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ORIGINAL ARTICLE

In vitro Evaluation of the Effects of Lycopene on Caspase System and Oxidative DNA Damage in High-Glucose Condition

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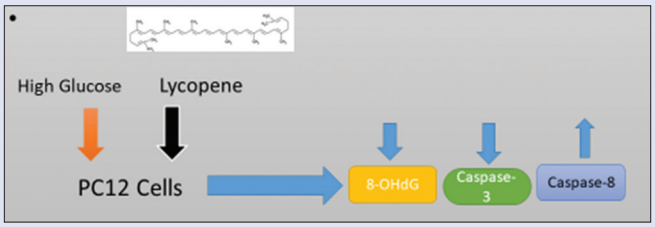
ABSTRACT

Aim and Background: The present study was planned to investigate the effects of lycopene, on the caspase-dependent apoptosis in high-dose glucose (HG)-treated PC12 cell line. PC12 cells were cultured *in vitro*. **Materials and Methods:** HG was prepared as G (250 mM), and lycopene was prepared as L1 (10 μM), L2 (20 μM), and L3 (40 μM). After 6 h of incubation, the cells were exposed to trypsin, and the samples were obtained with freeze/thaw method. Caspase 3, 8, 9; 8-hydroxy-2-deoxyguanosine (8-OHdG); and M30 were determined (enzyme-linked immunosorbent assay). **Results:** 8-OHdG increased in L3 ($P \leq 0.001$), whereas L1 caused a decrease in HG group ($P \leq 0.001$). Caspase-3 decreased significantly in L1, L2, and L3G compared to control ($P \leq 0.001$) group. Caspase-8 increased significantly in L1, L1G, L2G, and all L3 glucose groups ($P \leq 0.001$). There was no difference for Caspase-9. M30 was not affected by L and HG, which decreased significantly ($P \leq 0.001$). **Conclusion:** As a result, it was determined that, when PC12 cell line was treated with HG, lycopene application had effects on caspase enzymes and DNA damage.

Key words: Caspases, high glucose, *in vitro*, lycopene, oxidative DNA damage

SUMMARY

- Different doses of lycopene application on the high-glucose condition have reducing effect on oxidative DNA damage, caspase 3, and M30 which depends on apoptosis.



Abbreviations used: 8-OHdG: 8-hydroxy-2-deoxyguanosine; HG: High glucose; L1: Lycopene 10 μM; L1G: Lycopene 10 μM + 250 μM; L2: Lycopene 20 μM; L2G: Lycopene 20 μM + 250 μM; L3: Lycopene 40 μM; L3G: Lycopene 40 μM + 250 μM; M30: A general marker of apoptosis; MTT: 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide.

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INTRODUCTION

Due to the prolonged exposure of the organs to diabetes mellitus (DM)-induced hyperglycemia, carbohydrate, protein, and lipid metabolisms are impaired. In DM, there are complications that develop over time, especially due to oxidative stress. The most important goal in the treatment and prevention of diabetes is removing the hyperglycemia symptoms and preventing long-term complications. Scientific studies on traditional and herbal treatments are conducted in addition to hypoglycemic medicine and insulin treatment.[1-5]

Lycopene is a pigment of the carotenoid family found naturally in vegetables and fruits and the origin of the red color in tomatoes. There are studies that demonstrated antioxidant and hypoglycemic properties of lycopene. Hypoglycemic effects could be attributed to several mechanisms such as increasing the physical antioxidant capacity, stimulating insulin release, recovering the β-cell damage, and improving the action of insulin.[6-11] There are studies that demonstrated positive results in preventing hyperglycemia and complications by lycopene administration in experimental diabetes.[5,12,13]

Apoptosis, which is defined as programmed cell death, plays an active role in several pathological and physiological events, mainly in cellular generation–destruction. The cell in which the DNA damage and mutation occurs is removed by apoptosis. Caspase enzymes, which play a role in apoptosis mechanism, enable the cell to disappear by apoptosis.[14-16]

The present study was planned to investigate the effects of lycopene, an antioxidant carotenoid, on caspase-dependent apoptosis in the high amounts of glucose-treated PC12 cell lines.

MATERIALS AND METHODS

Cell material

In the present study, PC12 cells constructed with neuroendocrine tumor cells formed in the medullary region of the rat adrenal gland were used. The PC12 cell line is a tumoral line that develops from chromaffin cells with neuroendocrine properties. PC12 cells are commonly used to investigate neurotoxicity, neuronal repair, and the stages of neuroprotective process. PC12 cells are beneficial in distinguishing agents that allow longer neuronal lifecycle.[17,18]

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Preparation of the cells

The PC12 cells were cultured in *in vitro* conditions with regular passages of two to three times a week. Cells were incubated in RPMI 1640 culture medium that included 5% FBS, 20% 10 horse serum, 1% L-glutamine, 1% penicillin/streptomycin, and 0.0125% gentamicin in a humidified medium with 5% CO_2 and 95% air at 37°C.

