Research Article

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The Effects of *Ferula elaeochytris* on the Oxidant/Nitrosative Stress in Experimental Myoglobinuric Acute Renal Failure

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ABSTRACT

Free radicals and nitric oxide play an important role in the pathogenesis of Myoglobinuric Acute Renal Failure (mARF). Herein, we aimed to investigate the effects of *Ferula elaeochytris* on the oxidative-antioxidant system and nitrosative stress in experimental mARF. In our study, 56 Sprague-Dawley rats, equally divided in seven groups, were used as model organisms of experimentally induced mARF. A disease model of mARF was established with 50% glycerol in all groups except one. Moreover, 40 mg/kg and 80 mg/kg of *F. elaeochytris* root extract were administered intragastrically before and after model creation. The following were investgated in the kidney tissue: serum urea nitrogen, urea, creatinine, sodium and potassium levels of rats in all groups; urinary urea and creatinine levels, MDA, SOD, GPx, CAT, NO and nitrotyrosine parameters. In addition, histological changes in the kidney were recorder with electron microscopy. Significant changes were observed in the CAT, GPx and MDA values of all groups compared to the control group. Electron microscopy observations also supported these findings. Herein, we provide evidence regarding the limited protective and curative effects of *Ferula elaeochytris* on mARF through reduction of the oxidant/nitrosative stress.

Keywords: *Ferula elaeochytris,* Myoglobinuric Acute Renal Failure, Nitrosative Stress, Oxidative Stress.

1. Introduction

Acute renal failure (ARF), which is also characterized by acute loss of kidney function, is a disease that affects many organs and systems with symptoms such as regression or loss of renal functions within hours and days, inability to excrete nitrogenous residues due to kidney damage, and inability to maintain body fluid and electrolyte balance [1-2]. Rhabdomyolysis-induced myoglobinuric acute renal failure (mARF) is a uremic syndrome resulting from the release of cellular elements such as potassium cation ($K^{+)}$, phosphorous, uric acid, myoglobin and sarcoplasmic proteins into the circulation, after damage of the striated muscle cells (rhabdomyocytes) [3-5]. In addition, many studies highlight the importance of oxidative stress in the mARF pathogenesis [6-7].

Heme-containing respiratory proteins, myoglobin and hemoglobin (Hb), can oxidize biomolecules via peroxidase-like enzyme activity [8]. In myoglobin, oxidation of ferrous iron (Fe^{+2}) by molecular oxygen (O_2) to ferric cation (Fe^{+3}) leads to the generation of hydroxyl radicals (OH^{-}). This oxidative potential is neutralized by intracellular antioxidant molecules. However, escape of myoglobin from its cellular environment causes increased leakage of reactive oxygen particles and free radical-related cellular damage [9]. Although it could not be demonstrated in experimental models of mercuric chloride-induced ARF, studies in isolated proximal tubular systems using glutathione and deferoxamine, an iron chelator, showed that OH^{-}-related heme protein and free iron interaction plays a critical role in tubule damage [10-11].

The kidneys of animals with intramuscular injection of glycerol, which is the most commonly used model that best reproduces ARF due to rhabdomyolysis and increase H_2O_2 production compared to control [12]. It has been shown that reactive oxygen metabolites (ROM) formed in the glycerol model are largely mitochondria-derived and that inhibition of some steps of the respiratory chain can prevent kidney damage [13].

Ferula is a genus of approximately 220 species of perennial, segmented leafy and flowering plants of the parsley family. *Ferula* species are found in the arid regions of the eastern Mediterranean and Central Asia; 12 species are endemic in Anatolia [14-15]. It is known that these plants contain saponin, tannin, terpenes, starch, resins, essential oils (e.g. oleo-gum resin) and alkaloids [16-17].

Studies on some species of the genus *Ferula* have suggested that these plants have antioxidant, cytotoxic, antidiabetic, antimicrobial, antifungal, antispasmodic, antiulcerative and hepatoprotective effects, apart from their known aphrodisiac effects [18-19]. The species *Ferula elaeochytris* Korovin (also known as 'Çaksır') grows in Syria, Lebanon and the eastern Mediterranean region of Turkey such as Hatay, Adana and Mersin provinces [20].

