by about 21%), as compared to normal control group. Dietary interventions in these diabetic animals resulted in an improvement of PUFA/SFA ratio (by about 18% in fenugreek; 18% in onion; 23% in fenugreek + onion) in the cardiac tissue.

Influence on Cardiac Marker Enzymes

There was a significant decrease in the activities of circulatory cardiac marker enzymes of animal group with the dietary intervention of fenugreek, onion or their combination to diabetic rats, which was otherwise elevated by the diabetic condition (Table 3). The activities of AsAT and AlAT were decreased in fenugreek (15 and 14%, respectively)-, onion (17 and 18%, respectively)- and fenugreek + onion (32 and

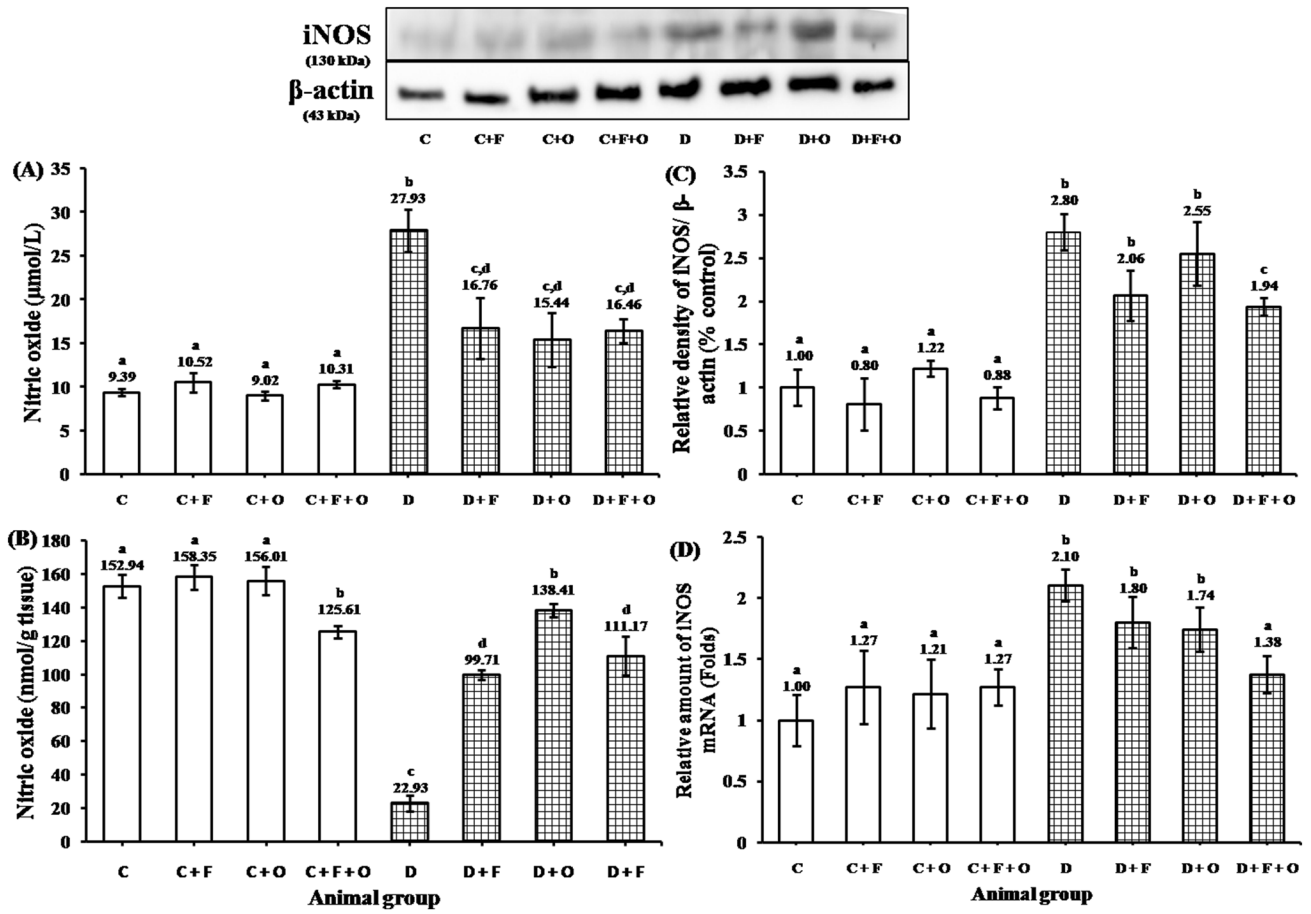

Fig. 3 Influence of dietary fenugreek seeds and onion on nitric oxide level in **a**. Plasma, **b** cardiac tissue, **c** cardiac iNOS protein and **d** mRNA expression in diabetic rats. Values are mean ± SEM of eight animals in each group. Each bar carrying different letters (a, b, c, d) is significantly different ($p < 0.05$). C, normal control; D, diabetic control; F, fenugreek; O, onion

33%, respectively)-fed groups, thus being additive in the latter group when compared with diabetic control. Feeding the combination of the two spices to diabetic rats did not have any additive effect when compared to their individual effects in the case of alkaline or acid phosphatase activity. Activities of LDH and CK-MB were decreased by 27 and 16% by fenugreek, 18% in onion and 54 and 34% in fenugreek + onion, amounting to a synergistic proportion in the latter. Increased