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Protective effect of lycopene against mercury-induced cytotoxicity in albino mice : Pathological evaluation

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Abstract: *The present study was carried out to evaluate the protective role of lycopene on cytotoxicity induced by mercury in albino mice. The animals were randomly divided into seven groups. Group I (control) were treated with tap water, Group II (positive control) were treated with 20 mg kg^{-1} d^{-1} lycopene, Group III were treated with 10 mg kg^{-1} body weight mercury, Group IV were treated with 10 mg kg^{-1} body weight mercury + 5 mg kg^{-1} d^{-1} lycopene, Group V were treated with 10 mg kg^{-1} body weight mercury + 10 mg kg^{-1} d^{-1} lycopene, Group VI were treated with 10 mg kg^{-1} body weight mercury + 15 mg kg^{-1} d^{-1} lycopene, Group VII were treated with 10 mg kg^{-1} body weight mercury + 20 mg kg^{-1} d^{-1} lycopene once a day for 20 consecutive days by oral gavage. The initial and final weights of all mice were measured by sensitive balance in order to investigate the effect of mercury and lycopene on the body weight of mice. Then, MN slides were prepared using the standard MN assay technique with Giemsa staining from erythrocyte cells of each mouse and were scored using binocular light microscope (Japan, Olympus BX 51). The results indicated that, all lycopene-supplemented lymphocytes showed a lower MN frequency than lymphocytes in only mercury-treated group. It was seen that lycopene had protective effect on MN particularly at 20 mg kg^{-1} d^{-1} dose when compared with the other doses. Besides, weight gain increased depending on dose of applied lycopene when compared with only mercury-treated group. In histopathological examinations, although it has been observed severe changes such as hemorrhage, hepatocyte degeneration and tubular degeneration of kidney in only mercury-treated group, there was an observable regression on the severity and account of these lesions in tissues of mice supplemented with different doses of lycopene. In vivo results showed that the lycopene supplementation decreases cytotoxicity induced by mercury and its protective role is dose-dependent.*

Key words: *In vivo micronuclei assay, Lycopene, Mercury toxicity, Pathology, Weight gain*
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Introduction

There are a serious rise in the environment of toxic chemicals and heavy metals. The accumulation of heavy metals in the body of organisms causes significant health risks (Joseph and Klinghardt, 2001). Mercury is one of the fairly harmful heavy metals for lives (Gul *et al*., 2004) and it has extremely toxic characteristic for organisms (Sharma *et al*., 2005). Mercury is a naturally occurring metal in organic (methyl mercury, ethyl mercury and phenyl mercury) and inorganic forms (mercury chloride) which possess different toxicity (ATSDR, 1999; Trasande *et al*., 2005). It is distributed throughout the environment by both natural sources and human activities (EPA, 2001). It is being widely used in fields such as the industrial, medical and agriculture. Mercury enters in the body from mining ore deposits, burning coal and waste and from manufacturing plants. This metal easily penetrates into tissues as kidney, lung, bloodstream, connective tissue, brain, adrenal and other endocrine glands (ATSDR, 1999; Joseph *et al*., 2001; Jagadeesan and Sankarsami Pillai, 2007) and it may be caused damages in all these tissues.

The use of certain substances may help in decreasing the cytotoxicity created by heavy metals (Sharma *et al*., 2005; Kavitha and Jagadeesan, 2006). A large number of compounds natural and synthetic have been evaluated for this aim (Pillai and Damodharan, 2007). Specially carotenoids were used to decrease these adverse effects in many *in vivo* and *in vitro* studies (Gulkac *et al*., 2004; Sendao *et al*., 2006). Carotenoids is a class of lipophilic pigments generally found in plants and they are strong antioxidants which are associated with scavenging of free radicals (Sendao *et al*., 2006). Supplementation of the human diet with antioxidants has been shown in numerous studies to reduce the proportion of DNA damage induced by various agents (Dusinska *et al*., 2003).

Lycopene has been shown to have the highest antioxidant activity among the carotenoids in cell protection against free radicals (Atessahin *et al*., 2006; Mure and Rossman, 2001). Lycopene is one of over 600 carotenoids found in nature. It is a natural pigment synthesized by plants and predominantly found in tomato, watermelon and grapefruit. Lycopene is an acyclic isomer of β-carotene with 11 conjugated double bonds, normally in the all-*trans* configuration (Bramley, 2000; Scolastici *et al*., 2007). It has been suggested that lycopene can prevent carcinogenesis by protecting vital biomolecules including DNA, proteins, enzymes and lipids (Scolastici *et al*., 2007). The anticancer activity of this carotenoid has been demonstrated in a large number of studies (Mahmooduzzafar *et al*., 2007; Atessahin *et al*., 2006; Mure and Rossman, 2001).

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Consequently, lycopene has been used as a food coloring agent for many years but recent studies have reported its antioxidant activity, free radical scavenging capacity and role to prevent some critical diseases, including cancers (Sendao *et al*., 2006; Atessahin *et al*., 2005). However, the protective mechanisms of lycopene are still not clear. The aim of the present study was to evaluate the protective role of lycopene on mercury-induced cytotoxicity in albino mice.

