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The effects of curcumin on the liver and remote organs after hepatic ischemia reperfusion injury formed with Pringle manoeuvre in rats

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Abstract. – BACKGROUND: We aimed to investigate the effects of curcumin on ischemia/reperfusion (IR) injury of the liver and distant organs resulting from liver blood flow arrest.

MATERIALS AND METHODS: Totally 40 rats, divided into four groups, each included 10 rats were used. Group I as only laparatomy, Group II laparatomy and curcumin application, Group III hepatic IR; and Group IV as hepatic IR and curcumin application group. Ischemia was generated by hepatoduedonal ligament clamping for 30 minutes and then reperfusion is started.

Curcumin capsules were opened and appropriate dose had been created within weighing scales. After calculations, the powder was diluted with saline. Fifteen minutes before the ischemia, curcumin was applied via oral gavage. Blood samples were taken from the animals for biochemical analysis at 60th minutes of the experiment in the first and second groups; 30 minutes after beginning reperfusion in the third and forth groups. Simultaneously, liver, lung and kidney tissues were sampled for biochemical and histopathological examinations.

RESULTS: Plasma malondialdehyde levels were found to be higher ($p < 0.001$), but total antioxidant activity values were not different in IR group compared with IR + curcumin group ($p > 0.05$). Biochemical and histopathological evaluation of tissue samples revealed that there were no differences in total antioxidant activity, total oxidant activity and histopathologic scores in IR + curcumin group compared with values of IR group ($p > 0.05$).

CONCLUSIONS: Curcumin did not reduce the effects of hepatic ischemia reperfusion injury on the liver and distant organs including kidneys and lungs significantly.

Key Words:

Hepatic ischemia-reperfusion, Curcumin, Liver, Kidney, Lung, Remote organs.

Introduction

Many surgical techniques with different periods of ischemia and reperfusion have been described. Liver ischemia/reperfusion (IR) injury may occur especially during large tumor resection and liver transplantation procedures. Oxygen deprivation causes development of ischemia and production of reactive oxygen radicals during reperfusion leading to further damage[1]. Although there are many studies designed to prevent damage in liver IR injury, they could not find the exactly suitable solution for preserving the organs from detrimental effects.

Curcumin (diferuloylmethane) is a fenolic compound which is basic pigment of *Curcuma Longa* plant, has anti-inflammatory, antioxidant and anti-carcinogenic effects[2-4] The antioxidant activity of Curcumin is comparable with antioxidant activity of vitamin C and E[2,5]. Some studies showed that curcumin reduces the harmful effects of oxidants on the vascular endothelial cells. Also curcumin's ability of lipid peroxidation inhibition was shown in animal models[6,7]. Induction of Phase II detoxification enzymes which are known to be protective agents from oxidative stress such as glutamylcysteine syn-

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thetase, glutathione S-transferases and NADPH/quinone oxido-reductase by curcumin have been reported[8].

This study was designed to investigate the protective effects of curcumin against hepatic and remote organ injury induced by hepatic IR formed with pringle maneuver. For this purpose, curcumin was given by oral gavage before experimental study and its activity was examined with biochemical markers and histopathological analysis of tissue samples of the liver, kidneys and lungs.

Materials and Methods

Experimental Animals

Forty male Wistar albino rats with average weight of 250 to 300 g were randomly selected for our experiment and purchased from Dicle University Dr. Sabahattin Payzin Health Sciences Application and Research Center. This project was approved by the Committee of Experimental Animals of Dicle University. All experimental procedures complied with the Guide for the Care and Use of Laboratory Animals. Rats were fed with water and standard rat chow in 12 hours day and night period at 25°C. Animals were fasted the night before the operation.

Surgical Procedures and Experimental Design

Anestesia provided by 50 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun®; Bayer AG, Leverkusen, Germany) via intramuscular injection and the experimental procedure was initiated. 10% povidone iodine solution (Betadine®) was performed for shaved skin cleansing. Hepatoduedonal ligament (v. porta, a. hepatica communis and common bile duct) was exposed. After placing a rubber band on top of hepatoduedonal ligament with 3.0 silk sutures around the turn has been suspended and the period of ischemia was initiated by performing Pringle maneuver. Following 30 minutes of ischemic period the suture was opened by loosening and 30-minute reperfusion period launched. At the end of this period, animals were sacrificed by sampling blood from the heart.

Animals were grouped as follows:

Grup I (Sham): Dissection of hepatoduodenal ligament was performed and no medication was given.

Grup II (Control): In addition to dissection, Curcumin was given at a dose of 200 mg/kg by oral gavage (according to T_{max}) 15 minutes before starting of the experimental study[9].

Grup III (IR): Thirty minutes after Pringle maneuver, reperfusion was performed 30 minutes and no drug was given.

