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ARAŞTIRMA / RESEARCH

Effect of *Ficus carica* (fig) seed oil administration on GSH levels, necrosis and cast formation in myoglobinuric acute kidney injury

Miyoglobinürik akut böbrek hasarında *Ficus carica* (incir) çekirdek yağı uygulamasının GSH düzeyleri, nekroz ve kast oluşumu üzerine etkisi

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Abstract

Purpose: In this study, the effect of applying different doses of *Ficus carica* (fig) seed oil obtained by cold pressing method on the kidney tissue and serum GSH level, as well as the formation of necrosis and cast in the experimental myoglobinuric acute kidney injury animal model created with glycerol was investigated.

Materials and Methods: 32 Wistar albino male rats weighing 460-540 g were randomly divided into four groups of 8 each. Sham Control, MAKI, MAKI+FC3, MAKI+FC6. Urea and creatinine levels of the groups were analyzed by biochemical method. Tissue necrosis level was determined by histological analysis of kidney tissue sections.

Results: While urea and creatinine levels increased significantly in the MAKI group compared to all groups, they were found to be lower in the high and low dose treatment groups with no significant difference between them. Tissue and serum GSH levels in the MAKI group were significantly decreased compared to all groups. In the MAKI+FC3 and MAKI+FC6 groups, an increase was detected in the tissue without dose difference, and in the serum only with high dose. The highest score in kidney tissue cast and necrosis levels were observed in the MAKI group, while significant improvements were detected in the treatment groups.

Conclusion: *Ficus carica*(fig) seed oil, provided improvement in morphological damage with improvement in functional damage and increase in antioxidative capacity.

Keywords:. Antioxidant, *Ficus carica* seed oil, myoglobinuric acute kidney injury, necrosis, reduced glutathione.

Öz

Amaç: Bu çalışmada, Gliserolle oluşturulmuş Deneysel Miyoglobinürük akut böbrek hasarı hayvan modelinde, soğuk pres yöntemiyle elde edilmiş olan *Ficus carica* (incir) çekirdeği yağının farklı dozlarda uygulanmasının, böbrek dokusu ve serum GSH düzeyi, ayrıca nekroz ve kast oluşumu üzerindeki etkisi araştırılmıştır.

Gereç ve Yöntem: Ağırlığı 460-540 gr arasında değişen, 32 adet Wistar albino erkek sıçan randomize 8'erli dört gruba ayrıldı. Sham Kontrol, MAKI, MAKI+FC3, MAKI+FC6. Grupların Üre ve kreatinin düzeyleri biyokimyasal yöntemle analiz edildi. Doku nekroz düzeyi böbrek doku kesitlerinin histolojik analizi ile belirlendi.

Bulgular: MAKI grubunda üre ve kreatinin düzeyi tüm gruplara göre anlamlı olarak artış gösterirken, yüksek ve düşük doz tedavi grubunda aralarında anlamlı fark olmazsızın düşük tespit edildi. MAKI grubu doku ve serum GSH düzeyi tüm gruplara göre anlamlı olarak azaldı. MAKI+FC3 ve MAKI+FC6 gruplarında, dokuda doz farkı olmaksızın, serumda ise yalnız yüksek doz ile artış tespit edildi. Böbrek dokusu kast ve nekroz düzeyinde en yüksek skor MAKI grubunda gözlenirken, tedavi gruplarında anlamlı düzeyde iyileşme tespit edildi.

Sonuç: *Ficus carica* (incir) çekirdeği yağı ise, fonksiyonel hasarda iyileşme ve antioksidatif kapasitede artış ile birlikte morfolojik hasarda iyileşme sağlamıştır.

Anahtar kelimeler: Antioksidan, *Ficus carica* çekirdek yağı, indirgenmiş glutatyon, miyoglobinürik akut böbrek hasarı, nekroz.

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INTRODUCTION

Myoglobinuric acute kidney injury (MAKI) is a structural and functional disorder that occurs in the kidney with the release of muscle cell contents into the circulation, mostly due to rhabdomyolysis that develops with severe muscle trauma[1,2]. The literal meaning of rhabdomyolysis is "striated muscle wasting"[2].

Typical metabolic changes accompanying rhabdomyolysis; hyperkalemia, metabolic acidosis, hypocalcemia or hypercalcemia, hyperuricemia, hyponatremia, and hyperphosphatemia with potential cardiac arrhythmia[3,4]. Myoglobin plays a role in kidney damage in 3 different ways, such as renal vasoconstriction, formation of intratubular debris, and direct toxicity to renal tubular cells. Hypovolemia and acidic urine increase the toxicity of myoglobin[5].