Materials and Methods

The present study has been carried out on 35 mice. Six weeks old male mice (*Mus musculus var. albino*) were used for *MN* analysis and determination of the alterations in body weight. Healthy mice were obtained from the Animal Research Center of Refik Saydam Hifzissiha Institute (Ankara, Turkey). Their mean body weight was 32.48±0.77 g. The mice were kept in metal cages with 5 albino mice per cage at 22°C (±3°C) temperature and a 12 hr light/dark cycle. Mice were acclimatized 1 week prior to the planned experiments and fed with standard pellet diet (Samsun Food Industry–Samsun, Turkey) *ad libitum*.

Product and chemicals: The lycopene was obtained from Vega Natural Products Ilt., Ltd., Konya-Turkey. Mercury (II) chloride (Sigma, Fluka) and Grunwald Giemsa (Sigma, Fluka) were purchased from Interlab A.S., Istanbul-Turkey.

Experimental design and exposure: Albino mice were divided into seven groups each consisting of five animals. Group I (control): In this group mice were treated with only tap water, Group II (positive control): treated with only 20 mg kg^{-1} d^{-1} lycopene, Group III: treated with only 10 mg^{-1} kg body weight mercury, Group IV: treated with 10 mg kg^{-1} body weight mercury + 5 mg^{-1} kg^{-1} d lycopene, Group V: treated with 10 mg kg^{-1} body weight mercury + 10 mg kg^{-1} d^{-1} lycopene, Group VI: treated with 10 mg kg^{-1} body weight mercury + 15 mg kg^{-1} body weight lycopene, Group VII: treated with 10 mg kg^{-1} body weight mercury (n: 5) + 20 mg kg^{-1} d^{-1} lycopene, once a day for 20 consecutive days by oral gavage. The mercury and lycopene solutions were prepared daily in distilled water. The dose of mercury in this study has been determined as 10 mg kg^{-1} body weight. This dose was chosen because it induced an increase in the frequency of cytotoxic damages which was essential for investigation of the protective role of lycopene (Inouye and Kajiwara, 1988; Chuu *et al*., 2001). The lycopene doses determined favourable to daily consumption amount that recommended by practitioners of nutritional medicine for support optimal health (Hsiao *et al*., 2005; Chin-Shiu *et al*., 2006).

MN assay: The *MN* assay was performed on erythrocytes. Blood samples were obtained from the tail vena of each mouse and 3-4 smears were prepared. 5 µl blood sample spread on slides, then cells on slides were fixed with methanol and stained with Grünwald Giemsa staining. From each smear, 1000 cells were examined under 100 magnification in binocular light microscope (Japan, Olympus BX 51) and *MN* cells were photographed under X500 magnification. For the scoring of *MN* the criteria as discribed were adopted Fenech *et al*. (2003).

Histopathological examinations: For histopathological examinations, tissue samples obtained from lung, heart, liver, kidney, stomach and intestine were fixed in 10% neutral buffered formalin solution. After the routine histopathological processing, tissue samples were embedded in paraffin wax and sectioned at 6 µm. All sections were stained with Hematoxylin and Eosin (H&E), and semiquantitatively assessed under the light microscope. Changes in the experimental histopathological parameters for different tissues were graded as follows: (–) no changes, (+), (++) and (+++) indicating mild, moderate and severe changes, respectively.

Statistical analysis: For the statistical analysis, differences between the groups were tested by analysis of variance (ANOVA) and Duncan test using SPSS for Windows version 10.0. The data were displayed as means ± SD and P values less than 0.05 were considered significant.

Results and Discussion

Table - 1: Frequency of micronucleus *(MN)* in mice erythrocyte cells treated with mercury and four daily doses of lycopene

Groups	Number of scored cell	Minimum	Maximum	Average ±SD
Group I	1000	1	3	2.20±0.84
Group II	1000	1	3	1.80±0.84
Group III	1000	53	55	54.20±0.84
Group IV	1000	48	49	48.60±0.55
Group V	1000	39	41	40.40±0.89
Group VI	1000	29	31	29.60±0.89
Group VII	1000	18	19	18.60±0.55

*Values presented as mean±SD (n=5). Group I: Control, Group II: positive control, Group III: mercury (10 mg kg^{-1} body weight), Group IV: mercury (10 mg kg^{-1} body weight) + lycopene (5 mg kg^{-1} d^{-1}), Group V: mercury (10 mg kg^{-1} body weight) + lycopene (10 mg kg^{-1} d^{-1}), Group VI: mercury (mg kg^{-1} body weight) + lycopene (15 mg kg^{-1} d^{-1}), Group VII: mercury (10 mg kg^{-1} body weight) + lycopene (20 mg kg^{-1} d^{-1})

Table - 2: Mean body weights in mice treated with mercury and four daily doses of lycopene

Groups	Initial weight (g)	Final weight (g)	Difference (g)
Group I	32.39±1.12	42.13±0.60	+ 9.74
Group II	32.57±0.44	44.00±0.60	+11.43
Group III	32.44±1.28	29.16±1.07	– 3.28
Group IV	32.42±0.55	33.40±0.55	+ 0.98
Group V	32.33±0.69	34.25±0.84	+ 1.92
Group VI	32.71±0.53	36.28±0.52	+ 3.57
Group VII	32.47±0.52	38.39±0.64	+ 5.92

