Protective Effects of Prunus armeniaca L (Apricot) on Low Dose Radiation-Induced Kidney Damage in Rats

Düşük Doz Radyasyon Alan Sıçanlarda Meydana Gelen Böbrek Hasarına Karşı Prunus armeniaca L (Kayısı)'nın Koruyucu Etkileri

ABSTRACT

OBJECTIVE: This experimental study was designed to evaluate radiation-induced kidney damage and the protective effect of apricot against it using histological parameters.

MATERIAL and METHODS: Rats were divided into 6 groups each containing 10 Sprague Dawley rats as follows: Regc: Rats on a regular diet (control diet) for 28 weeks; control group. Regx: Rats on a regular diet for 28 weeks, XRE on last day of eighth week. Aprc: Rats on an apricot diet for 28 weeks; control for no XRE. Aprx: Rats on an apricot diet for 28 weeks, XRE on last day of eighth week. Reg+Aprc: Rats on a regular diet for 8 weeks, followed by an apricot diet for the following 20 weeks; control. Reg + Aprx: Rats on a regular diet for 8 weeks, XRE on last day of eighth week, followed by an apricot diet for 20 weeks.

RESULTS: The kidneys of the control groups showed normal kidney histology, whereas Regx group showed major histopathological changes, such as glomerular collapse, hemorrhage, interstitial fibrosis and inflammatory infiltrates. The Aprx and Reg+Aprx groups showed smaller amounts of degeneration.

CONCLUSION: In conclusion, we suggest that agents with antioxidant properties such as apricot may have a positive effect in the treatment of renal diseases.

KEY WORDS: Radiation, Kidney, Apricot, Histopathology, Rat

ÖZ

AMAÇ: Bu çalışmada amacımız, antioksidan etkiye sahip Prunus armeniaca L (kayısı)'nın böbrek dokusu üzerine radyoprotektif etkisinin histopatolojik yöntemlerle araştırılmasıdır.

GEREÇ ve YÖNTEMLER: Çalışmamızda 10'ar adet Sprague Dawley cinsi sıçanlardan oluşan 6 grup kullanılmıştır. Kontrol (normal diyet) grubu: 28 hafta normal diyetle beslenen grup. Normal diyet+rad grubu: 20 hafta boyunca normal diyet alıp son 8 hafta normal diyet+ radyasyon alan grup. Kayısı diyeti alan grup: 28 hafta boyunca kayısı diyeti ile beslenen grup. Kayısı diyeti+Rad grubu: 20 hafta boyunca kayısı diyeti alıp son 8 hafta kayısı diyeti+radyasyon alan grup. Kayısı diyeti+normal diyet alan grup: 20 hafta boyunca kayısı diyeti alıp devam eden 8 hafta boyunca normal diyet alan grup. Rad+Kayısı diyeti alan grup: 20 hafta boyunca kayısı diyeti alıp devam eden 8 hafta boyunca normal diyet+ radyasyon alan grup.

BULGULAR: Çalışmamızda, kontrol gruplarında normal histolojik yapı mevcutken, radyasyon grubunda glomerüler hasar, hemoraji, intertisyel fibrozis ve infiltrasyon gibi histopatolojik değişikliklere rastlanmıştır. Diğer taraftan, Kayısı diyeti+Rad grubu ve Rad+Kayısı diyeti alan gruplarda bu dejenerasyonların azaldığı tespit edilmiştir.

SONUÇ: Sonuç olarak yaptığımız çalışmada radyasyona maruz kalındığında kayısı katkılı beslenmenin böbrek üzerine radyasyonun verdiği hasarı azalttığı tespit edilmiştir.

