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L-Arginine ameliorates cardiac left ventricular oxidative stress by upregulating eNOS and Nrf2 target genes in alloxan-induced hyperglycemic rats

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ABSTRACT

Hyperglycemia is independently related with excessive morbidity and mortality in cardiovascular disorders. L-Arginine-nitric oxide (NO) pathway and the involvement of NO in modulating nuclear factor-E2-related factor-2 (Nrf2) signaling were well established. In the present study we investigated, whether L-arginine supplementation would improve the myocardial antioxidant defense under hyperglycemia through activation of Nrf2 signaling. Diabetes was induced by alloxan monohydrate (90 mg kg^{-1} body weight) in rats. Both non-diabetic and diabetic group of rats were divided into three subgroups and they were administered either with L-arginine (2.25%) or L-NAME (0.01%) in drinking water for 12 days. Results showed that L-arginine treatment reduced the metabolic disturbances in diabetic rats. Antioxidant enzymes and glutathione levels were found to be increased in heart left ventricles, thereby reduction of lipid peroxidation by L-arginine treatment. Heart histopathological analysis further validates the reversal of typical diabetic characteristics consisting of alterations in myofibers and myofibrillary degeneration. qRT-PCR studies revealed that L-arginine treatment upregulated the transcription of Akt and downregulated NF-κB. Notably, transcription of eNOS and Nrf2 target genes was also upregulated, which were accompanied by enhanced expression of Nrf2 in left ventricular tissue from diabetic and control rats. Under these findings, we suggest that targeting of eNOS and Nrf2 signaling by L-arginine supplementation could be used as a potential treatment method to alleviate the late diabetic complications.

1. Introduction

The prevalence, incidence and mortality of all cardiovascular disorders (CVD) are two- to eightfold higher in persons with diabetes than in those without diabetes [1]. In reality, understanding the molecular relationship between diabetes and the development of heart disease is much more complex. Hyperglycemia induced reactive oxygen species (ROS) has been implicated as a potential molecular mechanism behind the onset and progression of diabetic cardiomyopathy and diabetic vascular complications, which are often associated with endothelial dysfunction [2]. Substantial evidence suggests the participation of ROS in diabetic cardiomyopathy which includes cardiac lipid peroxidation and functional or morphological damages to diabetic heart and this could be reduced by oral or systemic antioxidant administration [3].

The free radical nitric oxide ($NO^{\cdot}$) is one of the most widespread signaling molecules that participates in virtually every cellular function of the body. Nitric oxide (NO) is synthesized from the oxidation of L-arginine, by a family of nitric oxide synthase (NOS) enzymes. Endothelial NOS (eNOS) is the main contributor for circulating NO and this vascular endothelial nitric oxide is directly involved in regulation of endothelial function. Accumulating reports showed the importance of NO in endothelial dysfunction with a spectrum of related pathologies, which include hypertension [4], CVD [5] and atherogenesis [6]. Moreover, L-arginine is reported to have beneficial effects on several complications including pulmonary hypertension [7], type-1 diabetes [8], β cell neogenesis [9], insulin sensitivity [10], improvement of endothelial function [11] and reduction of fat mass in diabetic rats [12].

Nitric oxide involves in the activation of a transcription factor, nuclear factor-erythroid 2 (NF-E2)-related factor 2 also known as Nrf2. In turn, Nrf2 activates the expression of many antioxidant genes which include catalase, superoxide dismutase, UDP-glucuronosyltransferase, γ-glutamylcysteine synthetase, NAD(P)H quinone oxidoreductase 1 (NQO1), glutathione-S-transferase (GST), glutathione peroxidase (GSH-Px) and heme oxygenase-1 (HO-1) [13]. These Nrf2 regulated genes are characterized by the presence of a *cis*-acting element called antioxidant-responsive element (*ARE*), which lies within the regulatory region. The transcriptional up-regulation of the antioxidant genes through Nrf2/*ARE* signaling by NO is currently well established [14]. From this information it is

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apparent that increasing NO bioavailability and antioxidant status in myocardial tissue can be considered as a potential strategy to prevent the onset of hyperglycemia induced vascular dysfunction and CVD and is therefore of therapeutical interest.

