Letter to the Editor

Protective Effects of Lycopene on Furan-treated Diabetic and Non-diabetic Rat Lung

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We assessed the effects of furan and lycopene on the histopathological and biochemical changes on lungs, body and lung weights, and food consumption of rats. Furan and diabetes caused histopathological changes, increment in malondialdehyde levels, and decrease in antioxidant enzyme activities. Lycopene showed a protective effect against these damages, except for glutathione-S-transferase and glutathione peroxidase activities. Consequently, furan and diabetes resulted in lung toxicity. Our findings demonstrate that furan treatment resulted in more alterations in histology and biochemical parameters in diabetic rats and lycopene showed protective effects against these alterations.

Furan occurs in various processed foods. It is important to determine the harmful effects of furan, as it is known to affect both animals and humans[1]. It causes toxicological effects on the reproductive system in male rats[2].

Cells can reduce the pro-oxidative state through antioxidants. When exposed to toxicants, changes in the antioxidant enzyme activities occur. Hence, activities of these enzymes have been used to evaluate oxidative stress in the cells. Several chemicals may damage the cell membranes by inducing lipid peroxidation (LPO). Malondialdehyde (MDA) is an end product of LPO resulting from the interaction between ROS and cellular membranes[2-3].

Lycopene is a dietary carotenoid found in fruits. Recent studies have shown that lycopene is inversely associated with the risk of cancers and heart disease. Accumulated experimental evidence suggests that lycopene can inhibit oxidative damage in cell membranes[4]. In recent years, lycopene has been one of the most studied materials for its antioxidant property.

Diabetes is a major worldwide problem and a chronic metabolic disorder, with an incidence of 2.5%-3% in the world population. Therefore, in this study, we also used diabetic rats to determine the effects of furan and lycopene in diabetic individuals. Several reports have suggested the pathophysiological mechanisms of diabetes[5]. However, there are no sufficient data regarding pulmonary disease in diabetic patients.

The aims of this study were to determine the effect of furan on the lungs of diabetic and non-diabetic male rats and to assess whether these effects can be ameliorated by lycopene. We focused on the lungs because, although several studies have analyzed other tissues such as the liver and kidney in diabetes, data regarding the effects on lungs are scarce.

Male Wistar rats (300-320 g) were handled in accordance with the standard guide for the care and use of laboratory animals. The protocol was approved by the Çukurova University Animal Experiments Local Ethics Committee.

Furan (40 mg/kg body weight) and lycopene (4 mg/kg body weight) were dissolved in corn oil[1,4]. These doses were selected according to previous studies[1,4]. The animals were divided into eight groups (seven rats in each group): control, lycopene-treated, furan-treated, furan+lycopene-treated, diabetic control, diabetic lycopene-treated, diabetic furan-treated, and diabetic furan+lycopene-treated groups. After 28 d, the rats were sacrificed and dissected, and the lungs were isolated. Samples were stored at -80 °C until the analysis. Diabetes was induced by STZ injection. Rats with ≥300 mg/dL of glucose were selected for the diabetic group[5]. The body and lung weights and food consumptions of rats were measured by an automatic balance.

The MDA levels and superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), and glutathione peroxidase (GPx) activities were determined with a spectrophotometer, following the methods that were used in the study by Baş and Kalende[6].

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Tissues were fixed in formalin and then processed using a graded ethanol series. They were embedded in paraffin, cut (6-7 μm), and stained with hematoxylin and eosin (H&E). The sections were evaluated with a light microscope.

Data were analyzed using SPSS 20.0 for Windows. The experimental groups were compared using analysis of variance and Tukey's test. $P<0.05$ was considered statistically significant. The results are expressed as mean±standard error of the mean (SEM).

Body weights, lung weights, and food consumption did not show significant changes between the non-diabetic groups and also between the diabetic groups. When the diabetic and non-diabetic groups were compared, increases in lung weights and food consumption and decreases in body weights in diabetic rats were observed (Figure 1).

The enzyme activities and MDA levels were similar in both the control and lycopene groups. In the furan-treated group, there were decreases in the enzyme activities (GST 27.59%, CAT 31.99%, GPx 32.51%, and SOD 41.55%) and increases in the MDA levels (68.75%). When the furan group and the furan+lycopene group were compared, decreases in the MDA levels (14.41%) and increases in SOD (26.82%), CAT (10.95%), GPx (13.47%), and GST (21.43%) activities were observed.

