RESEARCH ARTICLE

The Effect of Lycopene Treatment on Oxidative DNA Damage of Experimental Diabetic Rats

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Abstract:

Objective:

Lycopene is a carotenoid with anti-inflammatory and antioxidant properties. The aim of this study was to determine the effects of lycopene on oxidative DNA damage levels in experimental diabetic rats.

Subjects and Methods:

Four experimental groups, each consisting of 7 rats, were prepared as Controls, Diabetes (D), Lycopene-treated diabetes (DL) and Lycopene (L). STZ (45 mg/kg) was administered to the diabetic groups intraperitoneally in a single dose. Lycopene was administered to the L and DL groups (10 mg lycopene/kg/day). The test procedure continued for four weeks. To understand the occurrence of diabetic conditions, serum glucose and HbA1c% in the whole blood were determined. The 8-OHdG levels, a marker of oxidative DNA damage, were determined in the blood serum.

Results:

Blood glucose and HbA1c% were higher in the DL group than in the control group and L group ($p < 0.05$) and lower in the D group ($p < 0.05$). 8-OHdG levels were higher in D group than the other groups ($p < 0.05$) while 8-OHdG levels in DL group were lower than D group ($p < 0.05$) and approximated to the control group.

Conclusion:

It can be suggested that lycopene may be described as a protective agent to prevent oxidative DNA damage originated from diabetes.

1. INTRODUCTION

Diabetes mellitus is a widespread disease with high morbidity and early mortality rates leading to vascular, renal, retinal or neuropathic disorders and acute metabolic complications due to prolonged hyperglycemia [1].

All the changes in the molecular integrity of the genetic material with the effect of exogenous or endogenous factors are referred as "DNA damage". Reactive oxygen species such as superoxide and hydroxyl radicals create various lesions which include 8-oxoguanine. It is known that oxidative stress induces oxidative DNA damage, which may lead to mutagenesis [2 - 4].

8-OHdG is the most commonly known oxidative DNA damage biomarker formed through free radicals and the

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direct photodynamic action. The increase in the amount of 8-OHdG depends on several pathological situations

including cancer, diabetes and hypertension as well as the aging process. 8-OHdG can be found in tissue, serum, urine and other biological materials [5 - 7]. The emergence of 8-OHdG as a measure of DNA oxidation has resulted in a new follow-up in the evaluation of oxidative DNA damage as frequently observed especially in diabetes, cardiovascular diseases and cancer [8].

Lipid peroxidation is known to increase in patients with diabetes. Oxidative stress plays an important role in Type 2 diabetes and pathogenesis of its complications. The studies conducted for this purpose reported that 8-OHdG is significant for the measurement of biomarkers such as malondialdehyde (MDA) and determining the status of oxidative stress in organisms [9].

Lycopene is thought to have a potential role as an effective antioxidant in the prevention of chronic diseases associated with oxidative stress, and thus attracts a serious scientific interest [10, 11]. Lycopene is a pigment belonging to the carotenoid family found naturally in many fruits and vegetables, especially in tomatoes and tomato products [12, 13]. In patients with Type 2 diabetes, consumption of tomato juice significantly reduced oxidized 'bad' cholesterol and significantly increased plasma lycopene levels [14].

This study has been designed to investigate the significant role of lycopene implementation on experimental diabetic rats, and the possible role of lycopene in the prevention of the complications of diabetes through observing oxidative DNA damage measurement of 8-OHdG levels.

2. MATERIALS AND METHODS

In this study, 28 male Wistar-Albino male rats weighing 200-250 g were used as materials. Rats were kept in cages in dark/light at a temperature of $22\pm2°C$ for 12 hours with permanent food and fresh water throughout the study (4 weeks). Experiments were conducted under the supervision of Van Yuzuncu Yil University Animal Experiments Local Ethics Committee.

The rats were randomly divided to control (C), diabetes (D), diabetes+lycopene (DL) and lycopene (L) groups with each group including 7 rats.

Control group: Only one dose was administered as Physiological serum was administered just one dose (i.p.).

