

Original Article

Anti-inflammatory effects of lycopene prevents cardiac dysfunction in streptozotocin-diabetic rats

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Abstract: Background: Recent evidences show that lycopene can lower the heart disease. Therefore, the aim of the present work was to evaluate the protective effects of lycopene on diabetic cardiomyopathy (DCM) through the control of the antioxidant status, its anti-inflammatory effect and efficacy against fibrosis in hearts of streptozotocin (STZ)-induced diabetic rats. Methods: Diabetes was induced in rats by intraperitoneal administration of STZ. After 8 weeks of lycopene treatment, the antioxidant status and oxidative stress were evaluated by the assessment of SOD activity, MDA content, and HO-1 protein expression. Proinflammatory cytokines such as tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6), and thromboxane synthase (TXS) were measured to evaluate the anti-inflammatory effect of lycopene. TGF- β 1, STAT4, STAT6, and SMAD3 protein expressions were evaluated to assess the effects of lycopene on myocardial fibrosis. Results: Lycopene treatment significantly enhanced the antioxidant status by increasing SOD activity and HO-1 protein expression, reduced the inflammatory response by decreasing TNF α , IL-6 and TXS expressions, and down-regulated the expression of TGF- β 1, STAT4, STAT6, and SMAD3 proteins in diabetic rats. Conclusions: Lycopene attenuated cardiac dysfunction by the increase of the antioxidant status, the decrease of inflammation and inhibition of myocardial fibrosis signaling molecules.

Keywords: Lycopene, diabetic cardiomyopathy, anti-inflammatory effect, antioxidants, fibrosis

Introduction

Diabetic cardiomyopathy (DCM) is one of the most serious cardiovascular complications in diabetic patients, which results in heart failure and high mortality. The increased DCM risk appears to be independent of atherosclerosis, hypertension, or other dysfunctions [1, 2]. Left ventricular dysfunction is an early sign of diabetic cardiomyopathy. Therefore, DCM has become an interesting subject of intensive investigation over the last 40 years. The mechanisms responsible for DCM are involved in hyperglycemia, increased oxidative stress [3-5], activation of various proinflammatory cytokines [6] and remodeling of the extracellular matrix in the heart [7]. Proinflammatory response lead to further oxidative stress and both these factors exacerbate the risk and progression of diabetic cardiomyopathy [8, 9]. In addition, inflammation could cause pathological myocardial remodeling and fibrosis, promoting heart failure development. Lycopene is a tetraterpene abundant in tomatoes and watermelons. Numerous studies show that this

unsaturated carotenoid has powerful beneficial effects on health. Indeed, it has powerful anti-inflammatory effects and is a strong antioxidant [10, 11], protecting cell membranes from lipid peroxidation as a free radical scavenger, and through stimulation of antioxidant enzymes activity [12]. Thus, lycopene exerts its protective effects against chronic diseases such as cardiovascular disease, and prostate cancer [13, 14]. Lycopene also improve cholesterol serum levels [15]. Therefore, our study aimed to assess the effects of lycopene on DCM in diabetic rats, exploring the mechanisms associated to its antioxidant and anti-inflammatory role, and the contributory pathways leading to myocardial fibrosis.

Materials and methods

Materials

Chloral hydrate, streptozotocin, and a secondary anti-rabbit antibody HRP conjugated (horse-radish peroxidase) were purchased from Sigma (Sigma Chemical Co, St. Louis, MO, USA). Rabbit