Although known medicinal plants, such as green tea, ginseng, black cumin, garlic, saffron, grape, ginger, pomegranate, and milk thistle, have been demonstrated to have a nephroprotective effect in experimental models of ARF [21], the potential effect of *Ferula elaeochytris* in ARF has not been explored yet. In our study, we aimed to investigate whether *F. elaeochytris* has any effect on kidney function, oxidative and nitrosative stress, and also to reveal any histopathological changes in the kidney tissue.

2. Material and Method

2.1. Animal model experimental design

Healthy Spraque Dawley female albino rats weighing 250-300g and averaging 3-4 months old were used in our experimental study. The present study is supported by the Mersin University Scientific Research Projects Unit (project coded 2017-2-TP3-2602). This study was performed according to the Mersin University Animal Experiments Local Ethics Committee (Decision of the Board dated 10/04/2017, meeting number 05 and number 09) approval. All experimental animals were obtained from Mersin University Experimental Animals Laboratory. The animals were dehydrated for only 16 hours during the animal model creation. All rats were fed with standard pellet rat chow in polycarbonate transparent cages and tap water, except for their 24-hour housing in metabolic cages, wherein they were fed with unsalted seeds and tap water. The mARF model was created by administering 8 mL/kg 50% glycerol to both rat hind extremities in an equal amount deep intramuscularly in the morning following a 16-hour period of thirst.

A total of seven experimental groups, eight rats per group, were formed by random selection from among the experimental animals:

Group 1 (Control): Animals were housed in metabolic cages for one day.

Group 2: 2 mL of saline (2 mL 0.9% NaCl) was given once intragastrically. The animals, which were kept for 1 hour, were left without water for 16 hours and the mARF model was created at the end of this period.

Group 3: 2 mL of *Ferula elaeochytris* root extract (40 mg/kg dissolved in 2 mL of saline) was administered once intragastrically. The animals, which were kept for 1 hour, were left without water for 16 hours and the mARF model was created at the end of this period.

Group 4: 2 mL of *F. elaeochytris* root extract (80 mg/kg dissolved in 2 mL of saline) was administered once intragastrically. The animals, which were kept for 1 hour, were left without water for 16 hours and the mARF model was established at the end of this period.

Group 5: The mARF model was created after a 16-hour period of thirst; animals kept for 1 hour were given 2 mL of saline intragastrically.

Group 6: The mARF model was established after a 16-hour period of thirst; 2 mL of *F. elaeochytris* root extract (40 mg/kg dissolved in 2 mL of saline) was administered intragastrically to the animals, which were kept for 1 hour.

Group 7: The mARF model was created after a 16-hour period of thirst. 2 mL of *F. elaeochytris* root extract (80 mg/kg dissolved in 2 mL of saline) was given intragastrically to the animals, which were kept for 1 hour.

The animals were kept in the metabolic cages for one day. At the end of the experiment, the subjects whose urines were collected for 24 hours were anesthetized with intraperitoneally administered xylazine (10 mg/kg) and ketamine (100 mg/kg). After ensuring that the subjects did not respond to painful stimuli, the abdominal cavity was opened, the kidneys were removed, and the subjects were sacrificed by drawing out intracardiac blood.

2.2. Ferula elaeochytris processing and extraction

Ferula elaeochytris, used in this study, was collected before flowering in Kahramanmaraş during the last week of May and the first week of June, cleaned, and left to dry in a cool, clean and sunless place. After drying, the roots were separated, cut into very small pieces by the grating method, and the extraction process was performed.

An amount of 100 g of finely powdered *F. elaeochytris* plant root was put into the prepared cartridge. The extraction process was continued for 4 hours with a 1 lt volume of Soxhlet extractor using approximately 1200 mL of methanol (99.7%) as solvent. The solvent of the extract obtained at the end of the period was removed with a rotavapor at 60 °C. Then, it was dried for 1 more hour in an oven heated to 60 °C and weighed. Extract yield was determined as 27%. All plant roots were processed in a similar way as above, in batches, and the plant extracts were collected and combined.

GC/MS analysis of the *F. elaeochytris* extract was performed. The initial temperature was set at 60 °C, the temperature rise rate was 3 °C /min, the final temperature was 325 °C, the analysis time was 78 min, the injection amount was 2.5 µl, the column properties were HP-5ms and carrier gas Helium was studied at a flow rate of 1 mL/min.