Diabetes group (D): 45 mg/kg single dose STZ (Sigma, USA) prepared in pH 4.5 citrate buffer was applied (i.p.). After 72 hours, blood samples were taken from the tail vein and glucose levels were measured with the PlusMED Accuro (Istanbul, Turkey) brand glucose meter. The rats with a blood glucose of 270 mg/dl and above were considered diabetic and included in the study.

Diabetes+lycopene group (DL): Blood glucose levels of 270 mg/dl and above were determined with a similar treatment in the diabetes group. Lycopene (redivivo^{TM} 10% FS, DSM, Nutritional Products Ltd., Basel, Switzerland) was dissolved orally in corn oil and administered orally at a dose of 10 mg/kg/day for 28 days.

Lycopene group (L): Lycopene was dissolved in corn oil, administered orally at a dose of 10 mg/kg/day for 28 days.

At the end of the 4-week trial, blood samples were taken from the left ventricle of the heart under ketamine anesthesia. In blood samples taken from the tail vein, glucose levels were determined, and HbA1c levels were determined in a blood serum with a commercial kit (Roche Diagnostics GmbH, D-68298 Mannheim, Germany) and an autoanalyzer (Hitachi-911, Roche Diagnostics, Indianapolis, IN). The oxidative DNA damage marker 8-OHdG was measured by EIA kit (Catalog Number: ADI-EKS-3501, Enzo Life Science) with ELISA (Anthus 20 rt brand, Austria).

The data from control and experimental groups were analyzed with a One-Way Variance analysis and the Post Hoc Tests (equal variances not assumed) was applied for multiple comparisons. Differences were considered significant at $p<0.05$.

3. RESULTS AND DISCUSSION

The results obtained are summarized in Table **1**.

Table 1. The results obtained for this study.

Parameters	Control group (S±SE)	Diabetes group (S±SE)	Diabetes+Lycopene group (S±SE)	Lycopene group (S±SE)
Glucose (mg/dl)	157.29±7.94a	516.71±29.38b	422.02±21.68c	147.57±9.21a
%HbA1c	1.53±0.03a	6.12±0.18b	3.99±0.08c	1.88±0.19a
8-OHdG (ng/mL)	37.45±1.10a	39.24±1.64b	37.76±1.52a	36.67±1.01a

Data were expressed as Mean±SE, Differences were considered significant at $P<0.05$, There are no differences between groups with same letter.

The highest blood glucose and HbA1c levels were determined in experimental diabetic (D) group ($p<0.05$), as expected. These diabetic markers in DL group were significantly higher than the control group and lycopene (L) group ($p<0.05$), but significantly lower than diabetic group ($p<0.05$). There was a statistically significant difference between control and L groups.

The oxidative DNA damage was the highest ($p<0.05$) in D group. No statistically significant difference was determined in 8-OHdG in control and L groups. 8-OHdG levels significantly decreased in DL group (following lycopene implementation) compared to the D group, and no significant difference was observed compared to control and L groups ($p>0.05$).

The increase of the prevalence of diabetes with morbidity and mortality requires additional development of preventive and therapeutic strategies. As an alternative to existing methods, new biomarkers are required to be used in the treatment, control and the determination of the risk. An indicator of intracellular oxidative stress which can be used as a potentially valuable biological marker in diabetes is 8-OHdG, a DNA oxidation product [15].

Experimental and epidemiological studies conducted recently reported that regular consumption of carotenoids (including lycopene) rich in fruits and vegetables has a significant role in the prevention of chronic diseases caused by oxidative stress [10, 13, 14].

Serum carotenoids including lycopene are closely related to Type 2 diabetes and decrease linearly in conditions that impair glucose metabolism [14]. In a study of serum lycopene levels in diabetic patients, Li *et al*. [10] reported lower lycopene levels in a diabetic group than the control group and a negative correlation between HbA1c and serum lycopene levels. Some researchers report that the beneficial effect of lycopene in the treatment and prophylaxis of diabetic complications is achieved by increasing the antioxidant capacity [10, 14]. In a study on STZ-induced experimental diabetic rats, Ali and Agha [16] reported hypoglycemic, hypolipidemic and antioxidant effects of lycopene at different doses. Kuhad and Chopra [17] identified the significance of lycopene administration in experimental diabetic rats as adjuvant therapy in the treatment of diabetic neuropathy. In our laboratory, a project on the effects of lycopene on pro-oxidant/total antioxidant status in diabetic rats using the same experimental method [18], lipid peroxidation increased in the diabetic group and total antioxidant status decreased compared to the control and lycopene groups ($p<0,001$). In the present study, blood glucose and HbA1c% levels were the highest ($p<0.05$) in experimental diabetic group and decreased significantly in diabetes+lycopene group ($p<0.05$), however, were still higher ($p<0.05$) compared to control and lycopene-treated groups. There was no statistically significant difference between control and lycopene groups.