level of cardiac damage marker Troponin-T (127%) in the plasma of diabetic rats was significantly restored at the end of the dietary regime by dietary fenugreek (38%), onion (31%) and fenugreek + onion (41%), compared to diabetic control group (Fig. 6).

The activities of cardiac marker enzymes AsAT, AlAT, alkaline phosphatase, LDH and CK-MB were decreased, while acid phosphatase activity was elevated under diabetic condition (Table 4). Feeding the combination of these two spices to diabetic rats did not have any additive effect when compared to the individual effects of fenugreek or onion on these. However, there was a tendency to normalize the condition with higher beneficial influence on the activities of AsAT (decrease by 10%) and acid phosphatase (increase by 19%), respectively, compared to diabetic control group.

Discussion

Globally, cardiovascular disease is a growing public health concern increasing at an alarming rate. About 17.5 million people die every year from cardiovascular disease, for which diabetes and hypertension are major risk factors [16]. Obesity and overweight, diabetes and hypertension, joined with malnutrition, are major health problems threatening the developing world. In view of the appreciable role of food and nutrition in the etiology of chronic diseases and in their prevention, effective nutritional practice for preventing or managing chronic diseases would be desirable. In this context, it would be most appropriate to evaluate the nutraceuticals potential of foods, especially the ones with hypoglycemic, hypolipidemic and antioxidant properties.