2.3. Sample extraction and analysis

2.3.1. Liquid samples

Blood Samples*:* The blood drawn from the heart of the animals under study was collected into gel tubes for biochemical analysis. In order to evaluate the kidney functions in the serum, urea (Biolab, France), creatinine (Biolab, France), BUN (Mybiosource, USA), Na^+ (Mybiosource, USA) and K^+ (Mybiosource, USA) levels were measured in an autoanalyzer with commercial kits.

Urine Samples: The urine samples were taken from subjects kept in metabolic cages for 24 hours; urea and creatinine levels were measured in an autoanalyzer with commercial kits (Elabscience, USA).

2.3.2. Tissue samples

In the extracted tissue samples, the protein levels were determined [22]; malondialdehyde (MDA) level [23] and catalase (CAT) activity [24] were measured spectrophotometrically. Superoxide dismutase (SOD) (Cayman 706002), glutathione peroxidase (GPx) (Cayman 703102), nitric oxide (NO) (Biosource BS2604161) and nitrotyrosine (3-NT) (Biosource MBS023877) analyses were performed with kits.

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2.4. Sample preparation for transmission electron microscopy

At the end of the experiment, the subjects were anesthetized with xylazine (10 mg/kg) and ketamine (100 mg/kg) intraperitoneally. After the abdominal cavity was opened, the kidneys were removed, and the subjects were sacrificed. The removed kidney samples were cut into pieces of 1 mm^3 from the cortex and fixed with fresh 2.5% glutaraldehyde solution for four hours and then they were washed with phosphate buffer in +4 °C. Then, the tissues were placed into a 1% solution of osmium tetroxide for post-fixation. After postfixation, the tissues were dehydrated in graded alcohol series and cleared with propylene oxide, and then embedded in epoxy resin for blocking. Tissue blocks were sliced using an ultramicrotome (Leica UCT-125, Leica Microsystems GmbH, Wien, Germany) and the sections were taken to copper grids and contrasted with uranyl acetate and lead citrate for five minutes. After contrasting, the sections were examined and photographed (Megaview III, Olympus GmbH, Germany) under a transmission electron microscope (JEOL JEM-1011, Jeol Ltd. Tokyo, Japan).

2.5. Statistical analyses

The Shapiro-Wilk test was used to examine whether the sample data follow a normal distribution. Analysis of variance (ANOVA) was applied to assess if there are differences across the averages of the 7 experimental groups. ANOVA was performed by assuming homogeneity of variances ($p>0.05$), where $p<0.05$ suggests violation of the homogeneity of variance assumption. Tukey' HSD was preferred as the *post-hoc* test for the pairwise group comparisons. Welch's correction was applied which assumes unequal means between groups ($p<0.05$). The Games-Howell test was used as the *post-hoc* test for the pairwise group comparisons. For both tests, p-value less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Biochemical results

The values referred to below are statistically significant, unless otherwise stated. The serum BUN, urea, creatinine, Na^+ and K^+ values are presented in Table 1.

The serum BUN values of the 2nd, 4th, 5th, 6th and 7th groups were determined to be higher than the 1st group ($p<0.05$). The serum BUN values of the 6th group compared to the 3rd group, as well as the 7th group compared to the 4th group, were found to be higher ($p<0.05$).

The serum urea levels of the 4th, 5th, 6th and 7th groups were shown to be higher than the 1st group

Table 1. BUN, urea, creatinine, Na^+ and K^+ values of serum sample

GROUPS	BUN (mg/dL) Mean ± SD	Urea (mg/dL) Mean ± SD	Creatinine (mg/dL) Mean ± SD	Na^+ (mmol/L) Mean ± SD	K^+ (mmol/L) Mean ± SD
1. Group	16.17 ± 0.98	34.57 ± 2.07	0.32 ± 0.06	142.00 ± 1.41	5.73 ± 0.49
2. Group	*26.19 ± 5.44	52.86 ± 14.87	0.33 ± 0.04	142.14 ± 1.57	0.39 ± 0.58
3. Group	22.37 ± 4.48	47.86 ± 9.58	0.30 ± 0.05	140.00 ± 1.73	**5.06 ± 0.34
4. Group	*19.51 ± 0.76	*42.29 ± 1.60	0.35 ± 0.08	*,**137.71 ± 2.50	*,**7.79 ± 0.25
5. Group	*180.57 ± 21.31	*386.43 ± 45.61	*5.19 ± 0.76	134.71 ± 5.02	7.11 ± 1.70
6. Group	*,•117.56 ± 59.08	*,•251.57 ± 126.46	3.10 ± 1.93	134.86 ± 6.44	5.24 ± 0.81
7. Group	*,••181.74 ± 45.75	*,••378.43 ± 95.28	*,••4.33 ± 1.12	*135.00 ± 4,12	6.37 ± 1.24