Grup IV (IR + Curcumin): In addition to procedures of Group 3, curcumin was given at a dose of 200 mg/kg by oral gavage (according to T_{max}), 15 minutes before starting of the ischemia period[9].

In each group, blood samples were obtained for biochemical analyses and tissue samples for biochemical and histopathological examinations at the end of the experimental procedures. Liver, both lungs, and kidneys were removed and tissue samples are retrieved. Plasma samples were obtained from blood centrifugation and transferred to plastic ependorf tubes with cover for biochemical analysis, stored at –80°C in deep freezer. Taken tissues were prepared for biochemical analyses, foreign tissue residues and blood were removed by washing with saline and thereafter tissues were transferred to plastic tubes with ependorf cover for biochemical analysis, stored at –80°C in freezer. The tissues taken for histopathological evaluation was put into plastic containers which include 10% formaldehyde solution.

Biochemical Analyses

Total antioxidant capacity (TAC) and malondialdehyde (MDA) analyses were performed in blood samples. Total oxidant status (TOA) and TAC analysis were performed in tissue samples. In addition, oxidative stress index (OSI) was calculated in tissue samples.

Homogenization of Tissues

Tissues which stored at –80°C were removed on the deep freezer and brought to the laboratory in dry ice. About 0.30 to 0.50 grams of tissue pieces were transferred into the tube and 2 ml of Tris-HCl buffer was added.

Tissues in the tube which placed into ice-filled plastic container were processed in the 50 mM pH 7.0 phosphate buffered saline (PBS) for 1-3 minutes on 14,000 rpm at homogenizer (Ultra

Turrax Type T8, IKA Labortechnic, Staufen, Germany). Homogenate was centrifuged for 30 minutes at + 4°C. Samples were taken from supernatant for TOA and TAC analysis.

Malondialdehyde (MDA) Analysis

MDA levels were evaluated by the method of Hammouda A el-R by measuring thiobarbituric acid (TBA) reactivity[10]. The sample and test tubes were prepared. Once 2.5 ml 10% (w/v) TCA solution was put to tubes after blank tube, 0.5 ml of distilled water and sample tube, 0.5 ml sample were put and they were mixed by vortex. After closing the mouth of the tubes, they were stored in 90°C water bath for 15 minutes. Tubes were cooled and then centrifuged for 10 minutes at 3000 × g then we received 2 ml from supernatant upon we added 1 ml from % 0,675 (w/v) TBA solution. Again, after waiting 15 minutes in 90°C water bath, tubes were cooled. The absorbance each sample were read against to blank at 532 nm. Serum MDA levels were calculated as μM/L by standard graphs which prepared with different concentrations of 1,1,3,3-tetramethoxypropane.

TOA Analysis

TOA Analysis is a full automatic colorimetric method which developed by Erel[11]. The color intensity that is related to the amount of the oxidants in the sample was measured spectrophotometrically. TOA values of the tissues were calculated to be nmol H_2O_2 equiv /mg protein.

TAC Analysis

This method is a fully automatic method developed by Erel[11,12] and is capable for measuring total antioxidant capacity of the body, against strong free radicals. TAC values of the blood were calculated to be μmol Trolox Equiv/L, TOA values of the tissues were calculated to be nmol Trolox Equiv/mg protein.

OSI Analysis

OSI is an indicator parameter of the degree of oxidative stress. Formulation is as follows:

$$OSI = (TOA/TAC) \times 100 \quad (13)$$

Histopathological Evaluations

Liver, kidney and lung tissue damage was scored mild to severe level. Tissues were put into the 10% formalin solution in paraffin blocks and it is prepared by slicing 4-μm sections. Tissues stained with hematoxylin-eosin and standard protocols were applied.

Hepatic ischemia-reperfusion injury were classified as follows; Grade 0, minimal damage or no damage; Grade 1, mild damage with cytoplasmic vacuolization and nuclear pycnosis; Grade 2, moderate damage with expanded nuclear pycnosis, cytoplasmic hypereosinophilia and loss of intercellular borders; Grade 3, severe damage with hemorrhage, neutrophil infiltration and severe necrosis with the disintegration of the hepatic adheres[14].

Lung injury secondary to hepatic IR injury were classified as follows; Grade 0, no damage; Grade 1, mild neutrophil leukocyte infiltration and mild-moderate interstitial congestion; Grade 2, moderate neutrophil leukocyte infiltration, perivascular edema formation and disintegration of the pulmonar structure; Grade 3, dense neutrophil leukocyte infiltration and absolutely destruction of pulmonary structure[15].