A comparison of the diabetic control group with the non-diabetic control group revealed higher MDA levels (50%) and lower enzyme activities (GST 15.52%, CAT 25%, GPx 22.65%, and SOD 26.64%). When we compared the diabetic control group with the diabetic lycopene group, the protective effects of lycopene were observed, except for GPx and GST activities. There were significant decreases in MDA levels (18.75%) and increases in SOD (17.43%) and CAT (15.86%) activities in the lycopene group. Furan treatment caused increases in the MDA levels (27%) and decreases in the enzyme activities (GST 32.65%, CAT 24.46%, GPx 34.86%, and SOD 31.33%) and lycopene showed protective effects against these damages. When the diabetic furan group and the diabetic furan+lycopene group were compared, significant decreases in the MDA levels (14.75%) and increases in SOD (16.62%), CAT (19.57%), GPx (33.03%), and GST (21.21%) activities were observed (Figure 2).

Normal lung alveoli structures were observed in the control (Figure 3A) and lycopene groups (Figure 3B). In the furan group, we detected emphysematous changes, thickened and increased connective

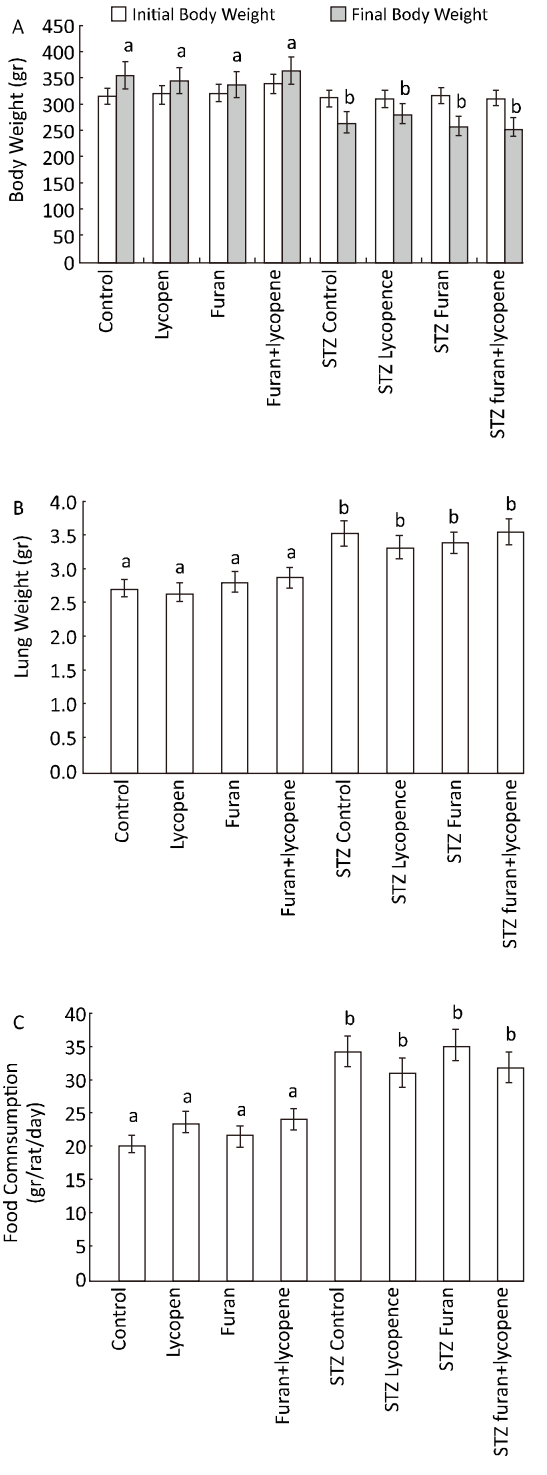

Figure 1. Effects of furan and lycopene on body weights (A), lung weights (B), and food consumption (C). Column superscripts with different letters represent significantly different values. Data represent the mean±SEM of seven samples. Significance at $P<0.05$.

tissue in the alveolar septa, and hemorrhage (Figure 3C). Emphysematous changes and thickened and increased connective tissue in the alveolar septa were also observed in the furan+lycopene group (Figure 3D), diabetic control group (Figure 3E), and diabetic lycopene group (Figure 3F). In the furan-treated diabetic rats, there was an increase in the severity of pathological changes. In the diabetic furan group, we detected emphysematous changes, thickened and increased connective tissue in the alveolar septa, hemorrhage, and desquamation of the epithelial lining of the terminal bronchiole (Figure 3G and 3H). Treatment with lycopene reversed these changes. Moderate hemorrhage and emphysematous changes were observed in the diabetic furan+lycopene group (Figure 3I).

