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## Lycopene ameliorates erectile dysfunction in streptozotocin-induced diabetic rats

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Diabetes mellitus (DM) is characterized by oxidative stress, which is one of the major pathophysiological mechanisms underlying diabetic erectile dysfunction (ED). Lycopene is one of the most potent antioxidants among the natural carotenoids. The present study was aimed to investigate whether lycopene could lower oxidative stress and attenuate ED in diabetic rats. Lycopene (10, 30, 60 mg/kg/d) was administered via intragastric intubation for 8 weeks to streptozotocin (STZ) (50 mg/kg, i.v.) induced diabetic rats. The results showed that chronic lycopene treatment significantly and dose dependently restored ED in diabetic rats by lowering blood glucose, reducing oxidative stress and up-regulating eNOS expression. These results indicated that lycopene treatment is potentially a new strategy for treating diabetic ED.

### 1. Introduction

Diabetes mellitus (DM) is the most common risk factor for the development of erectile dysfunction (ED) (Hirata et al. 2009). The prevalence of ED in diabetic patients is approximately 3 times more than that in general population (Richardson et al. 2002). As diabetic ED patients do not respond well to phosphodiesterase 5 inhibitors (PDE5i) (Rendell et al. 1999), more effective therapeutic strategies for the treatment of diabetic ED warrant further investigation.

One of the major pathophysiological mechanisms underlying diabetic ED is endothelial dysfunction (Liu et al. 2010), which mostly resulted from DM-induced oxidative stress damage in cavernosum tissue (Cai et al. 2000). Numerous clinical and laboratory studies have revealed that dietary carotenoids could reduce oxidative stress and therefore ameliorate endothelial dysfunction in DM condition (Zhao et al. 2011; Riccioni et al. 2009). Lycopene, mostly derived from tomatoes and tomato products, is one of the most potent antioxidants among the natural carotenoids (Di Mascio et al. 1990). It has a singlet-oxygen-quenching ability greater than that of  $\beta$ -carotene and Vitamin E by 47 and 100 times, respectively (Kuhad et al. 2008). Lycopene has been demonstrated to have many beneficial effects on human health, such as anti-oxidant, anti-cancer, anti-inflammatory and immunomodulatory activities (Kong et al. 2010). However, the ameliorative effect of lycopene on diabetic ED has not yet been reported. Therefore, the present study was designed to evaluate the effect of systemic treatment with lycopene on suppressing oxidative stress and ameliorating ED in streptozotocin (STZ)-induced diabetic rats.

### 2. Investigations and results

#### 2.1. General data

Table 1 shows body weight and blood glucose levels in the six groups. During the whole experimental period, diabetic rats showed a significant increase in blood glucose concentrations

and a decrease in body weight compared with those in normal control rats (all  $P < 0.01$ ). Chronic treatment with lycopene significantly and dose dependently decreased fasting blood glucose concentrations and increased body weight ( $P < 0.05$ - $P < 0.01$ ).

#### 2.2. Intracavernous pressure (ICP) and peak ICP/mean systemic arterial pressure (MAP) ratio

Table 2 shows the results of ICP and peak ICP/MAP ratio with and without the administration of lycopene. There were no significant differences in basic ICP and MAP among the six groups. The peak ICP and peak ICP/MAP ratio values in untreated diabetic group were significantly decreased compared with those in normal control group (all  $P < 0.01$ ). Chronic treatment with lycopene could significantly and dose dependently restore the peak ICP and peak ICP/MAP ratio values. However, the peak ICP and peak ICP/MAP ratio values in lycopene-treated diabetic rats did not return to the normal level ( $P < 0.05$ - $P < 0.01$ ).

#### 2.3. Malondialdehyde (MDA) concentrations and superoxidase dismutase (SOD) activities in the corpus cavernosum

As shown in Table 3, the MDA concentrations were significantly elevated and the SOD activities were significantly decreased in the corpus cavernosum of diabetic rats compared with those in the normal controls (all  $P < 0.01$ ). Chronic treatment with lycopene significantly and dose dependently attenuated the increased MDA content and reduced SOD activity in the corpus cavernosum ( $P < 0.05$ - $P < 0.01$ ).