ANAHTAR SÖZCÜKLER: Radyasyon, Böbrek, Kayısı, Histopatoloji, Sıçan

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Received : 16.11.2013
Accepted : 31.12.2013

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INTRODUCTION

Radiation exposure to the kidney causes acute and chronic injuries that could eventually lead to the development of fibrosis and end-stage organ failure (1). Rats are proper animals for the models to evaluate radiation-induced renal injury as their body structure is similar to that of humans (2). Radiation-induced renal injury is a widely used experimental model (3-5).

The dietary intake of vitamins, carotenoids and flavonoids, which are widely distributed in fruits, could be useful in protecting against nephrotoxicity (6-8). Apricot (Prunus armeniaca L.) is a fruit that has a high content of carotenoids, mainly β-carotene. β-Carotene is the source of provitamin A. A quantity of 250 g fresh or 30 g dried apricots provides 100% of the recommended daily allowance of provitamin A (9). Apricot also contains vitamins C and E and selenium (10). β-Carotene, vitamins C, E and selenium are well-known dietary antioxidants (11-13).

This experimental study was designed to clarify the role of oxidative stress in radiation-induced kidney damage and the possible protective effect of apricot against it, using histological parameters.

MATERIALS and METHODS

All experiments in this study were performed in accordance with guidelines for animal research from the National Institutes of Health and were approved by the Inonu University Ethics Committee on Animal Research (Ethic no: 2011 A-34).

Animals

Sixty adult male Sprague-Dawley rats weighing between 300 and 345 g were used in the study. All animals were kept at 22°C ± 2°C (room temperature) and 50% ± 10% humidity with a 12-hour light/12-hour dark cycle and were fed freely. For the 7-day acclimation period, rats were fed with regular rodent pellet diets and drinking water ad libitum. They were then divided into 6 groups (10 rats each) as follows:

- Regc: Rats on a regular diet (control diet) for 28 weeks; control group.
- Regx: Rats on a regular diet for 28 weeks, XRE on last day of eighth week.
- Aprc: Rats on an apricot diet for 28 weeks; control for no XRE.
- Aprx: Rats on an apricot diet for 28 weeks, XRE on last day of eighth week.
- Reg + Aprc: Rats on a regular diet for 8 weeks, followed by an apricot diet for the following 20 weeks; control.
- Reg + Aprx: Rats on a regular diet for 8 weeks, XRE on last day of eighth week, followed by an apricot diet for 20 weeks.

The Diets

Standard (regular) rat diet

A commercially available standard rat chow diet (Korkutelim Yem, Antalya, Turkey) was used in the control groups. The ingredients are given in Table I. For wheat, whole wheat, and crushed wheat was fed. The diet contained vitamins A, D, and E; calcium, phosphorus, and trace amounts of iron, manganese, copper, zinc, and cobalt.

Table I. Ingredient composition (gram per kilogram) of the standard diet and 20% apricot diet.

Ingredient	Standard diet (kilogram)	20% apricot diet (kilogram)
Corn	300	287.44
Wheat	154.81	–
Bran	149.87	69.3
Soya-48	260.38	380.85
Fish flour	80	80
Molasses	30	32.54
Marble	10.64	10.88
Salt	9.95	10
V-221	2.5	2.5
Syn-Met	1.77	2.48
Apricot	–	200

Apricot diet

Organic sun-dried apricots of the Kabaasi variety were used in this study. They were harvested from the Malatya region, a major apricot-producing province in Turkey. This variety was chosen because of its higher radical scavenging power and total phenolic content (14). After harvesting, the apricots were sun-dried for 14 days without any additives, then minced to 1 to 2-mm pieces and used in the study. A mixture of 20% apricots was prepared, and the diets were maintained to be isoenergetic with the regular rodent pellet diet. The amounts of wheat, bran, and soya-48 in the apricot diet were adjusted so that an adequate food supply for rat growth could be ensured while providing an isoenergetic status with the standard diet. The apricot diet had the same amounts of vitamins A, D, and E; calcium, phosphorus, and trace elements of iron, manganese, copper, zinc, and cobalt with the standard diet. Both diets had a metabolic energy of 11095 J/kg.

