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Effects of lycopene on plasma glucose, insulin levels, oxidative stress, and body weights of streptozotocin-induced diabetic rats

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Aim: To determine possible therapeutic effects of oral lycopene supplementation on plasma insulin levels, lipid peroxidation, blood glucose levels, and the antioxidant defense system of streptozotocin-induced diabetic rats.

Materials and methods: Classical biochemical methods were used to determine plasma malondialdehyde (MDA), nitric oxide (NO), glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) levels. Enzyme-linked immunosorbent assay was used to determine plasma insulin levels and reverse transcriptase-polymerase chain reaction was used to determine the levels of brain antioxidant enzymes (SOD, CAT, and GSH-Px).

Results: It was found that the diabetes-related increase in blood glucose levels was reduced by supplementation of lycopene over an 8-week period. Plasma NO levels and brain tissue GSH levels were meaningfully reduced in the treatment group compared to the diabetic group. In the hemolysate samples, it was determined that the treatment group's SOD, CAT, and GSH-Px activities significantly increased compared to the diabetic group. In the brain tissue homogenates, CAT and SOD activity did not show a significant change, whereas GSH-Px activity was increased in the treatment group compared to the diabetic group. SOD, CAT, and GSH-Px mRNA transcription levels were suppressed in the diabetic group compared to the control, and this suppression was stopped and increases were significantly induced by the supplementation of lycopene.

Conclusion: In this study, the oxidative damage and low insulin levels associated with diabetes were ameliorated with the administration of lycopene. The results of this study indicate that lycopene is an effective nutritional component to alleviate and/or prevent the complications of diabetes, and these findings can be used as a basis for future studies.

Key words: Oxidative stress, lycopene, antioxidant enzymes, mRNA, diabetes

Introduction

Diabetes mellitus is a chronic metabolic disorder and its incidence has been increasing all around the world. Several mechanisms are involved in the pathogenesis of diabetes and its complications. The most commonly accepted cause of diabetes is the oxidative damage that is caused by free radicals (1).

Recent studies showed an increasing use of medicinal plants or their extracts to ameliorate diseases (2,3). Lycopene is commonly found in many foods in nature. In this study, the effects of lycopene for overcoming oxidative damage in diabetes, which plays an important role in the development of diabetes, was investigated. The preventive effects of lycopene were also examined on diabetic disorders such as body weight loss, decreased insulin levels, and decreased plasma glucose levels.

Free radicals play an important role in plasma chemistry, biochemistry, and many other chemical processes within the human physiology (4). Free radical damage could be a reason for many diseases, including diabetes (5). When the number of free radicals increases to the point that they outnumber the antioxidants, they can attack the somatic cells

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and immune system (6). Antioxidants are molecules that neutralize the effects of free radicals by donating an electron to pair with the free radical's unpaired electrons. Healthy people have a balance between free radicals and antioxidants. However, it has been shown that people who have diabetes have higher levels of free radicals, which can cause diabetic complications (7). During diabetes, persistent hyperglycemia causes an increased production of free radicals, especially reactive oxygen species (ROS), for all tissues from glucose autoxidation and protein glycosylation (8,9). Damage of cellular organelles and enzymes and an increase in lipid peroxidation can be caused by high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms (1,10,11).

Carotenoids have protective effects against chronic diseases, which are thought to be caused by damage from free radicals. Carotenoids prevent oxidative damage in biological systems, such as damage to the cell membrane, DNA molecules, lipids, proteins, and other structures in the cell. Lycopene is a nonprovitamin A carotenoid with an open chain structure that has 11 linearly arranged conjugated double bonds. The antioxidant activity of lycopene is somewhat higher than that of β-carotene, but it is not present in as many foods as β-carotene (11). Lycopene is mainly found in red fruits and vegetables, such as watermelons, tomatoes, pink grapes, and apricots (12).

The overall objective of this research project was to investigate the possible antidiabetic and therapeutic effects of lycopene on diabetes in streptozotocin (STZ)-induced diabetic rats. The specific aims of the study were: 1) to investigate the effect of lycopene supplementation on blood glucose concentration, body weight, lipid peroxidation, and plasma insulin levels; 2) to investigate nitric oxide (NO), glutathione (GSH), and malondialdehyde (MDA) levels and the antioxidant enzyme [superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px)] levels in both blood and brain tissue samples; 3) and to investigate the effect of lycopene on the expression of the antioxidant enzyme genes (SOD, CAT, and GSH-Px) that regulate the antioxidative defense mechanisms in brain tissues.