Lycopene and diabetic cardiomyopathy

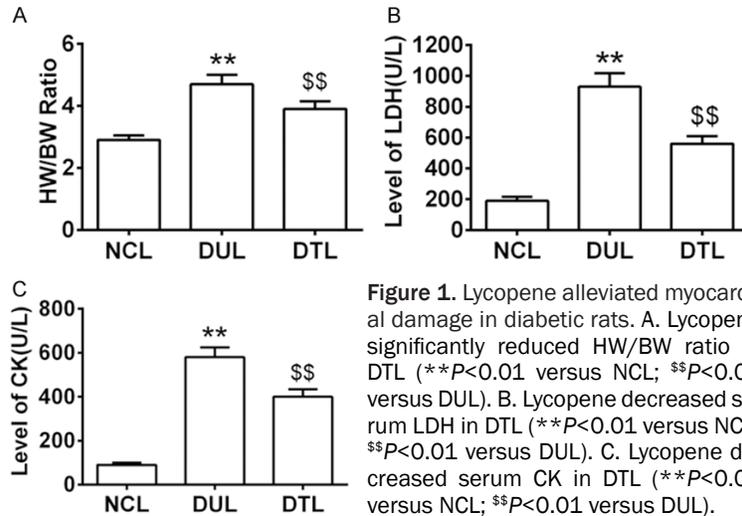


Table 1. Effects of lycopene on serum lipid profile (mean \pm SD)

	TC (mmol/L)	TG (mmol/L)	LDL (mmol/L)	HDL (mmol/L)
NCL	3.02 \pm 0.65	1.58 \pm 0.31	2.14 \pm 0.41	1.27 \pm 0.19
DUL	9.60 \pm 1.67**	4.34 \pm 0.98**	7.24 \pm 1.34**	0.35 \pm 0.10**
DTL	6.80 \pm 0.93\$\$	3.21 \pm 0.51\$	5.11 \pm 0.77\$	0.54 \pm 0.14\$

** $P < 0.01$ versus NCL; \$ $P < 0.05$, \$\$ $P < 0.01$ versus DUL; n=10 per group.

polyclonal antibodies β -actin, thromboxane synthase (TXS), signal transducers and activators of transcription 4 (STAT4), STAT6, SMAD3, and transforming growth factor beta 1 (TGF- β 1) were purchased from Bio Basic Inc (Canada). Heme oxygenase-1 (HO-1), tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6) were purchased from Wuhan Boster Bioengineering Limited Company (Wuhan, China).

Animals and treatment

Thirty healthy male Sprague-Dawley rats (190-230 g) were purchased from the animal experimental center of Wannan Medical College. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the Chinese National Institutes of Health. Animals were fed on standard chow, had free access to water, and were housed in a standard animal laboratory at room temperature (22 \pm 2 $^{\circ}$ C) on a 12-hour light/dark cycle for 2 weeks. Rats received an intraperitoneal injection of streptozotocin (STZ) at a dose of 70 mg/kg dissolved in cold sodium citrate buffer (0.1 mol/L natrium citricum buffer pH 4.5) for the induction of diabetes mellitus. The rats of the control group were treated with the same amount of buffer (normal control, NCL,

n=10). Diabetes was confirmed at 48 h after STZ injection by the evaluation of blood glucose concentrations (One Touch SureStep Meter, Life-Scan, Calif, USA) from tail vein samples. Diabetes was diagnosed as a result of a sustained elevated glucose concentration >16.7 mmol/L. Diabetic rats were randomly divided into two groups: diabetic rats without lycopene treatment (DUL, n=10), and diabetic rats with lycopene treatment (DTL) received lycopene (20 mg/kg per day) through an oral gavage tube. The three rats groups (NCL, DUL and DTL) had free access to water ad libitum. Eight weeks after

administration of lycopene, animals were anesthetized using sodium pentobarbital (30 mg/kg i.p.). Fasting blood samples and hearts were collected for further analyses.

Blood samples biochemical analyses

Blood samples were centrifuged at 1000 g to separate the serum, to evaluate its lipid profile. Serum total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) were measured using appropriated commercial kits and analyzed by a biochemistry analyzer according to the manufacturer's instructions. Serum creatine kinase (CK) and lactate dehydrogenase (LDH), biochemical markers of myocardial injury, were measured using an enzymatic colorimetric method (STAGO, France) according to the manufacturer's instruction and analyzed by a semi-auto biochemistry analyzer (Hlife Medical Instrument Co., Ltd, China).