Radiation Exposure

A conventional 200-kilovolt (peak), 20-ampere x-ray machine (Shimadzu, Kyoto, Japan) was used as the x-ray generator. Rats were irradiated at a distance of 80 cm with a dose rate of 0.5 Gy/min, delivering 0.2 Gy of x-ray irradiation to the genital region. Animals were immobilized in cages of adjustable length that accommodated the rat but prevented movement. All but the lower third of the body was covered with lead sheets. Rats were not medicated during XRE.

Histologic Evaluation

At the end of the study, the rats were sacrificed by ketamine anesthesia and the kidneys were quickly removed. The tissue samples were fixed in 10% formalin and were embedded in paraffin. Tissue sections were cut at 5μm, mounted on slides, stained with hematoxylin-eosin (H-E) for general kidney structure and Masson's trichrome for connective tissue. Sections were evaluated for the presence glomerular collapse, hemorrhage, interstitial fibrosis and tubulointerstitial injuries, such as inflammatory infiltrates. The microscopic score of each tissue was calculated as the sum of the scores given to each criteria. Scores were given as 0, none; 1, mild; 2, moderate and 3, severe for each criteria. Thus, the maximum score obtained was 12.

Tissues were examined using a Leica DFC280 light microscope and a Leica Q Win Image Analysis system (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

Statistical Analyses

Statistical analysis was carried out using the SPSS for Windows version 13.0 (SPSS Inc., Chicago, III., USA). All data are expressed as the arithmetic mean ± standart error (SE). Normality for continued variables in groups were determined by the Shapiro-Wilk test. One-way ANOVA and the post hoc Tamhane test were used to establish differences among groups for histopathological score and biochemical changes. $P < 0.05$ was regarded as significant.

RESULTS

Histopathological Assessment

The kidney of rats in the Regc group showed normal renal structure and there were no lesions (Figure 1A, B). Aprc and Reg + Aprc groups were similar to that of the control group (Figure 1C-F). However in the Regx group, the kidneys showed severe morphological damages. Glomerular collapse (Figure 2A), hemorrhage (Figure 2B), interstitial fibrosis (Figure 2C) were seen in this group. Moreover, tubulointerstitial injuries, such as inflammatory infiltrates (2D) were observed in Regx group. In Aprx and Reg + Aprx groups although the kidney tissue preserved its normal histological appearance, hemorrhage and and interstitial fibrosis still were marked in some areas (Figure 3A-D). The mean histopathological score was 0.10±0.14 in Regc group, 8.20±0.46 in Regx group, 0.30±0.15 in Aprc group, 5.30±0.39 in Aprx group, 0.30±0.15 in Reg + Aprc group, 6.20±0.51 in Reg + Aprx group. Significant differences were found between Regc and Regx group ($p<0.001$), Regx and Aprx group ($p<0.001$), and Regx and Reg + Aprx group ($p<0.001$).

The score of semiquantitative analysis in the kidney tissue is reported in Table II.

DISCUSSION

Radiation-induced renal damage is a widely recognized experimental model but the cytoprotective effect of apricot on histopathological damage has never been reported before. We observed that histological damage induced by radiation improved with the treatment of apricot

Radiation nephropathy has emerged as a significant complication of bone marrow transplantation and radionuclide radiotherapy and is a potential sequela of radiological terrorism and radiation accidents (15, 16). Medically, ionizing radiation may be used as the sole treatment or as adjuvant to chemotherapy or surgical management of neoplasia of different parts of the human body e.g. breast, cervix, bones, kidneys (17) and lungs, amongst others. Several authors reported radiation-induced

Table II. The results of semiquantitative histopathological assessment.

Parameter	Regc	Regx	Aprc	Aprx	Reg + Aprc	Reg + Aprx
Histopathological score	0.10±0.14	8.20±0.46[a]	0.30±0.15	5.30±0.39[b,c]	0.30±0.15	6.20±0.51[d,e,f]

[a]Significant increase ($P < 0.001$), vs. Regc group.