In rhabdomyolysis, due to the high amount of myoglobin transferred to the proximal tubule cells, the conversion capacity of iron to ferritin is exceeded, leading to intracellular ferrihemate accumulation, causing oxidative damage to the kidney cell. Decreased urinary pH due to metabolic acidosis resulting from acids secreted from damaged muscle cells further increases iron release from myoglobin[6]. Intratubular acidosis, which develops with low urine pH, paves the way for myoglobin and uric acid to precipitate in the tubule[7,3].

In the pathogenesis of MAKI, it has been reported that the decrease in total antioxidant capacity predisposes to the formation of oxidative damage due to free radicals[8]. It has been reported that antioxidative therapy in myoglobinuric AKI may exert protective effects by inhibiting lipid peroxidation in proximal tubular cells, as well as by inhibiting the redox cycle between ferric and ferrous myoglobin[9]. In addition, it has been shown that antioxidant applications after rhabdomyolysis are more effective[10].

Experimental models of acute tubular necrosis (ATN) that can mimic human ATN are created with 50% hypertonic glycerol in animals[11]. In rats, glycerol causes rapid myoglobulinuria, oliguria, and a marked reduction in glomerular filtration rate[12]. In glycerol-induced AKI, findings characterized by myoglobinuria, tubular necrosis, and increased renal vasoconstriction are observed[4].

Ficus carica is a fruit native to this region, widely grown on the Mediterranean coast[13]. *Ficus carica* fruits have been reported to contain high levels of polyphenols, flavonoids and anthocyanins[14]. It has been reported that the fruits of *Ficus carica* and its different parts have high antioxidant capacity due to the polyphenolic compounds they contain and especially the anthocyanins[15].

It has been determined that the main phenolic compound in figs is epicatechin[16]. An average of 1090-1100 mg of polyphenol is found in 100 g of fig dried fruit[17]. Dried fig seeds, 30% of which are oil; It contains 18.99% oleic, 33.72% linoleic, 32.95% linolenic, 5.23% palmitic, 8% stearic, 1.05% arachidonic fatty acids[18].

Güven et al. reported that fig seed oils in Turkey have fatty acids, tocopherol and phenolic contents (79.5mg/100g) as well as phytosterols (5.07%)[19]. Total tocopherol amount in fig seed was recorded as 4 g/kg[20]. Protective and therapeutic effects of *Ficus carica* and its various parts have been reported on many pathological models[21,22,23]

In the light of the information we have obtained, it is thought that the use of *Ficus carica* seed oil after MAKI will increase the antioxidant capacity and thus reduce the functional and morphological damage. The aim of this study is to investigate the effect of applying *Ficus carica* seed oil in different doses in an experimental MAKI model on tissue and serum GSH levels, necrosis and cast formations, together with functional damage in the kidney. In the field of health there are very limited scientific researches about *Ficus carica*. In this respect, our study is the first study in the literature to investigate the effects of Ficus carica seed oil in the MAKI model, and will contribute to the determination of supportive treatment alternatives for MAKI in the future.

MATERIALS AND METHODS

This experimental study was approved by the decision of Aydın Adnan Menderes University Animal Experiments Local Ethics Committee 64583101/2019/039 numbered and dated. 26/03/2019. 32 male wistar species rat was used that weighing 460-540 g, reared in Experimental Animal Production and Research Laboratory, kept in a circadian rhythm12 hours of light and 12 hours darkness of at 40-60% relative humidity, 22 ± 1 degree optimal temperature. The sample size was

established in accordance with similar studies in the literature. [24,25,26.] Standard rat chow and tap water were used for feeding the animals. The fig seed oil used was obtained from ONEVA company. Documents showing that the oil used is organic and detailed GC-MS and HPLC tables regarding its content are attached as files. The study was carried out at Aydın Adnan Menderes University Experimental Animal Production and Research Laboratory (Aydın, Turkey), Faculty of Medicine, Department of Physiology (By Derya İşler and Ferhat Şirinyıldız).