#### 2.4. Endothelial nitric oxide synthase (eNOS) mRNA and protein expression in the corpus cavernosum

As shown in Fig. 1 and Fig. 2, the eNOS mRNA and protein expression was markedly reduced in the corpus cavernosum of

**Table 1: Body weight and blood glucose**

	CON	DM	DM+LYCO(10)	DM+LYCO(30)	DM+LYCO(60)	CON+LYCO(60)
<b>Initial</b>						
Body weight(g)	223.8 ± 9.8	219.1 ± 11.3	224.5 ± 10.2	226.5 ± 9.7	224.4 ± 10.0	220.8 ± 9.5
Blood glucose (mM)	5.45 ± 0.73	5.36 ± 0.67	5.53 ± 0.71	5.48 ± 0.69	5.52 ± 0.80	5.47 ± 0.72
<b>8 weeks</b>						
Body weight(g)	394.3 ± 22.1 <sup>c</sup>	180.1 ± 13.1 <sup>a</sup>	200.8 ± 11.8 <sup>a</sup>	252.8 ± 11.0 <sup>a,c</sup>	303.1 ± 12.7 <sup>a,c</sup>	392.0 ± 25.6 <sup>c</sup>
Blood glucose (mM)	5.61 ± 0.91 <sup>c</sup>	20.83 ± 1.90 <sup>a</sup>	18.60 ± 1.93 <sup>a,b</sup>	14.26 ± 1.55 <sup>a,c</sup>	9.17 ± 1.08 <sup>a,c</sup>	5.50 ± 0.83 <sup>c</sup>

mM = mmol/L. All values are mean ± SD; n = 8 rats in all groups. <sup>a</sup> *P* < 0.01 vs. CON group; <sup>b</sup> *P* < 0.05 vs. DM group; <sup>c</sup> *P* < 0.01 vs. DM group

**Table 2: Evaluation of erectile function**

	CON	DM	DM+LYCO(10)	DM+LYCO(30)	DM+LYCO(60)	CON+LYCO(60)
Baseline ICP (mm Hg)	7.33 ± 0.48	7.31 ± 0.33	7.50 ± 0.42	7.45 ± 0.37	7.43 ± 0.40	7.48 ± 0.51
Peak ICP (mm Hg)	89.13 ± 6.28 <sup>c</sup>	42.50 ± 9.05 <sup>a</sup>	47.95 ± 8.94 <sup>a</sup>	55.73 ± 9.74 <sup>a,b</sup>	64.94 ± 9.17 <sup>a,c</sup>	89.40 ± 6.59 <sup>c</sup>
MAP (mm Hg)	112.8 ± 3.6	106.2 ± 3.9	108.8 ± 4.8	109.3 ± 4.0	106.5 ± 4.7	109.0 ± 5.6
Peak ICP/MAP	0.79 ± 0.03 <sup>c</sup>	0.40 ± 0.08 <sup>a</sup>	0.44 ± 0.09 <sup>a</sup>	0.51 ± 0.07 <sup>a,b</sup>	0.61 ± 0.08 <sup>a,c</sup>	0.82 ± 0.05 <sup>c</sup>

ICP = intracavernous pressure, MAP = systemic arterial pressure. All values are mean ± SD; n = 8 rats in all groups. <sup>a</sup> *P* < 0.01 vs. CON group; <sup>b</sup> *P* < 0.05 vs. DM group; <sup>c</sup> *P* < 0.01 vs. DM group

**Table 3: MDA concentrations and SOD activities**

	CON	DM	DM+LYCO(10)	DM+LYCO(30)	DM+LYCO(60)	CON+LYCO(60)
MDA (nmol/mg protein)	3.13 ± 0.41 <sup>c</sup>	6.17 ± 0.68 <sup>a</sup>	5.63 ± 0.49 <sup>a</sup>	4.96 ± 0.51 <sup>a,c</sup>	4.03 ± 0.48 <sup>b,c</sup>	3.00 ± 0.45 <sup>c</sup>
SOD (U/mg protein)	117.29 ± 10.80 <sup>c</sup>	66.78 ± 5.84 <sup>a</sup>	72.56 ± 4.78 <sup>a</sup>	76.94 ± 5.54 <sup>a</sup>	83.98 ± 7.46 <sup>a,c</sup>	119.19 ± 9.45 <sup>c</sup>