This study is an *in vivo* assessment of the effects on diabetic and non-diabetic rat lungs due to furan and lycopene treatment in terms of histopathological changes and oxidative stress. The characteristic symptoms of diabetes are increased food intake and loss of body weight. Increased food consumption due to the accumulation of glucose in the blood and increased excretion of glucose in the urine. A decrease in the body weight and increase in the organ weights have been shown previously[6]. Similarly, we observed reduction in the body weight and increment in lung weight and food consumption in the diabetic groups. Despite the increased food intake, we observed a decrease in the body weight because of lipolysis and gluconeogenesis. Organ weight increase in diabetes has been associated with increased synthesis and decreased degradation of extracellular matrix components[6].

MDA is a primary product of peroxidized fatty acids and hence, an increase in its level is an important indicator of LPO. Therefore, we measured the MDA levels for evaluating the oxidative stress caused by diabetes and furan. The MDA levels were significantly increased in the lung tissues treated with furan. This increase may be due to an increase in the free radicals resulting from the induction of oxidative stress[3]. MDA may also increase due to the adverse effects of furan on the fatty acids of cell membranes. A recent *in vivo* study showed that furan induced ROS generation and stimulation of LPO, supporting the role of oxidative stress in furan toxicity[7].

SOD, CAT, GPx, and GST are important cell protectors against oxidative stress. The antioxidant enzymes evaluated in this study are potential targets for chemical toxicity. Some studies have analyzed the antioxidant enzyme activities to determine the chemical

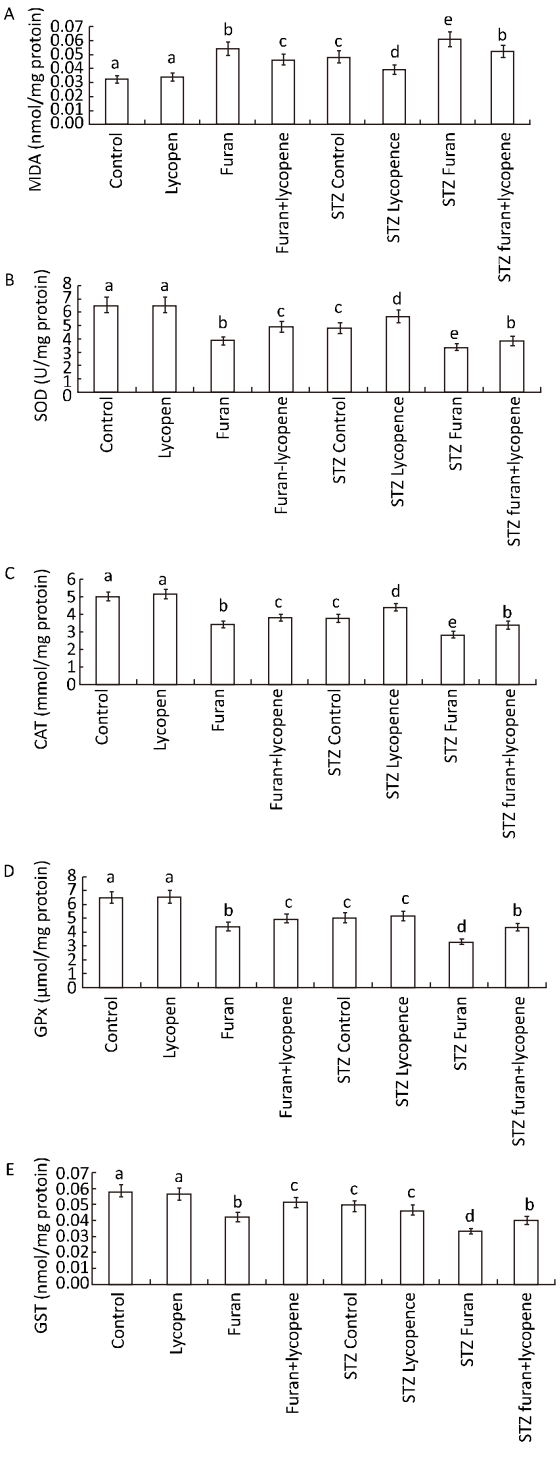

Figure 2. Effects of furan and lycopene on MDA levels (A) and SOD (B), CAT (C), GPx (D), and GST (E) activities of lung tissues. Column superscripts with different letters indicate significantly different values. Data represent the mean±SEM of seven samples. Significance at $P<0.05$.