Based on the well known interactions between eNOS and Nrf2 and intriguing links between L-arginine in the treatment of diabetes and vascular disease, we hypothesized that targeting eNOS, Nrf2 and their associated signaling would provide a more effective strategy to restore antioxidant defense in vascular disease. Thus, the present study was designed to explore the potentiality of L-arginine-NO pathway in the regulation of Nrf2 and its target genes against the hyperglycemia induced oxidant stress in alloxan induced type-1 diabetic myocardium.

2. Materials and methods

2.1. Animals and induction of diabetes

Albino rats of Wistar derived strain weighing about 280–320 g were used for this study. All the experiments were approved by Internal Research and Review Board, Ethical Clearance, Biosafety and Animal Welfare Committee of Madurai Kamaraj University. The level of care provided to the animals met the basic requirements outlined in the NIH guidelines. After an overnight fasting the rats were given a single i.p. injection of freshly prepared alloxan monohydrate (Sigma–Aldrich Inc., USA) dissolved in ice-cold saline, at a dose of 90 mg alloxan kg^{-1} body weight followed by 4 h post fasting period. Development of diabetes (blood glucose > 250 mg dl^{-1}) was verified on the third day of the experiment. Symptoms of diabetes were ascertained within a week of alloxan injection in terms of loss of body weight, polyphagia, polydipsia, polyuria and glycosuria.

2.2. Treatment protocol

Both non-diabetic and diabetic groups of rats were divided into three subgroups and they were administered either with L-arginine (2.25%) or L-NAME (0.01%) in drinking water for 12 consecutive days, after 7 days of alloxan injection. The control groups received only water. Each experimental group consisted of six animals. After the experimental period, the rats were fasted overnight and sacrificed. Fasting blood was collected and the heart was dissected out and the samples were stored at −70 °C.

2.3. Determination of blood plasma parameters

Plasma insulin level was estimated using ELISA kit (Monobind Inc. USA) according to the manufacturer's instructions. The level of plasma total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and triglycerides (TG) were determined using commercial kits (Agappe Diagnostics, India).

2.4. Fasting blood glucose (FBG) & oral glucose tolerance test (OGTT)

Blood samples were collected by tail vein puncture from overnight fasted animals and the blood glucose was estimated. The OGTT was performed by administering 50% of glucose solution to overnight fasted animals (1.5 g kg^{-1} body wt). Tail-vein blood samples were collected at 0, 30, 60 and 120 min after glucose administration and the glucose level was determined with a blood glucose monitoring system (Glucocard 01, Arkay Piramal Medical Pvt. Ltd, India).

2.5. Antioxidant marker assays and protein estimation

Heart left ventricular sections were minced and homogenized (10% w/v), separately, in ice-cold sodium, potassium phosphate buffer (pH 7.4) containing 1.15% KCl and the resultant supernatant was used for further assays. Protein content was estimated using the Bradford reagent (Sigma–Aldrich, USA). GST activity was measured by the protocol of Habig et al. [15] and GSH-Px activity was measured according to the method of Flohe and Gunzler [16]. Catalase activity was determined by the method developed by Aebi [17]. The lipid peroxidation was determined by estimating malondialdehyde (MDA) [18] and the amount of GSH was estimated by the method of Moron et al. [19].

2.6. Histopathological analysis

Specimens of heart tissues were formalin fixed, paraffin embedded and sectioned at 5 μM. Sections were stained with hematoxylin and eosin for evaluation.

2.7. RNA extraction and quantitative real-time PCR (qRT-PCR) analysis

Left ventricular RNA was extracted with the single step TRI Reagent (Sigma–Aldrich Inc. USA) and cDNA synthesis was performed with MMLV-reverse transcriptase. qRT-PCR was performed with SYBR Green PCR master mix (MBI-Fermentas, Lithuania). The oligos used in this study are listed in Supplementary Table 1. Real time cycling parameters: Initial activation step (95 °C—5 min), cycling step (denaturation 95 °C—20 s, annealing at 58 °C—20 s, and finally extension for 68 °C—20 s × 40 cycles), followed by a melting curve analysis to confirm specificity of the PCR. The C_t value was corrected by C_t reading of corresponding GAPDH controls. Data from three determinations (means ± SEM) are expressed as relative expression level. The reaction was performed in Mastercycler ep realplex^2 System (Eppendorf AG, Germany). The specificity of the PCR reaction was confirmed by agarose gel electrophoresis.