Experimental procedure

After the rats were randomly divided into 4 groups, the subjects were dehydrated for the last 24 hours, glycerol (85% Ph, Eur, BP. İSOLAB) administration as 8 ml/kg total volume for each subject, under light ether anesthesia, equally on both hind legs. and administered slowly intramuscularly in divided doses. After the first glycerol injection, animals were provided with a free diet and water intake.

Experimental groups

Sham Control: Intramuscular saline was applied to the lower extremity bilaterally. Distilled water was administered orally after 1, 24, and 48 hours. **MAKI:** Intramuscular 50% hypertonic glycerol 8 ml/kg was applied to the bilateral lower extremity. After 1, 24 and 48 hours, 3 ml/kg distilled water was administered orally. **MAKI+FC3:** Intramuscular 50% hypertonic glycerol 8 ml/kg was applied to the bilateral lower extremities. After 1, 24 and 48 hours, 3 ml/kg *Ficus carica* seed (Oneva Turkey) oil was administered orally. **MAKI+FC6:** Intramuscular 50% hypertonic glycerol 8 ml/kg was applied to the bilateral lower extremities. After 1, 24 and 48 hours, 6 ml/kg of *Ficus carica* seed oil was administered orally.

At 72nd hours, blood samples and tissue samples were taken by puncture under ketamine (Keta-Control Mefar Turkey) and xylazine (Rompun 2% Bayer Germany) anesthesia and the animals were sacrificed. After the blood samples were centrifuged at +4 ºC for 5 minutes at 3000 rpm, the serums were taken into eppendorf tubes and placed at -80 degrees to be used in biochemistry studies. The excised kidneys were divided into two longitudinally, then washed with physiological saline and placed at -80 degrees Celsius to be used in biochemistry studies. Biochemistry and histological analyzes were performed from the ipsilateral (right) kidney.

Biochemical analysis

Serum urea (Cat.No:201-11-0481 SunRed.) and creatinine (Cat.No: E0307Ra BT-LAB.) levels were determined according to the commercial elisa kit usage procedure. Tissue samples were homogenized in phosphate buffer to calculate GSH levels. Tissue homogenates were then centrifuged at 4000 rpm, 10 min, +4 °C and serum samples were used for analysis together with the obtained supernatants. Protein Determination; It was performed according to the Bradford method[27]. GSH results were obtained by dividing the amount of protein in ml. Tissue and Serum GSH Level Measurement was performed according to the manual procedure of the commercial Elisa Kit (Cat. No:E1101Ra BT-LAB.) The GSH values obtained in mg per milliliter were divided by the amount of protein in ml and the result values were obtained as ugGSH/ugprotein.

Histological analysis

It was carried out in the Research Laboratory of the Histology and Embryology Department of Faculty of Medicine. After the kidney tissue samples were fixed in 10% neutral formalin (37% extrapure UN 2209 Tekkim) solution at +4 °C for 24 hours, the samples were subjected to routine histological procedure. Tissues were followed overnight in a tissue tracking device, followed by 10% formalin followed by 80%, 90%, 100% ethanol series (dehydration), followed by xylol and paraffin. After the follow-up, the tissues were labeled and embedded in paraffin blocks. 5 µm sections were randomly obtained from the samples in paraffin blocks with a microtome (Leica RM 2135). These sections were stained with hematoxylin-eosin (Net Chemical Turkey Naroteks Turkey) according to the staining procedure[28] and closed with entellan. Images were taken with an Olympus DP20 digital camera mounted on an Olympus BX51 microscope. Randomized sections were taken from each subject after descending longitudinally to the middle part of the kidney. From these sections, images were taken in the cortex area with x100 magnification and counted according to histopathological parameters. Scoring data were obtained by calculating the ratio and percentage of structures containing damaged areas in the kidney section to all structures in the scanned area. Data obtained, 0=0-1%, 1=1−25%;

2=25−50%; 3=50−75%; It was scored as 4>75% by semi-quantitative method.

Statistical analysis

SPSS software was used for statistical analysis. (IBM version 26.0; SPSS Inc. Chicago, IL, USA)). In the analysis of biochemical parameters, the skewness-kurtosis coefficients and Kolmogrov-Smirnov/Shapiro Wilk tests, coefficients of variation, histogram, normal and detrended Q-Q graphs were scored and scored separately in order to determine the conformity of in-group variables to normal distribution in all groups. Variables were presented using the mean (± standard deviation), compared using the T test. Paired groups were compared using the The Kruskal-Wallis test was used to determine whether there was a difference between the groups. For the Kruskal-Wallis test, $p < 0.05$ was considered statistically significant. Then, all groups compared between the MAKI group and the treatment groups compared between each other by using Mann-Whitney U test. $P \leq 0.05$ was accepted for statistical significance, and p values were according to the MAKI group, ***, $p \leq 0.001$; **, $p \leq 0.01$; * denoted as $p \leq 0.05$.

