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# Furan-induced hepatotoxic and hematologic changes in diabetic rats: the protective role of lycopene

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Furan forms as a result of thermal treatment of food and induces harmful effects on organisms. In our work, lycopene, furan, and a combination of the two were given to diabetic male rats for 28 days. Hematological changes, total protein and cholesterol, triglyceride, and albumin levels, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and alkaline phosphatase activities of the serum, malondialdehyde levels, glutathione peroxidase, catalase, glutathione-S-transferase, superoxide dismutase activities, DNA damage in liver tissues and hepatic histopathological alterations were compared to a control group. There were significant changes in the liver function tests, DNA damage, activities of antioxidant enzymes, and malondialdehyde levels between diabetic control and non-diabetic control groups, between diabetic control and diabetic lycopene groups, and also between diabetic furan and diabetic control groups. In diabetic lycopene and diabetic furan + lycopene treated groups we designated the preventive effects of lycopene against diabetes and furan, however, on the analysed parameters only. In spite of some pathological alterations designated in diabetic furan treated group's liver, fewer pathological alterations were observed in furan+lycopene treated groups at the end of week 4. Consequently, lycopene significantly reduced furan- and diabetes-induced toxicity in rat liver.

KEY WORDS: *diabetes; DNA damage; lycopene; hepatotoxicity; oxidative stress*

Furan is a chemical largely used in industry. Humans may be exposed to it through consumption of thermally treated foods (1). Furan is also found in cigarette smoke and engine exhausts (2). It is also hepatotoxic and carcinogenic in animals; high incidence of cancers has been observed in animals, even at the lowest dose treated (1). Due to the prevalent use of furan, it is important to determine its toxic effects on organisms.

Diabetes mellitus is an endocrine disease with approximately 200 million people worldwide currently suffering from its complications (3). It causes morbidity and mortality due to the development of vascular complications like nephropathy and cardiovascular diseases. Owing to its quick global spread, diabetes mellitus is regarded as a considerable health problem (4).

Lycopene, a red–orange carotenoid pigment, has been examined in recent years and the results indicate that it is a highly effective antioxidant, possessing the capacity of free radical scavenging (5). It has many biochemical functions, some of which include antihyperlipidemic (6) and antiapoptotic effects (7). Recent experimental works have demonstrated that lycopene may prevent against cancers such as prostate cancer (5).

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The liver is an organ that activates and detoxifies several chemicals. Hepatotoxicity caused by these is observed through the changes in the liver marker profile and antioxidant enzymes such as, for example, superoxide dismutase (SOD), catalase (CAT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and pathological alterations (8, 9). Therefore, these parameters have been largely used as biomarkers (10).

Based on other previous findings that show the effects of lycopene against chemical toxicity (5, 11), in this study we evaluated lycopene's protective effects following furan exposure in diabetic rats. We investigated the parameters of oxidative stress (SOD, CAT, GPx, GST, MDA), hematological changes, liver function tests, DNA damage via comet assay and histopathological alterations of liver tissues after oral exposure to furan in diabetic rats.

## MATERIALS AND METHODS

### *Chemicals*

Furan, lycopene, streptozotocin (STZ) and all other chemicals were bought from Sigma-Aldrich Chemical Company. All chemicals used were of analytical grade. Lycopene and furan were dissolved in corn oil (12, 13).

*Animals and experimental design*

Animals used in this study were purchased from the Çukurova University Experimental Research and Application Centre. Male Wistar rats (300-320 g) were kept in plastic cages at 18-22 °C, 12 h light–dark cycle, standard diet and water ad libitum. The rats were treated in accordance with the standard guide for the care and use of animals and the instructions given by the Çukurova University Animal Experiments' Local Ethics Committee were followed throughout the experiment. After ten days of acclimatisation to the conditions of laboratory, the rats were divided into five groups (seven rats each) as follows:

Group I: control, received 1 mL $kg^{-1}$ body weight (bw) corn oil

Group II: diabetic control, received 1 mL $kg^{-1}$ bw corn oil

Group III: diabetic lycopene, treated with 4 mg $kg^{-1}$ bw lycopene (13)

Group IV: diabetic furan, treated with 40 mg $kg^{-1}$ bw furan (12)

Group V: diabetic furan+lycopene treated with 40 mg $kg^{-1}$ bw furan and 4 mg $kg^{-1}$ bw lycopene

In this study, furan and lycopene were given at 40 mg $kg^{-1}$ bw and 4 mg $kg^{-1}$ bw, respectively. The dose of furan was selected according to a previous study (12), and the dose of lycopene was chosen as proposed by Ateşşahin et al. (13). We selected these doses because Hamadeh et al. (12) treated rats with 40 mg $kg^{-1}$ furan and evaluated gene expression changes. In this study we wanted to evaluate whether these expression changes caused pathological and biochemical alterations or not.