[b]Significant increase ($P < 0.001$), vs. Aprc group.

[c]Significant decrease ($P <0.001$), vs. Regx group.

[d]Significant increase ($P < 0.001$), vs. Reg + Aprc group.

[e]Significant decrease ($P < 0.001$), vs. Regx group.

[f]Not significant increase ($P < 0.001$), vs. Reg + Aprc group.

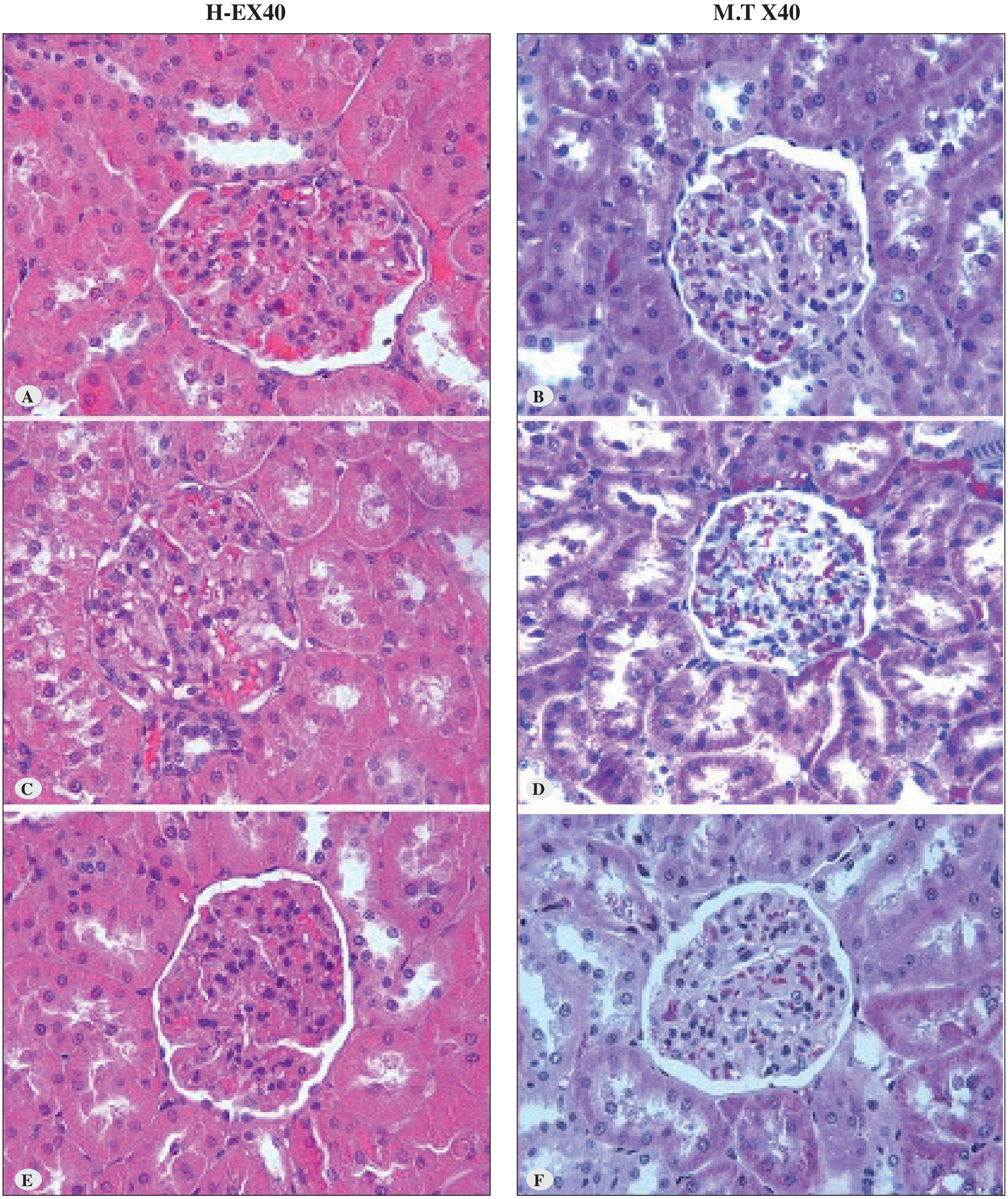

Figure 1: *Control **(A,B)**, Aprc **(C, D)** Reg + Aprc **(E, F)** groups normal kidney structure and there were no lesions.*