RESULTS

The mean serum creatinine level in the MAKI group was found to be significantly higher than in all groups ($P \leq 0.01$), and a decrease was found at the same level of significance in the MAKI+FC3 and MAKI+FC6 groups compared to the MAKI group ($P \leq 0.01$). It was not found statistically significant between MAKI+FC3 and MAKI+FC6 groups. While the mean serum urea level was found to be significantly higher in the MAKI group compared to all groups ($P \leq 0.01$), lower urea levels were found in the MAKI+FC3 and MAKI+FC6 groups compared to the MAKI group, and it was statistically significant ($P \leq 0.001$, $P \leq 0.01$). There was no significant difference between the different dose treatment groups. Comparative graph of mean serum creatinine and urea levels of all groups between groups is presented in Figure 1a and 1b.

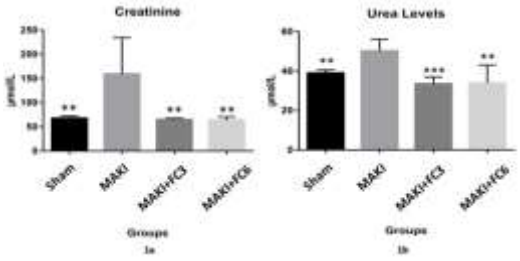

Figure 1. 1a: Graph of serum creatinine levels of all groups. 1b: Graph of serum urea levels of all groups. Significance levels of the differences of the groups according to the MAKI group.

While the mean tissue GSH level of the Sham group was higher than the MAKI group ($P \leq 0.001$), it was found to be higher in both different dose treatment groups compared to the MAKI group ($P \leq 0.01$). No significant difference was found between MAKI+FC3 and MAKI+FC6 groups. While the mean serum GSH level of the Sham group was higher than the MAKI group ($P \leq 0.01$), the MAKI+FC3 group did not show a significant difference compared to the MAKI group. The MAKI+FC6 group was found to be higher ($P \leq 0.05$) than the MAKI group. Comparative graph of mean tissue and serum GSH levels of all groups between groups is presented in Figure 2a and 2b.

In tissue microscopic sections, the level of necrosis in the sham group was significantly lower than in the MAKI group ($P \leq 0.001$), the MAKI+FC3 group was significantly lower than the MAKI group ($P \leq 0.001$), and the MAKI+FC6 group was again significantly lower than the MAKI group ($P \leq 0.01$) was found. When the treatment groups were compared within themselves, no significant difference could be shown between the MAKI+FC3 and MAKI+FC6 groups. In tissue microscopic sections, cast level was found to be significantly lower in the sham group compared to the MAKI group ($P \leq 0.001$), and the MAKI+FC3 group was not significant compared to the MAKI group. The cast level of the MAKI+FC6 group was found to be significantly lower ($P \leq 0.01$) compared to the MAKI group. The intergroup comparative graph of the mean necrosis and cast levels of all groups is presented in Figure 3a and 3b.

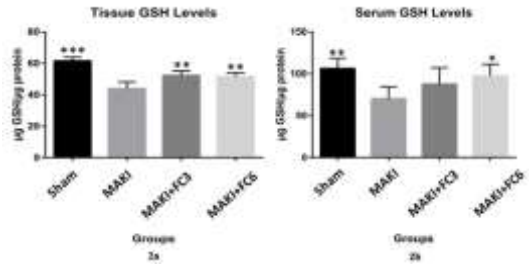

Figure 2. 2a: Graph of Tissue GSH levels of all groups. 2b: Graph of Serum GSH levels of all groups. Significance levels of the differences of the groups according to the MAKI group.

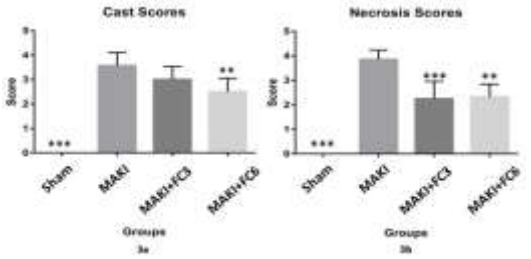

Figure 3. 3a: Graph of necrosis levels of all groups. 3b: Graph of cast levels of all groups.
Significance levels of the differences of the groups according to the MAKI group

When the kidney sections of the animals stained with hematoxylin-eosin H&E were examined under a light microscope; In the sham group, tubule and glomerular structures were normal, but no necrosis or cast formation was observed (Figure 4).