Furan and lycopene treatments were done via gavage. After 28 days, the animals were anesthetised with a combination of ketamine and xylazine and the livers were dissected. Blood samples were collected in tubes rinsed with an anticoagulant for the liver function assays of the serum. Liver tissues were ablated, cleaned, and washed for the investigations of the levels of MDA, activities of antioxidant enzymes, and light microscopic analyses.

Diabetes was induced in the second, third, fourth, and fifth groups by an injection of 55 mg $kg^{-1}$ STZ intraperitoneally. After two days, blood samples were gathered from tails. Blood glucose levels were measured and animals with a blood glucose level of ≥300 mg $dL^{-1}$ were approved as diabetic (14).

*Levels of malondialdehyde (MDA) and antioxidant enzyme activities*

MDA levels and antioxidant enzyme activities were measured using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). All measurements were done at +4 °C.

For analysing the levels of MDA, we relied on Ohkawa et al. (15). Absorbance was determined at 532 nm and concentrations were expressed as nmol mg $protein^{-1}$.

The hepatic CAT activity was determined following the method of Aebi (16) by measuring the hydrolysis of hydrogen peroxide absorbance at 240 nm. Liver samples were diluted with Triton-X-100 before determination of the CAT activity. The CAT activity was expressed as mmol mg $protein^{-1}$.

Marklund and Marklund's study (17) was used for determining the SOD activity. For control, pyrogallol solution was added to Tris buffer and results were recorded at 440 nm. Liver extract was treated with Triton X-100. The sample was then added to Tris buffer and mixed. The reaction was started by adding pyrogallol solution. Data were expressed as U mg $protein^{-1}$.

The GPx activity of the liver was measured using hydrogen peroxide as a substrate according to a study of Paglia and Valentine (18). The reaction was monitored at 240 nm. The activity was expressed as nmol mg $protein^{-1}$.

The GST activity of the rats' liver was determined by analysing the occurrence of 1-chloro 2,4-dinitrobenzene conjugate and glutathione (19). Absorbance was registered at 340 nm. The GST activity was described as μmol mg $protein^{-1}$.

*Hematological parameters*

Blood samples were analysed for red blood (RBC) and white blood cell (WBC) counts, hemoglobin, mean corpuscular volume (MCV), hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (PLT) using a Sysmex XT-2000i hematology analyser (Sysmex Co., Ltd., Kobe, Japan).

*Biochemical parameters*

Blood samples were taken from the heart and collected into sterile tubes. They were then centrifuged at 2000 *g* and the serum was separated. Levels of total protein and cholesterol, albumin, triglyceride, activities of aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase, and lactate dehydrogenase were analysed using a commercial spectrophotometer-enzymatic kit.

*Determination of DNA damage (Comet assay)*

Liver samples for the assessment of DNA damage were collected and processed immediately after the rats were killed. Tissue was homogenised, transferred into RPMI 1640 and centrifugated for 5 min. The alkaline comet assay was performed using a modified method described by Ozkan et al. (20).

Briefly, 150 μL of cell suspension per rat was mixed with 75 μL of 0.5 % low-melting point agarose in PBS at 37 °C. The sample was then pipetted onto a microscope slide pre-coated with a layer of 0.6 % normal-melting point

agarose. Two replicate slides per each rat were prepared. Gel was left to solidify on ice for 10 min. The slides were then immersed into a lysis solution (2.5 M NaCl, 100 mM $Na_2EDTA$, 10 mM Tris-HCl, 1 % Na-sarcosinate, pH 10 with 1 % Triton X-100 and 10 % dimethyl sulfoxide) overnight. After lysis, the slides were placed in a horizontal electrophoresis unit filled with cold freshly prepared denaturation/electrophoresis buffer (300 mM NaOH, 1 M $Na_2EDTA$, pH 13.0). DNA unwinding lasted for 20 min. Electrophoresis was performed for 20 min. at 25 V and 300 mA. Slides were washed in three changes of neutralisation buffer (0.4 M Tris-HCl, pH 7.5) at 5-min. intervals. Microgels were stained with ethidium bromide (2 μg $mL^{-1}$). Image analyses were done using BS 200 ProP, BAB Imaging System (Ankara, Turkey). Comets were captured under an epifluorescence microscope (BAB BS 200 ProP, Ankara, Turkey) at 20x and 40x magnification.

A total of 100 comets per sample per animal were scored randomly and pooled for each group of animals. To estimate DNA damage levels, three comet parameters were evaluated: tail DNA%, tail length, and tail moment.

*Histopathological studies*

Liver samples were fixed in formalin solution. They were hydrated with increasing ethanol series and embedded in paraffin. Sections (5-6 μm) were cut using a microtome (Leica RM2255, Germany) and stained with hematoxylin-eosin (H&E) for histological examinations. The sections were viewed and photographed using a light microscope (Olympus BX51, Japan) and a camera (Olympus E-330, Japan). They were evaluated for the degree of histopathological changes.

*Statistics*

The results of the experiment were expressed as the mean±SEM with seven rats in each group. Intergroup variation was analysed statistically using one-way analysis of variance and software version 20.0 SPSS for Windows, followed by a Tukey's test. Results were considered statistically significant when $P<0.05$.