Kidney injury secondary to hepatic IR injury were classified as follows; Grade 0, no changes; Grade 1, swelling of tubular cells, loosing of brush edges, from nuclear condensation which is showing nuclear looses consisting of one-third of tubular structures; Grade 2, addition for grade 1, nuclear looses ranging from two-thirds of tubular structures; Grade 3, changes including nuclear looses which effects more than two-thirds of tubular structures[16].

Statistical Analysis

Statistical analysis was performed by SPSS for Windows 11.5 (SPSS Inc., Chicago, IL, USA). Data was presented as mean (minimum, maximum) values for histopathological and biochemical values. Groups were compared by using the nonparametric Kruskal-Wallis test. Mann-Whitney U test was used for binary comparisons. Spearman correlation test was used for evaluation of the relationships between the parameters. $p < 0.05$ was considered significant.

Results

Evaluation of Serum MDA and TAC Levels

Serum MDA and TAC levels were significantly changed among the groups (respectively $p < 0.001$ and $p = 0.008$) (Figure 1). Serum MDA content, as an indicator of lipid peroxida-

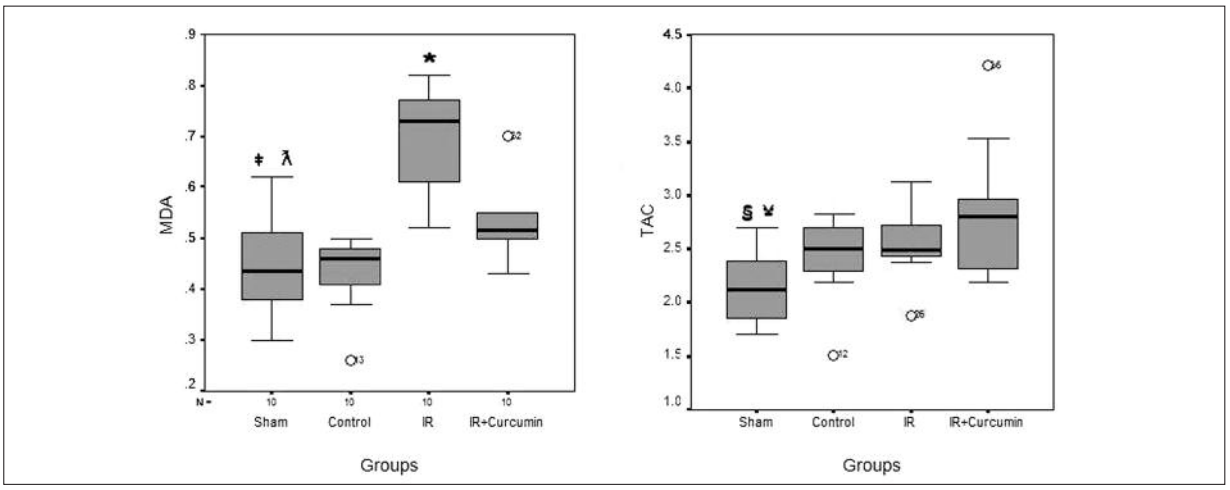

Figure 1. Serum MDA and TAC Levels in the groups. (MDA= Malondialdehyde, TAC= Total antioxidant capacity. ŧ$p < 0.001$ versus IR; ƛ$p = 0.043$ versus IR + Curcumin; *$p < 0.01$ versus IR + Curcumin; §$p = 0.015$ versus IR; ¥$p = 0.002$ versus IR + Curcumin).

tion, were significantly higher in IR group ($p < 0.001$) and IR + Curcumin group ($p = 0.043$) than Sham group. Serum MDA levels were significantly higher in IR group than IR + Curcumin group ($p < 0.001$). Serum TAC levels were significantly lower in Sham group than IR and IR + Curcumin groups (respectively $p = 0.015$ and $p = 0.002$). No significant differences were found in TAC levels among IR + Curcumin and IR group.

Evaluation of Tissue TAC, TOA and OSI Levels and Histopathological Scores in the Liver

TAC, TOA and histopathological score were significantly different when compared the groups (respectively $p < 0.001$, $p = 0.006$ and $p < 0.001$) (Figure 2). Liver TAC and TOA levels were significantly higher in IR (respectively $p < 0.001$ and $p = 0.005$) and IR + Curcumin groups (respectively $p < 0.001$ and $p = 0.004$) than Sham group. TAC and TOA levels of tissues in IR + Curcumin and IR groups were not different ($p > 0.05$). No significant differences were found in OSI levels among all groups. The liver histopathological scores were 0.30 ± 0.48 in Sham, 0.52 ± 0.67 in Control, 2.60 ± 0.51 in IR and 2.34 ± 0.47 in IR + Curcumin groups. The differences between IR and non-IR groups were significantly different ($p < 0.001$), but there was no significant difference between IR and IR + Curcumin groups (Figure 3).