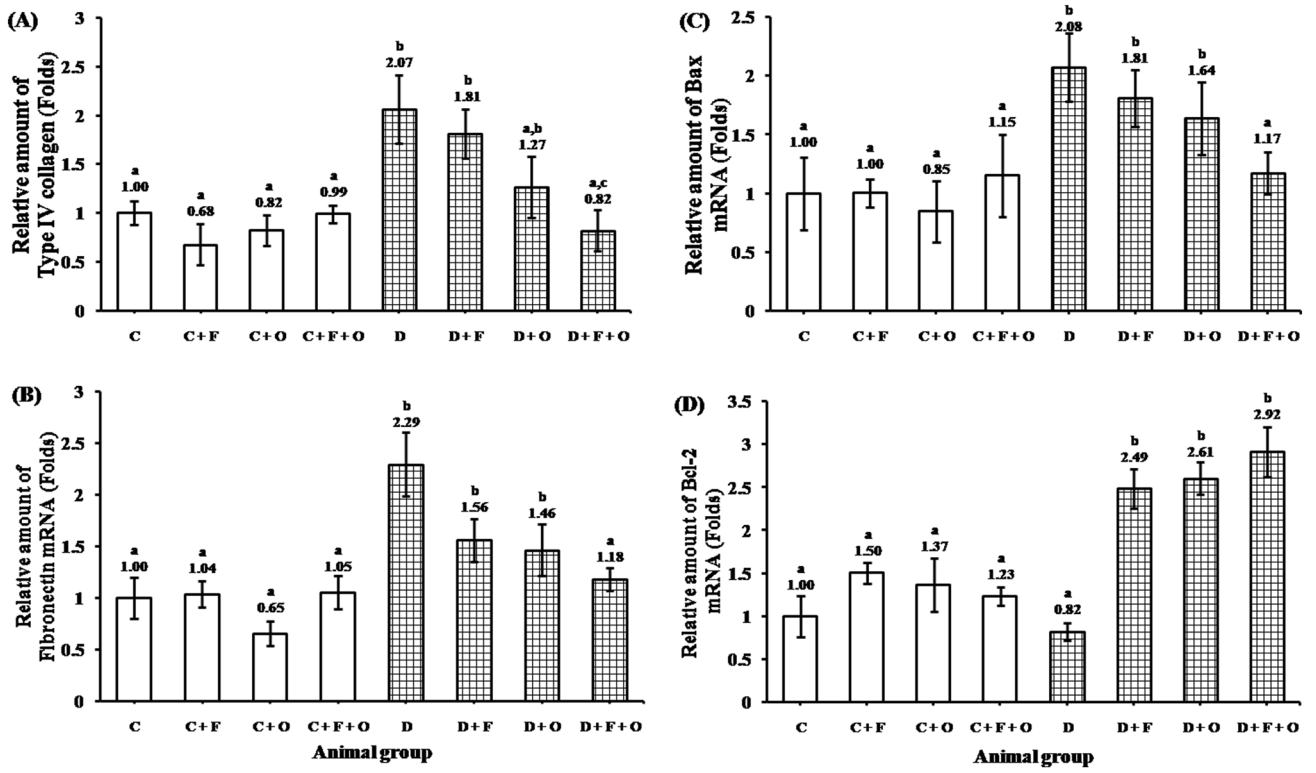

Fig. 4 Influence of dietary fenugreek seeds and onion on cardiac mRNA expression of **a**. Type IV collagen, **b** fibronectin, **c** Bax and **d** Bcl-2 in diabetic rats. Values are mean ± SEM of eight animals in each group. Each bar carrying different letters (a, b, c) is significantly different ($p < 0.05$). C, normal control; D, diabetic control; F, fenugreek; O, onion

The present study has evaluated the mechanism underlying cardio-protection by dietary fenugreek seeds and onion in addition to their hypoglycemic, insulinotropic and hypolipidemic influences. In a recent study, aqueous extract of fenugreek seeds at a dose of 0.5 to 1.0 g/kg body weight reduced heart rate, systolic blood pressure, diastolic blood pressure and mean arterial pressure in obese rats, indicating its potential in the management of dyslipidemia-induced cardiac complications [17]. Two separate studies with onion extract (400 mg/kg/day for 8 weeks) in fructose-fed rats [18] and red onion administration (10-40 mg/kg once daily for 14 consecutive days) to normotensive rats significantly reduced the hypertension [19].

The role of RAS in the development of diabetic cardiomyopathy is well recognized [20]. Their elevated level leads to cardiac hypertrophy, fibrosis and diastolic dysfunction in diabetes. Several studies have found that the levels of ACE were increased up to 24–40% in patients with diabetes [21]. Elevated level of serum ACE activity is reported in diabetic patients with retinopathy [22] and in diabetic animals [23, 24]. This elevation is claimed to have been caused by damage in vasculature as a result of diabetes. Our present study evidenced marked increase in plasma and cardiac ACE activity in diabetic rats. The observed up-regulated expression of cardiac ACE and AT_1 in diabetic rats is in agreement with a recent report [20]. Our study also evidenced for the first time that dietary fenugreek seeds and onion reduce the level of ACE in both cardiac and circulation and down-regulate the expression of ACE and AT_1 in cardiac tissue of diabetic animals. The highlight of our present investigation is that the combination of fenugreek seeds and onion produces a synergistic effect in ameliorating the biomarkers of vascular inflammation.

