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CARDIOPROTECTIVE EFFECTS OF CURCUMIN AGAINST DIABETES AND NICOTINE-COMBINED OXIDATIVE STRESS

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Abstract

Background: Diabetic cardiomyopathy (DCM) develop through oxidative stress-induced myocardial cell apoptosis that cause cardiac tissue damage resulting in hemodynamics disturbance while Cigarette Smoking (CS) is associated with a significant increase in the risk of recurrent ventricular tachyarrhythmia in ischemic and non-ischemic cardiomyopathy patients caused by oxidative stress. Curcuma longa extract (curcumin) is known to protect against hyperglycemia-induced oxidative stress. This brought in mind to investigate the probability of the crcumin ability to ameliorate the combined diabetes and smoking induced oxidative stress caused DCM

Materials and Methods: Diabetic rats were administered nicotine to investigate the effect of the combined oxidative stress of diabetes and nicotine. Moreover, curcumin was administered to examine its protective effect on possible oxidative stress induced diabetes and nicotine.

Results: Nicotine administration in a dose of 1.5 mg/kg to diabetic rats increased the oxidative stress. This occurs through elevation of plasma nitric oxide (NO) and upregulation of cardiac tissue inducible nitric oxide synthase (iNOS) and Endothelin-1 mRNA expressions, in addition to elevation of plasma triglycerides (TG), and LDL and reduction of HDL levels. Nicotine administration also reduced the cardiac tissue protective mechanism through reduction of plasma superoxide dismutase (SOD), cardiac tissue Erythropoietin (EPO), vascular endothelial growth factor (VEGF) isoforms and VEGF receptor mRNA expressions. These combined oxidative stresses were manifested by elevation of the plasma cardiac markers troponin I and creatine kinase (CK-MB). Supplementation of curcumin prevented the diabetic and nicotine-induced oxidative stress through reduction of plasma NO and iNOS and Endothelin-1 mRNA expressions to their control levels and elevation of plasma SOD and upregulation of cardiac tissue Erythropoietin (EPO), vascular endothelial growth factor (VEGF) isoforms and VEGF receptor mRNA expressions. This curcumin protective effect of the cardiac tissue was manifested by normalization of the plasma cardiac marker troponin I and CK-MB.

Conclusion: These results strongly confirmed that curcumin protected cardiac tissues from the combined oxidative stress induced by diabetes and nicotine.

Keywords: Curcumin, Cardiac oxidative stress, Nicotine, Diabetes

Introduction

The most diabetic associated cardiovascular complication is cardiomyopathy that cause mortality in diabetic patients (Lu et al., 2015). Cardiomyopathy is characterized by myocardial structural and functional disorders. Incidence of diabetes is associated with oxidative stress (Baynes 1991) and is linked to heart failure that is manifested by contractile dysfunction (Neubauer 2007). In the diabetic heart, hyperglycemia increased the production of reactive oxygen species that impose oxidative stress. This lead to the development and progression of DCM (Cai et al 2006). Oxidative stress has been considered the determinant marker for severity of heart failure (Hare 2001). Diabetic cardiomyopathy may develop through