2.8. Statistics

All the grouped data were statistically evaluated with SPSS Statistics (Version 19) software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. For all comparisons, differences were considered significant at a value of $P < 0.05$. All the results were expressed as mean ± SEM for six animals in each group.

3. Results

3.1. Effects of L-arginine and L-NAME treatment on general and metabolic parameters in diabetic and control rats

Compared to diabetic control, L-arginine and L-NAME treated controls showed significantly ($P < 0.005$ and $P < 0.005$ respectively) high level of total cholesterol. L-NAME treated diabetic group also showed significant increase ($P < 0.025$) in total cholesterol when compared to diabetic control. In comparison with non-diabetic control, triglycerides showed significant increase in L-NAME treated diabetic ($P < 0.025$) and control groups ($P < 0.025$). L-Arginine treatment has markedly increased the plasma insulin level in diabetic group ($P < 0.005$) compared to the diabetic control and this is consistent with the fasting blood glucose (FBG) level. L-NAME treatment has negatively affected the insulin level in diabetic animals compared to non-diabetic control ($P < 0.005$) and diabetic control ($P < 0.005$) groups. Significantly low level of FBG was obtained ($P < 0.005$) by L-arginine treatment in diabetic group. In

Table 1
Effect of L-arginine and L-NAME on general and blood plasma parameters.

	Non-diabetic control	Diabetic control	Con + L-Arg	Dia + L-Arg	Con + L-NAME	Dia + L-NAME
Food intake (g day^{-1})	34.25 ± 2.17	37.33 ± 2.64	38.08 ± 5.01	40.25 ± 2.96**	31.68±2.58$^{\#\#}$	36.50 ± 1.90
Water intake (ml day^{-1})	27 ± 6.77$^{\#\#\#}$	89 ± 8.38***	25 ± 6.98$^{\#\#\#}$	59 ± 8.65$^{***,\#\#\#}$	25 ± 5.90$^{\#\#\#}$	45 ± 8.71$^{***,\#\#\#}$
Weight Loss/Gain	+38.12 ± 8.88$^{\#\#\#}$	−32.33 ± 4.32$^{***,\#\#\#}$	+39.27 ± 4.57$^{\#\#\#}$	−17.88 ± 7.11$^{***,\#\#\#}$	+27.49 ± 6.45$^{**,\#\#\#}$	−16.54 ± 9.22$^{***,\#\#\#}$
Total Cholesterol (mg dl^{-1})	103.60 ± 8.50	98.60 ± 2.49	114.60 ± 10.28$^{*,\#\#\#}$	96.30 ± 4.04	117.30 ± 12.03$^{**,\#\#\#}$	112.60 ± 7.37$^{\#\#}$
Triglycerides (mg dl^{-1})	121 ± 8.10$^{**,\#\#}$	146 ± 13.20	134 ± 14.40	122 ± 15.17$^{\#\#}$	144 ± 17.00**	145 ± 11.59**
Low density lipoprotein (mg dl^{-1})	52 ± 3.05	63 ± 6.50**	85 ± 7.50$^{***,\#\#\#}$	67 ± 3.50***	72 ± 7.80$^{***,\#}$	66 ± 6.90***
High density lipoprotein (mg dl^{-1})	44 ± 20	42 ± 2.25	44 ± 1.73	45 ± 1.15$^{\#\#}$	41 ± 1.52**	46 ± 2.10$^{\#\#\#}$
Insulin (IμU ml^{-1})	8.61 ± 0.60$^{\#\#\#}$	4.25 ± 0.50***	4.15 ± 1.60***	6.19 ± 0.15$^{***,\#\#\#}$	5.2 ± 0.90***	1.89 ± 0.3$^{***,\#\#\#}$
Glucose(mg dl^{-1})	81.5 ± 9.60$^{\#\#\#}$	290 ± 15.50***	74 ± 8.40$^{\#\#\#}$	115.5 ± 9.70$^{***,\#\#\#}$	50.5 ± 6.80$^{***,\#\#\#}$	76.5 ± 7.90$^{\#\#\#}$