MDA = malondialdehyde, SOD = superoxidase dismutase. All values are mean ± SD; n = 8 rats in all groups. <sup>a</sup> *P* < 0.01 vs. CON group; <sup>b</sup> *P* < 0.05 vs. CON group; <sup>c</sup> *P* < 0.01 vs. DM group

diabetic rats compared to normal controls (*P* < 0.01). Administration of 60 mg/kg lycopene partly increased the mRNA expression of eNOS in corpus cavernosum of diabetic rats (*P* < 0.01, Fig. 1). Besides, administration of 30/60 mg/kg lycopene could also significantly restore the protein expression of eNOS in corpus cavernosum of diabetic rats (*P* < 0.01, Fig. 2).

### 3. Discussion

The present study investigated the possibly ameliorative role of lycopene on diabetic ED in STZ-induced diabetic rats. We demonstrated for the first time that chronic lycopene

administration can partially restore the impaired erectile function by lowering blood glucose and modulating oxidative stress/eNOS signaling cascade in STZ-induced diabetes rats, indicating the promising role of lycopene treatment on diabetic ED.

It is well known that DM is highly associated with an increased risk of ED in male patients. In animal studies, the ICP responding to nerve stimulation is a well accepted parameter to assess erectile function. In the present study, we observed the measurable reductions of the peak ICP and peak ICP/MAP ratio in a classic STZ induced rat model of type-1 DM. This result is accordance with that of many previous studies and confirms the close interaction between DM and ED (Hirata et al. 2009; Richardson et al. 2002; Liu et al. 2010; Yang et al. 2008).

The pathophysiology of diabetic ED is multi-factorial, of which endothelial dysfunction of the penile vascular tree is an important etiology (Bivalacqua et al. 2003). Actually, some mechanisms described for endothelial dysfunction are very likely to have parallels in ED (Maas et al. 2002). A considerable body of evidence implicates oxidative stress as a major etiologic factor in the pathogenesis of diabetic complications including endothelial dysfunction (Hidalgo-Tamola et al. 2008). DM can trigger oxidative stress and promote the production of reactive oxygen species (ROS) (Cai et al. 2000). ROS could contribute to endothelial dysfunction and ED by reacting with nitric oxide (NO) itself or by interfering with NO-mediated signaling cascade (Maas et al. 2002). In the present study, we observed the increased MDA content and reduced SOD activity in the corpus cavernosum of diabetic rats. The presence of oxidative damage and impaired antioxidant capacity is in parallel with the blunted erectile function in diabetic rats, supporting the possible interaction between oxidative stress and ED. Therefore, the antioxidant therapy might represent a potential approach to restore the impaired erectile function in the diabetic condition.

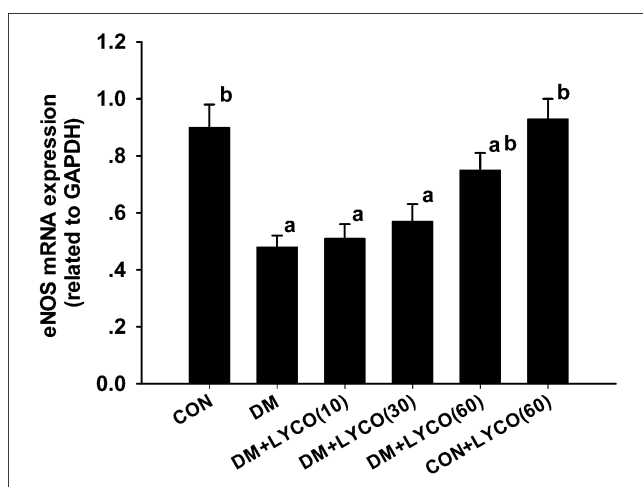


Fig. 1: mRNA expression of eNOS in the corpus cavernosum. All values are mean ± SD; n = 8 rats in all groups. <sup>a</sup> *P* < 0.01 vs. CON group; <sup>b</sup> *P* < 0.01 vs. DM group