oxidative stress-induced myocardial cell apoptosis that cause cardiac tissue damage resulting in hemodynamics disturbance (Sawyer et al., 2002 and Giordano 2005). Smoking is associated with a significant increase in the risk of recurrent ventricular tachyarrhythmia in ischemic and non-ischemic cardiomyopathy patients (Plank et al., 2014). Smoking is the cause of independent risk factor of cardiovascular diseases that may increase myocardial infarction incidence (Bernhard and Wang 2007). Increased rate of apoptotic cell death (Csiszar et al., 2005) caused by oxidative stress in addition to endogenous protective mechanism perturbations (Ferdinandy et al., 2007) were suggested to have a considerable role in cardiovascular aging. Vascular pathophysiology caused by cigarette smoking was suggested to be in part due to increased production of ROS (Csiszar et al., 2008). Cigarette smoking caused endothelial oxidative stress that results in NF-$_{K}$B activation and proinflammatory alterations in vascular function (Orosz et al., 2007). Renal hemodynamics disturbances were induced by nicotine replacement therapy similar to smoking (Ritz et al., 1998). A major part of chronic nicotine-induced intracellular ROS originates in the mitochondria (Arany et al., 2011). Through adversely affecting mitochondrial ROS production (Yang et al., 2007). The mechanism of chronic smoking-induced cardiovascular complications in diabetic patients still needs more elucidation especially at the molecular levels. The search for protection of the heart from such deleterious effect is continuous. Curcumin (a herbal extract) was reported to has anti-inflammatory effect through protecting the cellular component against reactive oxygen species (ROS) (Alison and Paul, 2000). Moreover, Curcumin has been demonstrated to have hepatoprotective effect against diethyl nitrosamine toxicity (Kadasa et al., 2015). More recently Curcumin intake was postulated to protect against coronary heart disease development through reducing oxidative damage and myocardium apoptosis (Liu et al., 2017). This motivates us to investigate the molecular mechanism of chronic administration of nicotine to streptozotocin induced diabetic rats and the possible protection against the combined oxidative stress of both diabetes and nicotine by curcumin.

Material and Methods

Animals

Twenty-four adult-male Wistar rats weighting 190-220 grams (65-70 days old) were purchased from animal house of college of Pharmacy, King Saud University, KSA. Animals were kept at 22°C and 55% humidity in animal house center of Taif University, with 12-h light:12-h dark cycle. The rats were fed a standard pellet diet, and water *ad libitum.* The present work was approved by the ethical committee of Taif University to conduct the research project NO. 1/434/2799

Formulation of animal food with the addition of curcumin

Curcumin powder added to the animal foods in a rate of 1.5 gm/kg of food. The pellets were powdered and crushed then curcumin powder was mixed well. Then a small volume of water was added and the mix was made to a paste then reformed to pellets again. For 4 days, the pellets were left to dry in air. The rate of curcumin and nicotine concentration addition was added based on body weight of rats (200 gm). The average food consumption was estimated for one week was found to be about 25-30gm/day and 25ml/day for water consumption respectively.

Experimental design

Rats were adapted for 1 week then divided into two equal groups (6 rats per each) as follow. The 1st group used as a negative control (injected citrate buffer, pH 4.5). The 2nd group (18 rats) were fasted for 8 hrs, after which they were injected intraperitoneal with a single dose of 60 mg/kg of streptozotocin (Sigma Chemical Co., St. Louis, MO, USA), freshly dissolved in citrate buffer (pH 4.5). After injection, rats were given 5% glucose drink overnight to avoid hypoglycemic shock. After 3 days, the fasting blood glucose levels were determined from tail blood sample using glucometer (Accu-Chek Aviva BG meter; Roche Diagnostics, Indianapolis, IN). Rats with glucose levels over 200 mg/100 ml considered diabetic and used for next experiments and divided into diabetic group (6 rats), diabetic-nicotine group (DN, 6 rats) supplied with nicotine (BDH. Chemicals LTD, Poole, England) in water (12 µg/ml to attain 1.5 mg/kg) and diabetic-nicotine-curcumin group (DNC, 6 rats) diabetic rats supplied with nicotine as in DN plus curcumin (1.5 gm/kg). Treatments were continued for 8 weeks after induction of diabetes. Random blood glucose levels were measured every week to confirm diabetes incidence. At the end of the experiment, rats were fasted for 8 hrs and decapitated after inhalation of diethyl ether. Blood samples were collected from retro-orbital venous plexuses of rats on heparinized tubes to obtain plasma. Heart samples were taken for RNA extraction and kept at -80ºC until RT-PCR analysis.

Biochemical analysis

Kits for biochemical assessments were purchased from Human diagnostic (GmbH-56205 wiesbaden Germany). Plasma troponin I, nitrous oxide (NO), Cholesterol, TG and HDL and creatine kinase (CK) were measured using commercial kits (United Diagnostic Industry, UDI, Dammam, Saudi Arabia). Plasma levels of superoxide dismutase (SOD)

was measured using kits from Jiancheng Institute of Bioengineering Company, Nanjing, China. All measurements were carried out based on the instruction manual of each kit and suppliers.

