

Effects of lycopene on oxidative stress and remnant liver histology after partial hepatectomy in rats

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Background/aims: Partial hepatectomy is performed for the treatment of mass lesions in the liver. Lycopene, which is a carotenoid, is present in various physiologic processes. In this study, the effects of lycopene administration in partially hepatectomized rats were evaluated by assessing various oxidant/antioxidant parameters, remnant liver histology and plasma nitric oxide levels. **Methods:** Thirty Wistar albino adult male rats were randomly divided into three equal groups as: Sham, Partial Hepatectomy and Lycopene-Administered + Partial Hepatectomy groups. Lycopene (4 mg/kg), which was dissolved in olive oil, was given to the rats per orally (via gavage tube) (0.1 ml) every day for 6 weeks before partial hepatectomy and for one week after partial hepatectomy. Tissue and blood samples were collected one week after partial hepatectomy. **Results:** Plasma malondialdehyde ($p<0.001$) and nitric oxide ($p<0.05$) levels in the lycopene-administered + partial hepatectomy group were significantly higher than in the partial hepatectomy group. Intraerythrocytic glutathione ($p<0.001$), plasma ($p<0.001$) and liver tissue Cu-Zn ($p<0.05$) superoxide dismutase levels of the lycopene-administered + partial hepatectomy group were significantly lower than in the partial hepatectomy group. **Conclusions:** Lycopene administration could be harmful by increasing oxidative stress after partial hepatectomy.

Key words: Partial hepatectomy, oxidative stress, lycopene, nitric oxide

Sıçanlarda parsiyel hepatektomi sonrası likopen uygulamasının oksidatif stres ve kalan karaciğer histolojisi üzerine etkileri

Amaç: Parsiyel hepatektomi karaciğerin kitlesel lezyonlarının tedavisinde uygulanmaktadır. Likopen bir karotenoidtir ve birçok fizyolojik mekanizmanın içinde yer alır. Bu çalışmada, sıçanlarda parsiyel hepatektomi sonrası likopen uygulamasının oksidan ve antioksidan parametreler, kalan karaciğer histolojisi ve plazma nitrik oksit düzeyi üzerine etkileri değerlendirildi. **Yöntem:** Otuz adet Wistar albino yetişkin erkek sıçan rastlantısal olarak üç eşit gruba ayrıldı: Sham grubu, parsiyel hepatektomi grubu, likopen verilen parsiyel hepatektomi grubu. Likopen (4 mg/kg) zeytin yağında çözüldü ve hepatektomi öncesi altı hafta ve hepatektomi sonrası bir hafta gavaj ile transoral olarak (0,1 ml) verildi. Doku ve kan örnekleri hepatektomiden bir hafta sonra alındı. **Bulgular:** Likopen verilen parsiyel hepatektomi grubunun plazma malondialdehit ($p<0.001$) ve nitrik oksit ($p<0.05$) seviyeleri parsiyel hepatektomi grubundan anlamlı derecede yüksekti. Likopen verilen parsiyel hepatektomi grubunun eritrosit içi glutatyon ($p<0.001$), plazma ($p<0.001$) ve karaciğer dokusu Cu-Zn ($p<0.05$) süper oksit dismutaz seviyeleri parsiyel hepatektomi grubundan anlamlı derecede düşüktü. **Sonuç:** Parsiyel hepatektomi sonrası likopen uygulaması oksidatif stresi artırarak zararlı olabilir.

Anahtar kelimeler: Parsiyel hepatektomi, oksidatif stress, likopen, nitrik oksit

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INTRODUCTION

Partial hepatectomy is performed for the treatment of mass lesions in the liver (metastases, primary liver tumors, hemangioma, etc.) or for living-donor liver transplantation (1). Hepatocytes undergo cell division after surgical removal of a portion of the liver. The liver has regenerative capacity for restoring it to normal size. Many different cell types that are present in the liver are active during the hepatic regeneration (2). The studies about partial hepatectomy have shown that initiation of the regenerative response depends on many factors (1). Endocrine and paracrine actions of growth factors and activation of specific proto-oncogenes and transcription factors are important during regeneration (3). The beneficial effects of many dietary compounds on liver regeneration have been shown (4).