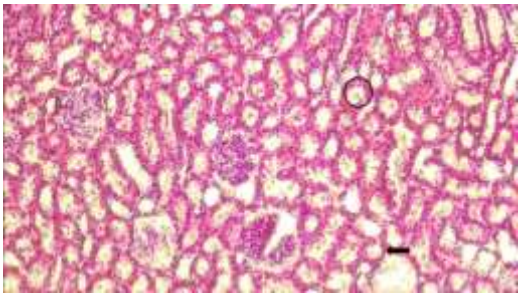

Figure 4. Sham group photomicrography.
Arrow: Normal distal tubule, Circle: Normal proximal tubule

In the MAKI group, in which the highest tubular damage score was observed, tissue organization was greatly disrupted, numerous necrotic areas in the tubule walls and significant dilatation in many tubules were observed. It was observed that the cast formations were mostly in the appearance of degenerated epithelial cells spilled into the lumen and in the form of larger stained areas. In addition, granular cast deposits were observed. In some distal tubule cells, degenerative changes with swelling and vacuolization in the cytoplasm, as well as some deformation of the glomerular structures were observed (Figure 5).

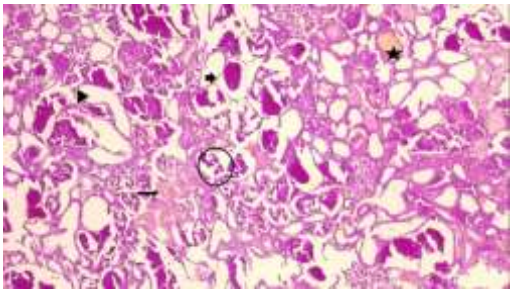

Figure 5. Photomicrography of the MAKI group.
Short arrow, tubular necrosis; circle, intratubular epithelial cell casts; star, myoglobin cast formation; arrowhead, tubular dilatation, long arrow, cytoplasmic swelling of the distal tubule wall

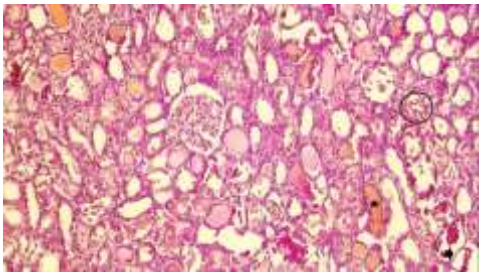

Figure 6. Photomicrography of the MAKI+FC3 group.
Short arrow, tubular necrosis; circle, intratubular epithelial cell casts; star, myoglobin cast formation.

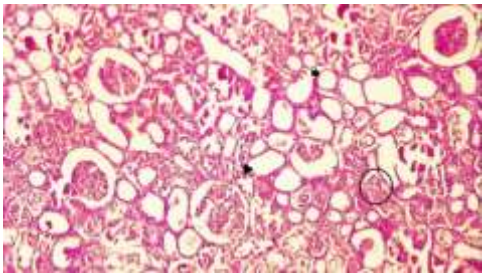

Figure 7. Photomicrography of the MAKI+FC6 group.
Short arrow, tubular necrosis; circle; intratubular cell casts; arrowhead, tubular dilation

While necrosis and cast formation were observed in less areas in the treatment groups compared to the MAKI group, it was observed that the cast formations were mostly hyaline or myoglobin accumulations in the MAKI+FC3 group (Figure 6).

In the MAKI+FC6 group, it was observed that the cast formations were mostly in the form of intraluminal epithelial casts, although less in area than in the MAKI group (Figure 7).

The macroscopic view of the kidney sections of all groups is presented in Figure 8.