Materials and methods

Animal model and sample preparation

Twenty-four male Wistar rats (n = 6 per group), 2 months of age and weighing between 150 and 250 g, were allocated randomly and equally as control (C), diabetic (D), lycopene-supplemented diabetic (D + L), and lycopene-supplemented control (L) groups. For this study, permission was issued by the ethical committee of Mustafa Kemal University.

After overnight fasting, diabetes was induced in the rats by a single intraperitoneal injection of STZ (Sigma) at a dose of 45 mg/kg. The STZ was dissolved in 0.1 M cold sodium citrate buffer, pH 4.5 (13). The rats were allowed to drink a 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Plasma glucose levels were measured with an Accu-Check Go strip test in a glucometer. Blood sugar levels were measured before and after 48 h of STZ induction. After 48 h of STZ induction, the rats whose blood glucose levels were ≥300 mg/dL were considered as diabetic. The rats of the control and diabetic groups were fed without any supplements except sunflower oil, which was used for dissolving the lycopene, while 4 mg/kg body weight of lycopene was administrated orally, daily, to the diabetic + lycopene group and the lycopene group for 8 weeks. The dose of lycopene that was used in this study was selected on the basis of previous studies (11,14). Plasma glucose levels and the body weights of the animals were measured weekly for the duration of the study.

The lycopene was suspended in sunflower oil and administered to the rats at a dose of 4 mg/kg body weight, once a day for 8 weeks. The control and diabetic rats received the same volume of sunflower oil. The diabetic + lycopene and the lycopene control rats were given the same volume of lycopene. The rats were anesthetized by an intramuscular injection of 50 mg/kg of ketamine, and blood was taken by puncturing the heart ventricle at the end of the experiment. Blood samples were centrifuged at 3000 rpm for 20 min and the plasma was separated. Red blood cells that remained on the bottom of the tubes were washed with a phosphate buffer, pH 7.4, and the samples were then kept at –20 °C until they were analyzed. The hemoglobin concentrations of the erythrocytes were measured using the method described by Drabkin (15).

MDA, CAT, SOD, GSH-Px, and NO assays

The brains were removed from each rat, washed with an ice-cold physiological saline (PBS) buffer of pH 7.0 containing protease inhibitor (Pi) mixture (Sigma), and used for biochemical studies. Tissue samples were homogenized in an ice-cold PBS buffer, pH 7.0, containing a complete Pi mixture. The homogenates were centrifuged at 4 °C at 15,000 rpm for 20 min, and the soluble fraction was retained. The protein concentrations of the supernatants were measured using the method described by Bradford (16), using bovine serum albumin as a standard. The degree of lipid peroxidation was assessed by measuring the MDA levels in the plasma and brain tissue samples (17). CAT enzyme activity in the red blood cells and homogenates was measured using the speed reduction of hydrogen peroxidase (18). Total SOD activity in the homogenates and plasma samples was determined according to the method of Sun et al. (19). The reduced GSH concentrations in the homogenates and plasma samples were determined according to the method of Sedlak and Lindsay (20). The NO concentration in the plasma and brain tissue samples (homogenates) was determined indirectly by measuring the nitrite levels based on the Griess reaction (21). The GSH-Px activity in the erythrocytes and homogenates was measured according to the method of Paglia and Valentine (22). Blood plasma insulin levels were measured with the enzyme-linked immunosorbent assay (ELISA).

mRNA extraction and RT-PCR

In the brain tissues, the mRNA expression levels of the antioxidant enzymes (CAT, SOD, and GSH-Px) were determined by reverse transcriptase-polymerase chain reaction (RT-PCR). The RNA was isolated using TRI Reagent (Sigma). The RNA's purity was then checked by spectrophotometer with 260-nm and 280-nm filters. The RNA having a 260/280 measurement of ≥1.8 was studied. Moreover, to ensure the presence of RNA, the RNA structures were observed under an ultraviolet (UV) light that was run in 1% agarose gel (electrophoresis). From each sample, 1 μg of RNA was taken and complementary DNA (cDNA) was produced using reverse transcriptase. Next, Taq polymerase, buffers, and oligonucleotides (primers) were added to 1-μL cDNA samples. Primers were specific for each PCR and 100 ng per sample was used. The base sequences and their product sizes are given for each primer, GSH-Px (23), CAT, and SOD (24) in Table 1. β-Actin was used as a housekeeping (control gene) primer (25). PCR amplification was done using a thermal cycler. Before the PCR products were captured under the UV light, they were run in 1.5% agarose gel. The digital photos were assessed with the DigiDoc-It image analyzer program. The mRNA transcription levels were determined by performing normalization of the control gene. The data were analyzed using SPSS 9.05 for Windows for one-way ANOVA and post hoc multiple comparison tests.