Analysis of gene expression

For preparation of total RNA, 50 mg of heart tissues were homogenized in 1ml QIAzol (QIAGEN Inc., Valencia, CA) and RNA was extracted as stated before (Alkedaide et al 2016). For cDNA synthesis, mixture of 2 µg of total RNA were denatured and reverse transcribed in a total volume of 20 µl as stated before (Alkedaide et al 2016). For Semi-quantitative Polymerase chain reaction (PCR), mRNA expression of some genes markers of heart damage and integrity were examined using corresponding specific primers (table 1). Of these examined genes, are erythropoietin (EPO), nitric oxide synthase (iNOS), vascular endothelial growth factor (VEGF) isoform (A), VEGF receptor 2 (VEGF R2) and endothelin-1. The used primers were synthesized by Macrogen (Macrogen Company, GAsa-dong, Geumcheon-gu. Korea). PCR was conducted as stated in our previous study (Alkediade et al 2016). As a reference, expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was used. PCR products were run on gel 2 % agarose (Bio Basic INC. Konrad Cres, Markham Ontario) gel in TE (Tris-EDTA) buffer. The gel was stained with ethidium bromide. PCR products were photographed under UV light. The intensities of the bands were quantified using NIH image program (http://rsb.info.nih.gov/nih-image).

Table 1: Primers and PCR conditions used for the tested genes.

Gene	Product size (bp)	Annealing Temp.	Direction (5'-3')	Primer sequence
G3PDH	309	52°C	Sense	AGATCCACAACGGATACATT
			Antisense	TCCCTCAAGATTGTCAGCAA
EPO	530	60°C	Sense	TACGTAGCCTCACTTCACTGCTT
			Antisense	GCAGAAAGTATCCGCTGTGAGTGTTC
iNOS	340	55°C	Sense	TCTGTGCCTTTGCTCATGAC
			Antisense	CATGGTGAACACGTTCTTGG
Endothelin-1	727	57.5°C	Sense	ATCATCTGGGTCAACACTC
			Antisense	GAATCTCCTGGCTCTCTG
VEGR isoforms	514 462 330	58°C	Sense	GACCCTGGTGGACATCTTCCAGGA
			Antisense	GGTGAGAGGTCTAGTTCCCG
VEGFR2	500	57°C	Sense	ACGGGGCAAGAGAAATGAAT
			Antisense	GCAAAACACCAAAAGACCAC

Statistical analysis

Data of current study were analyzed using analysis of variance (ANOVA) and Schefft's protected least-significant difference test using SPSS software (SPSS version13.0, IBM, Chicago, IL, USA) with $P < 0.05$ regarded as statistically significant. Results were expressed as means ± standard error (SE).

Results

Protective effect of curcumin on biochemical measurements related to cardiac activity

Plasma levels of troponin I, creatine kinase (CK-MB), TG and LDL were increased in diabetic rats than control and was further increased with nicotine administration while returned to nearly the normal control levels in the rats group administered curcumin. Plasma NO was increased in diabetic rats compared to control and reduced in the diabetic rats administered nicotine and further reduced in the diabetic nicotine rats administered curcumin. SOD was found to be significantly decreased in diabetic rats compared to control which could be due to oxidative stress induced by diabetes. Reduction of this antioxidant enzyme levels became more severe in diabetic rats treated by nicotine and improved by curcumin supplementation (Tables 2).

Table 2: Plasma changes in biochemical parameters related to cardiac activity during diabetes and after administration of nicotine and/or curcumin.