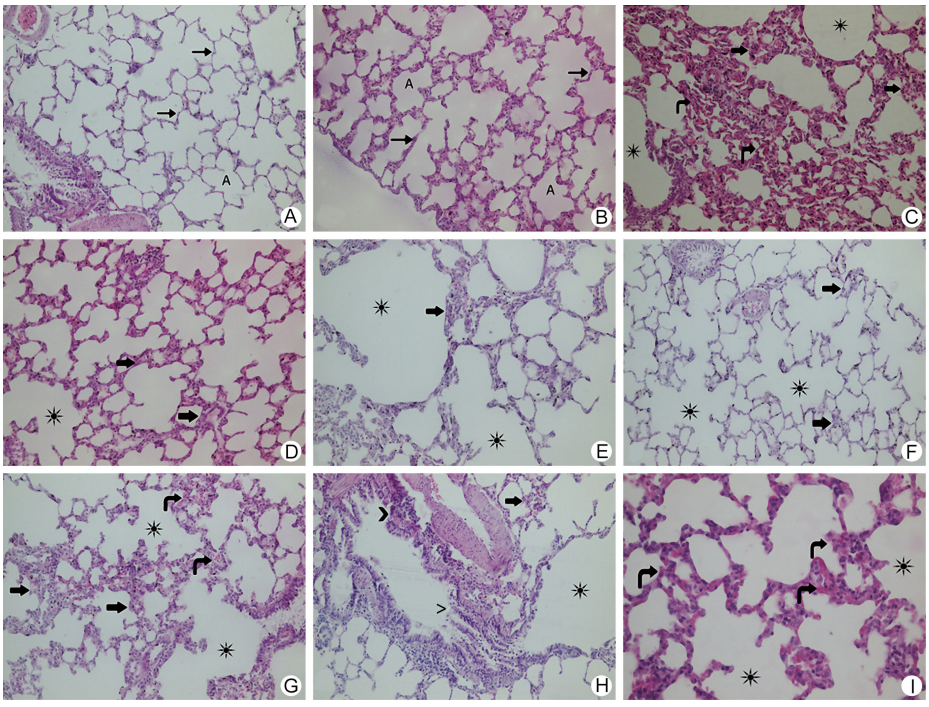

Figure 3. (A) Lung section of control and lycopene groups (B) showing normal alveoli (A) and normal alveolar septa (→), 200×. Furan group (C) showing emphysematous changes (✱), thickened and increased connective tissue in the alveolar septa (➔), and hemorrhage (curled arrow), 200×. Furan+lycopene group (D) showing emphysematous changes (✱) and thickened and increased connective tissue in the alveolar septa (➔), 200×. Lung sections of diabetic control (E) and diabetic lycopene (F) groups showing emphysematous changes (✱) and thickened and increased connective tissue in the alveolar septa (➔), 200×. Diabetic furan group (G and H) showing emphysematous changes (✱), thickened and increased connective tissue in the alveolar septa (➔), hemorrhage (curled arrow), and desquamation of the epithelial lining of the terminal bronchiole (>) (G-200×, H-400×). Diabetic furan+lycopene group (I) showing moderate hemorrhage (curled arrow) and emphysematous changes (✱), 400×.

effects. Alterations in the enzyme activities are caused due to exposure to chemicals. Hence, assessment of these enzyme activities may provide important information about oxidative stress[6-7]. In our study, furan treatment caused reductions in SOD, CAT, GPx, and GST activities. A probable reason for these reductions could be the increased formation of free radicals, which is supported by the levels of MDA obtained in this study.

In this study, furan treatment also induced pathology in the rats. The adverse effects of furan have also been proven in previous studies. Gill et al.[8] demonstrated that furan induces histopathological changes in the liver. Karacaoğlu et al.[9] also showed the harmful pathological effects of furan in the pancreas and adrenal cortex.

Extreme ROS formation induces harmful effects in the organs, including histopathological changes and differences in antioxidant enzyme activities and biochemical parameters[5]. Baş and Kalender demonstrated that diabetes increases LPO, alters the activities of antioxidant enzymes, and causes histological alterations[6]. We also observed decreases in SOD, CAT, GPx, and GST activities, increases in MDA levels, and histopathological changes in diabetic animals. Reduced antioxidant levels as a result of increased free radical production in diabetes have been previously reported[10]. Lycopene showed protective effects against diabetes in terms of the SOD and CAT activities, MDA levels, and histopathology of lungs.

The antioxidant activity of lycopene has been extensively evaluated on the basis of its ability to scavenge free radicals and protect cells against oxidative damage[4]. In this study, lycopene demonstrated protective effects against the damages caused by furan. It reduced the histopathological changes and MDA levels and

increased the antioxidant enzyme activities. Lycopene also showed protective effects against diabetes, except for GPx and GST activities. In another study, lycopene showed preventive effects against cisplatin-induced testicular damage[4].

In summary, furan treatment caused increase in the MDA levels, decrease in enzyme activities, and histopathological changes in lungs. It also resulted in more harmful effects in the diabetic groups versus the non-diabetic groups. However, lycopene reversed these effects by its antioxidant property. Lycopene was also able to protect against diabetes-induced oxidative damage, except for GST and GPx activities.

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