ACE mediates fibrosis by stimulating the synthesis of extracellular matrix (ECM) components, apoptosis/proliferation, infiltration of inflammatory cells and the release of inflammatory cytokines and growth factors [25]. The increased gene expression of ECM components, such as collagen, fibronectin and of TGF-β1, causes cardiac fibrosis and promotes cardiac stiffness, leading to diastolic dysfunction [26]. In our present study, dietary fenugreek seeds and onion significantly countered the over-expression of type IV collagen and fibronectin in cardiac tissue, indicating the molecular mechanism underlying cardio-protective influence. In support of this, these dietary interventions have previously showed significant modulation of histopathological changes, increased glycogen and collagen fibers as a measure of fibrosis under diabetic condition [9]. The antiapoptotic influence

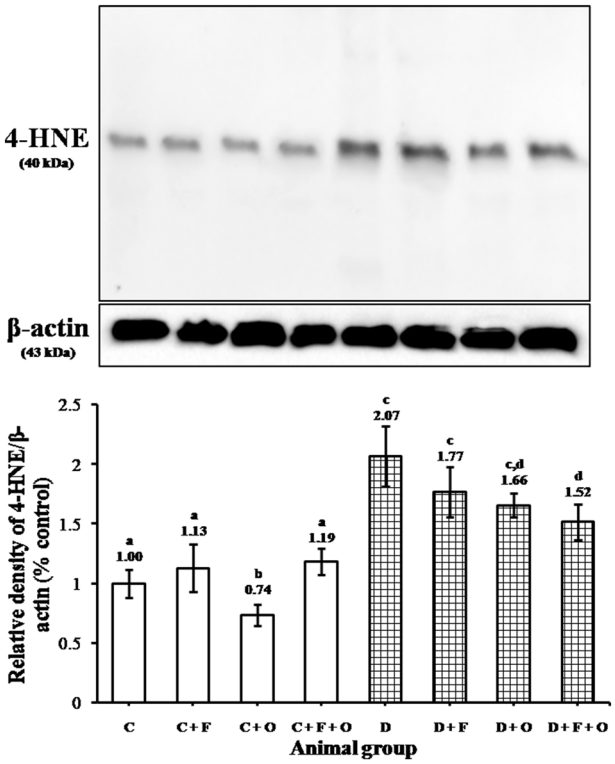

Fig. 5 Influence of dietary fenugreek seeds and onion on cardiac 4-HNE protein expression in diabetic rats. Values are mean ± SEM of eight animals in each group. Each bar carrying different letters (a, b, c, d) is significantly different ($p < 0.05$). C, normal control; D, diabetic control; F, fenugreek; O, onion

was assessed by examining the level of Bax and Bcl-2 mRNA expression. In response to pro-apoptotic stimuli, Bax can translocate from cytosol to mitochondria, resulting in mitochondrial cytochrome-C discharge and stimulation of caspase-9 and caspase-3. Activated caspase-3 will cleave inhibitor of caspase-activated deoxyribonuclease, and the release of caspase-activated deoxyribonuclease from the complex leads to DNA fragmentation [27]. Dietary fenugreek seeds and onion protect against the myocardial apoptosis by partially restoring the mRNA expression of Bax and Bcl-2. Thus, blocking RAS either by inhibiting ACE or blocking AT_1 receptor has clearly demonstrated the positive outcome on this diabetic complication.

Nitric oxide (NO) plays an important role in the protection against the onset and progression of cardiovascular complication. The underlying pathology for most cardiovascular diseases is atherosclerosis, which in turn is associated with endothelial dysfunction. Any disturbance in NO bioavailability leading to a loss of the cardio-protective actions is thought to be the result of endothelial dysfunction. The protective role of NO includes regulating the blood pressure and vascular tone, inhibition of platelet aggregation, leukocyte adhesion and prevention of smooth muscle cell proliferation [28]. NO is normally produced by endothelial nitric oxide synthase (eNOS) under normal physiological condition, but is also produced by inducible nitric oxide synthase (iNOS) in the target tissues under oxidative stress. NO is very reactive; increased expression of iNOS induces the formation of NO in diabetic heart. Meanwhile, an elevated level of ROS, while simultaneously interacting with NO, leads to its decreased bioavailability in heart and also produce peroxynitrite ($ONOO^-$), leading to myocardium injury. Several researchers have shown that although the expression of iNOS was up-regulated, the level of nitric oxide metabolites was decreased or unchanged in the myocardium under diabetic condition [29–31], which conforms to our observation. However, circulating NO level was significantly increased in diabetic rats compared to normal control. Our data are similar to the recent report on patients with type 2 diabetes, which suggested that NO synthesis in erythrocyte was driven by L-arginine catabolism [32]. There is no study so far on the beneficial influence of dietary fenugreek seeds, onion and fenugreek + onion which down-regulated the iNOS expression with restored nitric oxide metabolites in the cardiac tissue. An elevated level of NO metabolites in circulation was significantly countered by all these three dietary interventions with the highest benefit from the combination.