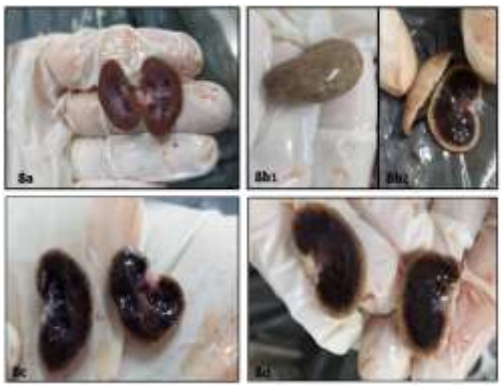

Figure 8. All group makroscopic views. 8a: Sham group, 8b1-8b2: MAKI group, 8c:MAKI+FC3 group, 8d: MAKI+FC6 group

DISCUSSION

In our study, the effects of low-dose and high-dose *Ficus carica* seed oil on parameters such as urea and creatinine levels, which are markers of kidney damage after 72 hours, and GSH levels in tissue and serum, were investigated in the MAKI model we created with glycerol injection. 72 hours after glycerol administration, blood serum urea and creatinine levels increased, GSH levels decreased in tissue and serum, cellular damage, necrosis and cast formation in kidney tissue were observed. In the groups given *Ficus carica* seed oil, a decrease in elevated serum urea and creatinine values without difference between high and low doses, an increase in tissue GSH levels in a non-dose dependent manner, and an increase in serum GSH values with high doses were detected. A non-dose-dependent decrease in necrosis rates was observed in kidney tissue.

Experimental studies in the MAKI model show that urea and creatine levels increase after glycerol administration. In the study of Tsai et al. serum BUN and creatinine values increased continuously at 1, 3, 6, 9, 12, 24 and 48 hours from glycerol until 48th hour[29]. In the study of Al Asmari et al. the increase in serum BUN and creatinine levels at 72nd hour is parallel to our study[30]. Again, very high urea and creatinine values were reported at the 96th hour in the MAKI model created with glycerol[31]. In a study in which the recovery phases of myohemoglobinuric acute renal failure performed with, 50% glycerol were examined, significant decreases were observed in BUN values, which increased up to 2-6 days after the glycerol injection in animals on the 6th day. It has been reported to only 3 out of 47 animals return to baseline BUN values at 72nd and 144th hours[32]. In the study conducted by Fernandez et al. it was shown that the increase in serum urea and cratinine levels in the acute renal failure model created with 10 ml/kg 50% glycerol was more than the 24th hour in the 72nd hour[8]. In another study, in a rhabdomyolysis model created with 50% glycerol at doses of 7 ml/kg, higher urea and creatinine values were reported 96 hours after glycerol injection, similar to our increase rates at 72nd hour, compared to the control group[40]. Al Laham reported high urea and creatinine levels up to the 6th day in (acute renal failure) ARF induced by 50% glycerol[33]. Moggio et al. reported that in a MAKI model in which they evaluated kidney functions and tissue damage simultaneously, the BUN level peaked at 72nd hour after glycerol injection, and the acute effects of kidney damage decreased at 240 hours, and the healing process began[34]. The high urea and creatinine values at the 72nd hour reported in the studies support our idea that we should set the 72nd hour as the time to terminate the study.

Although Fernández-Fúnez et al. reported a significant decrease in the total antioxidant level at the 24th hour and a spontaneous increase at the 72nd hour with glycerol althought a significant decrease was observed in the GSH level at the 72nd hour in the glycerol group in our study[8]. In our study, it is seen that glycerol causes depletion of tissue and serum GSH stores. Studies have also shown that the decrease in GSH and structural damage in MAKI continue for a long time, and that significant decreases in tissue GSH levels have been reported even 6 days after glycerol[33]. The decrease in serum GSH level in the glycerol group in our study is similar to the study of Vlahovic et al.[35]. In our study, it is thought that damage to other organs, such as the liver, may also be effective in the decrease in glycerol and serum GSH levels. As a matter of fact, it is

known that acute renal damage due to rhabdomyolysis causes multiple damage in other organs[36,37].

Abul-Ezz et al. reported that differences in GSH levels between groups were parallel to the histopathological results of acute kidney injury (AKI)[38]. In parallel with the findings of renal tubular damage and necrosis in our study, Al Asmari et al. detected extensive tubular damage in the cortex and medulla region of the kidney in animals with AKI[30]. Studies report that tubular damage starts very early and continues for a long time. As a matter of fact, in the study of Nara et al. in MAKI, it was reported that the early period findings of renal tubular injuries observed with myoglobin accumulations and larger stained areas were observed to increase at the 1st and 3rd hours[39]. The histopathological findings that we detected at 72 hours in our study are consistent with the study of Tajik et al. in which tissue section samples taken at 96th hour showed necrosis between 50-100% in the glycerol group[40]. In the study of Moggio et al. kidney tissue damage begins to deteriorate at the 12th hour after the functional parameters begin to deteriorate, necrotic areas appear after 12 hours, cast formations and necrotic areas with casts to the tubule lumen reach their peak at the 72nd hour, at the 240th hour. On the other hand, it was reported that tissue organization was largely recovered, but healing was prolonged up to 720 hours[34]. These long-term findings strengthen the idea that we can maximize functional and structural kidney damage at 72nd hour.