*Values presented as mean±SD (n=5). Group I: Control, Group II: positive control, Group III: mercury (10 mg kg^{-1} body weight), Group IV: mercury (10 mg kg^{-1} body weight) + lycopene (5 mg^{-1} kg^{-1} d), Group V: mercury (10 mg kg^{-1} body weight) + lycopene (10 mg kg^{-1} d^{-1}), Group VI: mercury (10 mg kg^{-1} body weight) + lycopene (15 mg kg^{-1} d^{-1}), Group VII: mercury (10 mg kg^{-1} body weight) + lycopene (20 mg kg^{-1} d^{-1})

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Table - 3: Statistically comparision of data belonging to micronucleus *(MN)* frequency and body weight determined in treatment group mice at the end of 20th day

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
MN frequency	2.20±0.84^{f}	1.80±0.84^{f}	54.20±0.84^{a}	48.60±0.55^{b}	40.40±0.89^{c}	29.60±0.89^{d}	18.60±0.55^{e}
Bodyweight	42.13±0.60^{b}	44.00±0.60^{a}	29.16±1.07^{f}	33.40±0.55^{e}	34.25±0.84^{e}	36.28±0.52^{d}	38.39±0.64^{c}

*Values presented as mean±SD (n=5). Means denoted with different superscripts are within the same line are statistically significant. Group I: Control, Group II: positive control, Group III: mercury (10 mg kg^{-1} body weight), Group IV: mercury (10 mg kg^{-1} body weight) + lycopene (5 mg kg^{-1} d^{-1}, Group V: mercury (10 mg kg^{-1} body weight) + lycopene (10 mg kg^{-1} d^{-1}), Group VI: mercury (10 mg kg^{-1} body weight) + lycopene (15 mg kg^{-1} d^{-1}), Group VII: mercury (10 mg kg^{-1} body weight) + lycopene (20 mg kg^{-1} d^{-1})

Table - 4: Histopathological evaluation of mice treated with mercury and four daily doses of *Lycopene*

Histopathological change	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Hyperemia and emphysema of the lung	–	–	+ + +	+ + +	+ +	+	+
Hyperemia in the liver	–	–	+ + +	+ + +	+ +	+	+
Hemorrhage in the liver	–	–	+ + +	+ ++	+ +	+	+
Hepatocyte degeneration	–	–	+ + +	+ + +	+ +	+	+
Hyperemia in myocardial tissue	–	–	+ + +	+ + +	+ +	+	+
Hemorrhage in miyocardial tissue	–	–	+ + +	+ + +	+ + +	+	+
Gastric hyperemia	–	–	+ + +	+ + +	+ + +	+ +	+ +
Gastric hemorrhage	–	–	+ + +	+ + +	+ + +	+ +	+
Intestinal hyperemia	–	–	+ + +	+ + +	+ +	+ +	+
Intestinal hemorrhage	–	–	+ +	+ +	+ +	+	+
Renal hyperemia	–	–	+ + +	+ + +	+ +	+ +	+ +
Renal hemorrhage	–	–	+ + +	+ + +	+ +	+	+
Tubular degeneration of kidney	–	–	+ + +	+ + +	+ +	+	+

*Group I: Control, Group II: positive control, Group III: mercury (10 mg kg^{-1} body weight), Group IV: mercury (10 mg kg^{-1} body weight) + lycopene (5 mg kg^{-1} d^{-1}), Group V: mercury (10 mg kg^{-1} body weight) + lycopene (10 mg kg^{-1} d^{-1}), Group VI: mercury (10 mg kg^{-1} body weight) + lycopene (15 mg kg^{-1} d^{-1}), Group VII: mercury (10 mg kg^{-1} body weight) + lycopene (20 mg kg^{-1} d^{-1}), - (no change), + (mild change),++ (moderate change), +++ (severe change)

MN analysis: Microscopic examination of erythrocyte cells showed that there were only several the *MN* formation in lymphocyte cells of mice in control and positive control groups. The *MN* frequency of lymphocytes belonging to positive control group was fairly similar to control group ($p>0.05$). Mercury treatment showed a significant increase in the number of *MN* formation (Fig. 1). Detailed information related with the *MN* frequency was showed in Table 1, 3. The highest frequency of *MN* was observed in mice treated with only mercury and least frequency of *MN* was observed in mice treated with different dose of lycopene. The animals treated with different lycopene doses showed a significant reduction ($p<0.05$) in the frequency of *MN* when compared with the animals treated with only mercury. *MN* frequency decreased with rising of the lycopene doses. There was a significant dose-effect relationship between the *MN* frequency and lycopene. *MN* frequency in the mice treated with 20 mg $^{-1}kg^{-1}d^{-1}$ doses of lycopene was lower than those in mice treated with the 5, 10 and 15 mg $^{-1}kg^{-1}d^{-1}$ doses of lycopene. There was a statistically significant difference between treatment groups and control groups for *MN* frequency ($p<0.05$). Besides, there was statistically significant difference among 5-20 mg $kg^{-1}d^{-1}$ doses of lycopene ($p<0.05$).