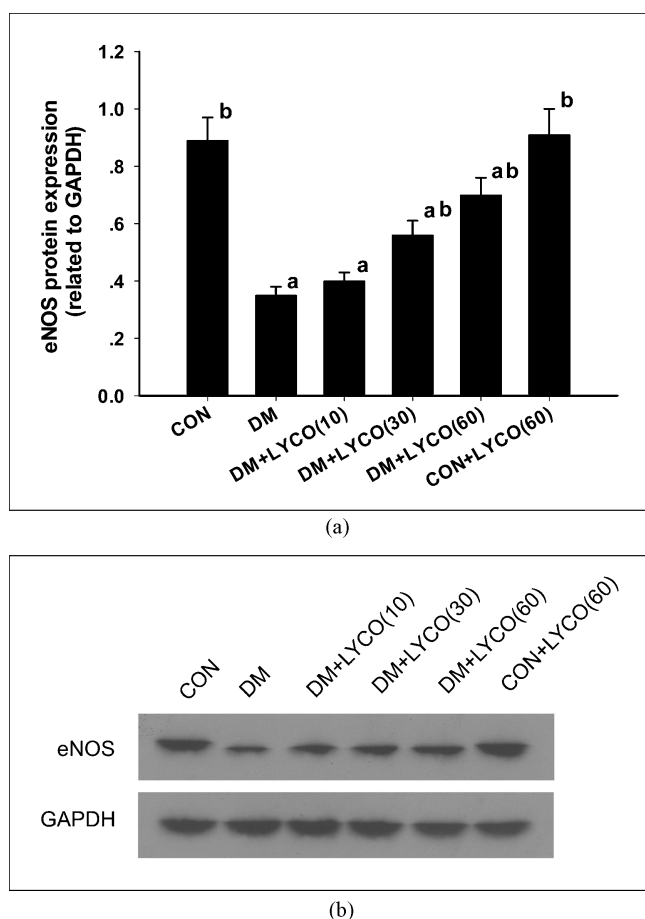


Fig. 2: Protein expression of eNOS in the corpus cavernosum (A) Western blot bands were quantified by densitometry and data were presented as eNOS/GAPDH ratios. (B) Representative western blot bands. All values are mean  $\pm$  SD; n = 8 rats in all groups. <sup>a</sup>  $P < 0.01$  vs. CON group; <sup>b</sup>  $P < 0.01$  vs. DM group

Nutritional supplementation with natural carotenoids is believed to have the ability to inhibit the tissue damage deriving to oxidative stress. As one of such natural carotenoids, lycopene is a powerful broad-range antioxidant that naturally present in tomatoes and tomato products (Di Mascio et al. 1990). Lycopene is an open-chain hydrocarbon containing 11 conjugated and 2 non-conjugated double bonds arranged in a linear array (Ali et al. 2009). The high number of conjugated dienes of lycopene makes it a powerful antioxidant among the natural carotenoids, with a free radical-scavenging ability twice as high as that of  $\beta$ -carotene and 10 times higher than that of  $\alpha$ -tocopherol (Zhu et al. 2011). Lycopene can also reserve SOD by reducing xanthine oxidase (Kuhad et al. 2008). The current study demonstrated that lycopene treatment significantly and dose dependently ameliorated ED in diabetic rats, accompanied by a decrease of the MDA levels and an increase of SOD activities in the corpus cavernosum. These results indicated that lycopene, through its anti-oxidative property, mediated at least a portion of it ameliorative effect on diabetic ED. In addition, the blood glucose concentrations in diabetic rats were significantly reduced by lycopene treatment, which is consistent with many previous studies (Ali et al. 2009; Zhu et al 2011; Kuhad et al. 2008). Therefore, we cannot rule out that lycopene ameliorates diabetic ED partly due to lowering blood glucose concentrations.