Parameter	Control	Diabetes (D)	Diabetes + Nicotine (DN	Diabetes + Nicotine + Curcumin (DNC)
Troponin I (ng/ml)	0.6 ± 0.06	0.8 ± 0.02*	1.1 ± 0.03#	0.43 ± 0.08 $
CK MB (ng/ml)	2.7±0.2	3.32± 0.03*	5.8±0.04#	3.7±0.18$
TG (mg/dL)	52.3 +8	63.3 ±12.5*	81.7+4.5 #	61.7±14$
LDL (mg/dL)	504.8±12.5	640.8±8.4*	882.8±34#	589 ± 22$
HDL (mg/dL)	18.7±1.2	15.3±0.5	11.3±1.2 #	21± 0.9 $
NO (µmol/L)	22.7±1.7	62 ± 4.7*	39±0.7 #	28.7± 0.3 $
SOD (U/mL)	11.67 ± 0.16	7.94± 0.17*	5.80 ± 2#	10.73±0.3$
GPx U/ml	115.67 + 3.8	85.67 + 3.3*	69 + 0.7 #	105.3 + 9. $
MDA (nmol/ml)	3 +0.8	4.4 + 2.1*	8.74+ 1.2 #	3.63+ 0.16 $

*Values are represented as means ± SE for four rats. Values with symbol * are significantly at P<0.05 relative to control. Values with symbol # are significantly at P<0.05 relative to D. Values with symbol $ are significantly at P<0.05 relative to DN.*

Protective effect of curcumin on cardiac Erythropoietin (EPO) and iNOS mRNA *expression*

In the cardiac muscle the mRNA expression levels of EPO show reduction in diabetic rats and in diabetic rats administered nicotine while curcumin administration to diabetic nicotine rats caused EPO mRNA expression to increase even higher than control rats (Figure 1A). The levels of cardiac tissues iNOS mRNA expression was increased only in the diabetic rats administered nicotine while curcumin supplementation to rats group administered nicotine reduced the iNOS expression nearly to the control levels (Figure 1B).

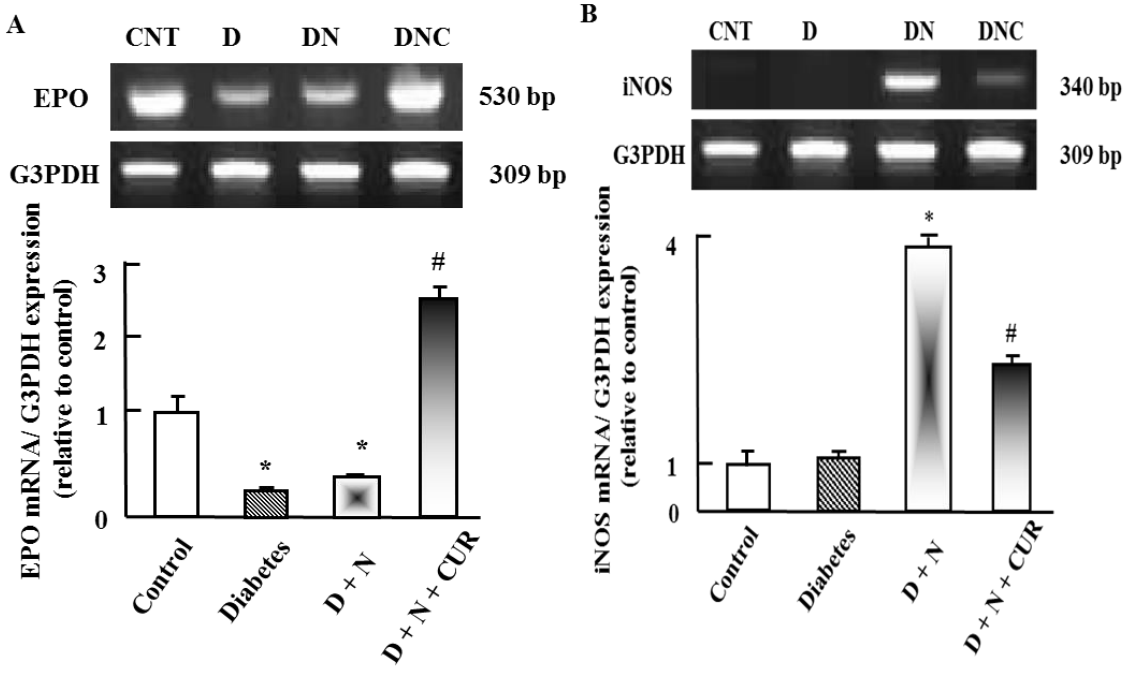

Figure 1: *Protective Effect of curcumin on mRNA expression of EPO and iNOS in Cardiac tissues: Total RNA was extracted from heart tissues and the expressions of Erythropoietin (EPO) (A) inducible nitric oxide synthase (iNOS) (B) were semi-quantified by using RT-PCR. Values are means ± SE of 6 rats. *p < 0.05 Vs Control rats, §* p < 0.05 Vs diabetic rats, # p < 0.05 Vs DN treated rats. The treatment groups are: Control (CNT) Diabetes (D), Diabetes and nicotine (DN) and diabetes plus nicotine plus curcumin (DNC).*