Values are given as mean ± SEM for groups of six animals each. Comparison with Non-diabetes control: $^{*}P < 0.05$; $^{**}P < 0.025$; $^{***}P < 0.005$. Comparison with diabetic control: $^{\#}P < 0.05$; $^{\#\#}P < 0.025$; $^{\#\#\#}P < 0.005$. Values are statistically significant at $P < 0.05$.

Table 2
Level of GST, GSH-Px, catalase, GSH and TBARS in left ventricular heart tissue of control and experimental rats.

Groups	Non-diabetic control	Diabetic control	Con + L-Arg	Dia + L-Arg	Con + L-NAME	Dia + L-NAME
GST	18.49 ± 1.74$^{\#\#\#}$	9.85 ± 1.02***	17.49 ± 2.01$^{\#\#\#}$	18.08 ± 1.78$^{\#\#\#}$	16.25 ± 1.65$^{*,\#\#\#}$	13.01 ± 1.18$^{***,\#\#\#}$
GSH-Px	35.4 ± 1.80$^{\#\#\#}$	26.2 ± 0.60***	41.0 ± 1.70$^{***,\#\#\#}$	38.1 ± 1.40$^{***,\#\#\#}$	29.2 ± 0.60$^{***,\#\#}$	28.2 ± 0.60***
Catalase	17.5 ± 0.90$^{\#\#\#}$	6.25 ± 0.45***	16.1 ± 0.50$^{**,\#\#\#}$	15.2 ± 1.04$^{***,\#\#\#}$	14.1 ± 1.20$^{***,\#\#\#}$	10.7 ± 0.98$^{***,\#\#\#}$
Glutathione	4.16 ± 0.72$^{\#\#\#}$	2.28 ± 0.6***	3.72 ± 0.31$^{\#\#\#}$	3.28 ± 0.74**	3.78 ± 0.54$^{\#\#\#}$	1.99 ± 0.23***
TBARS	0.745 ± 0.09$^{\#\#\#}$	3.05 ± 0.11***	1.59 ± 0.12$^{***,\#\#\#}$	2.05 ± 0.13$^{***,\#\#\#}$	1.62 ± 0.10$^{***,\#\#\#}$	3.5 ± 0.12$^{***,\#\#\#}$

Values are given as mean ± SEM for groups of six animals each. Comparison with Non-diabetes control: $^{*}P < 0.05$; $^{**}P < 0.025$; $^{***}P < 0.005$. Comparison with diabetic control: $^{\#}P < 0.05$; $^{\#\#}P < 0.025$; $^{\#\#\#}P < 0.005$. Values are statistically significant at $P < 0.05$. GST – nmol of CDNB conjugated min^{-1} mg protein^{-1}, Catalase-nmol of H_2O_2 consumed min^{-1} mg protein^{-1}, GSH-Px-oxidized NADPH nmol min^{-1} mg protein^{-1}, GSH-μg/g of tissue. TBARS – MDA/mg of protein.

contrast, the L-NAME treatment also reduced the blood glucose level ($P < 0.005$) than L-arginine treated group, compared to diabetic control (Table 1).

3.2. L-arginine supplementation improves left ventricular antioxidant defence and reduces lipid peroxidation

As evidenced by the results, alloxan intoxication significantly attenuated the myocardial antioxidant enzyme activities compared to the corresponding control (Table 2). In L-arginine treated diabetic rats, the activities of tissue antioxidant enzymes GST, GSH-Px, and catalase demonstrated a significant increase ($P < 0.005$), compared to the diabetic control, which reflects restoration of the antioxidant enzyme systems to near-normal values. Furthermore, the L-NAME treatment also significantly increased the GST ($P < 0.005$) and catalase ($P < 0.005$) activity in diabetic rats. The results of our study showed that diabetic condition has reduced the level of GSH and L-arginine administration, significantly improved ($P < 0.005$) the amount present in the heart ventricle homogenate, compared to the diabetic control. TBA-reactivity was recorded as high in diabetic control ($P < 0.005$) compared to the corresponding control group. Similar results were obtained in L-NAME treated groups which is comparable to the result of diabetic control and showed significant increase ($P < 0.005$) of TBA-reactivity compared with the non-diabetic control. Treatment with L-arginine could not totally abolish the hyperglycemia induced peroxidation and showed a modest TBA-reactivity in corresponding control also (Table 2).