## RESULTS

None of the rats died during the experimental period. Blood glucose levels of diabetic rats were 300 mg $dL^{-1}$ or greater throughout the experiment (Table 1).

*MDA levels and antioxidant enzyme activities*

Diabetes decreased the activities of GPx, SOD, GST, and CAT in the liver. Application of furan further decreased enzyme activities (Figures 1-4) ($P<0.05$). We observed an increase in the activities when lycopene was applied both in the diabetic lycopene and diabetic furan+lycopene groups compared with the diabetic control and diabetic furan groups, respectively.

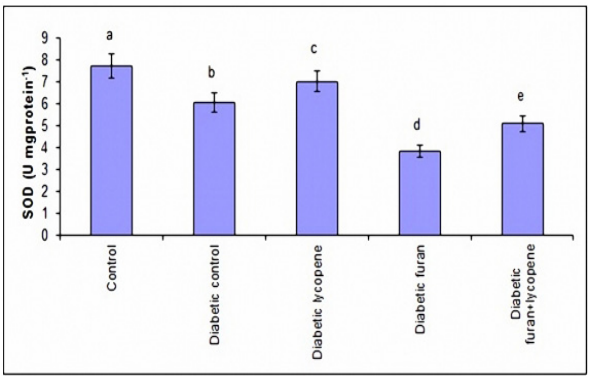


**Figure 1** *SOD activities (U mg protein$^{-1}$) in the liver of rats treated with furan (40 mg kg$^{-1}$ bw) and lycopene. Each bar represents mean±SEM of seven animals in each group. Columns superscripted with different letters are significantly different. Significance at* $P<0.05$

Levels of MDA were identified in liver tissues of rats. All groups showed an increased MDA level compared to controls. We observed more elevated results in the diabetic furan group than in the diabetic control group ($P<0.05$). In the diabetic lycopene and diabetic furan+lycopene groups we designated the preventive effect of lycopene on the inspected parameters (Figure 5).

*Hematological parameters*

Significant changes were not found in the levels of hemoglobin, RBC, hematocrit, MCHC, MCV, and MCH when all groups were compared one to the other. At the end of the 4$^{th}$ week, when the diabetic control group was crosschecked against controls, there was a significant increment in WBC and platelet counts. These counts were significantly lowered when the diabetic lycopene group was compared with the diabetic control. When furan was administered to diabetic rats, we observed an increase in the changes of WBC and platelets. Lycopene showed preventive effects against furan-induced alterations in WBC and platelet counts (Table 2).

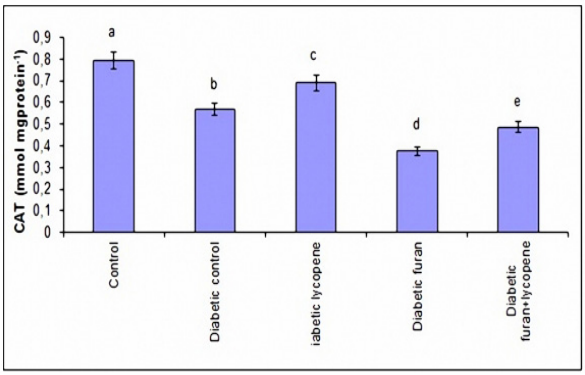


**Figure 2** *CAT activities (mmol mg protein$^{-1}$) in the liver of rats treated with furan (40 mg kg$^{-1}$ bw) and lycopene on. Each bar represents mean±SEM of seven animals in each group. Columns superscripted with different letters are significantly different. Significance at* $P<0.05$

**Table 1** *Blood glucose levels (mg dL$^{-1}$) of control and experimental groups*

| Groups | Blood Glucose (mg dL$^{-1}$) | | |
|---|---|---|---|
| | day 1 | day 14 | day 28 |
| Control | 96.2±7.7 | 115.9±14.2 | 94.6±13.8 |
| Diabetic control | 417.8±13.3 | 421.3±18.6 | 414.3±20.4 |
| Diabetic lycopene (4 mg kg$^{-1}$ bw) | 381.7±19.2 | 392.8±10.5 | 384.3±12.7 |
| Diabetic furan (40 mg kg$^{-1}$ bw) | 408.9±11.2 | 406.4±13.7 | 411.5±10.8 |
| Diabetic furan+lycopene | 386.2±11.4 | 401±8.25 | 397.4±21.6 |

*Values are mean±SEM of seven rats in each group. Significance at* P<*0.05*

*Hepatic function tests and lipid profile*

In the diabetic control group we observed significant increases in AST, LDH, ALT, and ALP activities, triglyceride, and total cholesterol levels, along with decreases in the levels of total protein and albumin as compared with the control. When furan was administered to diabetic rats, an increase in the levels of the above mentioned parameters, except in triglyceride and total cholesterol levels was found. Lycopene showed preventive effects against both diabetes and furan-induced alterations in hepatic function tests but not in the lipid profile (Table 3).