Results

Beginning from week 5, a significant diabetes-related decrease ($P < 0.05$) in body weight was observed, but lycopene administration had no significant effect

Table 1. The primers used in the PCR and PCR conditions (F: forward primer, R: reverse primer).

Gene	Primers	Product (bp)	PCR program	Cycles
β-Actin	F-CATCGTCACCAACTGGGACGA R-CGTGGCCATCTCTTGCTCGAAG	466	Initial: 95 °C, 10 min / annealing: 95 °C, 1 min – 55 °C, 70 s – 72 °C, 100 s / final: 72 °C, 10 min	35
GSH-Px	F-CTCTCCGCGGTGGCACAGT R-CCACCACCGGGTCGGACATAC	290	Initial: 94 °C, 5 min / annealing: 94 °C, 30 s – 60 °C, 60 s – 72 °C, 30 s / final: 72 °C, 5 min	32
CAT	F-GGCAGCTATGTGAGAGCC R-CTGACGTCCACCCTGACT	116	Initial: 94 °C, 5 min / annealing: 94 °C, 30 s – 55 °C, 30 s – 72 °C, 15 s / final: 72 °C, 5 min	35
SOD	F-GTTCCGAGGCCGCCGCGCGT R-GTCCCCATATTGATGGAC	192	Initial: 94 °C, 5 min / annealing: 94 °C, 30 s – 55 °C, 30 s – 72 °C, 20 s / final: 72 °C, 5 min	35

Table 2. Effects of lycopene supplementation on the change of body weight (g) in rats (mean ± standard error).

Week	Control group	Diabetic group	Diabetic group + lycopene	Lycopene group
1	134.33 ± 2.82	143.60 ± 8.80	140.40 ± 2.99	148.00 ± 3.07$^{¥,\#}$
2	144.67 ± 2.59	143.20 ± 7.42	137.80 ± 1.96	159.67 ± 3.88$^{¥,\#}$
3	146.33 ± 3.02	140.40 ± 7.90	134.00 ± 2.55	163.50 ± 3.18$^{¥,\#}$
4	147.67 ± 3.07	139.60 ± 5.61	135.20 ± 2.03$^{¥}$	170.00 ± 2.86$^{*,\#}$
5	153.83 ± 2.73	139.20 ± 6.72$^{¥}$	133.20 ± 2.58$^{¥}$	170.67 ± 2.75$^{¥,\#}$
6	159.33 ± 2.29	130.20 ± 7.17*	130.20 ± 2.76*	174.50 ± 2.88$^{¥,\#}$
7	161.50 ± 3.02	132.00 ± 7.01*	131.80 ± 2.76*	178.50 ± 2.49$^{¥,\#}$
8	170.00 ± 2.93	137.40 ± 6.02*	135.60 ± 0.68*	184.17 ± 2.55$^{¥,\#}$

$^{*}P < 0.001$, $^{¥}P < 0.05$ compared to the control. $^{\#}P < 0.05$ compared to the STZ-diabetic group.

on body weight loss (Table 2). The blood glucose levels significantly ($P < 0.001$) increased as a result of diabetes in the diabetic group, which was suppressed by the lycopene administration during the 8-week period in the treatment group (Table 3). The plasma and brain tissue GSH levels and the plasma NO levels were increased due to diabetes. The GSH and NO levels in the plasma and the GSH levels in the brain tissue of the treatment group were lower than in the diabetic group (Tables 4 and 5). The ELISA results indicated that diabetes caused a significant decrease in plasma insulin concentrations (Table 5), and this was significantly ($P < 0.001$) reversed by the oral supplementation of lycopene. The lipid peroxidation (MDA) levels were significantly increased in both the brain tissue and the blood samples of the diabetic group compared to the control group. This increase had been significantly ($P < 0.005$) lowered by the lycopene supplementation in the brain tissue samples, but there was no significant effect of lycopene in the blood samples. The SOD, CAT, and GSH-Px activities in the erythrocytes were increased in the diabetic + lycopene group (Table 6). The GSH-Px, SOD, and CAT activities were significantly ($P < 0.005$) increased in the brain tissues of the diabetic group compared to the control, but the lycopene administration did not have any significant effect in lowering the activities of the enzymes to the levels of the control group.

Table 3. Plasma glucose concentrations (mg/dL; mean ± standard error).