3.3. Improvement of oral glucose tolerance by L-arginine and L-NAME treatment in diabetic animals

In diabetic control group, the blood glucose levels at 0, 30, 60 and 120 min were much higher and remained constant. The glucose level of diabetic rats treated with L-arginine showed a gradual increase (582.5 ± 35.7 mg dl^{-1}) at 1 h and rapid decrease towards the FBG after 2 h. The diabetic rats treated with L-NAME also showed a gradual increase of blood glucose level at 30 min (265 ± 17.5 mg dl^{-1}) however, it slowly reached the FBG level (113 ± 6.8 mg dl^{-1}) at 2 h. The OGTT curve was not significantly changed compared to non-diabetic control group, which suggested that L-arginine or L-NAME treatment did not influence glucose tolerance in normal rats (Supplementary Fig. 1).

3.4. Effect of treatment on morphological changes in left ventricular cardiomyocytes

Histological assessments of left ventricular cardiac segments of control sample (Fig. 1A) showed unremarkable changes. The diabetic control myocardium was identified by disorganized myocardial fibers with degeneration and necrosis of myocytes (Fig. 1B). Both control and diabetic rats treated with L-arginine (Fig. 1C and D) showed mild edema and focal areas of fiber degeneration. L-NAME treatment formed mild edema in the heart muscle fibers (Fig. 1E) and diabetic rats treated with L-NAME (Fig. 1F) showed muscle fiber with edema, congested blood vessels and focal mononuclear inflammatory cells.

3.5. Impact of L-arginine and L-NAME treatment on relative gene expression of Akt, Caspase 3 (Casp3) and NF-κB in left ventricular diabetic myocardium

In order to assess the effect of endogenously produced NO, we evaluated the expression of *Akt*, *Casp3* and *NF-κB* genes by qRT-PCR (Fig. 2). Significant activation of *Akt* mRNA transcription was observed in L-arginine treated diabetic ($P < 0.005$) group relative to non-diabetic control, which indicates the activation of the survival pathway by L-arginine treatment (Fig. 2A). In contrast, both