Results of biomarkers of serum samples were given as mean±standard deviation
*Significant differences in the levels of BUN, urea, creatinine, Na+ and K+ in other groups and 1. group ($p<0.05$).
**Significant differences in the levels of Na+ and K+ in other groups and 2. group ($p<0.05$).
•Significant differences in the levels of BUN and urea in other groups and 3. group ($p<0.05$).
••Significant differences in the levels of BUN, urea and creatinine in other groups and 4. group ($p<0.05$).

(p<0.05). The serum urea values of the 6th group versus the 3rd group and the 7th group versus the 4th group were found to be higher (p<0.05).

Regarding the serum creatinine levels, in the groups under study, serum creatinine values of the 5th, 6th and 7th groups were determined to be higher than the 1st group (p<0.05), whereas the serum creatinine level of the 7th group was higher than the 4th group (p<0.05). When the differences between the 6th group and 3rd group serum creatinine values were examined, a difference was observed between the groups, but it was not statistically significant.

The serum Na^+ value in group 4 was found to be lower compared to the 1st and 2nd groups (p<0.05). Likewise, the Na^+ value of the 7th group was lower than the 1st group (p<0.05). However, a difference was observed in the Na^+ values between the other groups, but it was not statistically significant though.

When the serum K^+ values were examined, the value of the 4th group was determined to be lower than the 1st and 2nd groups (p<0.05). In a similar fashion, the serum K^+ value of the 3rd group was lower than the 2nd group (p<0.05).

The urea and creatinine values detected in urine samples taken from the rats under study are presented in Table 2.

The urine urea value was shown to be lower in the 5th group than the 1st group (p<0.05), whilst the one in the 7th group was determined to be lower compared to the 1st and 4th groups (p<0.05).

The urinary creatinine level was found to be higher in the 4th group and lower in the 5th group, as compared to the 6th group (p<0.05). In addition, the urinary creatinine value of the 7th group was determined to be lower than the 4th group (p<0.05).

CAT, SOD, GPx activities and MDA, NO and 3-NT levels were detected in the kidney tissue samples of rats. Except for the SOD enzyme activity of the 3rd group, CAT and SOD enzyme activities of all groups were shown to be lower, whilst GPx enzyme activities were higher compared to the 1st group (p<0.05). In addition, the GPx enzyme activity of the 7th group was found to be lower than the 4th group (p<0.05) (Table 3).

The MDA values of all groups were determined to be higher than the 1st group (p<0.05), whereas the value of the 3rd group was also shown to be higher compared to the 2nd group (p<0.05) (Table 3).

The NO activity of all groups except the 7th group was found to be higher compared to the 1st group (p<0.05); the NO activity of the 7th group was lower than the 4th and 5th groups (p<0.05) (Table 3).

Regarding the 3-NT levels, the 5th group was determined to be lower than the 1st group and the 7th group was determined to be lower than the 6th group (p<0.05). (p<0.05). The 3-NT level of the 6th group was found to be lower than the 5th group (p<0.05) (Table 3).

3.2. TEM analysis

The TEM images are shown in Fig. 1-6, where X[number] indicates magnification. In the control group, glomerulus (Fig. 1A) and tubule epithelial cells (Fig. 2A and B) in the kidney tissues had normal morphological features. The podocytes and the foot processes of these cells (pediceles), the endothelial cells lining the glomerular capillaries and the glomerular basement membrane were normal. Normal mesangial cells were observed in the intercapillary space in the glomerulus.