Body weight: The results related with the weight gain were given in Table 2 , 3. These data showed that mercury treatment significantly prevented the weight gain of mice. The highest weight gain was observed in positive control group at the end of experimental period. The least body weight was observed in mice treated with only mercury. In control and positive control groups, the weights of all mice increased about 9.74, 11.43 g according to initial weight, respectively. The weights of mice exposed to only mercury showed decrease of about 3.28 g according to initial weight. But, lycopene supplement caused again rising in body weight when compared with mice exposed to only mercury. In mice supplemented with 5, 10, 15 and 20 mg $^{-1}kg^{-1}d^{-1}$ doses of lycopene, the body weight increased about 0.98, 1.92, 3.57, 5.92 g according to initial weight, respectively. A parallel correlation was determined between increase in lycopene doses with increase in weight gain. And also, these increases were statistically significant between 10-20 mg $kg^{-1}d^{-1}$ doses of lycopene ($p<0.05$). But, there was no statistically significant difference between 5-10 mg $kg^{-1}d^{-1}$ doses of lycopene ($p>0.05$).

Pathology: Detailed information related with the histopathological findings was showed in Table 4.

No histopathological changes were observed in Group Iand Group II (Fig.2).:

There were severe histopathologial lesions in Group III. Hyperemic, hemorrhagic and degenerative changes were mainly lesions. Many blood vessels and capillaries were hyperemic in heart, lung, liver, kidney, stomach and intestines. There was emphysema in the alveoli and interalveolary capillaries were hyperemiz in the lung. Hyperemic vessels and hemorrhagic foci

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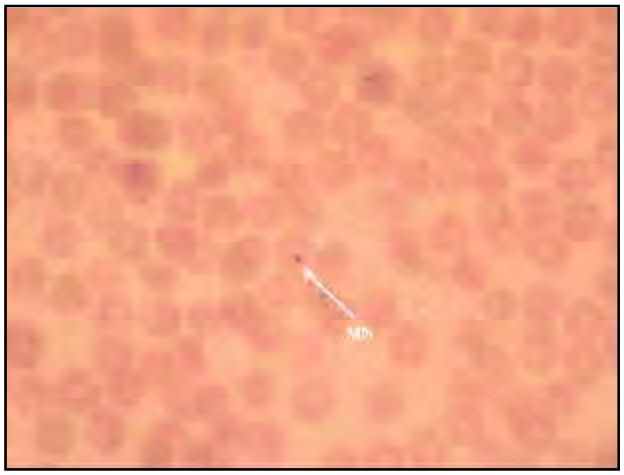

Fig. 1: The appearance of erythrocyte cells in blood tissue. Arrow: a micronucleated erythrocyte. Giemsa staining. Magnification, X500

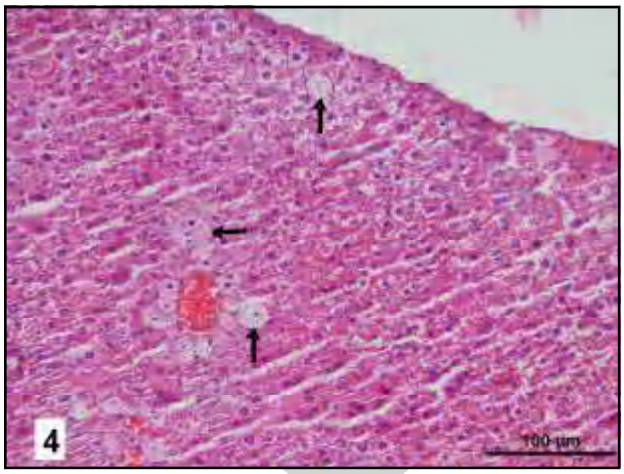

Fig. 4: Liver in group III exposed to 10 mg kg^{-1} body weight mercury. Presence of characteristic severe hepatocyte degeneration because of mercury toxicosis. Arrows show swelling of the cytoplasm and picnotic nuclei. H&E

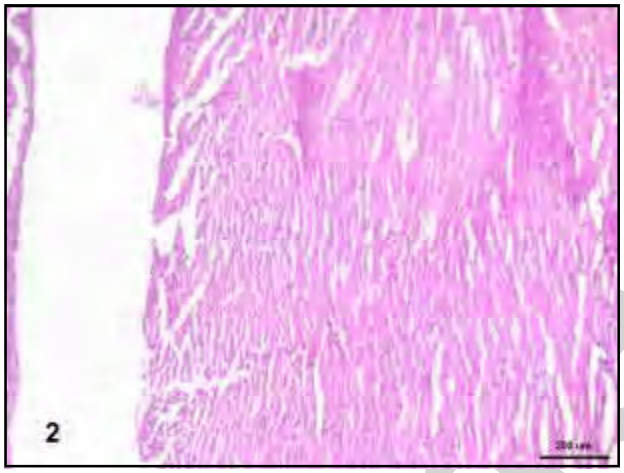

Fig. 2: Heart in positive control group at the end of 21st day. Absence of histopathological changes. H&E