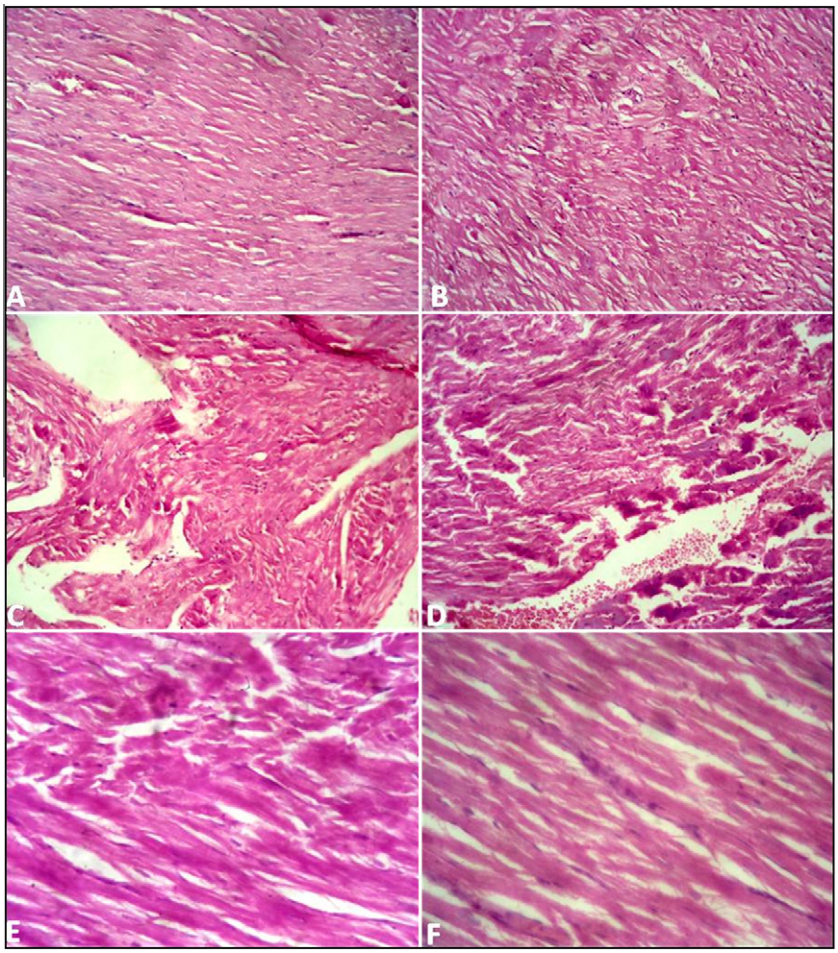

Fig. 1. Histopathology. Representative photomicrographs of left ventricular sections obtained from myocardial tissues of experimental and control rats. (A) Non-diabetic control; (B) diabetic control; (C) Con + L-Arg; (D) Dia + L-Arg; (E) Con + L-NAME; (F) Dia + L-NAME (400×).

L-arginine and L-NAME treated diabetic groups significantly upregulated the expression ($P < 0.005$) of *Casp3* mRNA relative to non-diabetic control (Fig. 2B). Consistently the expression of *NF-κB* also showed significant elevation in diabetic control ($P < 0.005$) and L-NAME treated diabetic and non-diabetic groups ($P < 0.005$), relative to non-diabetic control (Fig. 2C).

3.6. L-arginine accelerates relative gene expression of eNOS, Nrf2 and its target genes in left ventricular myocardial tissue

The Nrf2 regulated genes glutamate cysteine ligase (*GCL*), *HO-1* and *NQO-1* were chosen for the gene expression analysis to understand the influence of L-arginine treatment on the antioxidant defense system. The expression of *Nrf2* was significantly low in diabetic control ($P < 0.005$) and both L-arginine treated control and diabetic groups showed 4.5 and 5-fold increased expression ($P < 0.005$ and $P < 0.005$ respectively), relative to the non-diabetic control (Fig 2E). L-NAME treatment did not affect the expression of *Nrf2* mRNA in diabetic rats. The expression of *GCL* ($P < 0.005$), *HO-1* ($P < 0,005$) and *NQO-1* ($P < 0.005$) were found to be high, which indicates that the L-arginine treatment enhances the expression of *Nrf2* regulated enzymes (Fig. 2F–H). L-arginine treatment could also improve the left ventricular *eNOS* expression up to 3.5-fold in control ($P < 0.005$) as well as diabetic animals ($P < 0.005$), which are significant. L-NAME treatment resulted in maintenance of basal level expression of *eNOS*, which can be compared to untreated control (Fig. 2D).

4. Discussion

In the present study we found that L-arginine supplementation decreased the weight loss in diabetic rats. The total cholesterol and triglycerides levels were decreased by L-arginine treatment and increased by L-NAME treatment. The earlier reports suggest that L-arginine treatment could alter the lipid metabolism [20] and the beneficial effects of L-arginine–nitric oxide pathway, in part lie on hypocholesterolemic effect [21].

The FBG level showed significant decrease in both L-arginine and L-NAME treated diabetic groups. In OGTT the diabetic animals treated with L-arginine showed a gradual increase and a rapid decrease in blood glucose level. However, after glucose load, the blood glucose level of diabetic animals treated with L-NAME slowly reached the level of FBG. Our finding thus, confirms that administration of L-NAME impairs insulin secretion and resulted in reduced glucose tolerance in rats and humans and it attenuates the insulin-mediated increase in glucose uptake by muscles, including heart [22]. It should be noted that in diabetic animals, the level of insulin was restored to near normal level by L-arginine treatment but the L-NAME treatment decreased the plasma insulin level. The previous results showed a marked fall in plasma NO and insulin level, but a simultaneous elevation in glucose, lactate, ketone bodies and lipid peroxide levels in alloxan induced diabetic animals [8].