Podocytes, glomerular basement membrane, glomerular endothelial cells and mesangial cells generally exhibited normal ultrastructural features in the kidney tissues in the pre-model (Fig. 2A) and post-model control groups (Fig. 2D), namely the pre-model 40 mg (Fig. 2B) and pre-model 80 mg (Fig. 2C), as well as the corresponding post-model 40 mg (Fig. 2E) and post-model 80 mg (Fig. 2F) treatment groups.

In the pre-model control group, although tubular epithelial cells lining the renal tubules generally had normal morphological features (Fig. 3A and B), cellular enlargement - due to intracellular edema - was observed in tubule epithelial cells in some tubules (Fig. 3C and D).

In the pre-model 40 mg treatment group, the tubules appeared generally normal (Fig. 4A), but in some tubular epithelial cells intracellular edema was observed (Fig. 4B). In the pre-model 80 mg treatment group, tubules had normal ultrastructural features (Fig. 4C).

In the post model control group, some tubular epithelial cells exhibited normal morphological features (Fig. 5A). In some tubules, it was observed that the

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Table 2. Urea and creatinine values of urine sample.

Groups	Urea (mg/dL) Mean ± SD	Creatinine (mg/dL) Mean ± SD
1. Group	4350.57 ± 2049.17	60.96 ± 36.33
2. Group	6598.00 ± 2801.39	116.82 ± 33.87
3. Group	6499.17 ± 1881.27	103.43 ± 38.90
4. Group	5788.43 ± 849.72	*157.21 ± 54.64
5. Group	*1762.25 ± 1495.16	*438.65 ± 779.02
6. Group	701.25 ± 147.47	14.12 ± 2.61
7. Group	*,**901.00 ± 608.37	19.73 ± 12.92

Results of biomarkers of urine samples were given as mean±standard deviation
*Significant differences in the levels of biomarkers of urine samples in other groups and 1. group (p<0.05).
**Significant differences in the levels of urea 7. group and 4. group (p<0.05).

Table 3. MDA, CAT, SOD, GPx, NO and 3-NT values in renal tissue sample

Groups	MDA (nmol/g protein) Mean ± SD	CAT (U/mg protein) Mean ± SD	SOD (U/mg protein) Mean ± SD	GPx (U/mg protein) Mean ± SD	NO (µmol/mg protein) Mean ± SD	3-NT (nmol/g protein) Mean ± SD
1. Group	2.23 ± 0.22	21.50 ± 5.30	0.31 ± 0.09	0.92 ± 1.47	0.46 ± 0.09	2.87 ± 0.36
2. Group	*7.40 ± 0.64	*5.20 ± 0.53	*0.06 ± 0.01	*6.17 ± 1.39	*2.23 ± 0.70	2.36 ± 0.22
3. Group	*4.39 ± 0.45	*,**5.92 ± 0.30	0.16 ± 0.16	*5.68 ± 1.25	*1.46 ± 0.45	2.56 ± 0.22
4. Group	*7.02 ± 0.65	*5.80 ± 0.57	*0.05 ± 0.00	*4.50 ± 0.35	*2.07 ± 0.37	2.62 ± 0.06
5. Group	*7.59 ± 0.63	*5.26 ± 0.47	*0.11 ± 0.05	*7.11 ± 1.63	*2.62 ± 0.90	*2.25 ± 0.20
6. Group	*7.16 ± 0.54	*5.17 ± 0.63	*0.05 ± 0.01	*6.59 ± 1.02	*1.39 ± 0.41	2.81 ± 0.16
7. Group	*6.87 ± 0.60	*5.01 ± 0.48	*0.06 ± 0.01	*,•7.63 ± 0.43	•,••1.09 ± 0.47	••2.48 ± 0.11

Results of renal tissue samples were given as mean±standard deviation
*Significant differences in the levels of MDA, CAT, SOD, GPx, NO and 3-NT in other groups and 1. group (p<0.05).
**Significant differences in the levels of such as MDA in 3 group and 2. group (p<0.05).
•Significant differences in the levels of GPx and NO in 7 group and 4. group (p<0.05).
••Significant differences in the levels of NO and 3-NT in other groups and 5. group (p<0.05).