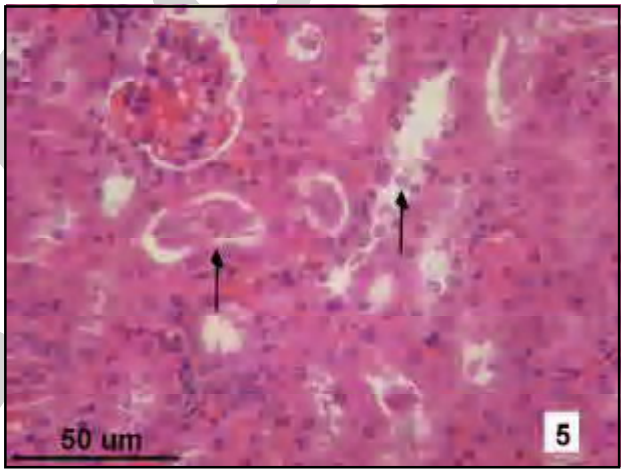

Fig. 5: Kidney section prepared from mercury-treated mice in group III (section prepared on day 21). A section shows glomerular hyperemia, tubular degenerations and albuminoid content (arrows). H&E

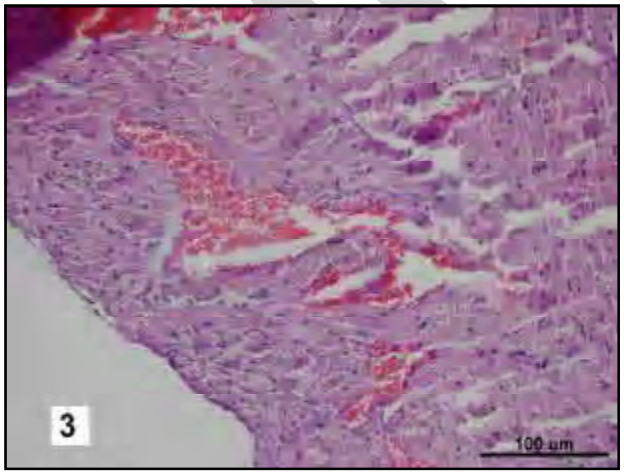

Fig. 3: Heart in group III treated with 10 mg kg^{-1} body weight mercury. Note the presence of severe dilatations in the vessels and hyperemia. H&E

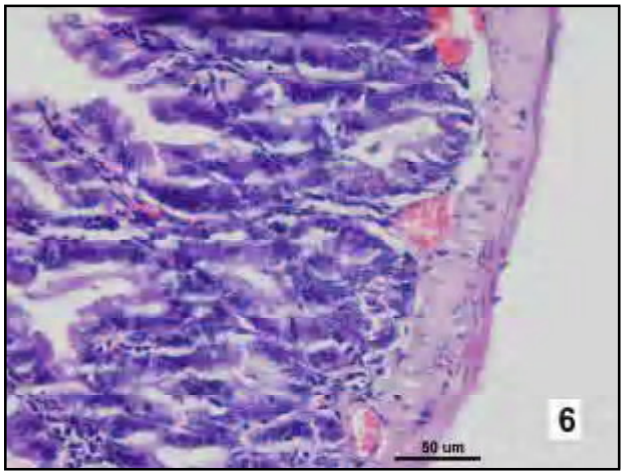

Fig. 6: A mouse intestine histopathology in mercury-treated group at 6 weeks of age. A section shows characteristic hyperemic vessels. H&E

were detected in the myocard (Fig. 3). In the liver, central veins and sinusoids were dilated because of hyperemia. Many hepatocytes were swollen and pycnotic nuclei were detected in periacinary hepatocytes (Fig. 4). In kidney, there was hyperemia in the medulla. Tubular dilatation were detected and they were filled with albuminoid content (Fig. 5). Hyperemic vessels and hemorrhagie were also detected in the gastric and intestinal propria (Fig. 6). Hyperemic, hemorrhagic and degenerative lesions were seen also in Group IV. Tubular degeneration of the kidney (Fig. 7) and degenaration of the hepatocytes lesions were also observed in this group. Besides, there was prominent hyperemia and hemorrhage in the myocard (Fig. 8).

Histopathological changes such as hyperemia and hepatocyte degeneration in Group V were similar to Group III and IV. However, there was small amount decreasing severity of the hemorrhagic lesions. Besides, hepatocyte was observed as a noticeble regression severity of the degeneration of the hepatocytes when it compared with Group III (Fig. 9)

A significant regression in the severity of the lesions was observed in Group VI. Especially, the rate of the hepatocyte degeneration and tubular degeneration significantly decreased in this group (Fig. 10). In additon to this findings, small hemorrhagic foci were seen in these tissues.

The regression in the severity of histopathological lesions were prominent also in Group VII . The rate of the degenerative changes in the liver and kidney were significantly decreased. With limited hepatocyte degeneration, there was a prominent regression in the severity of the hepatocyte degeneration in the liver. Kupffer's cell reaction was apparent (Fig. 11). Besides, there was a prominent regression in the severity of the tubular lesions in the kidney. Albuminoid content in the tubular lumens was not generally observed in this group (Fig. 12).