The aliphatic hydrocarbon lycopene, which is found abundantly in tomato and tomato-based products, is one of the 600 known naturally occurring carotenoids. Recently, lycopene received particular attention as a result of studies indicating it is a highly efficient antioxidant and has a singlet-oxygen and free radical scavenging capacity (5). Thus, in this study, the effects of lycopene administration in partially hepatectomized rats were evaluated by assessing various oxidant/antioxidant parameters, liver histopathology and plasma nitric oxide (NO) levels.

MATERIALS AND METHODS

This study was conducted according to the guidelines of the Animal Care Review Board of Istanbul University, Cerrahpasa Medical Faculty, in accordance with National Legislation and the Council Directive of the European Communities on the Protection of Animals Used for Experimental and Other Scientific Purposes (L358/1, November 24, 1986). Thirty adult male Wistar albino rats weighing 200-250 g were obtained from the Cerrahpasa Medical Faculty Experimental Animal Production and Research Laboratory. The rats were kept in standard colony cages (15x25x40 cm) (3 or 4 rats per cage) under controlled conditions including temperature of 28 °C, light (10/14 h light/dark) and humidity 50% - 55%. The animals were fed with standard rat chow and tap water ad libitum.

Experimental Design

Thirty adult male Wistar albino rats were randomly divided into three equal groups. Group1:

Sham operation (S) group (n = 10); Group2: Partial Hepatectomy (PH) group (n = 10); and Group 3: PH + lycopene-treated (PHL) group (n = 10). Lycopene (4 mg/kg), which was dissolved in olive oil (0.1 ml), was given to the rats per orally (via gavage tube) every day in the morning for 6 weeks before partial hepatectomy and one week after partial hepatectomy. The sham and the PH groups received equal volume per oral 0.09% NaCl solution at the same time. The liver tissue samples and the blood samples were collected one week after partial hepatectomy. Under ether anesthesia, thoracotomy and laparotomy were performed. The heparinized blood samples were taken from the right ventricle after thoracotomy. The plasma and liver tissue samples were stored at -70 °C for the biochemical analysis. The liver tissue samples were collected immediately and fixed in 10% formaldehyde solution for histopathological analyses.

Surgical Procedure

The rats were given ether anesthesia in a closed jar. Induction time took 2-3 minutes (min). The abdominal wall was cleansed with povidone iodine solution after shaving. Median abdominal incision was performed. The left and median lobes of the liver were exposed with the standard 70% hepatectomy technique (6). The peduncle of the left and median lobe was resected with 4/0 silk initially. The right and caudate lobes of the liver were left in place in all of the rats. The abdominal incision was closed with 2/0 silk continuous sutures as a single layer. Only laparotomy was performed in Group S (sham). No rat died during the experiment.

Biochemical Procedure

The liver tissue samples were weighed and homogenized with 0.15 M KCl. The tissue homogenates were sonicated twice with a 30-second (s) interval at moderate intensity. After completion of sonication, the homogenates prepared for malondialdehyde (MDA) measurement were centrifuged at 3000 rpm for 10 min whereas the ones prepared for NO determination were centrifuged at 15000 rpm for 15 min to obtain supernatants. All procedures were performed with ice cooling. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), total protein, albumin, and NO levels were measured only in plasma.

Assay of AST, ALT, LDH, Albumin, and Total Protein

AST, ALT, LDH, total protein, and albumin levels were measured by enzymatic methods using com-

mercial kits (Olympus, Hamburg, Germany) on Olympus AU800 analyzer.

Assay of Glutathione (GSH)

Reduced GSH concentration was determined according to the method of Beutler *et al.* (7) using metaphosphoric acid for protein precipitation and 5-5'-ditiobis-2-nitro benzoic acid for color development. GSH concentrations were expressed mg/g hemoglobin (Hb) in erythrocytes and micromol/mg protein in liver tissue. Hemoglobin concentration was determined by the cyanomethemoglobin method (8).