Antioxidant status and oxidative stress evaluation

Serum was separated from fasting blood samples. Serum malondialdehyde (MDA) content

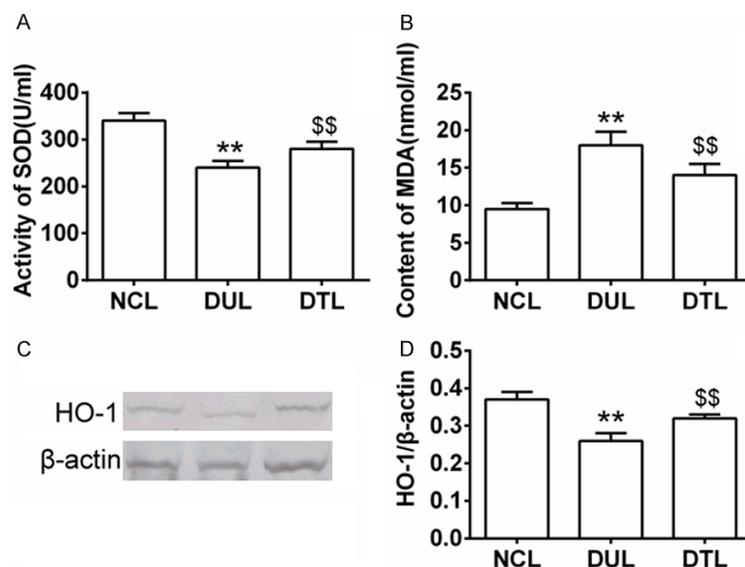


Figure 2. Lycopene enhanced the serum antioxidant status and reduced oxidative stress in diabetic rats. A. Serum SOD activity was significantly positively modulated by lycopene treatment in DTL (** $P < 0.01$ versus NCL; \$\$ $P < 0.01$ versus DUL). B. Lycopene treatment reduced serum MDA level in DTL (** $P < 0.01$ versus NCL; \$\$ $P < 0.01$ versus DUL). C. HO-1 protein expression. D. Lycopene increased HO-1 level in DTL (** $P < 0.01$ versus NCL; \$\$ $P < 0.01$ versus DUL).

and superoxide dismutase (SOD) activity were detected using a 722 visible spectrophotometer (Shanghai INESA Scientific Instrument Co., Ltd, China) according to the kit instructions (NanJing JianCheng Bioengineering Institute, China).

Western blotting

Left ventricle tissues (0.15 g) were homogenized for 1 min in ice-cold lysis buffer (1.5 ml) containing 100 mM sodium pyrophosphate, 50 mM HEPES, 100 mM NaF, 10 mM Na_3VO_4 , 10 mM EDTA, and 2 mM PMSF (phenylmethanesulfonyl fluoride). The lysate was centrifuged at 11,500 g for 15 min at 4°C. Equal amounts of proteins from the supernatants were separated using a 12% sodium dodecyl sulfate polyacrylamide running gel and 5% stacking gel. After the electrophoresis, the separated proteins were transferred on 0.2 μm nitrocellulose membranes. Membranes were blocked with 5% nonfat milk in TBS-T buffer (pH 7.5) containing 10 mM Tris, 150 mM NaCl, 0.1% Tween20 for 1.5 hours at room temperature. Next, the membranes were incubated overnight at 4°C with the following rabbit polyclonal antibodies: β -actin (1:1000), TGF- β 1, STAT4, STAT6,

SMAD3, TXS (1:1000) (Bio Basic Inc. Canada), HO-1 (1:300), TNF- α (1:400), IL-6 (1:400) (Wuhan Boster Bio-engineering Limited Company, China) in TBS-T containing 5% nonfat milk. After extensive washing, the membranes were incubated with a secondary anti-rabbit antibody (1:9,000) for 1 h. After rinsing with TBS-T for four times, a DAB kit (Bio Basic Inc. Canada) was used to detect proteins bound to membranes, according to the manufacturer's protocol.

Statistical analysis

Results were expressed as mean \pm SD. Differences between the three groups were analyzed by SPSS16.0 statistics software using one-way ANOVA and the least significant difference (LSD) test.

Values of $P < 0.05$ were considered statistically significant.

Results

Effects of lycopene on rats' general physiological status

Blood glucose concentrations in rats receiving STZ were consistently higher than 16.7 mmol/L. Hyperglycemia rats showed the typical symptoms of diabetes including, but not limited to, increase in water consume, reduction in body weight, and polyuria. Treatment with lycopene reduced these symptoms. In addition, diabetic rats showed an increase in heart weight/body weight (HW/BW) ratio, considered a DCM marker, and lycopene significantly decreased this ratio (**Figure 1**).