*Histological changes*

As revealed by the pathological assessment, both diabetes and furan treatment induced liver injury in rats (Figure 6). In diabetic rats treated with furan there were extensive damages in the liver sections. Eosinophilic cytoplasm, vascular congestion, and hemorrhage were observed in the diabetic control group. Vascular congestion, mononuclear cell infiltration, dilation of sinusoids, hemorrhage, degeneration of hepotocytes were observed in the liver following furan treatment. In addition, in diabetic furan rats, severe histopathological changes were determined. Lycopene alleviated the harmful effects of both diabetes and furan (Figure 6). Table 4 shows the scores of histological alterations in all groups.

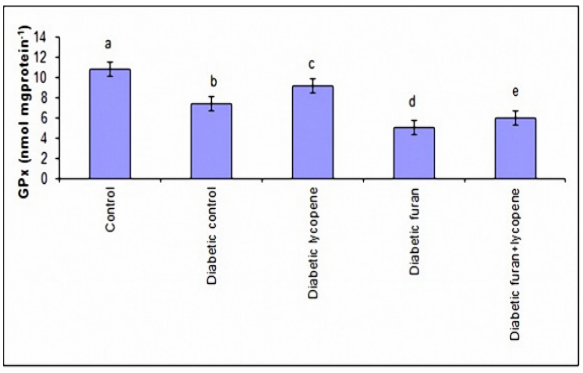


**Figure 3** *GPx activities (nmol mg protein$^{-1}$) in the liver of rats treated with furan (40 mg kg$^{-1}$ bw) and lycopene on. Each bar represents mean±SEM of seven animals in each group. Columns superscripted with different letters are significantly different. Significance at* P<*0.05*

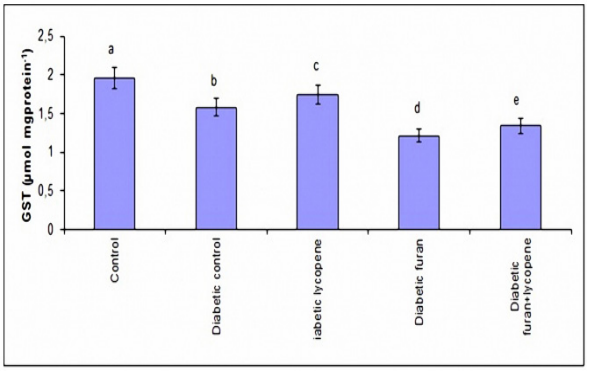


**Figure 4** *GST activities (µmol mg protein$^{-1}$) in the liver of rats treated with furan (40 mg kg$^{-1}$ bw) and lycopene. Each bar represents mean±SEM of seven animals in each group. Columns superscripted with different letters are significantly different. Significance at* P<*0.05*

*Primary DNA damage in liver cells*

The mean tail length and tail DNA % significantly increased with diabetes and furan treatment. A decrease in these values was observed in the diabetic furan+lycopene and diabetic lycopene groups compared with the diabetic furan and diabetic control groups, respectively (Table 5). The types of DNA damage observed using the comet assay are shown in Figure 7 for the control and diabetic groups.

## DISCUSSION

The comprehensive purpose of our study was to identify the effects of furan and lycopene on the liver of diabetic rats at the point of oxidative damage.

Data which were obtained from the present study ascertained that furan treatment and diabetes caused an increase in the activities of ALP, LDH, ALT, and AST of rats. In a work by Farokhi et al. (21), increased ALT and AST activities were also detected in diabetic rats. This increase may be due to liver dysfunction and a breakdown in enzyme synthesis which changes the liver's membrane permeability (9). Furan-exposed and diabetic animals also revealed lower total protein and albumin levels. The albumin level may decrease in rats as a disorder of the liver function after furan exposure; there are studies confirming that chemicals cause a reduction of albumin and total protein levels (22). In previous studies, researchers observed that diabetes caused an increase in the serum levels of total

**Table 2** *Effects of subacute (28 days) treatment with furan (40 mg kg$^{-1}$bw) and lycopene (4 mg kg$^{-1}$ bw) on hematological parameters of Wistar rats*