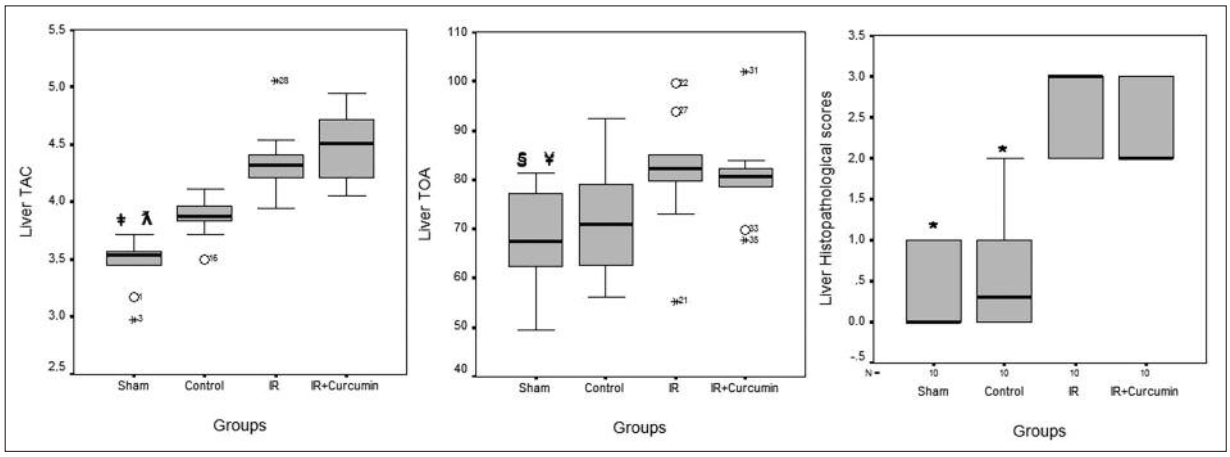

Figure 2. Liver TAC, TOA levels and histopathological scores in the groups. (TAC = Total antioxidant capacity, TOA = Total oxidant activity. ŧ$p < 0.001$ versus IR; ƛP < 0.001 versus IR + Curcumin; §$p = 0.005$ versus IR; ¥$p = 0.004$ versus IR + Curcumin; *$p < 0.001$ versus IR and IR + Curcumin).