Effects of lycopene on CK and LDH

Circulating markers of cardiac damage, such as CK and LDH, were evaluated to estimate the presence and level of injury. The results showed that CK and LDH levels were significantly increased in DUL compared with NCL. Lycopene treatment reduced the increase of CK and LDH levels in DTL when compared with DUL (**Figure 1**).

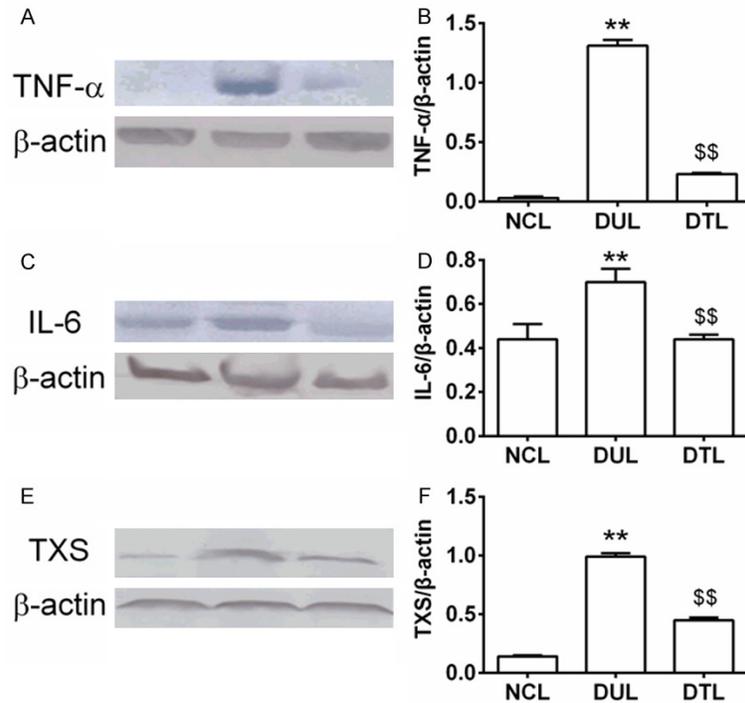


Figure 3. Lycopene enhanced anti-inflammation of diabetic rats. Lycopene decreased the expression and relative level of TNF- α (A and B), IL-6 (C and D), TXS (E and F) in DTL (** $P < 0.01$ versus NCL; \$\$ $P < 0.01$ versus DUL).

when compared with NCL. Treatment with lycopene reduced the amount of TNF- α and IL-6 proteins in DTL compared with DUL rats. TXS catalyzes the production of thromboxanes from its substrate prostaglandin endoperoxide. Our result showed that TXS protein expression was significantly increased in DUL compared with NCL. Lycopene treatment decreased its amount in DTL (Figure 3).

Effects of lycopene on fibrosis

To explore the mechanism regulating STZ-induced cardiac dysfunction and beneficial effects of lycopene, fibrogenic factors such as TGF- β 1, STAT4, STAT6, and SMAD3 were assessed in hearts of normal rats, and diabetic rats with or without lycopene

Effects of lycopene on serum lipids profile

As shown in Table 1, diabetes resulted in a lipid metabolism disorder. TC, TG, and LDL serum levels were significantly increased in DUL compared with NCL, while HDL level was decreased. Lipids profile was improved by lycopene treatment in DTL when compared with DUL.

Effects of lycopene on antioxidants and oxidative stress

MDA content, a product of lipid peroxidation, was increased, while SOD activity, an antioxidant, was reduced in DUL compared with the level of the same markers in NCL. Treatment with lycopene partly recovered SOD activity and decreased MDA content in DTL serum. The level of the antioxidant protein HO-1 was also significantly reduced in DUL myocardial tissues compared with NCL, while its level was significantly increased in DTL myocardial tissues due to lycopene treatment (Figure 2).

Effects of lycopene on inflammatory

TNF- α and IL-6 protein expression was significantly increased in DUL myocardial tissues

treatment. As shown in Figure 4, sustained hyperglycemia increased TGF- β 1, STAT4, STAT6, and SMAD3 expressions in DUL. Lycopene treatment partially reduced their expressions in DTL hearts.