Based on the results of the current study, L-arginine significantly improved the level of antioxidant marker enzymes found in left

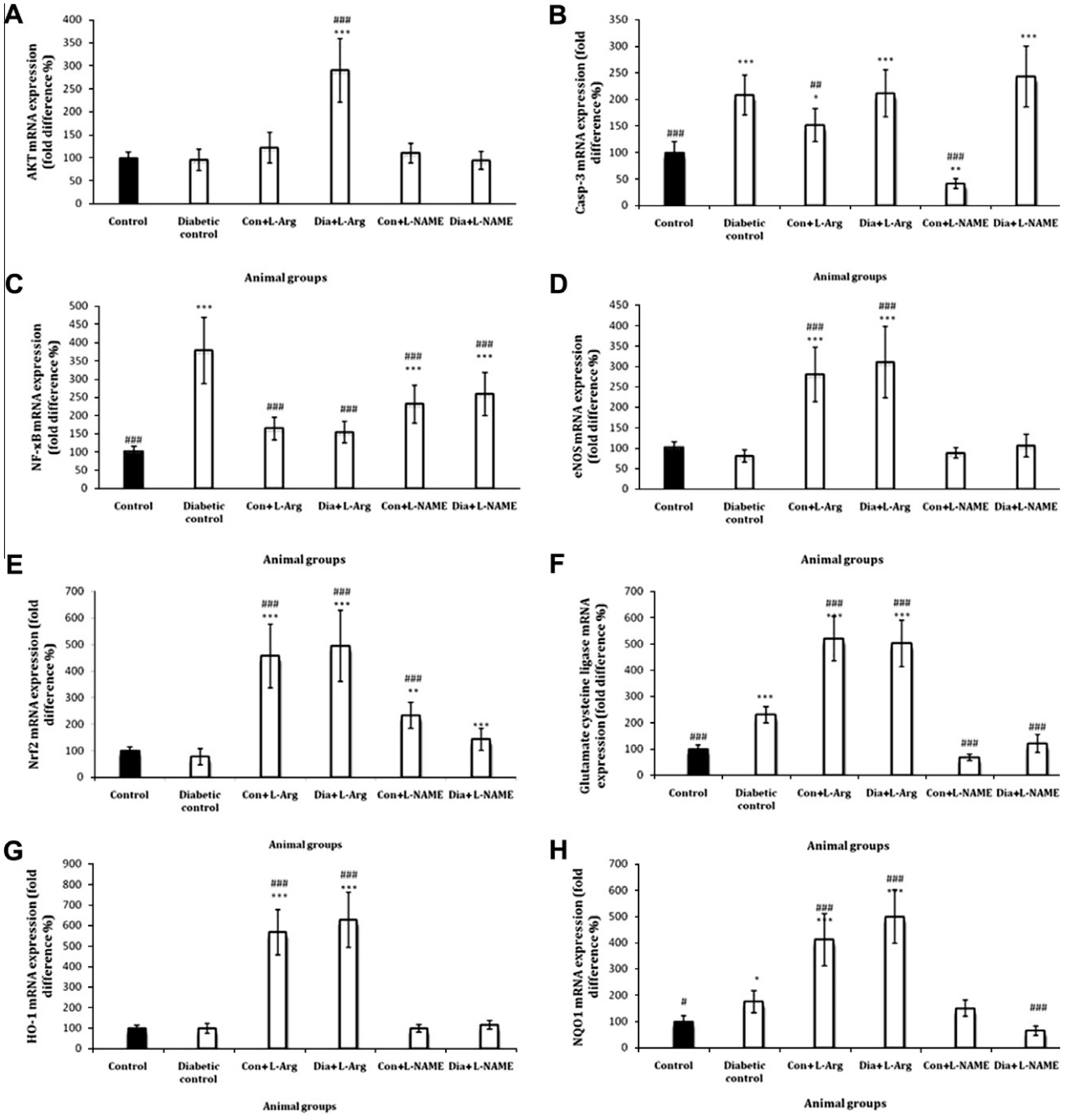

Fig. 2. Quantitative RT-PCR analysis. Relative average expression of candidate genes from analyzed groups normalized with GAPDH expression detected by qRT-PCR. Values are given as mean ± SEM for groups of six rats each. Comparison with non-diabetic control: $^{*}P < 0.05$; $^{**}P < 0.025$; $^{***}P < 0.005$. Comparison with diabetic control: $^{\#}P < 0.05$; $^{\#\#}P < 0.025$; $^{\#\#\#}P < 0.005$. Values are statistically significant at $^{*}P < 0.05$.