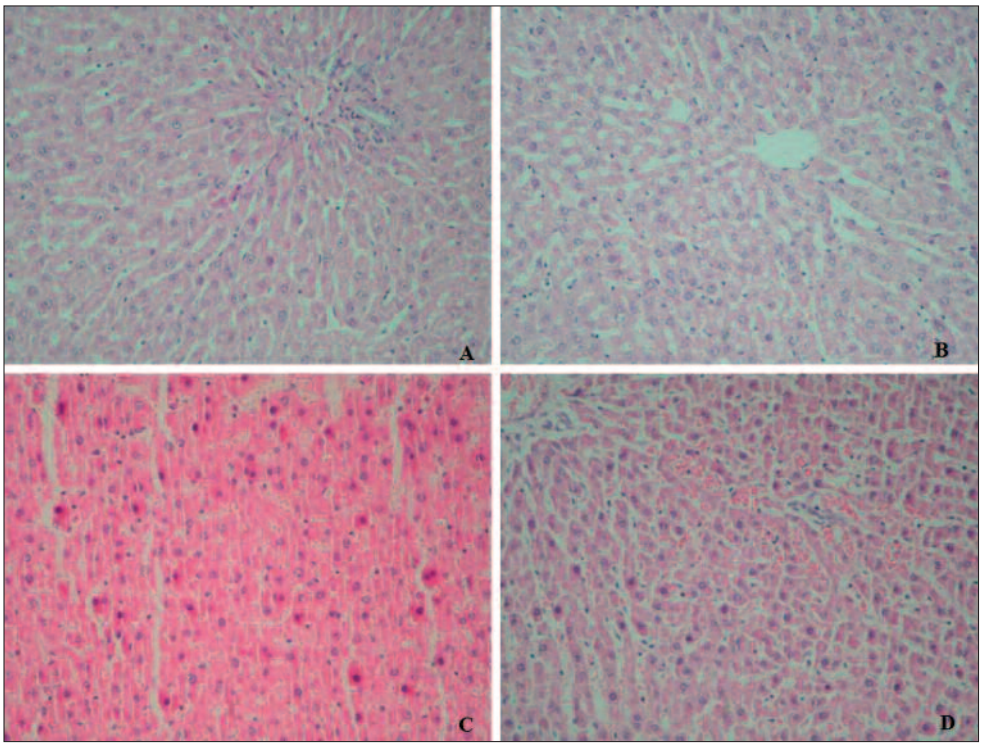

Figure 3. Effects of curcumin on hepatic tissue injury after IR evaluated by histological examination. In groups Sham and Control, Focal nuclear picnosis and cytoplasmic vacuolation in some hepatocyte are seen (***A*** and ***B***, respectively) (H&E stain, × 200). ***C,*** In group IR, Cytoplasmic hypereosinophilic changes, nuclear picnosis, loss of intercellular borders, necrobiosis and necrosis in many hepatocytes and haemorrhage in some disordered areas in the liver tissue can be seen (H&E stain, × 200). ***D,*** In group IR + Curcumin, Cytoplasmic hypereosinophilic changes, nuclear picnosis, loss of intercellular borders and necrobiosis in some hepatocytes and haemorrhage in a few disordered areas in the liver tissue are seen (H&E stain, × 200).

Evaluation of Tissue TAC, TOA, OSI Levels and Histopathological Scores in the Kidneys

TOA and histopathological scores were statistically different among the groups (respectively $p = 0.001$ and $p < 0.001$), but the differences in TAC levels between the groups were not significant ($p > 0.05$) (Figure 4). TOA levels were statistically lower in Sham group than IR and IR + (respectively $p = 0.001$ and $p = 0.001$). TOA levels of tissues in IR + Curcumin and IR groups were not different ($p > 0.05$). OSI levels were

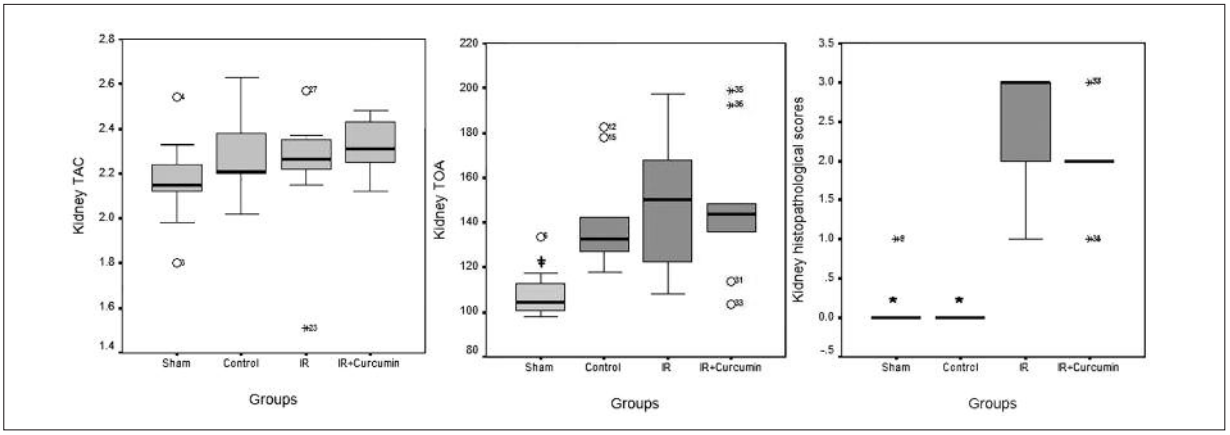

Figure 4. Kidney TAC, TOA levels and histopathological scores according to the groups. (TAC = Total antioxidant capacity, TOA = Total oxidant activity. ᵗp=0.001 versus IR and IR + Curcumin; *$p < 0.001$ versus IR and IR + Curcumin).

found to be lower in Sham group than controls, IR and IR + Curcumin groups (respectively $p = 0.015$, $p = 0.035$ and $p = 0.015$). However no significant difference was found in OSI levels between IR + Curcumin and IR groups. The kidneys histopathological scores were 0.20 ± 0.42 in Sham, 0.00 ± 0.00 in Control, 2.60 ± 0.69 in IR and 2.00 ± 0.66 in IR + Curcumin groups. The differences between IR and non-IR groups were significant ($p < 0.001$), but there was no significant differences between IR and IR + Curcumin groups (Figure 5).

Evaluation of Tissue TAC, TOA, OSI Levels and Histopathological Scores in the Lungs

TAC, TOA and histopathological score were significantly different when compared the groups (respectively $p < 0.001$, $p = 0.008$ and $p < 0.001$) (Figure 6). Lung TAC and TOA levels were significantly lower in Sham group than IR (respectively $p = 0.002$ and $p < 0.001$) and IR + Curcumin groups (recpectively $p = 0.002$ and $p = 0.002$). TAC and TOA levels of tissues in IR + Curcumin and IR groups were not different ($p > 0.05$). No significant differences were found in OSI levels among all groups. The lungs histopathological scores were 0.10 ± 0.32 in Sham, 0.68 ± 0.67 in Control, 1.66 ± 0.47 in IR and 1.23 ± 0.41 in IR + Curcumin groups. The differences between lungs histopathological scores of IR and non-IR groups were significant ($p < 0.001$), but there was no significant differences between IR and IR + Curcumin groups (Figure 7).