Discussion

In the present study, our results showed that lycopene attenuated diabetic cardiomyopathy. Lycopene reduced the expression of inflammatory mediators such as TXS, TNF- α and IL-6, while increased SOD activity and HO-1 protein expression, both working as antioxidants. Furthermore, we investigated the effects of lycopene on myocardial fibrogenic markers. Lycopene significantly reduced TGF- β 1, STAT4, STAT6 and SMAD3 expressions. These data suggested that the beneficial effects of lycopene on DCM might be associated with its antioxidant and anti-inflammatory role, as well as its ability on preventing fibrosis.

Oxidative stress, which results from excessive reactive oxygen species (ROS) formation and reduced antioxidant capacity, is considered as an important factor in DCM development and

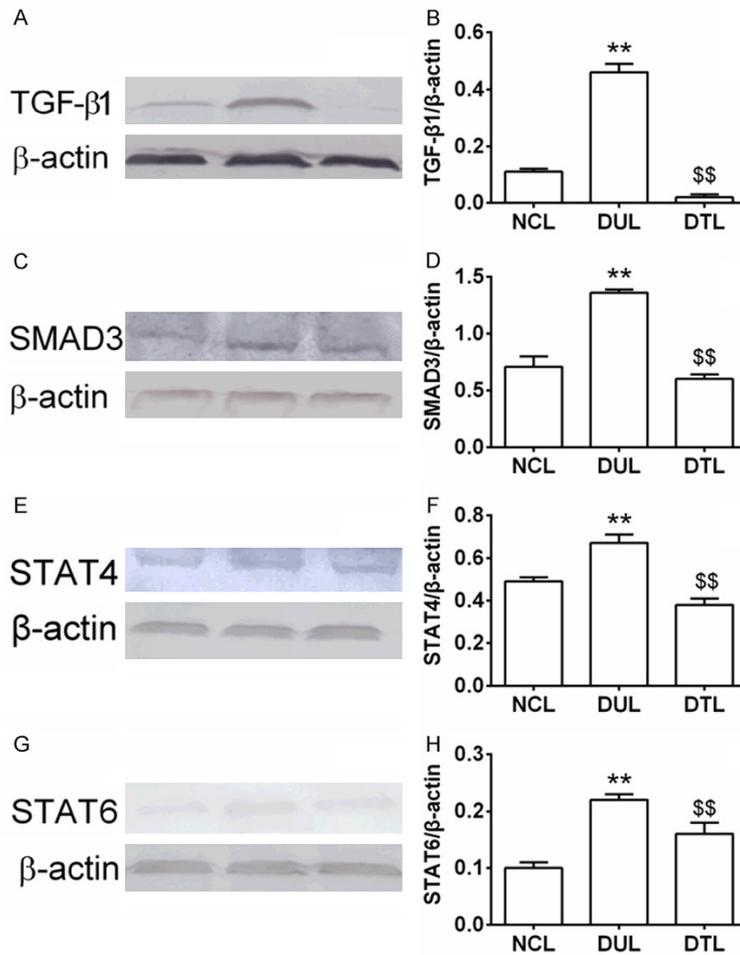


Figure 4. Lycopene reduced the expressions of fibrogenic factors. Lycopene decreased the expressions of TGF-β (A and B), SMAD3 (C and D), STAT4 (E and F), and STAT6 (G and H). (** $P < 0.01$ versus NCL; \$\$ $P < 0.01$ versus DUL).