Metabolically, heart in diabetes is characterized by diminished glucose utilization and increased fatty acid oxidation, resulting in lipid accumulation in the myocardium [33]. The hyperlipidemia in diabetes resulting in altered fatty acid composition would affect the cardiac function. The fluidity and functionality of the cardiac membrane are influenced by the length and degree of unsaturation of fatty acyl chains [34]. In the present study, total PUFA of cardiac tissue was decreased with a concomitant increase in SFA and that arachidonic acid (20:4) content in membrane lipids was particularly low in diabetic rats. The decreased PUFA in diabetes is because of a decrease in Δ5 and Δ6 desaturase activities [35] or auto-oxidation of PUFA to the lipid peroxidation product 4-HNE [36]. The present study revealed that dietary fenugreek seeds, onion and fenugreek + onion significantly increased the total PUFA content, particularly arachidonic acid, along with down-regulated expression of 4-HNE protein and SFA in cardiac tissue. This could be the result of the hypolipidemic effect of fenugreek seeds or onion [5, 37], while their combination achieved a higher beneficial effect. The hypolipidemic property of fenugreek seeds is attributable to the soluble fiber galactomannan, flavonoids and saponins present in them [4]. *S*-Methyl cysteine sulfoxide and *S*-allyl cysteine sulfoxide are the principal sulfur compounds of onion responsible for the hypolipidemic effect besides flavonoids [5].

To evaluate the extent of cardiac damage by STZ-induced diabetes, we measured AlAT, AsAT, LDH, phosphatases, CK-MB and specifically Troponin-T. When myocardium

Table 2 Effect of dietary fenugreek seeds and onion on cardiac fatty acid composition (%)