The relative importance of endothelial-derived NO from eNOS for maintaining normal endothelial function and erection in the penile has recently been elucidated (Bivalacqua et al. 2003). A decrease in NO bioavailability resulting from decreased

eNOS expression or activity underlies the pathophysiology of cavernous vascular endothelial dysfunction and ED (Zuo et al. 2011). Therefore, it would be reasonable to hypothesize that the reduced expression of eNOS in the corpus cavernosum of diabetic rats observed in the present study may also contribute to diabetic ED. We demonstrated that the eNOS mRNA and protein expression was significantly improved by chronic lycopene treatment in diabetic rats. In a more recent study, Zhang et al. revealed that the antioxidant treatment with quercetin can significantly up-regulate eNOS expression in the corpus cavernosum of diabetic rats (Zhang et al. 2011). These findings are in accordance with the present study and outline the key role of antioxidant treatment with lycopene on the activation of eNOS cascade and the attenuation of ED in diabetic condition.

We are aware of several important limitations to the present study. First, the STZ induced rat model of type-1 DM used in the present study is different from type-2 DM, which is the predominant form of DM in clinical practice. In addition, our findings are still preliminary and might not be directly related to humans.

In conclusion, we revealed the ameliorative effect of long-term chronic lycopene treatment to enhance erectile function in diabetic rats by lowering blood glucose, reducing oxidative stress and up-regulating eNOS expression. These results indicated that lycopene treatment is potentially a new strategy for treating DM-induced ED.

## 4. Experimental

### 4.1. Animals

Male Sprague-Dawley rats weighing 200–250 g were obtained from the experimental animal center of Yanan University. The rats were kept at  $20 \pm 2^\circ\text{C}$  with a 12 h light/dark cycle and were given free access to chow diet and tap water. All procedures were performed according to the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85–23, revised 1996).

### 4.2. Experimental protocol

Diabetes was induced by intravenous injection of STZ (50 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) freshly dissolved in sterile sodium citrate buffer (0.1 M, pH 4.5; Shanghai Sangon Biotech, Shanghai, China). The control rats received citrate buffer only. The rats were considered to be diabetic and used for the study if they had fasting blood glucose  $> 16.7$  mM at three days later after injection of STZ, as detected by the Abbott Optium Xceed system (Abbott Diabetes Care, Alameda, USA). The rats were then randomly divided into 6 groups of 8 animals each: (1) control group (CON, control rats treated with vehicle), (2) diabetic group (DM, diabetic rats treated with vehicle), (3) low/medium/high dose lycopene-treated diabetic group (DM + LYCO(10)/DM + LYCO(30)/DM + LYCO(60), diabetic rats treated with 10 mg/kg, 30 mg/kg and 60 mg/kg lycopene, respectively; North China Pharmaceutical Group Corporation, Shijiazhuang, China) and (4) high lycopene dose-treated control group (LYCO(60), control rats treated with 60 mg/kg lycopene; North China Pharmaceutical Group Corporation, Shijiazhuang, China). Lycopene was dissolved in 0.5 ml corn oil (Sigma-Aldrich, St. Louis, MO, USA) and administered via intragastric intubation for 8 weeks.

### 4.3. Evaluation of erectile function

Erectile function was assessed by measuring peak ICP response to electric stimulation of the cavernous nerve and the ratio of peak ICP/MAP. Under anesthesia with urethane (1.2 g/kg), each rat was placed on a heating pad to maintain body temperature. A PE-50 tube was inserted into the left carotid artery via a midline neck incision to measure the MAP. A lower abdominal midline incision was performed and the pelvic ganglion and cavernous nerves were exposed using a surgical microscope. A stainless steel bipolar electrode connected to an electrical pulse stimulator was placed onto the cavernous nerve. Next, the penile crus were exposed through a sagittal perineal incision. A 25-gauge needle connected to a polyethylene-50 tube filled with heparin (250 U/ml) solution was inserted into the penile crus for measurement of ICP. The stimulus parameters were: current 2 mA, frequency 20 Hz, pulse width 0.2 ms, and duration 40 s. Three electro-stimulations were replicated at intervals of 10 min. The ICP was measured and recorded

with a multi-channel bio-signal collection processing system (RM6240B, Chengdu Instrument Factory, Chengdu, China) Then the rats were sacrificed using pentobarbital (200 mg/kg, i.p.), following which corpus cavernosum was harvested for analysis.