Assay of Cu-Zn Superoxide Dismutase (SOD)

Cu-Zn SOD activity was determined by the method of Sun *et al.* (9). The assay involves inhibition of nitroblue tetrazolium (NBT) (Sigma Chemical Co., St. Louis, MO, USA) and reduction with xanthine-xanthine oxidase (Sigma Chemical Co., St. Louis, MO, USA) that was used as a superoxide generator. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50%.

Assay of Malondialdehyde (MDA)

Lipid peroxidation was ascertained by the formation of MDA, which was estimated by the thiobarbituric acid method (10). One volume of sample was mixed thoroughly with two volumes of a solution of trichloroacetic acid (TCA) (30%), thiobarbituric acid (TBA) (0.75%) and 5M hydrochloric acid (HCl). Tubes were placed in boiling water for 15 min and centrifuged at 3000 rpm for 10 min. The supernatant was read at 535 nm in a spectrophotometer. The amount of MDA was calculated using an extinction coefficient ($1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$). Plasma and tissue MDA levels were expressed as nmol/ml and nmol/100 mg protein, respectively. Protein concentration was determined by the Lowry method (11).

Assay of Nitric Oxide

Nitric oxide (NO) was measured as its stable metabolites nitrate (NO_3^-) and nitrite (NO_2^-). Nitrate was first reduced by nitrate reductase to nitrite, and then nitrite was determined spectrophotometrically by the Griess reaction (12) (Roche, Cat No 1 756 281). Plasma NO concentrations were expressed as $\mu\text{mol/L}$.

Histopathological Procedure

One lobe of the liver was removed after collection of blood and cut into longitudinal sections 2-4 mm

in thickness. Liver tissue slices were then fixed in 10% buffered formalin and embedded in paraffin. Each section in 4 μm thickness was stained with hematoxylin and eosin for light microscopic assessment. An arbitrary scope was given to each microscopic field at a magnification of 20x, 40x and 100x. Cellular lipoidosis, lipid deposition, cellular swelling, focal necrosis, mitosis, increase in Kupffer cell count, inflammation in portal area, presence of granuloma, and evaluation of central vein and surrounding liver parenchyma were the principle criteria for the histopathological evaluation.

Statistical Analysis

All data are expressed as means and standard deviations (means $\pm$ SD) and 95% confidence intervals. Data were compared between groups using one-way ANOVA and post-hoc Scheffe test. SPSS 12.0 (SPSS: Statistical Package for the Social Sciences) was used for assessing the significance of differences between groups. $p < 0.05$ was considered significant.

RESULTS

The results of the biochemical parameters are summarized in Table 1 and Table 2. Weights of the livers and hematocrit levels of the experimental groups are summarized in Table 3. AST, albumin levels and liver tissue MDA were increased significantly after partial hepatectomy ($p < 0.001$). Liver tissue GSH levels ($p < 0.01$) and weight of liver ($p < 0.001$) were significantly decreased after partial hepatectomy. ALT ($p < 0.001$), AST ($p < 0.001$), LDH ($p < 0.001$), MDA ($p < 0.001$), and NO ($p < 0.05$) levels of the PHL group were significantly higher than in the PH group in plasma. Cu-Zn SOD and intraerythrocytic GSH levels of the PHL group were significantly lower than in the PH group ($p < 0.001$) in plasma. MDA, albumin, LDH, AST, and ALT levels of the PHL group were significantly higher than in the Sham group ($p < 0.001$) in plasma. Liver tissue MDA levels of the PHL group were significantly higher than in the PH group ($p < 0.001$). Liver tissue Cu-Zn SOD levels of the PHL group were significantly lower than in the PH group ($p < 0.05$). Liver tissue Cu-Zn SOD levels of the PHL group were significantly lower than in the Sham and PH groups ($p < 0.05$). There were no significant differences between the groups in hematocrit levels. There were no significant differences in histopathological evaluation between PH (Figure 1) and PHL (Figure 2) groups (Table 4).