| Parameters \ Groups | Control | Diabetic control | Diabetic lycopene | Diabetic furan | Diabetic furan+lycopene |
|---|---|---|---|---|---|
| RBC (M mm$^{-3}$) | 7.78±0.98$^{a}$ | 8.43±0.68$^{a}$ | 7.78±0.98$^{a}$ | 8.26±0.78$^{a}$ | 8.39±0.57$^{a}$ |
| Hemoglobin (g dL$^{-1}$) | 15.60±0.89$^{a}$ | 14.20±0.54$^{a}$ | 14.10±0.68$^{a}$ | 14.80±0.55$^{a}$ | 14.70±0.95$^{a}$ |
| Hematocrit (%) | 41.30±2.92$^{a}$ | 40.40±3.92$^{a}$ | 43.10±3.57$^{a}$ | 42.20±3.97$^{a}$ | 40.82±2.79$^{a}$ |
| WBC (K mm$^{-3}$) | 5.19±0.37$^{a}$ | 8.71±0.22$^{b}$ | 7.12±0.31$^{c}$ | 9.89±0.46$^{d}$ | 8.99±0.24$^{e}$ |
| MCH (pg) | 16.80±2.91$^{a}$ | 18.00±1.15$^{a}$ | 18.20±1.93$^{a}$ | 20.20±3.62$^{a}$ | 19.30±2.55$^{a}$ |
| MCV (fL) | 49.90±4.82$^{a}$ | 54.28±3.47$^{a}$ | 50.95±3.72$^{a}$ | 51.65±3.89$^{a}$ | 52.71±5.03$^{a}$ |
| MCHC (g dL$^{-1}$) | 30.44±3.82$^{a}$ | 35.16±2.67$^{a}$ | 33.13±0.84$^{a}$ | 30.48±3.02$^{a}$ | 33.18±0.83$^{a}$ |
| Platelet (K mm$^{-3}$) | 705.27±13.11$^{a}$ | 852.17±22.81$^{b}$ | 756.13±21.62$^{c}$ | 947.52±30.69$^{d}$ | 893±13.26$^{e}$ |

*Values are mean±SEM of seven rats in each group. Significance at* P*<0.05. Within each column, means superscripted with different letters are significantly different*

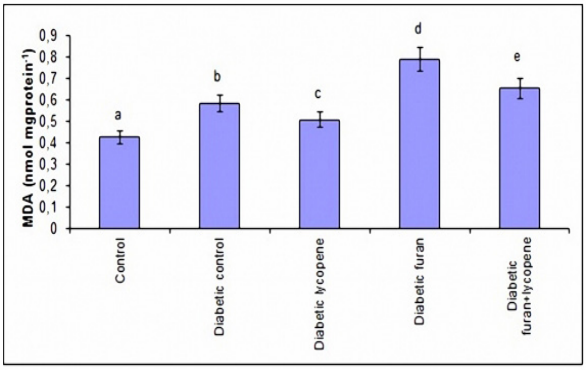


**Figure 5** *MDA levels (nmol mg protein$^{-1}$) in the liver of rats treated with furan (40 mg kg$^{-1}$ bw) and lycopene. Each bar represents mean±SEM of seven animals in each group. Columns superscripted with different letters are significantly different. Significance at* P*<0.05*

cholesterol and triglyceride (23). Thus, increased parameters can be due to the effects of diabetes on the permeability of membranes of hepatic cells (24). Lycopene showed protective effects against furan and diabetes-induced changes in hepatic function tests. However, we did not determine the effects of furan and lycopene on total cholesterol and triglyceride levels.

Chemicals can change hematological parameters (25). In the present study, furan and diabetes enhanced WBC counts. This increase may point to the activation of the immune system of rats (25). The increase in WBC counts of animals is in agreement with the data of Mahour and Saxena (26). It is clear from our observation that PLT counts in rats treated with 40 mg kg$^{-1}$ body weight of furan differed from the control. A significant inrease in PLT counts was found in the furan treated group. Chemicals can induce histopathological damages (27) and these damages can stir up an increase in PLT and WBC counts (22). Our histopathological results support this probability in the furan treated group. The results obtained from Sultan et al. (28) indicated that diabetes mellitus imparted negative effects on various hematological attributes.

Several studies suggest that oxidative stress appears to be the key determinant of toxicity induced by furan and diabetes both *in vivo* and *in vitro* (29, 30). Recent *in vivo* studies in furan treated animals showed reactive oxygen species (ROS) formation and stimulation of lipid peroxidation (LPO) promoting the function of oxidative stress in the toxicity of furan (31, 32). Furan-caused oxidative stress adds substantially to liver injury. Excessive ROS and raised LPO are some of the unwanted effects of furan (32). An increased MDA level, end product of LPO, is a major determinant of LPO (33). Incremental MDA was determined in the liver of furan-exposed rats. Selmanoğlu et al. (34) also reported enhanced MDA levels in furan toxicity in their work.

Mammalian cells have antioxidants and antioxidant enzymes to prevent tissues from oxidative damage.

**Table 3** *Effects of subacute (28 days) treatment with furan (40 mg kg$^{-1}$ bw) and lycopene (4 mg kg$^{-1}$ bw) on the liver function tests and lipid profile of Wistar rats*