#### 4.4. Measurement of MDA concentrations and SOD activities in the corpus cavernosum

One segment of corpus cavernosum was made into 5% tissue homogenate in ice-cold 0.9% NaCl solution. Then a supernatant was obtained from the tissue homogenate by centrifugation (3000g, 4 °C, 10 min). The MDA concentrations were determined in the form of thiobarbituric acid reactive substance (TBARS) (Ohkawa et al. 1979) using a commercial kit (Jiancheng Bioengineering, Nanjing, China) according to the manufacturer's instructions. Following the commercial kit manual (Jiancheng Bioengineering, Nanjing, China), the SOD activities were determined by monitoring the inhibition of the autoxidation of hydroxylamine (Kono et al. 1978). The MDA concentrations and SOD activities were normalized to the weight of a strip of the corpus cavernosum and expressed as nmol/mg protein and U/mg protein, respectively.

#### 4.5. Quantitative real-time PCR

One segment of corpus cavernosum was homogenized and total RNA was isolated using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription (RT) was performed in a 20 µl reaction system with 4 µg total RNA treated by RNase-free DNase I (TAKARA Biotechnology, Dalian, China). Quantitative real-time RT-PCR was performed using the TAKARA TP800 PCR Thermal Cycler Dice Real Time system (TAKARA Bio Inc, Shiga, Japan). The polymerase chain reaction (PCR) primers and probes for TaqMan analysis were purchased from TAKARA Biotechnology Company (Dalian, China). The primers are as follows (Wang et al. 2009): sense primer 5' GTGGTTTGCCTTGGTCAACCTAATG 3' and anti-sense primer 5' GGACATTTTCGGACTCACATTGCG-3' for eNOS; sense primer 5' CCATGGAGAAGGCTGGG 3' and anti-sense primer 5' CAAAGTTGT-CATGGATGACC 3' for GAPDH. Relative quantitative evaluation of target gene levels was based on standardizing RNA levels by correcting for GAPDH levels in the same sample.

#### 4.6. Western blot analysis

One segment of corpus cavernosum was homogenized in RIPA buffer (Sangon Biotech, Shanghai, China) and centrifuged at 3000 g for 10 min at 4 °C. Protein concentration was quantified using BCA protein assay kit assay kit (Pierce Biotech Inc., Rockford, IL, USA). Protein extracts (50 µg/lane) were separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane and blocked. After incubation at room temperature for 1 h with 5% skimmed milk (Dingguo Biotech, Beijing, China) in Tris-buffered saline (TBS) containing 0.1% Tween 20 (TBST), the membranes were incubated overnight with primary antibody against eNOS (diluted 1:2,000; Abcam, Cambridge, UK) and GAPDH (diluted 1:2,500; Abcam, Cambridge, UK). Subsequently, the nitrocellulose membrane was washed twice for 10 min with TBST and incubated for 2 h with the appropriate diluted horseradish peroxidase (HRP)-conjugated secondary antibody. The immunoreactions were visualized by enhanced chemiluminescence (ECL) system (Pierce Biotech Inc., Rockford, IL, USA) according to the manufacturer's instructions. The protein signals were quantified by scanning densitometry using the PDI Imageware System (Huntington Station, New York, USA). The results from each experimental group were expressed as relative integrated intensity compared with GAPDH.

#### 4.7. Statistical analysis

All data are expressed as means ± SD. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey post hoc test for multiple comparisons. SPSS 13.0 (SPSS Inc, IL, USA) statistical software was used for all statistical analyses.  $P < 0.05$  was considered to be statistically significant.

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