Table 1. Plasma levels of the biochemical parameters

Experimental groups	ALT (IU/L)	AST (IU/L)	LDH (IU/L)	Albumin (g/L)	Total protein (g/L)	MDA (nmol/mg protein)	Cu-Zn SOD (U/ml)	Intraerythrocytic GSH (mg/g hemoglobin)	NO (μmol/L)
Sham	26±4.21	36.83±4.32	692.33±36.97	3.28±0.50	7.40±0.25	2.10±0.18	21.74±1.95	3.65±0.14	21.21±2.22
PH	32.90±3.32	148±23.45***	651.40±102	4.25±0.43***	6.67±0.48***	2.30±0.28	23.44±2.52	4.26±0.56	22.30±2.26
Lycopene +PH	72.44±23.69***,###	398.78±61.12***,###	1240.56±393.14***,###	4.13±0.39***	6.37±0.28***	3.45±0.54***,###	19.19±1.82###	3.51±0.37###	25.89±3.14#

• Significant differences between S group and other group defined with (*)

• Significant differences between PH group and other group defined (#)

(*): p<0.05, (**): p<0.01, (***): p<0.001

(#): p<0.05, (##): p<0.01, (###): p<0.001

Table 2. Liver tissue levels of the biochemical parameters

Experimental groups	MDA (nmol/mg protein)	GSH (μmol/mg protein)	Cu-Zn SOD (U/mg protein)
Sham	1.26±0.29	8.31±0.76	8.75±0.31
PH	38.55±3.73***	6.33±1.13**	7.91±1.34
Lycopene + PH	48.36±6.10***,###	6.34±1.72**	6.23±2.02**,#

• Significant differences between S group and other group defined with (*)

• Significant differences between PH group and other group defined (#)

(*): p<0.05, (**): p<0.01, (***): p<0.001

(#): p<0.05, (##): p<0.01, (###): p<0.001

Table 3. The liver weights and the hematocrit levels of the experimental groups

Experimental groups	Weights of the liver (g)	Hematocrit (%)
Sham	8.95±0.61	34.13±2.85
PH	6.29±0.34 ***	34.50±3.25
Lycopene + PH	7.83±0.66###	35.50±3.07

• Significant differences between S group and other group defined with (*).

• Significant differences between PH group and other group defined (#).

(*): p<0.05, (**): p<0.01, (***): p<0.001

(#): p<0.05, (##): p<0.01, (###): p<0.001

Table 4. Scores of the histopathological evaluation of the liver damage

Groups	Score
Sham	0±0
PH	1,1±0,34
Lycopene + PH	1,6±0,42

DISCUSSION

Improved knowledge of the functional anatomy of the liver with sophisticated perioperative care and patient selection are the contributory factors of successful liver surgery (13-15). However, liver failure following partial hepatectomy still occurs and remains incompletely understood. The funda-

mental parameters lay the groundwork for liver failure following partial hepatectomy, such as functional mass of the remnant liver, the age of the patient and the presence of pre-existing liver disease such as cirrhosis, chronic hepatitis and fatty liver disease. These situations interfere with the normal regenerative pathways or initiate apoptotic pathways, resulting in a net loss of functional hepatocytes (16). Increased oxidative stress during the early phase of liver regeneration had been observed as a cause of surgery and a reactive response of the reduced organ to compensate for the extra functional load (17-19). We observed significantly increased oxidative stress and liver function tests levels and significantly decreased liver tissue GSH levels after partial hepatectomy. Hepatic lipid peroxidation peaks at 24 hours after partial hepatectomy when GSH content is minimum (19,20). It has also been addressed that oxidative stress is reduced before cell division (19,21,25). Increased oxidative stress could diminish the regeneration process.

Lycopene, which is the most prominent carotenoid in tomatoes, is the most potent *in vitro* antioxidant among the carotenoids (22). Jamshidzadeh et al. (23) had suggested that tomato extracts or only lycopene could be effective against oxidative