Effects of fig seed oil on intestinal and liver pathological models; reported in animal studies. In Orak's study, the effects of fig seed oil on small intestine ischemia-reperfusion injury due to experimental mesenteric artery occlusion were investigated by giving 3 ml/kg and 6 ml/kg doses for 10 days. In this study, a non-dose-dependent increase in tissue CAT and GSH levels was obtained in the groups given fig seed oil in intestinal tissue, and a significant improvement was observed in histopathological parameters, more prominently in the high dose group[41]. In our study, an increase in GSH level was observed similarly in the groups to which we gave fig seed oil, a significant difference was obtained in the high-dose group, moreover, it was observed that the histopathological parameters, which we determined similarly with this study, improved more in the high-dose group. In a similar study conducted by Şirinyıldız, the effects of fig seed oil in an experimental colitis animal model induced by TNBS were investigated and compared separately at 3 ml/kg and 6 ml/kg doses for 3 days and 15 days. It has been reported that the level of SOD increases in a dose and time-dependent manner, high dose is more effective in increasing GPx activity, similar to our study, and long-term application without dose difference is effective in the increase in CAT level[42]. Again, in this study, the support of antioxidant parameters with the improvement in histopathological findings is consistent with our study, and the level of necrosis was shown to be dose- and duration-dependent here. In our study, while an increasing improvement in cellular morphology was noted at the cast level with dose, we could not statistically show the dose-dependence of necrosis recovery, because the kidney tissue cellular morphology was affected by the physiopathological processes in the MAKI model or by the effect of *Ficus carica* seed oil on different systemic parameters (ion balances) that we do not know yet. etc.) may show secondary precision change. In Yeşilçayır's study, hepatotoxicity induced by paracetamol in the liver was significantly increased in the high dose group of fig seed oil administered at doses of 3-6 ml/kg for 7 days, and centrilobular necrosis was not observed in the liver in both dose groups, which is similar to the necrosis findings we obtained in the kidney tissue[43]. However, unlike in our study, although the level of necrosis decreased in both dose treatment groups, it was not completely reset.

In these studies, the protective effects of fig seed oil have been shown in general. Due to the unpredictability of the MAKI model due to its formation mechanism and the superiority of the effect of the antioxidative application after MAKI[10], the therapeutic effects were investigated in our study, although the findings we obtained overlap with other in vivo studies. In the light of this information, it has been shown that fig seed oil increases antioxidant capacity and improves morphological damage along with functional improvement in the kidney.

One of the limitations of our study is that the presence of findings such as systemic hyperkalemia, hypocalcemia and hyponatremia in MAKI due to muscle damage could not be supported by the differences in serum K^+, Na^+ and Ca^{++} levels in the kidney damage and functional recovery. In addition, supporting functional damage with urine urea and creatinine values may provide more predictable findings for future studies. However, the kidney

damage development findings we obtained and the macroscopic and microscopic structural improvement and functional recovery findings we observed with *Ficus carica* seed oil are in line with our hypothesis and this forms a basis for further studies.

In conclusion, our study is the first study in the literature to examine the effects of *Ficus carica* seed oil in the MAKI model. The results prove that *Ficus carica* seed oil provides an increase in antioxidant capacity and healing of tissue damage. Further studies and investigation of protective efficacy in different parameters are recommended.

Yazar Katkıları: Çalışma konsepti/Tasarımı: ROE, Dİ; Veri toplama: FŞ, Dİ; Veri analizi ve yorumlama: Dİ, FŞ; Yazı taslağı: FŞ, Dİ; İçeriğin eleştirel incelenmesi: ROE, FŞ; Son onay ve sorumluluk: Dİ, FŞ, ROE; Teknik ve malzeme desteği: FŞ, ROE; Süpervizyon: ROE; Fon sağlama (mevcut ise): yok.
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