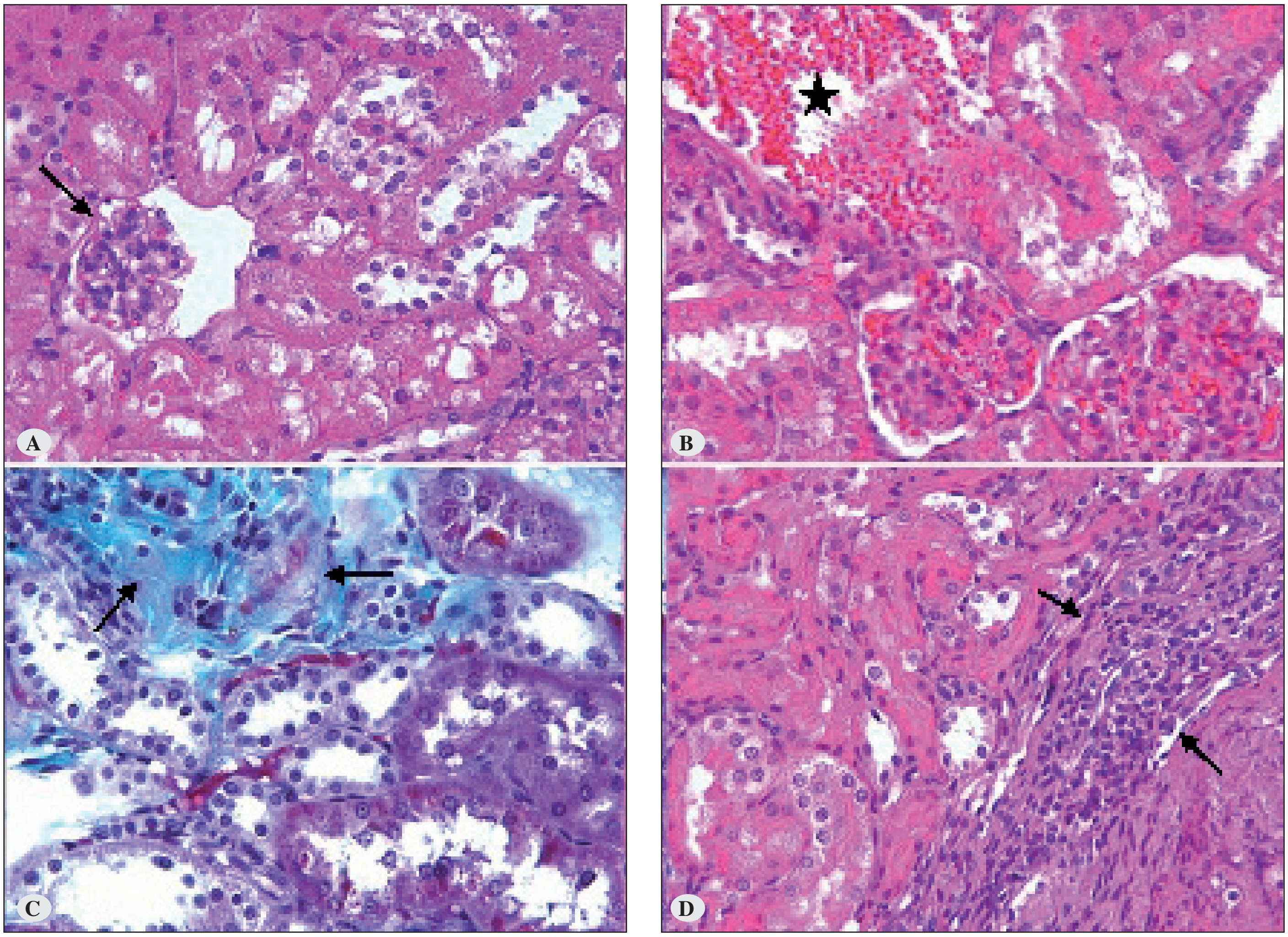

Figure 2: *Regx group* ***(A)*** *the appearance of glomerular collaps (arrow) H-E; x40,* ***(B)*** *visible hemorrhage. (asterisk) H-E; x40,* ***(C)*** *notice the interstitial fibrosis (arrows) Masson's Trichrome ; x40,* ***(D)*** *visible inflammatory cell infiltration (arrows) H-E; x40.*

kidney injury in rats (18-20). Cohen et al. defined radiation nephritis as kidney injury and impairment of its function by ionizing radiation, which can occur after irradiation of one or both kidneys (21). The kidneys are highly radiosensitive organs, but radiation-induced injury occurs in proportion to the radiation dose and volume of renal tissues exposed, and this may lead to renal failure. Kaldır M et. al. reported diffuse intertubular fibrosis and tubular atrophy (2). Lıu Dian-ge and Wang Tie-min et al. (18) showed radiation resulted in glomerulosclerosis, interstitial fibrosis, hemorrhage and inflammatory infiltrates in radiation induced kidney injury. In the present study, we detected glomerulosclerosis, hemorrhage and tubulointerstitial injuries, such as inflammatory infiltrates, tubular dilatation and interstitial fibrosis.

Apricot is a fruit which has a high β-carotene content (22). Carotenoids are naturally occurring pigments in plants that are involved in light-harvesting reactions and protection of plant organelles against singlet oxygen-induced damage. Carotenoids have singlet oxygen and peroxy radical-quenching properties because of their long chain of conjugated double bonds. β-Carotene may have additive benefits due to its ability to be converted to vitamin A. β-Carotene is the major plant source of vitamin A (23, 24). Apricot also contains vitamins C and E and Selenium (25). Vitamin C is considered to be one of the most powerful, least toxic natural antioxidants. It is water soluble and has cofactor activity (26). Vitamin E and Selenium also have antioxidant properties (27, 28). Apricot was shown to have nephroprotective effects in different models of kidney toxicity A research on the therapeutic effects of apricot on the kidney damage induced by methotrexate in rats revealed that glomerulosclerosis and apoptosis findings significantly decreased in Apricot treatment group (29).

This study has some limitations especially in practical application to the human. The 20% apricot is not a realistic diet