| Parameters \ Groups | Control | Diabetic control | Diabetic lycopene | Diabetic furan | Diabetic furan+lycopene |
|---|---|---|---|---|---|
| Total protein (g dL$^{-1}$) | 7.18±0.24$^{a}$ | 6.22±0.18$^{b}$ | 6.76±0.21$^{c}$ | 4.64±0.37$^{d}$ | 5.71±0.22$^{e}$ |
| Albumin (g dL$^{-1}$) | 4.56±0.19$^{a}$ | 4.02±0.07$^{b}$ | 4.24±0.11$^{c}$ | 3.03±0.08$^{d}$ | 3.54±0.07$^{e}$ |
| AST (U L$^{-1}$) | 37.21±1.62$^{a}$ | 45.60±1.47$^{b}$ | 41.57±2.02$^{c}$ | 53.20±3.17$^{d}$ | 48.82±1.09$^{e}$ |
| ALT (U L$^{-1}$) | 5.02±0.27$^{a}$ | 7.43±1.14$^{b}$ | 5.73±0.31$^{c}$ | 9.94±0.59$^{d}$ | 8.96±0.33$^{e}$ |
| ALP (U L$^{-1}$) | 14.08±2.91$^{a}$ | 21.08±2.27$^{b}$ | 17.92±0.73$^{c}$ | 31.22±1.13$^{d}$ | 26.81±3.01$^{e}$ |
| LDH (U L$^{-1}$) | 45.81±3.62$^{a}$ | 59.89±2.01$^{b}$ | 53.67±4.12$^{c}$ | 74.06±5.04$^{d}$ | 65.71±3.16$^{e}$ |
| Triglyceride (mg dL$^{-1}$) | 27.04±3.02$^{a}$ | 35.16±2.41$^{b}$ | 33.73±2.84$^{b}$ | 34.46±3.24$^{b}$ | 33.88±1.98$^{b}$ |
| Total cholesterol (mg dL$^{-1}$) | 702.86±19.71$^{a}$ | 822.74±22.38$^{b}$ | 801.13±11.55$^{b}$ | 837.59±33.71$^{b}$ | 835.1±28.76$^{b}$ |

*Values are mean±SEM of seven rats in each group. Significance at* P*<0.05. Within each column, means superscripted with different letters are significantly different*

**Table 4** *Grading of the histopathological changes in the liver sections*

| Groups / Pathology | Control | Diabetic control | Diabetic lycopene | Diabetic furan | Diabetic furan+lycopene |
|---|---|---|---|---|---|
| eosinophilic cytoplasm | - | ++ | + | + | + |
| vascular congestion | - | ++ | + | ++ | + |
| hemorrhage | - | ++ | - | +++ | ++ |
| mononuclear cell infiltration | - | - | - | + | - |
| dilation of sinusoids | - | - | - | +++ | ++ |
| degeneration of hepotocytes | - | - | - | ++ | + |

*Scoring was done as follows: (-) none, (+) mild, (++) moderate, (+++) severe*

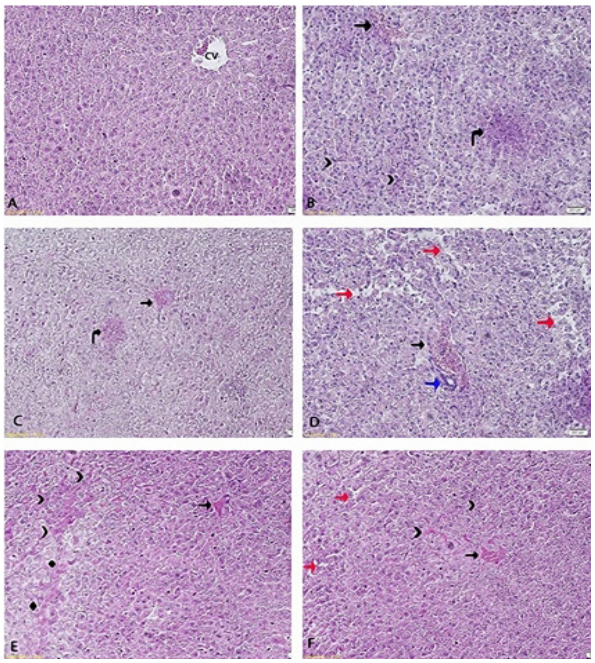


**Figure 6** *(A) Liver section of control rats, CV: central vein 200X. (B) Liver sections of diabetic control rats showing (curled arrow) eosinophilic cytoplasm, (black arrow) vascular congestion, (>) hemorrhage 200X. (C) Liver sections of diabetic lycopene treated rats showing (curled arrow) eosinophilic cytoplasm, (black arrow) vascular congestion 200X. (D, E) Liver sections of diabetic furan treated rats showing (black arrow) vascular congestion, (blue arrow) mononuclear cell infiltration, (red arrow) dilation of sinusoids, (>) hemorrhage, (♦) degeneration of hepotocytes 200X. (F) Liver sections of diabetic furan+lycopene treated rats showing (>) hemorrhage, (red arrow) dilation of sinusoids, (black arrow) vascular congestion 200X*

Alteration in the activity of antioxidant enzymes can be regarded as a sensitive marker of the response of cells to oxidative stress (35). Aiming to disclose the effects of furan and lycopene, we determined alterations in intracellular antioxidant enzyme activities. As crosschecked against the control group, treatment of rats with 40 mg $kg^{-1}$ furan and diabetes induced decreases in the enzyme activities of CAT, GST, GPx, and SOD. Nevertheless, these decreases were reduced by the treatment with lycopene (4 mg $kg^{-1}$). From these data, the changes of the GST, SOD, CAT, and GPx activities resulting from the treatment with lycopene clearly point to the competence of the defence system of cells to respond to an oxidative stress agent. Alterations of antioxidant enzyme activities observed in this work may be due to the production of ROS. Selmanoğlu et al. (34) reported that treatment of rats with furan showed changes in antioxidant enzyme activities. Connections between chemical treatment and alterations in antioxidant enzyme activities have been proven (36).