The data obtained from the evaluation of the blood glucose and HbA1c% levels prove that lycopene implementation in experimental diabetic rats may have beneficial effects on impaired glucose metabolism. In fact, the data suggesting that lycopene implementation in the treatment and prophylaxis of diabetic complications is effective by increasing the antioxidant capacity supports this idea [10, 16].

As a result of the statistical evaluation of 8-OHdG levels obtained in this study, oxidative DNA damage was observed the highest ($p<0.05$) in the experimental diabetic group. There was no statistically significant difference in 8-OHdG in control and lycopene groups. 8-OHdG levels in diabetes+lycopene group significantly ($p<0.05$) decreased following the lycopene implementation compared to diabetic group and approximated to control and lycopene-treated groups.

According to some researchers, DNA damage in diabetic individuals increases almost three times compared to the controls [19, 20]. In our study, significantly higher 8-OHdG levels in diabetes group than the other experimental groups were consistent with the literature. Lycopene as an antioxidant increases the total antioxidant potential and decreases oxidative damage by catching reactive oxygen species. Furthermore, supplemental lycopene regulates gene functions

and intracellular communication, stimulates hormones and the immune response or regulates the metabolism, thus reduces the risk of chronic disease [21, 22].

Many studies investigating the effects of lycopene implementation on the oxidative DNA damage reported that there were positive effects of lycopene on DNA damage [23]. Carotenoids can inhibit DNA damage in experimental animals and humans, which is closely related to the dosing [24, 25]. Lycopene implementation has been determined to decrease oxidative DNA degradation and urinary 8-OHdG levels [26], as by both completely inhibiting the creation of Comet and reducing 8-OHdG levels [27]. Moreover, in this study, although 8-OHdG levels in the diabetic group have increased substantially than all other experimental groups ($p < 0.05$), it has been determined that 8-OHdG levels in DL group were considerably lower than diabetes. 8-OHdG levels being significantly lower in lycopene-treated diabetic group (DL) and being close to the control and lycopene groups was regarded as a data supporting the positive effects of lycopene reducing the DNA damage. As a result, although blood glucose and HbA1c% levels in lycopene-treated diabetic group (DL) have increased compared to the control and lycopene-treated groups, it was determined significantly lower than the diabetic group. The decrease in glucose and HbA1 levels after lycopene treatment may be an evidence for restoring the islet cells damaged by streptozotocin, and likewise, 8-OHdG levels can be an indicator for prevention by lycopene.

CONCLUSION

Although 8-OHdG levels significantly increased in diabetic group than in the other experimental groups, lycopene implementation has beneficial effect on this level to the control and lycopene groups. It is thought to have beneficial effects for the protection of genetic material in diabetic individuals and hence for the treatment and prevention of complications due to diabetes. 8-OHdG levels in the lycopene group and the control group were considered as a benchmark supporting the fact that the lycopene dose used is within reliable limits for this study. This study can be considered as a preliminary study for future studies for different doses, times and other oxidation parameters can be used to explore the possible role of carotenoids in the pathogenesis, treatment and protection of insulin resistance and diabetes.

FUNDING

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Experiments were conducted under the supervision of Van Yuzuncu Yil University Animal Experiments Local Ethics Committee.

HUMAN AND ANIMAL RIGHTS

No humans were involved in this study. The reported experiments were in accordance with the standards set forth in the 8th Edition of Guide for the Care and Use of Laboratory Animals (http:// grants.nih.gov/grants/olaw/Guide-for-thecare-and-use-of-laboratory-animals.pdf).

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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