Fatty acids	C	C + F	C + O	C + F + O	D	D + F	D + O	D + F + O
14:0	0.50 ± 0.06^{a}	0.56 ± 0.02^{a}	0.61 ± 0.02^{b}	0.63 ± 0.05^{b}	0.42 ± 0.07^{c}	0.48 ± 0.05^{c}	0.49 ± 0.06^{c}	0.55 ± 0.04^{a}
14:1	0.71 ± 0.05^{a}	0.71 ± 0.04^{a}	0.74 ± 0.07^{a}	0.77 ± 0.01^{a}	0.62 ± 0.02^{b}	0.65 ± 0.05^{b}	0.66 ± 0.02^{b}	0.70 ± 0.03^{a}
16:0	9.65 ± 0.34^{a}	10.44 ± 0.21^{a}	9.52 ± 0.32^{a}	8.06 ± 0.20^{b}	14.05 ± 0.57^{c}	9.98 ± 0.34^{a}	9.12 ± 0.12^{a}	9.99 ± 0.43^{a}
16:1	0.66 ± 0.03^{a}	0.68 ± 0.01^{a}	0.69 ± 0.08^{a}	0.77 ± 0.06^{a}	1.71 ± 0.19^{b}	0.97 ± 0.13^{a}	0.91 ± 0.07^{a}	0.58 ± 0.02^{a}
18:0	19.54 ± 0.91^{a}	18.56 ± 0.78^{a}	19.73 ± 1.54^{a}	18.16 ± 0.87^{a}	14.91 ± 1.78^{b}	17.65 ± 1.63^{a}	18.18 ± 1.12^{a}	18.24 ± 1.22^{a}
18:1	6.18 ± 0.78^{a}	4.43 ± 0.90^{b}	8.86 ± 1.13^{c}	6.59 ± 1.23^{a}	9.24 ± 1.61^{c}	6.36 ± 0.88^{a}	7.98 ± 0.67^{c}	5.06 ± 0.85^{b}
18:2	25.78 ± 2.35^{a}	24.68 ± 2.51^{a}	21.40 ± 1.90^{b}	24.63 ± 3.63^{a}	28.85 ± 2.78^{c}	$26.96 \pm 2.79^{a,c}$	$26.52 \pm 3.51^{a,c}$	$25.15 \pm 1.31^{a,c}$
18:3	2.96 ± 0.12^{a}	2.75 ± 0.23^{a}	2.78 ± 0.10^{a}	2.64 ± 0.11^{a}	1.04 ± 0.09^{b}	1.76 ± 0.04^{c}	1.99 ± 0.06^{d}	2.98 ± 0.10^{a}
20:0	0.51 ± 0.01^{a}	0.62 ± 0.01^{a}	0.61 ± 0.02^{a}	0.69 ± 0.01^{a}	3.89 ± 0.09^{b}	1.17 ± 0.13^{c}	0.79 ± 0.10^{a}	0.57 ± 0.04^{a}
20:4	24.00 ± 2.10^{a}	26.11 ± 1.58^{b}	24.10 ± 1.53^{a}	26.31 ± 1.49^{b}	15.77 ± 2.64^{c}	22.39 ± 1.89^{d}	21.74 ± 1.53^{d}	24.50 ± 1.75^{a}
20:5	1.00 ± 0.03^{a}	0.37 ± 0.01^{b}	2.07 ± 0.89^{a}	0.57 ± 0.02^{b}	0.50 ± 0.04^{b}	0.84 ± 0.03^{c}	0.66 ± 0.01^{c}	1.10 ± 0.06^{a}
22:0	0.50 ± 0.02^{a}	0.91 ± 0.05^{b}	0.90 ± 0.02^{b}	1.00 ± 0.06^{b}	0.34 ± 0.01^{a}	0.98 ± 0.02^{b}	0.89 ± 0.03^{b}	1.31 ± 0.07^{c}
22:1	3.98 ± 0.23^{a}	2.47 ± 0.13^{b}	2.95 ± 0.42^{c}	4.32 ± 0.35^{a}	7.58 ± 0.78^{d}	5.09 ± 0.90^{e}	5.94 ± 0.64^{f}	2.71 ± 0.28^{b}
22:5	0.79 ± 0.06^{a}	1.99 ± 0.21^{b}	1.24 ± 0.20^{c}	0.57 ± 0.19^{a}	0.62 ± 0.09^{a}	0.82 ± 0.06^{a}	0.57 ± 0.10^{a}	1.29 ± 0.05^{c}
22:6	1.49 ± 0.28^{a}	2.74 ± 0.35^{b}	2.09 ± 0.65^{a}	2.99 ± 0.15^{b}	0.74 ± 0.10^{c}	1.39 ± 0.42^{a}	1.61 ± 0.12^{a}	2.23 ± 0.43^{a}
24:1	2.03 ± 0.61^{a}	2.14 ± 0.55^{a}	2.00 ± 0.41^{a}	2.22 ± 0.11^{a}	1.23 ± 0.08^{b}	2.98 ± 0.19^{c}	2.90 ± 0.23^{c}	3.51 ± 0.30^{d}
PUFA	60.71	60.33	60.45	61.31	55.02	59.13	59.46	60.08
SFA	39.57	39.83	39.84	39.61	45.49	41.34	41.49	40.39
PUFA/SFA	1.53	1.51	1.52	1.55	1.21	1.43	1.43	1.49

Values are mean ± SEM of eight animals in each group. Means within the same row (in each parameter) carrying different superscripts (a, b, c, d, e, f) are significantly different ($p < 0.05$)

C, normal control; D, diabetic control; F, fenugreek; O, onion

Table 3 Effect of dietary fenugreek seeds and onion on circulatory marker enzymes in diabetic rats