Discussion

During hepatic resections, intraoperatif bleeding has been reported as related to morbidity and mortality[17]. Continuous or intermittent vascular clamping of the hepatoduedonale ligament, called as "Pringle manoeuvre" is performed in

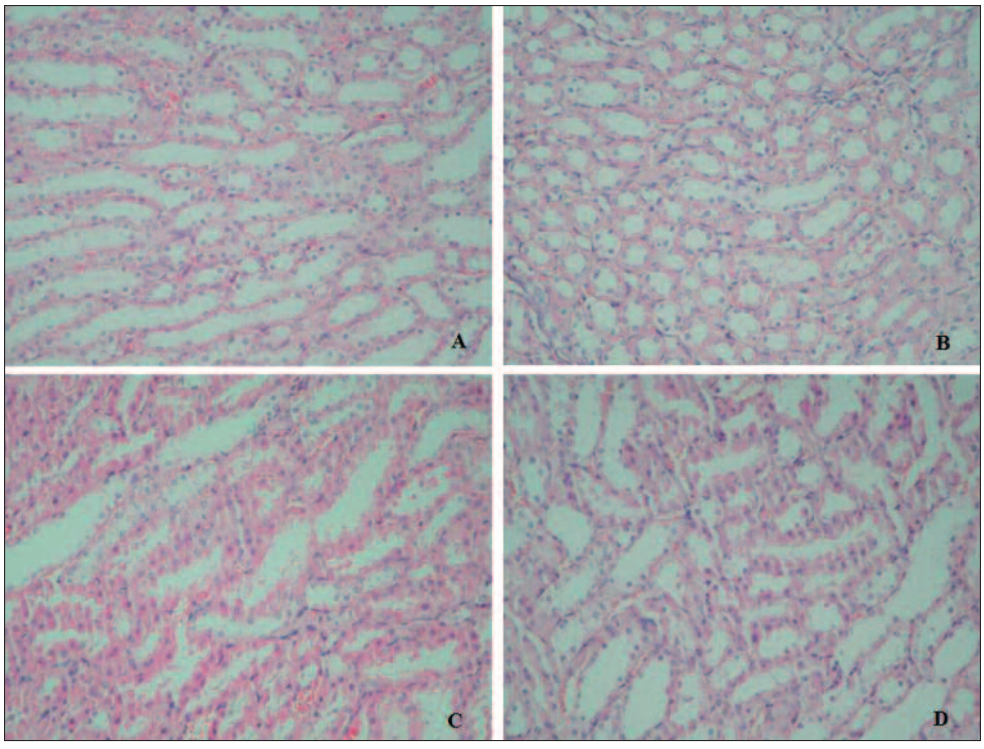

Figure 5. Effects of curcumin on kidney tissue injury after IR evaluated by histological examination. ***A,*** In group Sham, Mild hidropic changes in some tubular cells and normal histomorphological appearance in the other sites can be seen (H&E stain, × 200). ***B,*** In group Control, Normal morphological architecture in the renal tubules (H&E stain, × 200). ***C,*** In group IR, Cellular swelling, loss of brush borders, nuclear condensation and nuclear loss in the renal tubular cells is evident (H&E stain, × 200). ***D,*** In group IR + Curcumin, Cellular swelling, loss of brush borders, nuclear condensation and nuclear loss in the renal tubular cells can be seen. Note that some renal tubules have nearly normal histomorphological architecture with only moderate cellular swellings (H&E stain, × 200).