The long-term exposure to high amounts of organic and inorganic mercury can perennially damage the brain, kidney, liver, central nervous system, gastro-intestinal and developing fetus. Short-term exposure to high amounts of mercury vapors may cause effects including lung damage, nausea, vomiting, diarrhea, increases in blood pressure and heart rate, skin rashes, thyroid dysfunction and eye irritation. There are also cancer data available for mercury. Mercuric chloride increases several types of tumors in rats and mice. Chronic dietary exposure of mice to methylmercury resulted in significant rises in the percentages of kidney tumors in mice.

Besides, data are available on developmental effects in guinea pigs, hamsters and monkeys (ATSDR, 1999; EPA, 2001; Tiwari and Bhattacharya, 2004). Mercury induces the formation of reactive oxygen species (ROS) such as hydrogen peroxides. These ROS enhances the sequential iron (Fe) and copper (Cu)-induced production of lipid peroxides and the highly reactive hydroxyl radicals. These lipid peroxides and hydroxyl radicals may cause the DNA, chromosome and cell membrane damages and thence destroy the cell (Sharma *et al*., 2005).

In recent years, genetic monitoring of populations exposed to potential mutagens is an early warning system for genetic disease as cancer. The most frequently used genetic endpoints is chromosomal aberrations and *MN* frequency (Celik and Akbas, 2005). The *MN* test is a reliable technique for the evaluation of mutagenic effects induced by chemical agents (Mozdarani and Kamali, 1998). In present study, cytotoxicity induced by mercury using as an indicator the *MN* assay in albino mice was detected. The result indicated that no statistical difference was observed between *MN* scores in erythrocytes obtained from the control and positive control groups. There were several the *MN* formation in lymphocytes of mice belong to either groups. However, results revealed a significant increase in frequency of *MN* in peripheral blood erythrocytes obtained from mice treated with mercury. These data are in agreement with the previous reports obtained on the potential effects of mercury. For example, the genotoxic effects of mercury were studied using the *MN* assay in V79 Chinese hamster fibroblasts. Eventually, a statistically significant increase in *MN* frequency observed at the 0.05 μM mercury concentration dose (Thomas *et al*., 2004). In another study, *Cyprinus carpio* fish, exposed to 2, 20 and 200 mg concentrations of mercury indicated that for 2 mg^{-1} kg^{-1} body weight concentration of mercury, there was no significant increase in frequency of *MN*, but at 20 and 200 mg kg^{-1} body weight mercury concentrations there was a significant increase in *MN* frequencies (Nepomuceno *et al*., 1998). As a result, all these findings may be associated with a loss of genetic material. Namely, *MN* is cytoplasmic chromatin masses with the appearance of small nuclei that originate from chromosome fragments or whole chromosomes that are not included in the main daughter nuclei during metaphase or anaphase phase of cell division. Hence, the *MN* formation causes loss of genetic material. A number of studies have shown that mercury (II) chloride binds to DNA and causes strand breaks *in vitro*. Mercury (II) chloride has also been shown to rise in chromosomal aberrations in Chinese hamster ovary cells *in vitro* (World Health Organization, 2005; Howard *et al*., 1991).

The frequency of *MN* in all groups supplemented with lycopene was much lower than those treated with only mercury. In mice treated with lycopene the *MN* frequency decreased parallel with the increasing of lycopene doses. The results indicate that lycopene could reduce mutagenic effects of mercury.

In this study we also investigated the changes in body weight of mercury and lycopene applied mice. As a result significant differences were observed in weight gains of mice treated with mercury when compared with the control and positive control groups. The mean body weights of mice in all mercury treatment groups were lower than those of the controls. The highest level of body weight was observed in positive control group mice and least level of body weight was observed in mice treated with only mercury. Supplementation with different doses of lycopene was associated with an increase in weight gain. Supplementation with different doses of lycopene was associated with an increase in weight gain. In lycopene-supplemented group, the largest effect of supplementation was seen at 20 mg kg^{-1} d^{-1} lycopene dose, 5,10,15 and 20 mg kg^{-1}

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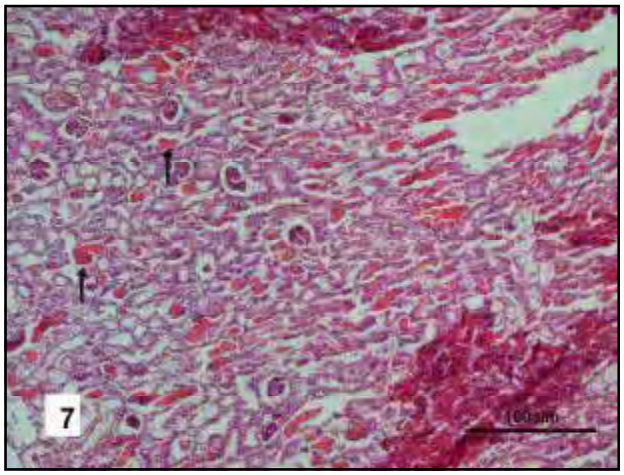

Fig. 7: Liver section in group IV treated with 10 mg kg^{-1} body weight mercury + 5 $mg^{-1}kg^{-1}$ d lycopene. Arrows show the dilatated tubules filled by albuminoid content. H&E