Day	Control group	Diabetic group	Diabetic group + lycopene	Lycopene group
1	129.3 ± 8.7	113.8 ± 4.7	112.2 ± 5.0	133.5 ± 2.8
2	137.2 ± 7.5	448.2 ± 18.7*	469.4 ± 12.0	127.3 ± 35.9
9	89.3 ± 8.4	384.8 ± 28.2*	382.4 ± 20.5^{f}	90.0 ± 2.8
16	107.7 ± 12.6	432.0 ± 46.6*	408.4 ± 36.3^{f}	114.0 ± 6.7
23	115.2 ± 5.6	407.2 ± 58.5*	336.2 ± 58.0^{f}	118.0 ± 5.6
30	94.8 ± 4.5	398.2 ± 50.6*	251.4 ± 27.0^{f}	99.1 ± 5.8
37	103.0 ± 7.2	386.4 ± 46.7*	244.2 ± 15.5^{f}	104.0 ± 5.2
44	97.3 ± 4.2	408.4 ± 28.4*	274.8 ± 23.5^{f}	97.0 ± 3.8
51	81.8 ± 16.2	408.2 ± 52.2*	249.8 ± 35.8$^{\#}$	96.3 ± 5.0
58	103.5 ± 3.3	424.2 ± 37.5*	331.2 ± 38.6^{f}	94.7 ± 3.5

$^{*}P < 0.001$ compared to the control. $^{\#}P < 0.05$, $^{f}P < 0.001$ compared to the STZ-diabetic group.

Table 4. Effect of lycopene administration on the glutathione peroxidase (GPx), glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) in brain tissue samples (mean ± standard error).

	Control	Diabetic group	Diabetic group + lycopene	Lycopene group
GSH-Px (U/mg protein)	0.94 ± 0.039	1.37 ± 0.045*	1.77 ± 0.061^{f}	1.17 ± 0.037*,#
GSH (nmol/g protein)	477.85 ± 7.69	496.50 ± 3.13*	428.79 ± 4.93^{f}	488.97 ± 3.78#
MDA (nmol/g protein)	4.9 ± 0.14	6.93 ± 0.2*	5.17 ± 0.085^{f}	7.02 ± 0.2*, f
SOD (U/mg protein)	2.39 ± 0.14	2.90 ± 0.005*	2.94 ± 0.11#	2.52 ± 0.11*,#
CAT (k/g protein)	0.17 ± 0.012	0.25 ± 0.014*	0.26 ± 0.012#	0.22 ± 0.016*,#

*P < 0.005 compared to the control. #P < 0.05, fP < 0.005 compared to the STZ-diabetic group.

The β-actin gene is a constitutive gene that is used for the normalization (in the basis of the band densities) of mRNA transcription levels. In the RT-PCR analyses, it was found that the CAT gene mRNA transcription levels were suppressed by 7.6% in the diabetic group compared to the control group; they were stimulated in the lycopene-supplemented diabetic group by 187.2% (P < 0.05) compared to the diabetic group (Figure). The mRNA transcription levels of the GSH-Px gene were suppressed by 16.8% in the diabetic group compared to the control group, and they were stimulated by 333.5% (P < 0.05) in the D + L group compared to the diabetic group (Figure). Similarly, the SOD mRNA transcription levels were suppressed by 12.4% in the diabetic group compared to the control, and they were stimulated by 170.1% (P < 0.05) in the diabetic + lycopene group compared to the diabetic group (Figure).

Discussion

This study indicated that diabetes caused a significant decrease in body weight; however, there were no significant effects of lycopene on body weight loss (Table 2). These findings are consistent with the studies of Mellert et al. (26), Sindhu et al. (27), and Duzguner et al. (11). Lipolysis and gluconeogenesis are the 2 main reasons for weight loss during diabetes (27,28). Diabetes-increased blood glucose levels were suppressed by the lycopene during the 8-week period (Table 3). Similarly, Duzguner et al. (11) determined that during 3 weeks of lycopene administration, the blood glucose levels in diabetic rats were decreased. Diabetes caused a significant decrease in the plasma insulin concentration (Table 5) due to the damage caused by the cytotoxic effects of STZ in the pancreatic β cells. This was reversed by the oral administration of lycopene. It can be concluded that the induction of free radicals caused a dysfunction of pancreatic β cells and resulted in the reduction of insulin secretion. Lycopene decreases oxidative stress and the damage on essential components of the cells such as lipids, proteins, and DNA by capturing and reducing free radicals (29,30).

Lipid peroxidation (MDA) levels were significantly increased by diabetes. This finding is in agreement

Table 5. Effect of lycopene administration on plasma MDA, NO, GSH, and insulin in blood/serum samples (mean ± standard error).