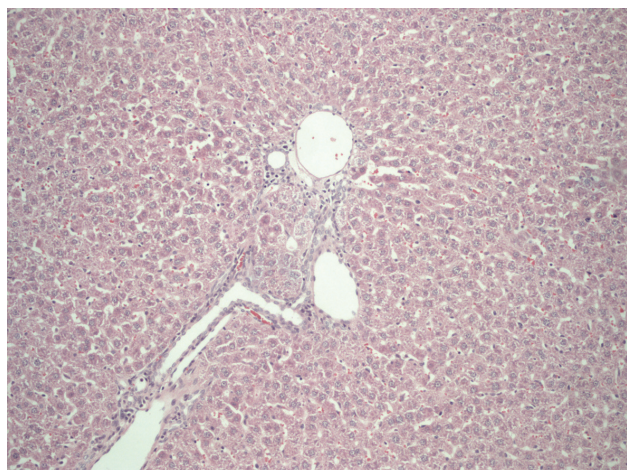


Figure 1. The liver tissue of the PH group. Hematoxylin-eosin dye 20x

stress-induced damage by chemicals or drugs to some organs. This suggestion was also supported by other authors (24). Lycopene supplementation did not cause any differences in plasma AST, ALT or intraerythrocytic GSH levels in recent studies (26). Tomato extract was found to be effective against oxidative stress due to acetaminophen-induced hepatotoxicity (23). We observed increased lipid peroxidation, increased liver enzymes levels and diminished antioxidant response in the lycopene-treated hepatectomized group. Dose-response effects of lycopene on selected drug-metabolizing and antioxidant enzymes in the rats were investigated previously (27). It has been reported that lycopene has antioxidant and histologically cytoprotective effects on different tissues, such as heart and skeletal muscle tissue at doses of 2-8 mg/kg/day (26). We administered a 4 mg/kg/day dose of lycopene. Profitable effects of this dose of lycopene were reported in recent studies (28,29). As an unforeseen circumstance, we observed no beneficial effects of lycopene on liver regeneration in the histopathological evaluation. The antiproliferative effects of lycopene were also reported before (5,39). Lycopene might have an antiproliferative effect on rapidly regenerating or dividing tissues, such as liver or tumoral lesions. It has been suggested that liver regeneration is a process that takes place under conditions of low oxidative stress (19). Increased oxidative stress by lycopene administration in hepatectomized rats could diminish the regeneration process in the liver.

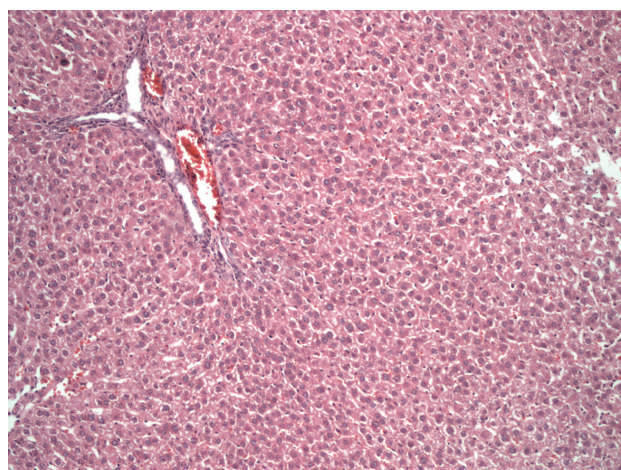


Figure 2. The liver tissue of the PHL group. Hematoxylin-eosin dye 20x

The role of NO seems to be controversial because in some models of inflammation, it has been shown that tissue dysfunction or injury could occur after inhibition of NO (30). It has also been reported that NO treatment supports liver regeneration, improves liver function and has hepatoprotective effects (31,32). NO can mediate a number of physiological and pathological reactions (33). NO could initiate production of reactive oxygen species after partial hepatectomy and during liver regeneration. Cantré et al. (31) had reported that inhibition of the release of NO decreased liver damage. We observed increased NO levels with increased lipid peroxidation levels. NO may act both as a cytotoxic agent and a cytoprotective agent, the main determinants being its concentration and the environment (34,35).

The antioxidant and antiproliferative actions of lycopene and other lycopeneoid molecules have been demonstrated *in vitro*. It has been reported that tomatoes, containing or not containing lycopene, have a higher potential than lycopene to attenuate and/or to reverse oxidative stress-related parameters (36-38). We administered only lycopene, not tomato extract, and performed an *in vivo* study.

In conclusion, based on our results, we can suggest that lycopene administration may be harmful by increasing oxidative stress in partially hepatectomized rats. Further studies are needed to define the effects of lycopene or tomato extract on the oxidant and antioxidant status after liver resection.

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