Protective effect of curcumin on cardiac VEGF isoform and VEGF Receptor mRNA expression

In the cardiac muscle, the mRNA expression levels of ***VEGF*** isoform were decreased in diabetic rats and further decreased in diabetic rats administered nicotine. While in the diabetic rat groups supplemented with curcumin in addition to nicotine, the VEGF isoforms mRNA expression was increased even than control levels (Fig.2A). We further measured the VEGF receptor in the cardiac muscle that show reduction in the diabetic rats and further reduction in the diabetic rats administered nicotine while curcumin supplementation prevented this reduction and increased its expression even than control levels (Fig 2B).

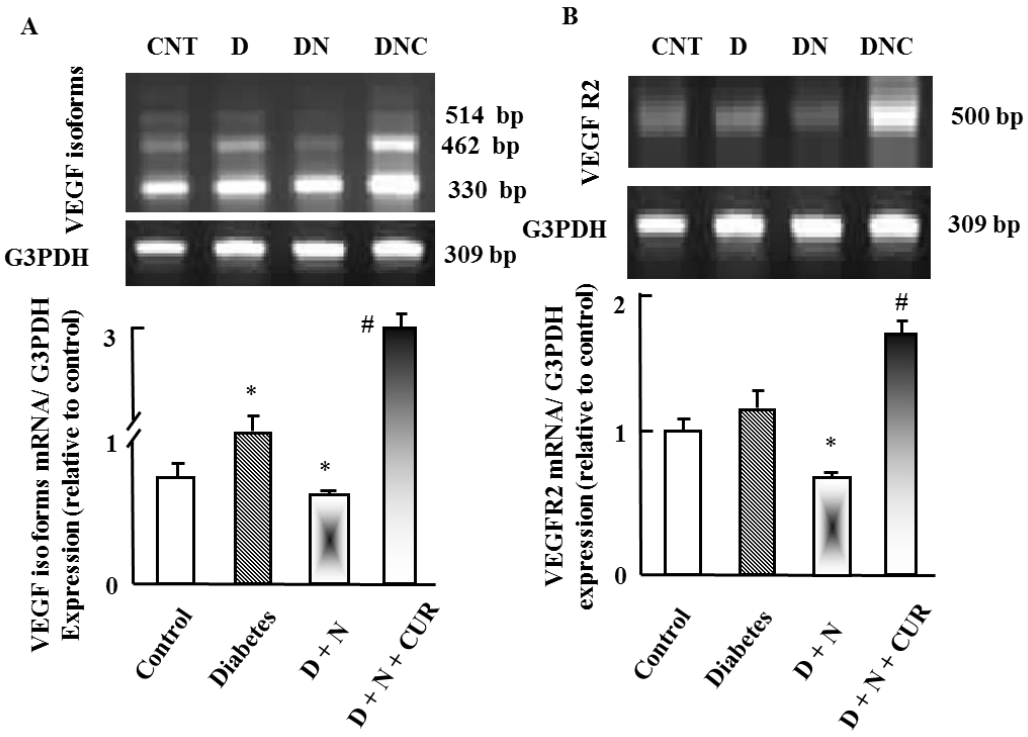

Figure 2: *Effect of curcumin on mRNA expression of VEGF isoforms and VEGF receptor 2 in Cardiac tissues: Total RNA was extracted from heart tissues and the expressions of vascular endothelial growth factor (VEGF) (A), VEGF receptor 2 (VEGF R2) (B) were semi-quantified using RT-PCR. Values are means ± SE of 6 rats. *p < 0.05 Vs Control group, §* p < 0.05 Vs diabetic rats, # p < 0.05 Vs DN treated rats. The treatment groups are: Control (CNT) Diabetes (D), Diabetes and nicotine (DN) and diabetes plus nicotine plus curcumin (DNC).*

Protective effect of curcumin on cardiac Endothelin-1 mRNA expression

In the cardiac muscle, the mRNA expression levels of endothelin-1 was decreased in diabetic rats than control and highly induced in the diabetic rats administered with nicotine while curcumin administration to diabetic nicotine rats caused endothelin-1 mRNA expression to return nearly to the control expression levels (Figure 3A).