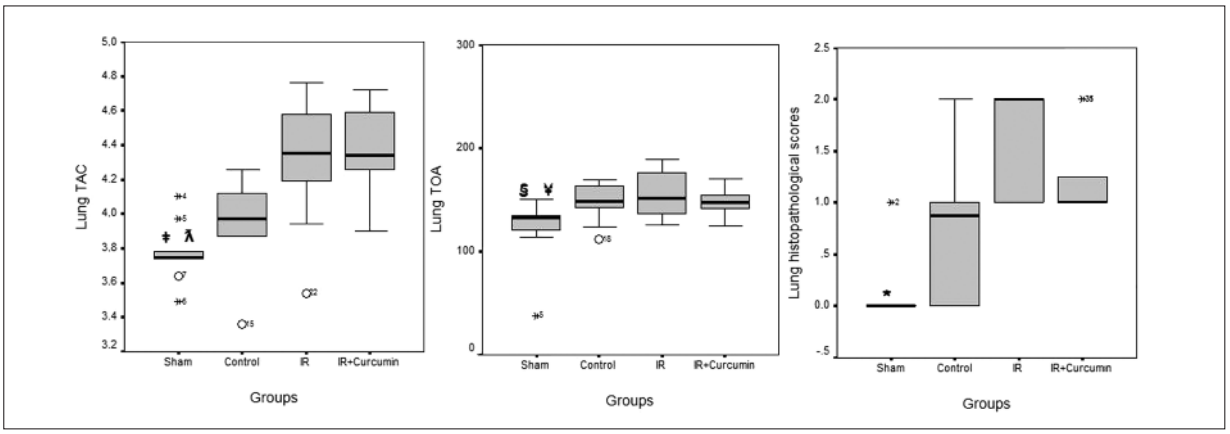

Figure 6. Lung TAC, TOA levels and histopathological scores according to the groups. (TAC = Total antioxidant capacity, TOA = Total oxidant activity. $^{\text{ŧ}}p = 0.002$ versus IR; $^{\lambda}p = 0.002$ versus IR + Curcumin; $^{\S}p < 0.001$ versus IR; $^{¥}p = 0.002$ versus IR + Curcumin; $*p < 0.001$ versus IR and IR + Curcumin).

order to reduce the blood loss[18]. However, Pringle manoeuvre causes IR liver injury and intestinal congestion[17]. Hypoxia and reoxygenation are considered to be the main factors leading to tissue damage during the IR period[19]. Free oxygen radicals arise during reperfusion is thought to be the cause of inception of cellular events which include inflammation, necrosis, hepatocellular damage and apoptosis[20].

Various mechanisms have been proposed to explain the liver IR injury. Kuppfer cells are activated abnormally by free oxygen radicals. Secre-

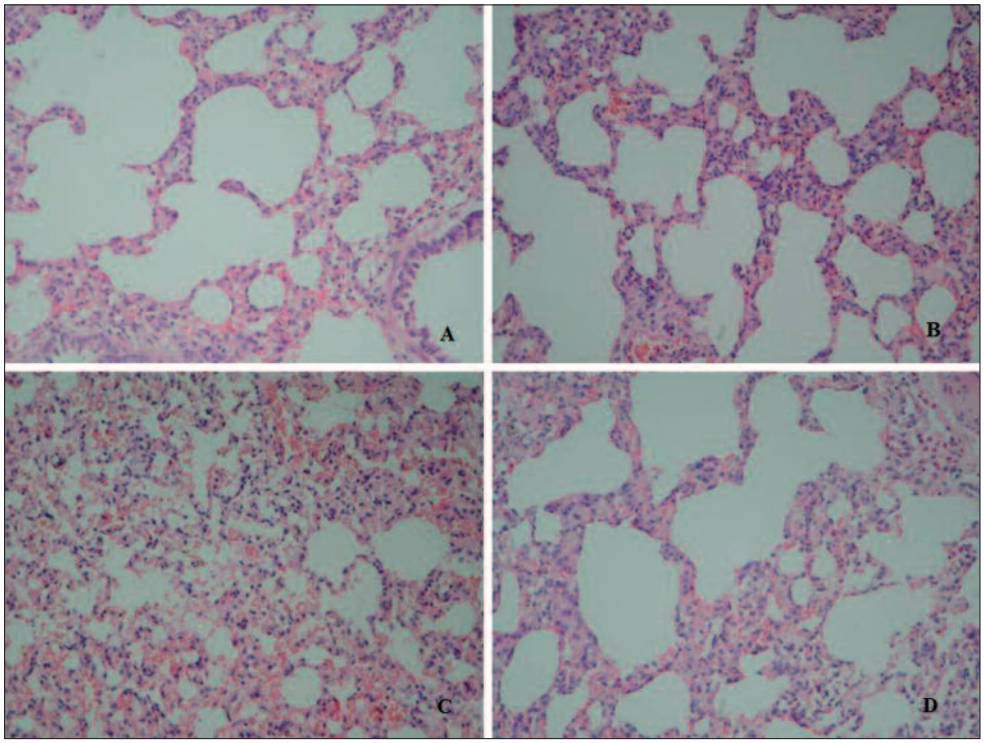

Figure 7. Effects of curcumin on lung tissue injury after IR evaluated by histological examination. ***A,*** In group Sham, Mild PNL infiltration and mild to moderate interstitial congestion in the lung tissue (H&E stain, × 200). ***B,*** In group Control, Mild PNL infiltration and moderate interstitial congestion in the lung tissue (H&E stain, × 200). ***C,*** In group IR, Interstitial inflammation, perivascular edema and haemorrhage with disintegration of the parenchimal lung architecture (H&E stain, × 200). ***D,*** In group IR + Curcumin, Mild to moderate PNL infiltration and interstitial congestion in the lung tissue (H&E stain, × 200).

tion of inflammatory cytokines and proteolytic enzymes from these cells are thought to play a key role in liver IR[4,21]. However, free oxygen radicals may start oxidative stress mechanisms through by lipid peroxidation[21].