Microscopic findings in the liver were severe with furan treatment and diabetes, similar to the findings in previous reports (2, 21, 37). Furan induced vascular congestion, mononuclear cell infiltration, dilation of sinusoids, hemorrhage, and degeneration of hepatocytes in this study. These histopathological changes coincide with alternations in biochemical and hematological parameters in the present study. Adverse effects of furan have been proven in previous studies too. Gill et al. (38) determined that furan induced histopathological changes in the liver and kidney. Also, in

**Table 5** *Effect of furan (40 mg $kg^{-1}$ bw) and lycopene (4 mg $kg^{-1}$ bw) on liver DNA damage in non-diabetic and diabetic rats as estimated by the alkaline comet assay*

| DNA parameters | Control | Diabetic control | Diabetic Lycopene | Diabetic Furan | Diabetic Furan + Lycopene |
|---|---|---|---|---|---|
| Tail DNA (%) | $48.80\pm3.27^{a}$ | $75.77\pm4.60^{b}$ | $73.06\pm2.16^{c}$ | $94.06\pm1.54^{d}$ | $85.34\pm6.80^{e}$ |
| Tail length | $16.50\pm0.71^{a}$ | $67.00\pm7.78^{b}$ | $42.50\pm26.16^{c}$ | $147.00\pm21.26^{d}$ | $52.00\pm9.90^{e}$ |
| Tail moment | $8.052\pm1.02^{a}$ | $31.34\pm20.03^{b}$ | $73.06\pm2.16^{c}$ | $138.92\pm22.21^{d}$ | $44.71\pm11.98^{e}$ |

*Values are mean±SEM of seven rats in each group. Superscripted letters indicate significant differences ($P<0.05$) among diabetic rats exposed to furan and lycopene. Within each column, means superscripted with different letters are significantly different ($P<0.05$)*

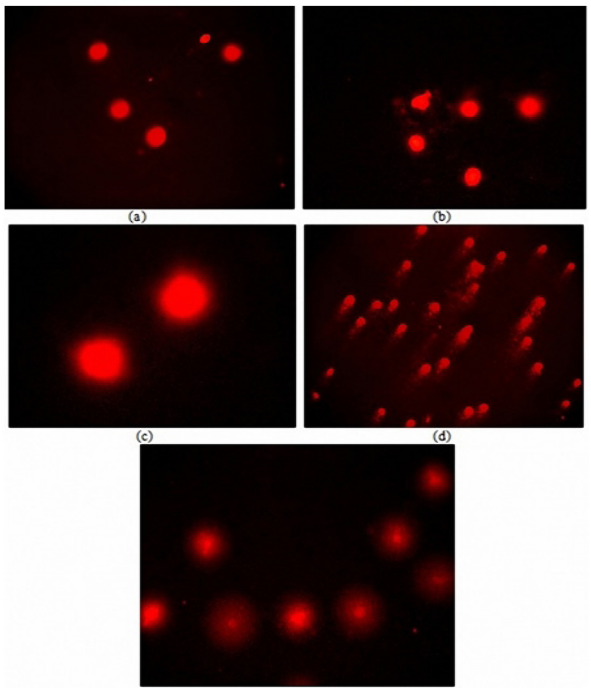


**Figure 7** *Photomicrographs of liver nuclei observed after the alkaline comet assay procedure in rats treated with furan (40 mg kg$^{-1}$ bw) exposure and/or lycopene and in the control group. (a) control, (b) diabetic control, (c) diabetic lycopene, (d) diabetic furan, (e) diabetic furan + lycopene*
*Stained with ethidium bromide. Photomicrographs have been acquired by BS 200 ProP, BAB Imaging System (Ankara, Turkey) under magnification 20X (a, b, d, e,) and 40X (c)*

a study by Karacaoğlu et al. (39), furan caused harmful effects in the pathology of pancreas and adrenal cortex.