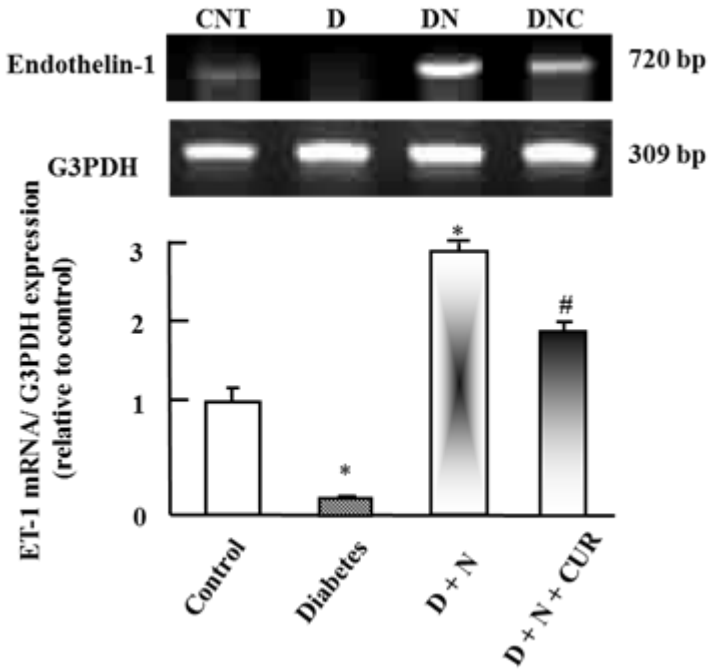

Figure 3: *Effect of curcumin on mRNA expression of endothelin-1 (ET-1) in Cardiac tissues: Total RNA was extracted from heart tissues and the expressions of ET-1 was semi-quantified using RT-PCR analysis. Values are means ± SE of 6 rats. *p < 0.05 Vs Control group, §* p < 0.05 Vs diabetic group, # p < 0.05 Vs DN treated rats. The treatment groups are Control (CNT) Diabetes (D), Diabetes and* nicotine (DN) and diabetes plus nicotine plus curcumin (DNC).

Discussion

The current study confirmed protective effect of curcumin against oxidative stress induced by diabetes and nicotine. As known, hyperglycemia is accompanied by an increase in production of ROS that impose oxidative stress and plays a role in the pathogenesis of DCM (Cai et al., 2006). In the current study, the combined damaging effect of diabetes and nicotine on the cardiac muscle may operate through inducing oxidative stress through increasing nitric oxide production (table 2) through inducing iNOS mRNA expression (Fig.1 B) and decreasing the scavenging and protective mechanism through decreasing SOD (table 2) and downregulation of EPO expression levels (Fig.1 A). Superoxide overproduction in the cellular systems is a marker for diabetic cardiomyopathy (Kain et al., 2011). SOD enhances dismutation of superoxide radicals (Moussa 2008). The reduction of SOD in diabetic rats in our study and other (Badole et al., 2015), imply that diabetic oxidative stress operates through SOD reduction. Indeed, inability of the different body antioxidant mechanism to scavenge reactive oxygen species (ROS) due to overproduction and/or reduction of the body anti-oxidant defense mechanisms lead to oxidative stress.