In recent years, some regional drugs which have been pleiotropic biological activity are used for supplementary and alternative treatment of different diseases[22]. Curcumin (diferuloylmethane) is one of them and it is active component of ribosome of *Curcuma longa*. Curcumin is an important intracellular agent which has wide pharmacological activity that is involved anti-inflammatory, antioxidant, anti-carcinogenic and bactericidal effects[3,4,19,22]. *In vitro* studies are shown that curcumin is a effective cleaner for reactive oxygen radicals and reactive nitrogen species. But *in vivo* studies, not fully understand effects of curcumin; a directly antioxidant agent or not[19]. In another study, curcumin is shown to be a potent inhibitor of cytochrome P450 and glutathione S-transferase enzymes in rat liver[23]. Reyes-Gordillo et al[24] showed that curcumin prevents NF-kB activation on acute liver damage, too. Additionally, Curcumin reduces the formation of proinflammatory cytokines Tumor necrosis factor-α, interferon-γ, interleukin-6 and interleukin-1β[22,24,25] correlates with damage by lipid peroxidation of membranes. Histopathologically, areas of necrosis and hemorrhage and the neutrophil infiltration were observed in study groups but this findings were more common in IR group. Free oxygen radicals cells leads a main role in hepatic injury, especially early stages[21]. Oxidative stress is activated the mechanism leading to the synthesis of proinflammatory cytokines and cell adhesion molecules[30]. Therefore, these results are suggested for a moderate positive effect of curcumin on hepatic injury but this effect shows low level of significance.

Hepatic ischemia reperfusion injury leads to distant organ damage as well as liver damage. The development of acute renal failure after major hepatic IR is extremely common (40-85%), resulting in high mortality and morbidity in perioperative period[30]. It is suggested that hepatic IR is reasonably for damage to kidney tissue[31]. Park et al[30] reported that hepatic ischemia reperfusion not only caused by rapid infiltration of neutrophils in the liver, also in the kidney it is postulated in a similar way. Activated neutrophils release cause of tissue damage with through arachidonic acid metabolites, free oxygen radicals and neutrophil elastase. In the present study, kidney TAC levels increased and TOA levels decreased with the administration of curcumin. And also, OSI were lower in IR + Curcumin group than IR group. Histopathologically, IR and IR + Curcumin groups had swelling of tubular cells, brush edge loss, nuclear condensation showing nuclear loss and nuclear loss extending from two out of three of tubular structures. In addition, in IR group, nuclear losses included more than two out of three of tubular structures but this difference was not significant.

Curcumin had limited effect in lung injury which occurred after hepatic IR. Active neutrophils which released after hepatic IR are an important source of free oxygen radicals, and free oxygen radicals play a main role of lung injury. Oxidative and inflammatory mediators released into the systemic circulation afterwards IR. Pulmonar microvascular field is primary target of these mediators[31]. During IR injury, the pro-inflammatory mediators are released and destroy integrity of the endothelium. It increases permeability of protein and deteriorate of lung endotelium[32]. Chen et al[33] reported that in rats, curcumin decreased the activity of iNOS which increases inflammatory cytokines, eosinophile sequestration and airway hypersensitivity in lung injury according to aspiration. Ota et al[34] demonstrated that the ventilation is the main factor of determining lung injury in the model of hepatic ischemia reperfusion also lung injury didn't occur in the ventilation of low tidal volume but were seen more in the ventilation of high tidal volume. In this study, we demonstrated that higher levels of TAC and lower levels of TOA and OSI in IR + Curcumin group than IR group in the lung tissue. Histopathologically, mild-moderate neutrophil leukocyte infiltration with interstitial congestion in the lung tissue was detected in IR and IR + Curcumin groups, and also the formation of perivascular edema and fragmented pulmonary structures were accompained to IR group. But as stated in the literature, there were no signicant difference between IR and IR + Curcumin group. This result can be attributed that there was no change in animal ventilation volumes and curcumin was not effective on the changes.

Conclusions

Hepatic ischemia reperfusion injury formed by pringle maneuver leaded to damage in liver, and distant organs, such as kidney and lung. Admin-

istration of curcumin did not significantly reduce the damage of the liver and distant organs. Further comparative experimental and clinical studies are needed to evaluate the use of curcumin for nutritional support, especially at the preoperative period of liver surgery.

Acknowledgements

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Conflict Interests

The Authors declared no conflict of interests.

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