Diabetes and complications of diabetes are associated with increased oxidative stress (3). Shanmugam et al. (40) and Suresh et al. (29) observed that diabetes increased LPO and changed antioxidant enzyme activities. There are also studies confirming that diabetes causes histological alterations in different tissues (40). In this study there are alterations in antioxidant enzyme activities, MDA levels, haematological, and biochemical parameters in the diabetic groups compared to controls. DNA is known to be targeted by various chemicals whose activity results in genotoxic effects (41, 42). This study was also performed to observe the genotoxic potential of furan and the protective role of lycopene in liver tissues of diabetic rats. It was observed that the tail lengths were elongated with diabetes and furan treatment. Furan causes sister chromatid exchanges in vitro in Chinese hamster ovary cells and furan exposure induces chromosomal aberrations in both Chinese hamster ovary cells and B6C3F1 mouse bone marrow cells (43). Diabetes induces oxidative stress in cells resulting in the formation of toxic products such as MDA and 4-hydroxynonenal. Both aldehydes, MDA and 4-hydroxynonenal, can cause mitochondrial enzyme damage and DNA breaks. 4-hydroxynonenal can play roles in toxic pathways such as the induction of caspases and the laddering of genomic DNA (44). Also, it is clear that furan-treated diabetic rats are more sensitive than diabetic rats without furan treatment. This situation is supported by a previous study (45). In addition, Apaydin et al. (4) indicated in their study that lead nitrate treated diabetic rats were more sensitive than diabetic rats without lead nitrate treatment.

In order to overcome the extreme levels of ROS, cells have enzymes like SOD, GPx, GST, and CAT. Even so, these protective systems cannot exactly oppose the destructive effects of ROS (35). Additional antioxidants can advance to increase the defence systems of cells. For this reason, these antioxidants are necessary to cope with excessive ROS production. Recent studies showed that lycopene, which is an antioxidant, significantly prevented the chemical-induced changes in antioxidant enzyme activities, MDA levels, and liver marker enzymes (13, 46). In addition, Matos et al. (47) determined in their study that oxidative DNA damage was reduced with lycopene treatment in rats *in vivo* and in cell culture. These findings are supported in our data. Also, the results showed that lycopene substantially decreased the levels of serum hepatic function parameters, WBC, and platelet counts in furan and diabetes-treated animals and improved the histopathologic changes in the liver of rats. Lycopene becomes protective towards the examined parameters because of its antioxidant properties, like in other studies (46, 48). It may be an indirect scavenger of ROS or it can boost antioxidant enzyme activities; therefore it may prevent the toxicity produced by furan and diabetes. Carotenoids are well known scavengers of singlet oxygen and its other excited species. During singlet oxygen quenching, energy is transferred from singlet oxygen to the lycopene molecule, converting it to the energy-rich triplet state. The trapping of other ROS, like OH•, $NO_2$• or peroxynitrite, in contrast, leads to oxidative breakdown of the lycopene molecule. Thus, lycopene may protect *in vivo* against oxidation of lipids, proteins, and DNA (13).

Considering the data obtained from this study we can say that furan and diabetes cause liver damages by way of oxidative stress. Due to the generation of ROS, both cause damage to cell membranes and DNA, as confirmed by the comet assay, as well as histopathological alterations. Our findings suggest that lycopene applied at the tested concentration could protect rat liver against furan and diabetes-induced toxicity but not completely. The promissing results obtained in this study speak in favour of further investigation using other lycopene doses and exposure scenarios to prove its usefulness as a cyto/genoprotective agent.

*Conflict of Interest*

No conflict of interest is declared by the authors.

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**Hepatotoksične i hematološke promjene u dijabetičkih štakora izazvane furanom: zaštitna uloga likopena**

Furan se stvara toplinskom obradom hrane i djeluje štetno na organizam. U ovom smo istraživanju dijabetičke mužjake štakora tretirali likopenom, furanom i kombinacijom tih dviju tvari tijekom 28 dana. Procijenjene su hematološke promjene, ukupne razine proteina, kolesterola, triglicerida i albumina, aktivnosti alanin aminotransferaze, aspartat aminotransferaze, laktat dehidrogenaze i alkalne fosfataze u serumu, razine malondialdehida, aktivnosti glutation peroksidaze, katalaze, glutation-S-transferaze i superoksid dizmutaze te oštećenje DNA u tkivu jetara i hepatičke histopatološke promjene u odnosu na kontrolnu skupinu. Utvrđene su značajne promjene u testovima funkcije jetara, razinama oštećenja DNA, aktivnostima antioksidacijskih enzima i razinama malondialdehida između dijabetičke kontrolne skupine i dijabetičke skupine tretirane likopenom te između dijabetičke kontrolne skupine i kontrolne skupine bez dijabetesa, između dijabetičke kontrolne skupine i dijabetičke skupine tretirane likopenom te između dijabetičke skupine tretirane furanom i dijabetičke kontrolne skupine. U dijabetičkoj skupini tretiranoj likopenom i dijabetičkoj skupini tretiranoj i furanom i likopenom utvrdili smo preventivne učinke likopena, ali samo u odnosu na analizirane parametre. Unatoč patološkim promjenama koje su utvrđene u jetrima dijabetičke skupine tretirane furanom, manje ih je utvrđeno u skupinama koje su tretirane furanom i likopenom na kraju četvrtoga tjedna. Zaključak je istraživanja da je likopen značajno smanjio toksičnost koju je prouzročio furan i dijabetes u jetrima štakora.

KLJUČNE RIJEČI: *dijabetes; likopen; hepatotoksičnost; oksidacijski stres; oštećenje DNA*