Erythropoietin exerts a cardio-protective effect against infarction and ischemia-reperfusion injury (Calvillo et al 2003; Moon et al., 2003). The downregulation of EPO expression in the cardiac tissue by diabetes and nicotine may imply that they exert their oxidative stress through reducing the cardiac muscle protective mechanism. This oxidative stress exerted by diabetes and nicotine on the cardiac tissue may be the cause in the elevation of plasma creatine kinase (CK-MB) and Troponin I (table 2). Diabetic animals showed increased levels of (CK-MB) as reported by Edet et al (2009) and our findings. Diabetes enhanced the expression of the cardiac damage markers like troponin I (Kain et al., 2011). When cardiac damage occurs, cardiac myocyte release troponin into circulation (Hassan et al., 2009). In our study diabetes, increased plasma troponin that was further increased with nicotine administration (table 2).

The addition of curcumin protected the cardiac muscle from the damaging effect of diabetes and nicotine. This curcumin protection is manifested by reducing the oxidative stress through reducing the NO production and down regulation of the iNOS mRNA expression. It also protects the heart from the diabetes and nicotine-oxidative stress through the increase in plasma SOD and upregulation of EPO mRNA expression and normalization of diabetes and nicotine elevated LDH, TG, normalization of the diabetes and nicotine-reduced plasma HDL. EPO production is primarily stimulated by hypoxia that increases serum EPO levels up to several hundred-fold depending on severity of hypoxia (Ebert and Bunn 1999). hypoxia response element (HRE) was demonstrated in the 3′- flanking region of the human EPO gene (Semenza et al., 1991) hypoxia-inducible factors (HIFs), are the master transcription factors for oxygen-dependent EPO gene regulation (Franke et al., 2013) HIF-1α mRNA levels increase dramatically in response to hypoxia or ischemia in heart (Lee et al., 2000) . Hydroxylation and subsequent ubiquitination of HIF-1a is suppressed under hypoxic conditions, resulting in HIF-1a protein accumulation without changes in HIF-1a mRNA levels (Stockmann and Fandrey 2017).

Hyperglycemia was demonstrated to enhance production of methylglyoxal (MGO) that was recently reported to enhance the degradation of HIF-1α protein (Ramalho et al., 2017) this may explain EPO downregulation in diabetic rats in our study due to diabetes induced degradation of HIF. Moreover, Nanocurcumin ameliorated hypoxia-induced hypertrophy and apoptosis in H9c2 cells by stabilizing HIF-1α protein and accumulation (Nehra et al., 2015). This could be the mechanism through which curcumin induces EPO expression in the current study.

This protective effect is manifested by normalization of the diabetes and nicotine-induced plasma CK-MB and Troponin I. VEGF is well known for its ability to stimulate proliferation of endothelial cells in vitro and neovasculogenesis in the ischemic heart in vivo (Zisa et al., 2009; Gao et al., 2007). The down regulation of the VEGF isoforms and VEGF receptor mRNA expression by diabetes and nicotine may be one of the pathway through which they stressed the cardiac muscle. Curcumin upregulated mRNA expression of the VEGF isoforms and VEGF receptor could be a pathway through which curcumin protect the cardiac muscle from the combined oxidative stress exerted by diabetes and nicotine.

During myocardial experience ischemia and reperfusion endothelin levels are significantly increased in the coronary and the ischemic area tissues (Ling et al., 2014). The diabetes plus nicotine-induced endothelin mRNA in the cardiac tissue indicates that their combined oxidative stress may increase the probability of ischemic area in the cardiac tissues. However, the addition of curcumin nearly normalized the induced endothelin-1 mRNA expression caused by nicotine and diabetes which indicates the protective effect of curcumin against the diabetes and nicotine oxidative stress.

Conclusion

The current study confirmed that both diabetes and nicotine showed combined oxidative stress on the cardiac tissues through increasing in ROS production, NO production, and upregulation of cardiac tissue iNOS mRNA expression diabetes and nicotine decreased the protective mechanism through decreasing plasma SOD and EPO mRNA expression in cardiac muscle. They increased the main cardiac biomarkers (Troponin and CK-MB). These deleterious effects were ameliorated by curcumin administration. Curcumin protects cardiac tissues from oxidative stress through normalization of troponin and creatine kinase cardiac biomarkers.

Conflict of Interest: Authors declare that there are no competing interests.

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