progression [16-19]. Sustained hyperglycemia results in ROS overproduction by several mechanisms such as glucose auto-oxidation, shifts in redox balances, decreased tissue concentrations of low molecular weight antioxidants [4, 20], which damage various organs including heart. Decreased SOD levels in diabetes are considered a consequence of a deteriorated antioxidant defense system [21]. HO-1 possesses a cytoprotective role due to its antioxidant and anti-inflammatory abilities [22]. Numerous experimental evidences indicate that antioxidant treatments can improve DCM from type 1 and type 2 diabetic animals by inhibiting ROS generation [5, 23-25]. It has been widely demonstrated that lycopene is a potent antioxidant. In this study, CK and LDH were increased in serum of diabetic rats. These two parameters are considered as cardiac damage markers, since they represent power-

ful and sensitive predictors of increased cardiac complications [26]. Furthermore, our results showed that SOD activity and HO-1 expression level were significantly decreased, while MDA level, a production of lipid peroxidation, was increased in diabetic rats. However, lycopene treatment reduced CK and LDH levels, as well as MDA content, while increased SOD activity and HO-1 expression level, suggesting that lycopene alleviated cardiac damage by reducing oxidative stress and enhancing the antioxidant system.

Inflammation is another important diabetic feature [27]. Hyperglycemia-induced oxidative stress increase the expression of inflammatory cytokines such as TNF-α, IL-6, and IL-1β in diabetic myocardium [28, 29]. In addition, ROS generation activates inflammatory and apoptotic signaling pathways which are correlated with diabetic cardiomyopathy, and accelerates the myocardial fibrosis [28, 30, 31]. Thromboxane synthase (TXS) modulates the inflammatory response by catalyzing prostaglandin endoperoxide into thromboxanes [32], which enhances inflammatory response. These phenomena have remarkable effects such as induction of various cardiovascular diseases through platelet activation and vasoconstriction regulation [33]. Previous studies have confirmed that thromboxane production is involved in cardiovascular diseases such as coronary artery syndrome, atherosclerosis, and vessel remodeling [34]. Indeed, as a confirmation of its involvement, the use of TXS inhibitors result in an improvement of the cardiovascular system through their anti-inflammatory properties [35]. The present study indicated that diabetes induced an inflammatory response through up-regulation of inflammatory cytokines such as TNF-α, TXS and IL-6. Our further results suggested an anti-inflammatory role of lycopene

since it decreased TNF- α , TXS and IL-6 expressions.

Signal transducers and activators of transcription (STATs), a family composed of 7 proteins, are activated by growth factors and cytokines through phosphorylation, which transmit signals from the extracellular compartment to the nucleus, regulating gene expression. In addition, Janus kinase/signal transducers and activators of transcription (JAK/STATs) are involved in cell proliferation and differentiation of various type of cells [36]. STAT4 plays a critical role in modulating the inflammatory responses in various related diseases such as diabetes and cardiovascular disease [37, 38]. STAT6 has been shown to play an important role in the development of allergic airway eosinophilic inflammation and disease [39, 40]. Previous studies indicated that JAK/STAT signaling pathway can cause myocardial hypertrophy and fibrosis in diabetes [41]. In this study, our results showed that lycopene treatment down-regulated the expression of STAT4 and STAT6, which are actually involved in inflammation. Transforming growth factor (TGF- β) is a key mediator of fibrosis in various tissues [42]. It activates the SMAD signal transduction pathway via phosphorylating serine residues in SMAD2 and SMAD3 [43]. Activated SMADs translocate to the nucleus and modulate the expression of several genes, including connective tissue growth factor (CTGF), critically involved in fibrotic diseases [44, 45]. Increasing evidence shows that activated SMAD2/3 cascade exerts a pivotal role in extracellular matrix protein expression [46]. Moreover, recent findings support the role of SMAD3 in fibrogenesis signaling pathway induced by many fibrogenic factors such as TGF- β 1 and advanced glycation end products [47]. Indeed, reduced SMAD3 expression alleviates fibrosis in many diseases, such as hypertensive cardiac remodeling and pulmonary fibrosis [48, 49]. Inflammatory response accelerates the myocardial fibrosis in diabetes [30]. Our study indicated that lycopene treatment reduced the expression of TGF- β 1 and SMAD3. Therefore, lycopene inhibitory effect on fibrosis might be associated with its ability to regulate inflammatory signaling pathways.

Conclusion

The present study suggested a role of lycopene in the improvement of DCM in dia-

betic rats. The beneficial effect of lycopene might be related to its anti-inflammatory ability through STAT-related pathways, as well as myocardial fibrosis inhibition. These findings indicated that lycopene might be therapeutically used in patients with DCM.

Acknowledgements

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Disclosure of conflict of interest

None.

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