Cytotoxicity (3-[4,5-Dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide cell viability test)

Cells that were allowed to propagate under adequate conditions were treated with trypsin-ethylenediaminetetraacetic acid and removed from the flask base when they coated 80% of the flask surface. The cells were counted three times with thoma slides and seeded in 96-well culture dishes (2000 cells per well) for the MTT cell viability test. They were then incubated for 24 h at 37°C in a CO_2 incubator. After the incubation, the medium on the cells was removed. Lycopene and glucose that were prepared in different concentrations were treated at the rate of 100 µl per well in the dose that was determined for IC_{50} value, and a dose was divided into three wells over the cells. After 6 h, 10 µl MTT solution was added to each well.

The culture dishes were incubated in a CO_2 incubator at 37°C for 4 h to convert MTT stain into water-insoluble formazan crystals. To dissolve the formazan crystals formed by living cells, 100 µl MTT lysis solution was added to each well. Pipetting was conducted to dissolve the formazan crystals completely. Finally, the optical densities of the cells were read at 570 nm in the enzyme-linked immunosorbent assay (ELISA) instrument. Control cell viability that was not treated with the test substance was accepted as 100%, and the test cell viability rates were expressed as percentages.

Agent applications

Cells were cultured in poly-L-lysine-coated 12-well plates with 350 µl of medium suspension (500,000 cells per well). The cells were allowed to adhere for 24 h, and then the medium on the top was removed. Concentrations of high glucose concentration in 250 mM and 10 (L1), 20 (L2), and 40 µM (L3) serum-free medium lycopene concentrations were combined, and the doses were applied to a total of 16 wells [Table 1].

Table 1: Experimental groups

Groups	
Control	
L1	10 µM Lycopene
L2	20 µM Lycopene
L3	40 µM Lycopene
Glucose	250 mM Glucose
L1G2	10 µM Lycopene - 250 mM Glucose
L2G2	20 µM Lycopene - 250 mM Glucose
L3G2	40 µM Lycopene - 250 mM Glucose

After 6 h, lysis was conducted with the freeze-thaw method, and the suspension was centrifuged at 3000 rpm for 20 min.

Biochemical analyses

Analysis of caspase 3, 8, and 9 was conducted in sterile tubes with the secreted components of the surface cell culture. After centrifuging (2000–3000 rpm) for about 20 min, the supernatant collected on the surface was carefully collected. Approximately 1 million cells/ml were used to examine the cellular components. Phosphate-buffered saline (pH 7.2–7.4) was used to adjust the cell suspension density. To reveal the cellular components, the freeze-thaw cycle was repeated and centrifuged (2000–3000 RPM) for approximately 20 min.

Oxidative DNA damage (8-hydroxy-2-deoxyguanosine [8-OHdG]) levels were determined with DNA Damage ELISA kit (EKS-350, Enzo Life Sciences (ELS) AG, Lausen, Switzerland).

Statistical analysis

Descriptive statistics for the studied properties were expressed as median, mean, standard deviation, and minimum and maximum values. Kruskal–Wallis test was used to determine whether there was any difference between the groups based on these properties. Dunnet's multiple comparison test was used to identify different groups. The statistical significance level was accepted as 5%, and SPSS Version 22.0 (IBM Türk Ltd, Istanbul, Turkey) statistical software was used for calculations.

RESULTS

The difference between group averages indicated with different letters was significant.

It was determined that the 8-OHdG concentrations decreased in all lycopene groups plus high-dose glucose (HG) (L1G, L2G, and L3G) ($P \leq 0.001$) [Table 2].

Analysis of caspase 3 demonstrated that there was a significant decrease in L1, L2, and L3G groups when compared to the control [Table 2].

Significant increases were observed in caspase 8 in L1, L1G, L2G, and L3G groups compared to control. The lowest caspase 8 was observed in the G group compared to other glucose groups [Table 2].

Direct and cross L and glucose applications did not lead to any variations in caspase 9 enzyme, and M30 protein levels were not affected by lycopene and HG treatment [Table 2].

DISCUSSION

In addition to the pharmacological treatments available in DM treatment, more individualized methods with adverse effects are being investigated. The plants are among the most frequently researched material in the field due to their active ingredients. Previous studies demonstrated that lycopene protects the cells against oxidative DNA damage and reactive oxygen species (ROS).[5,7,9]