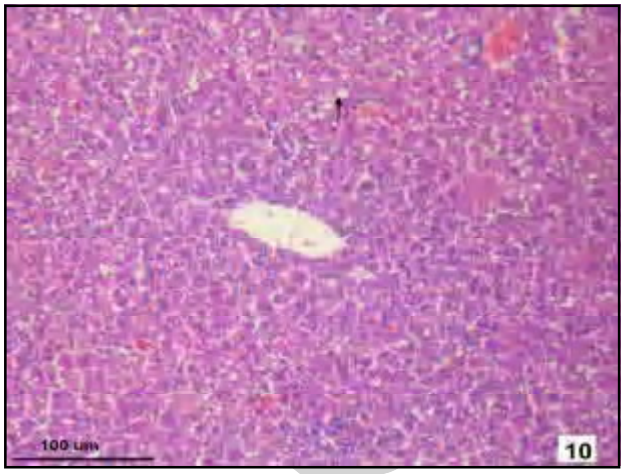

Fig. 10: Liver in group VI treated with 10 mg kg^{-1} body weight mercury + 15 $mg^{-1}kg^{-1}$ d lycopene. A section shows hyperemia in capillaries and some sinusoids. Arrow notes swelling of cytoplasms of some hepatocytes. H.E.

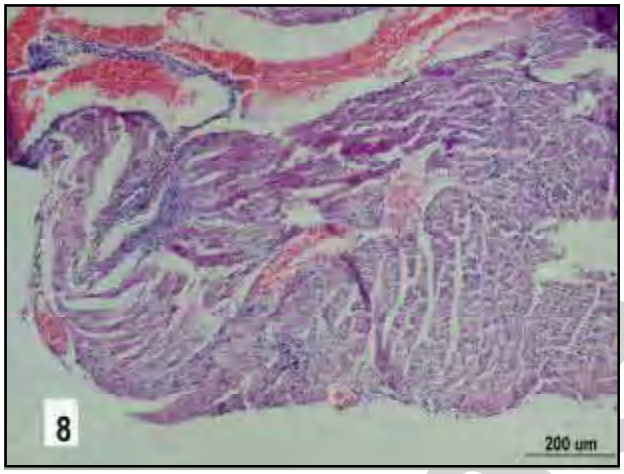

Fig. 8: Heart section in group IV treated with 10 mg kg^{-1} body weight mercury + 5 $mg^{-1}kg^{-1}$ d lycopene. A section shows severe hyperemia and hemorrhagia. H&E

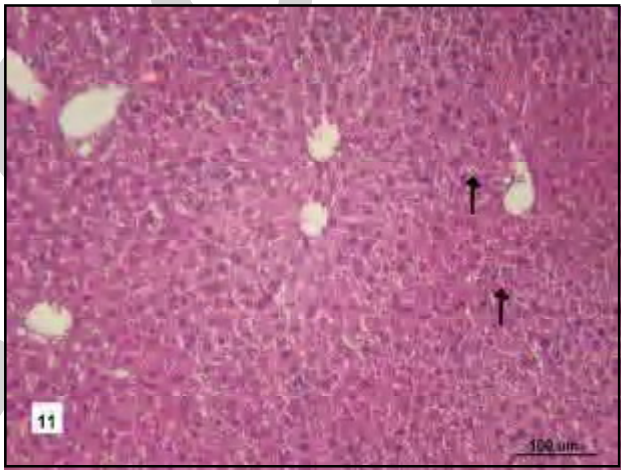

Fig. 11: Liver section in group VII treated with 10 mg kg^{-1} body weight mercury + 20 $mg^{-1}kg^{-1}$ d lycopene. A section shows decrease in the rate of the degenerative hepatocytes. Arrows note increase of the Kupffer's cells. H&E

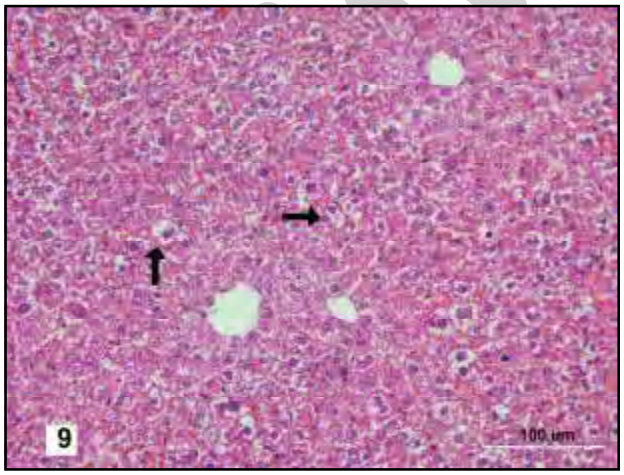

Fig. 9: Liver in group V treated with 10 mg kg^{-1} body weight mercury + 10 $mg^{-1}kg^{-1}$ d lycopene. Arrows show the presence of swelling in the hepatocyte cytoplasm. H&E