Animal group	AsAT	AlAT	Alkaline phosphatase	Acid phosphatase	LDH	CK-MB
C	89.94 ± 1.59^{a}	52.94 ± 1.84^{a}	13.10 ± 2.21^{a}	12.08 ± 0.26^{a}	121.86 ± 3.62^{a}	244.31 ± 10.29^{a}
C + F	81.00 ± 0.35^{a}	54.93 ± 2.19^{a}	10.94 ± 0.07^{a}	12.54 ± 0.52^{a}	83.68 ± 1.74^{b}	217.57 ± 0.85^{b}
C + O	84.65 ± 1.88^{a}	57.13 ± 1.00^{a}	10.36 ± 0.24^{a}	11.10 ± 0.67^{a}	116.72 ± 5.32^{a}	197.02 ± 1.19^{b}
C + F + O	78.39 ± 1.76^{a}	59.93 ± 2.35^{a}	9.86 ± 0.02^{a}	10.29 ± 0.81^{a}	110.94 ± 3.35^{a}	226.15 ± 9.43^{a}
D	98.02 ± 5.48^{b}	85.55 ± 7.20^{b}	69.33 ± 8.47^{b}	6.44 ± 0.06^{b}	164.17 ± 5.32^{c}	320.99 ± 8.53^{c}
D + F	83.48 ± 2.11^{a}	73.59 ± 6.54^{c}	38.61 ± 3.31^{c}	8.86 ± 0.55^{c}	120.45 ± 10.74^{a}	268.25 ± 9.62^{d}
D + O	81.16 ± 0.93^{a}	70.17 ± 4.83^{c}	37.79 ± 1.63^{c}	9.32 ± 0.66^{a}	134.13 ± 12.26^{d}	312.49 ± 5.90^{c}
D + F + O	66.68 ± 1.12^{c}	57.20 ± 3.37^{a}	34.86 ± 5.74^{c}	9.69 ± 0.38^{a}	74.64 ± 6.74^{e}	210.87 ± 30.58^{a}

Values are mean ± SEM of eight animals in each group. Specific activity units: (IU/L). Means within the same column (in each parameter) carrying different superscripts (a, b, c, d, e) are significantly different ($p < 0.05$)

AsAT, aspartate amino transferase; AlAT, alanine aminotransferase; LDH, lactate dehydrogenase; CK-MB, creatine phosphokinase-MB; C, normal control; D, diabetic control; F, fenugreek; O, onion

is metabolically damaged, these markers are released into extracellular fluid. A significant rise in the activity of these marker enzymes in plasma accomplished by their concomitant depletion in cardiac tissue confirmed the incidence of myocardium damage. Fenugreek seeds and onion were found to ameliorate the effect of STZ-induced pathological changes in cardiac tissue, indicating their efficiency as cardio-protectants which can protect cardiomyocyte membrane integrity. This must have resulted from significantly reduced concentration of ROS, lipid peroxides and is supported by histopathological examination of myocardium in a recent report [9]. Diabetic rats treated with dietary fenugreek seeds, onion or fenugreek + onion significantly reduced the level of cardiac damage markers in circulation. The latter intervention sometimes produced an additive effect, particularly in the case of AlAT and AsAT or even synergistic effect especially in the case of LDH and CK-MB. The elevated level of the potent biomarker for myocardial necrosis and

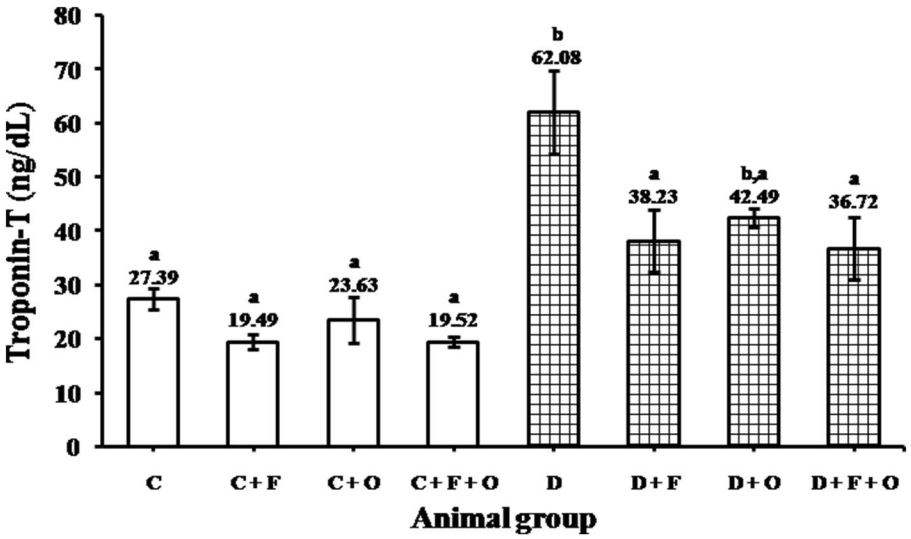

Fig. 6 Influence of dietary fenugreek seeds and onion on plasma Troponin-T level in diabetic rats. Values are mean ± SEM of eight animals in each group. Each bar carrying different letters (a, b) is significantly different ($p < 0.05$). C, normal control; D, diabetic control; F, fenugreek; O, onion