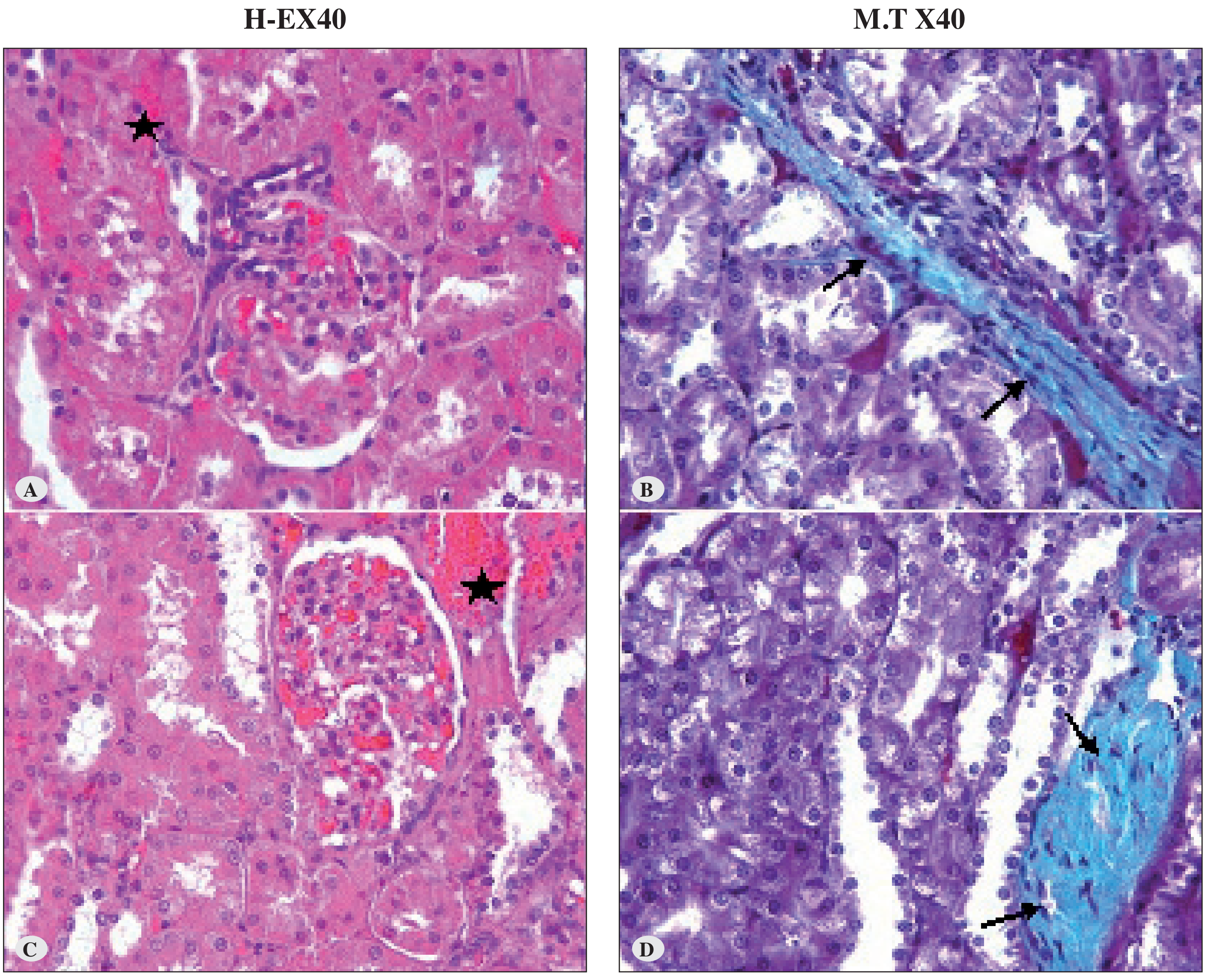

Figure 3: *Aprx **(A, B)** and Reg+ Aprx **(C, D)** groups. Hemorrhage (asterisks) and intertisial fibrosis still were present (arrows).*

option for humans. Yet, concentrated forms or habitual ingestion in smaller portions may be investigated for beneficial effects. Another limitation is the diversity of apricot varieties that differ in phenolic and carotenoid contents. Also, commercial processing of the fruit necessitates addition of preservative, which may alter the antioxidant capacity. Fresh fruit, on the other hand, is available only seasonally. Yet, sun- dried forms are available throughout the year and would be free of most additives (30).

In this study, we found that the detrimental effects of low-dose radiation on kidney tissue are ameliorated by Prunus armeniaca L (apricot), introduced either before or after exposure. In conclusion, we suggest that agents with antioxidant properties such as apricot may have a positive effect in the treatment of renal diseases.

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