Table 2: Biochemical results of groups

Groups	8-OHdG (nm/mg)	Caspase 3 (nm/mg)	Caspase 8 (nm/mg)	Caspase 9 (nm/mg)	M30 concentrations (nm/mg)
Control	1.439±0.107^{a}	1.551±0.265^{a}	1.147±0.056^{a}	1.679±0.078	1.718±0.072^{a}
HG	1.402±0.037^{a}	1.586±0.118^{a}	1.144±0.155^{a}	1.609±0.124	1.466±0.192^{b}
L1	1.355±0.047^{a}	1.172±0.188^{b}	1.409±0.086^{c}	1.483±0.082	1.627±0.091^{a}
L2	1.349±0.021^{a}	0.692±0.368^{c}	1.207±0.138^{a,b}	1.514±0.152	1.652±0.136^{a}
L3	1.597±0.086^{a}	1.486±0.038^{a}	1.315±0.081^{b,c}	1.581±0.063	1.641±0.038^{a}
L1G	1.370±0.111^{b}	1.641±0.018^{a}	1.516±0.183^{c}	1.607±0.024	1.643±0.289^{a}
L2G	1.125±0.022^{c}	1.559±0.107^{a}	1.521±0.08^{c}	1.659±0.112	1.648±0.049^{a}
L3G	1.207±0.048^{c}	1.276±528^{b}	1.475±0.094^{c}	1.625±0.073	1.420±0.401^{b}

The difference between group averages indicated with different letters is significant. HG: High glucose; 8-OHdG: 8-hydroxy-2'-deoxyguanosine

Lycopene can protect kidney cells in experimental diabetes by inhibiting the nuclear factor-κB signaling pathway against inflammation and alleviating oxidative stress.[19]

The metabolic stress induced by glucose auto-oxidation, advanced glycosylation, end-product formation, and hyperglycemia is the cause of oxidative stress in diabetic individuals. Oxidative stress is the most important cause of DNA damage. 8-OHdG is known as an oxidative DNA damage marker and is the most commonly observed and the best known marker formed in the DNA by endogenous or exogenous ROS, which is produced during normal oxidative metabolism. Increase in the amount of 8-OHdG, which demonstrates oxidative DNA damage, occurs due to the destruction of nuclear and mitochondrial DNA by free radicals.[20,21]

8-OHdG, a product of DNA oxidation, is also an indicator of intracellular oxidative stress which could be used as a potentially valuable biomarker in HG environments, for example, in diabetes.[22] In the present study, it was determined that 8-OHdG concentrations were reduced in all HG groups that were treated with low-dose lycopene and in medium- and high-dose lycopene groups.

These are the useful models in cell signaling. The PC12 cell line is an important model in neurobiological and neurochemical studies and the investigation of several signals.[23,24] It was determined that oxidative DNA damage did not change significantly due to HG effect in this cell line, but significantly decreased in lycopene + HG group when compared to the control.

In this study, it was observed that the application of HG treatment in the PC12 cell line significantly affected caspase 3 and 8 in the apoptotic pathway and after lycopene was added, caspase 3 enzyme did not demonstrate a significant difference when compared to the control. Caspase 8 enzyme generally increased in lycopene + HG groups. M30 levels, a general marker of apoptosis, were lower only in highest lycopene + HG groups when compared to the control group.

Studies on the significance of lycopene as a protective agent in experimental diabetic patients reported that apoptosis proliferated in other cell lines where lycopene was treated against HG-induced apoptosis, and treatment of different doses of lycopene resulted in an increase in proliferation in all doses when compared to the control.[25,26] Lycopene can regulate blood glucose, insulin, and insulin intolerance by inhibiting STAT3 signal and Srebp-1c gene expression.[27] It was determined that lycopene had a protective effect on glucose oxidase-based oxidative stress-induced apoptosis in pancreatic acinar AR42J cells.[28]

Studies on the anti-apoptotic effects of lycopene on oxidative stress caused by certain physiological conditions were also conducted.[29-38] It was considered that lycopene has a protective effect through p53 inhibition, reduction in caspase 3, inhibition of the apoptotic signal pathway by the induction of the increase in Bcl-2 and Bax expressions, and the resulting increase in the antioxidant capacity.[35]

ROS threatens continuous integrity and proper functioning of the cellular DNA. Carotenes, which also include lycopene, protect against antioxidants by scavenging free radicals that cause DNA damage and through DNA repair mechanisms.[36-38] Supplemental lycopene administration has a DNA-protective effect by both completely inhibiting Comet formation and reducing 8-OHdG levels.[39] It was suggested that, in cells treated with lycopene, which is known to have protective effects against DNA degradation, lipid peroxidation is inhibited, the 8-oxodGuo increase is reduced, and this might be an evidence of its tumor-protective effect by promoting lycopene oxidative destruction.[20]

Furthermore, it was observed that low doses of lycopene are effective as an antioxidant in human prostate cancer cell culture, whereas high doses promote DNA damage.[40]

CONCLUSION

It was observed that oxidative DNA damage decreased with different doses of lycopene application, especially in HG-treated groups. In the present study, it was determined that caspase enzymatic activities, especially caspase 3 and 8 enzymatic activities, which are apoptotic indicators and M30 levels, increased in some doses in HG groups and the highest lycopene dose decreased caspase 3 and M30. Based on the fact that lycopene treatment was effective in certain doses on the prevention of HG-induced oxidative DNA damage and apoptosis, it was concluded that it is worth investigating lycopene in more detail using the doses determined for this cell line and different system parameters.

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Conflicts of interest

There are no conflicts of interest.

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