Table 4 Effect of dietary fenugreek seeds and onion on cardiac marker enzymes in diabetic rats

Animal group	AsAT[1]	AlAT[1]	Alkaline phosphatase[2]	Acid phosphatase[2]	LDH[2]	CK-MB[2]
C	0.405 ± 0.018^{a}	1.40 ± 0.049^{a}	0.145 ± 0.017^{a}	0.131 ± 0.014^{a}	1.228 ± 0.028^{a}	3.21 ± 0.20^{a}
C + F	0.427 ± 0.017^{a}	1.38 ± 0.065^{a}	0.185 ± 0.037^{a}	0.132 ± 0.003^{a}	1.170 ± 0.012^{a}	3.14 ± 0.32^{a}
C + O	0.424 ± 0.019^{a}	1.42 ± 0.086^{a}	0.161 ± 0.002^{a}	0.131 ± 0.001^{a}	1.179 ± 0.014^{a}	3.08 ± 0.06^{a}
C + F + O	0.410 ± 0.001^{a}	1.38 ± 0.057^{a}	0.179 ± 0.018^{a}	0.136 ± 0.001^{a}	1.264 ± 0.013^{a}	3.82 ± 0.19^{b}
D	0.387 ± 0.003^{a}	1.14 ± 0.015^{b}	0.121 ± 0.006^{b}	0.189 ± 0.003^{b}	1.110 ± 0.008^{b}	2.31 ± 0.36^{c}
D + F	0.390 ± 0.005^{a}	1.24 ± 0.005^{b}	0.154 ± 0.050^{a}	0.166 ± 0.008^{b}	1.301 ± 0.029^{a}	2.54 ± 0.78^{c}
D + O	0.426 ± 0.002^{a}	1.37 ± 0.032^{a}	0.159 ± 0.018^{a}	0.165 ± 0.009^{b}	1.316 ± 0.016^{a}	2.52 ± 0.37^{c}
D + F + O	0.427 ± 0.006^{a}	1.33 ± 0.006^{c}	0.152 ± 0.014^{a}	0.153 ± 0.004^{c}	1.218 ± 0.015^{a}	2.50 ± 0.29^{c}

Values are mean ± SEM of eight animals in each group. Specific activity units: 1-µg pyruvate released/min/mg protein; 2-µmol/min/mg protein

Means within the same column (in each parameter) carrying different superscripts (a, b, c) are significantly different ($p < 0.05$)

AsAT, aspartate amino transferase; AlAT, alanine aminotransferase; LDH, lactate dehydrogenase; CK-MB, creatine phosphokinase-MB; C, normal control; D, diabetic control; F, fenugreek; O, onion

Troponin-T in diabetes was significantly countered by all the three dietary interventions indicating importance of these spices against myocardial injury in diabetic condition.

Conclusion

For the first time, this study has demonstrated that fiber-rich fenugreek seeds and sulfur compound-rich onion elicit the cardio-protective influence in experimental diabetes. In this study, we observed that dietary intervention with fenugreek seeds and onion significantly blocked renin–angiotensin system (RAS), which is accomplished with down-regulated ECM, apoptotic, iNOS expressions in the cardiac tissue and nitric oxide metabolites in circulation. The administration of these spice compounds reduced the level of oxidative stress by inhibiting lipid peroxidation (reduced 4-HNE formation) possibly through the increased antioxidant status. Marker enzymes of cardiac damage were significantly restored by these dietary interventions. The results of present investigation suggested that a higher beneficial influence was achieved by the combination of these two spices, sometimes amounting to an additive or even synergistic effect in attenuating oxidative stress in the cardiac tissue of diabetic rats.

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Compliance with Ethical Standards

Conflicts of interest The authors have no conflict of interest.

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