	Control	Diabetic group	Diabetic group + lycopene	Lycopene group
MDA (μmol/mL)	3.16 ± 0.17	19.38 ± 0.55*	20.22 ± 0.45^{f}	23.79 ± 0.52*,#
NO (μmol/L)	37.94 ± 0.75	61.32 ± 0.90*	53.45 ± 1.20^{f}	66.70 ± 0.96*,#
GSH (nmol/mL)	652.86 ± 8.67	664.14 ± 9.34¥	654.47 ± 11.18#	703.91 ± 5.88¥,#
Insulin (ng/mL)	4.28 ± 0.83	0.12 ± 0.02*	1.91 ± 0.22^{f}	4.16 ± 0.71¥,f

*P < 0.001, ¥P < 0.05 compared to the control. fP < 0.001, #P < 0.05 compared to the STZ-diabetic group.

Table 6. Effect of lycopene administration on red blood cell GPx, SOD, and CAT (mean ± standard error).

	Control	Diabetic group	Diabetic group + lycopene	Lycopene group
CAT (k/g Hb)	4.21 ± 0.61	8.89 ± 0.64*	11.94 ± 0.92[f]	8.90 ± 0.69[#]
GSH-Px (U/g Hb)	0.83 ± 0.016	0.68 ± 0.023[¥]	1.03 ± 0.054[f]	0.94 ± 0.035[f]
SOD (U/g Hb)	118.74 ± 6.89	215.58 ± 8.23*	248.42 ± 5.69[f]	150.55 ± 4.39[f]

*P < 0.001, [¥]P < 0.01 compared to the control. [f]P < 0.005, [#]P < 0.05 compared to the STZ-diabetic group.

with the findings of Duzguner et al. (11). Dixon et al. (31) noted that carotenoids can significantly decrease plasma MDA levels. This finding is not in agreement with the results of the present study. It was determined that lycopene caused a significant decrease of MDA levels in the brain tissues, but not in the plasma (Tables 4 and 5). The increase in NO formation may play a role in the damage of the β cells during the development of diabetes (32). Seven et al. (33) determined that the plasma GSH concentration of STZ-induced diabetic rats increased compared to the control group. Similarly, this study showed that both the NO and GSH levels in the plasma and the GSH levels in brain tissue of the diabetic group were elevated compared to the control group. These increases were lowered in the treatment group compared to the diabetic group (Tables 4 and 5). The SOD activities showed no alterations in the plasma samples or the brain tissue samples among diabetic and treatment groups. This finding is consistent with that of Sindhu et al. (27). Additionally, Sindhu et al. (27) suggested that hyperglycemia is able to generate ROS, which can either inhibit or have no effect on antioxidant enzyme activities (i.e. SOD). The mRNA transcription levels of the antioxidant enzymes (SOD, CAT, and GSH-Px) were down-regulated by diabetes, and all of them were up-regulated by lycopene administration.

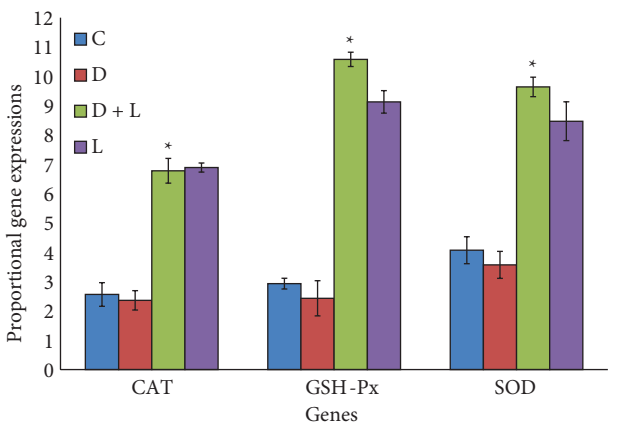

Figure. Proportional gene expression levels of the CAT, GSH-Px, and SOD genes obtained from brain tissues (normalization process based on the average density values of the PCR product bands that were obtained on agarose gel and the density of the target gene, i.e. SOD, CAT, and GSH-Px, proportioned to the density of the control gene, β-actin). *P < 0.05 compared with the STZ-diabetic group. C: control group, D: diabetic group, D + L: lycopene-supplemented diabetic group, and L: lycopene-supplemented control group.

It was demonstrated that diabetes mellitus caused oxidative damage and lipid peroxidation in the brain tissue and decreased the plasma insulin levels, and these effects were significantly ameliorated by the lycopene administrations. The results suggested that lycopene has a significant role in silencing diabetic disorders by its antioxidative effects.

It is concluded that lycopene treatment should be considered in the treatment of diabetic complications and hyperglycemia. Lycopene supplementation can be beneficial for humans in order to reduce the harmful effects of diabetes, such as oxidative damage and decreased plasma insulin concentrations and blood glucose levels.

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