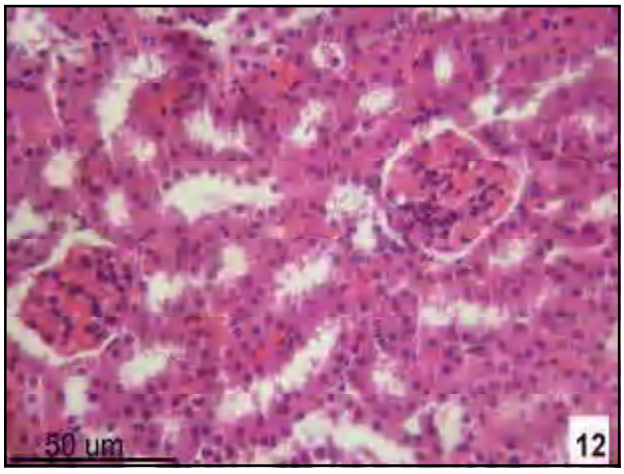

Fig. 12: A Mouse kidney histopathology in group VII treated with 10 mg kg^{-1} body weight mercury + 20 mg $kg^{-1}d^{-1}$ lycopene. A section shows glomerulary hyperemia and dilate tubules without albuminoid content. H&E

d^{-1} dose of lycopene caused 14, 17, 24 and 31% increase of body weight according to group treated with only mercury, respectively. This information is parallel with other data available so far. Many epidemiological studies showed that there is a clear relationship between the weight decrease and exposure to mercury. For example, dose-related decreases in body weight when compared with the controls were reported in male and female rats exposed to 1.25, 2.5, 5, 10, or 20 mg kg^{-1} body weight of mercury. In a similar study, when rats were exposed to mercury (II) chloride by gavage or subcutaneous injection for up to 11 weeks, the rate of body weight gain decreased after 20 days and actual weight loss occurred after 65-70 days (W HO, 2005). These results may related to the metabolism rate of the mice. Besides, the another cause of this weight loss may be related with digestion system damage. In a previous study reported that the third of the mucosal surface of the stomach was thickened and necrotic in mice exposed to difference doses of Allyl Isothiocyanate (NTP, 1982). It has been also reported that chemical agents are cause severe corrosive injury to the mouth, throat, esophagus and stomach, with bleeding, perforation, scarring, or stricture formation as potential sequelae (ATS DR, 2007). This situation may reduced water and food consumption in mice and mice loss weight. In the present study, prominent histopathological changes such as hyperemia (in lung, myocard, liver, stomach, intestine and kidney tissues), hemorrhagia (in myocard, liver, stomach, intestinal tissues) and degenerative changes (hepatic and renal tissues) were observed in only mercury-treated group. In groups supplemented with lycopene, there was no histopathological change similar to control group. There was seen a prominent decreasing in the severity of the hyperemic, hemorrhagic and especially degenerative changes in the liver and kidney tissues of mice supplemented with 15 and 20 mg kg^{-1} d^{-1} doses of lycopene.

All these results suggest that lycopene may prove useful in reducing of the toxic effects induced by chemical agents as mercury. The protective effect of lycopene on mercury-induced damages may be attributed to its antioxidant activity. The researches found that antioxidants are significant molecules that act as free radical scavengers and they trap the free radicals and give up own electrons. Thus, antioxidants, molecules as protein, lipid, enzyme, chromosome and DNA were protected against free radical oxidation (Feri, 1994; Halliwell *et al*., 1995). Our data suggest that the protective effect of lycopene on cytotoxicity may be the result of its interaction with free radicals such as hydrogen peroxide and nitrogen dioxide radicals (Mure and Rossman, 2001). Lycopene scavenges reactive superoxide radical, organic peroxyradicals and nitric oxide more efficiently than other carotenoids. The radical quenching ability of lycopene appears to depend on the number of conjugated double bonds (11 compared to 9 in β-carotene) (Mure and Rossman, 2001). It has been suggested that lycopene can prevent carcinogenesis by protecting vital molecules including DNA. It was also reported that this carotenoid can be useful that scavenges of reactive oxygen species (ROS), at the regulation of detoxification system, at the inhibition of cell proliferation and at the modulation of signal transduction pathway (Scolastici *et al*., 2007). Besides, many studies results indicated that the carotenoids as lycopene can be decreased the frequency of damages in cells of different organisms. For example, Atessahin *et a*l. (2006) investigated the protective effect of lycopene on cisplatin (CP)-induced spermiotoxicity in rats. As a result, they showed that lycopene have a possible protective effect against cisplatin–induced spermiotoxicity. In a similar study, it has been examined possible protective effect on chromosomal damages induced by the antitumor drug cisplatin (cDDP) in rat bone marrow cells. The results suggest that this carotenoid may prove useful in reducing of the chromosomal damages induced by cisplatin (Sendao *et al*., 2006). In another study, it has been investigated the antigenotoxic and antimutagenic effects of lycopene in Chinese Hamster ovary cells treated with hydrogen peroxide, methylmethanesulphonate, or 4-nitroquinoline-1-oxide. The results showed that lycopene reduced the frequency of micronucleated cells induced by the three mutagens and this chemopreventive activity was depending on the lycopene concentrations (Scolastici *et al*., 2007).

In our study, it could be concluded that lycopene acts as a potential antioxidant that prevents cytotoxicity induced by mercury in albino mice. In other words, supplementation with lycopene resulted in beneficial effects against cytotoxicity and especially this effect is related with dose of lycopene. So from these results it can be concluded that lycopene supplementation might be a good candidate for the standing apart from hazardous environmental factors.

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