ventricular tissue of diabetic rats. However, the glutathione level showed moderate improvement by L-arginine treatment. The L-NAME treated diabetic animals showed drastic fall in glutathione level, which iterate inhibition of NO bioavailability and fails to provide antioxidant defense to cardiac tissue. Lipid peroxidation is a well-established mechanism of oxidative damage and the measurement of MDA provides a convenient index of lipid peroxidation. We also show that L-arginine treatment decreases the left ventricular lipid peroxidation which could be associated with a partial elevation in heart total antioxidant capacity. As expected, the L-NAME treatment failed to attenuate the lipid peroxidation and there was no improvement in overall antioxidant status.

The histological assessment of left ventricle cardiac myocytes of diabetic control showed disorganized myocardial fibers and necrosis of myocytes, which confirms the free radicals mediated damage. Moreover, L-arginine treatment reduced the inflammation and the fibrotic scars in diabetic samples, which confirms the beneficial effects of L-arginine. Excessive NO has deleterious effects through ROS production and $ONOO^{\cdot}$ formation. Accordingly, the L-arginine treated control and diabetic rats showed mild edema, which could be a result of overdose of L-arginine. L-NAME treated non-diabetic rats showed mild edema and diabetic rats showed tiny scars, edema, congested blood vessels and focal mononuclear inflammatory cell which indicate that the myocardium has

minimum infarction when compared to non-diabetic control. This can be an attributed to defective NO synthesis that may contribute to the structural changes in the coronary vasculature and myocardium in certain pathological conditions *in vivo* [23].

PKB/Akt signaling is believed to promote proliferation and increased cell survival whereas, caspases are crucial effectors in the cell death pathways. NF-κB, which is a redox-sensitive transcription factor and an inflammatory marker, appears to be regulated by NO. In our experiments, the significant activation of *Akt* mRNA transcription in diabetic rats indicates the increased survival of myocytes by L-arginine treatment. Our results also showed that *NF-κB* expression was reduced in L-arginine treated diabetic samples, but significantly increased in L-NAME treated groups and diabetic control. Inconsistently, the quantitative results of *Casp3* mRNA showed a significant expression in diabetic and non-diabetic groups of L-NAME as well as L-arginine also. Taken together, these data clearly show that L-arginine protects the cardiac myocytes by upregulating the *Akt* signaling and reducing the expression of *NF-κB*, but failed to compromise the expression of *Casp3*. The probable explanation for this unexpected result is unoptimised dose of L-arginine as revealed by histopathological evaluation of this study.

In our study, the L-arginine treatment was found to increase the relative gene expression of eNOS. The enhanced expression of eNOS, in turn, activates the mRNA expression of *Nrf2* in left ventricular heart tissue of diabetic rats. The L-arginine also enhanced the relative gene expression of Nrf2 associated genes *GCL*, *NQO1* and *HO-1*, which are very critical antioxidant enzymes for proper redox homeostasis to protect cells from irreversible oxidative damage. In conclusion, our results clearly demonstrate that administration of NO precursor improves the antioxidant status of left ventricular myocardial tissue, even under challenging hyperglycemic condition, which is essentially required to overcome diabetic cardiomyopathy and endothelial dysfunction produced by potent free radicals. Despite all of these beneficial effects, knowledge on key components of the transcriptional regulation of *NF-κB*, *Casp3* and other inflammatory genes needs to be understood. Overall, the L-arginine-NO pathway and Nrf2 signaling may provide a viable therapeutic target in the control of cardiac diseases resulting from hyperglycemia induced inflammatory stimuli.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.10.064.

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