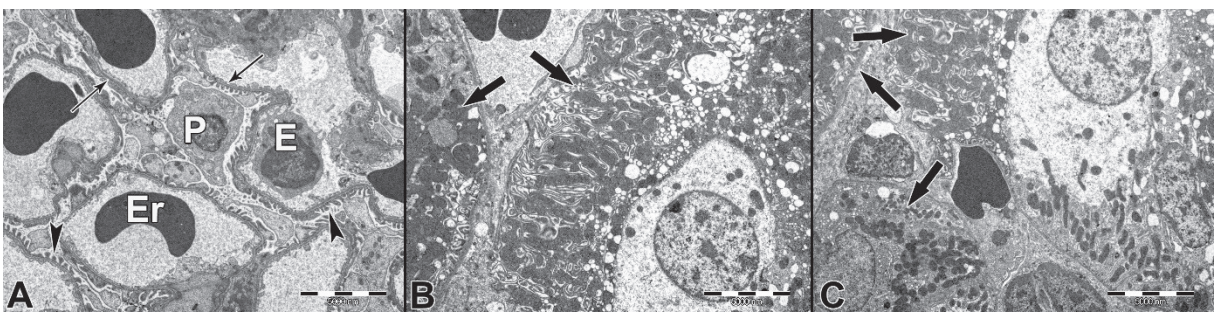

Figure 1. Control group. Glomerular (A) and tubule epithelial cells (B and C) with normal morphological features. Endothelial cell (E), podocyte (P), pedicels of podocyte (arrowhead), erythrocyte (Er), glomerular basement membrane (thin arrow), tubule epithelial cells (thick arrow). A, B and C: X5000.

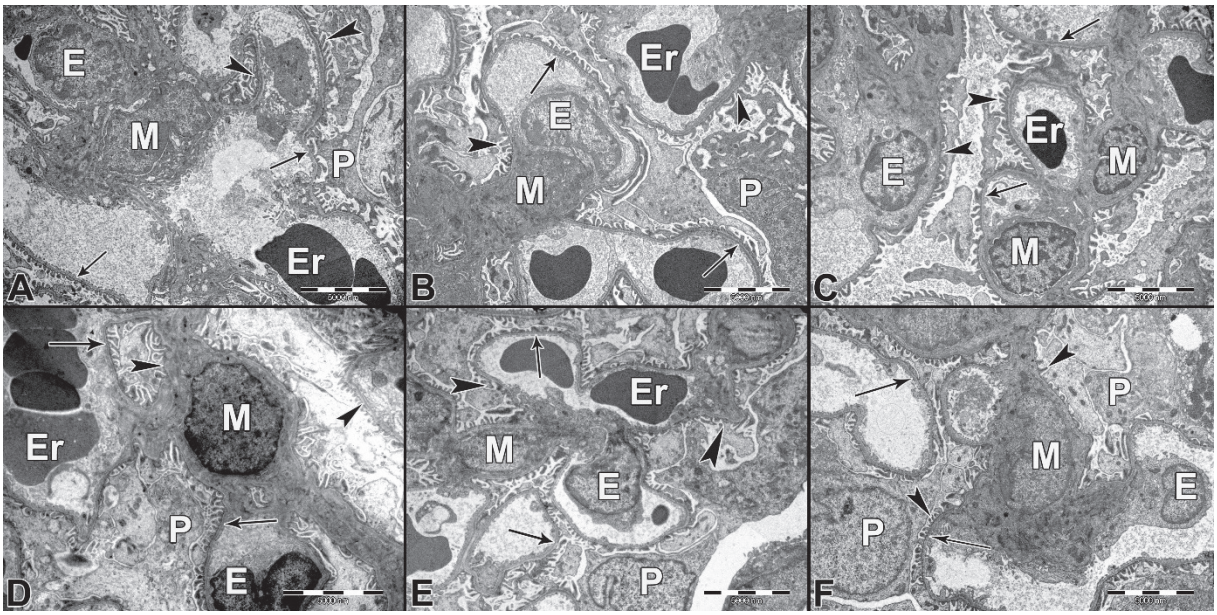

Figure 2. Glomerular with normal morphological features in group A. Pre-model control group, B. Pre-model 40 mg treatment group, C. Pre-model 80 mg treatment group, D. Post model control group, E. Post model 40 mg treatment group, F. Post model 80 mg treatment group. Endothelial cell (E), podocyte (P), pedicels of podocyte (arrowhead), erythrocyte (Er), glomerular basement membrane (thin arrow), mesangial cell (M). A-E and F: X5000.

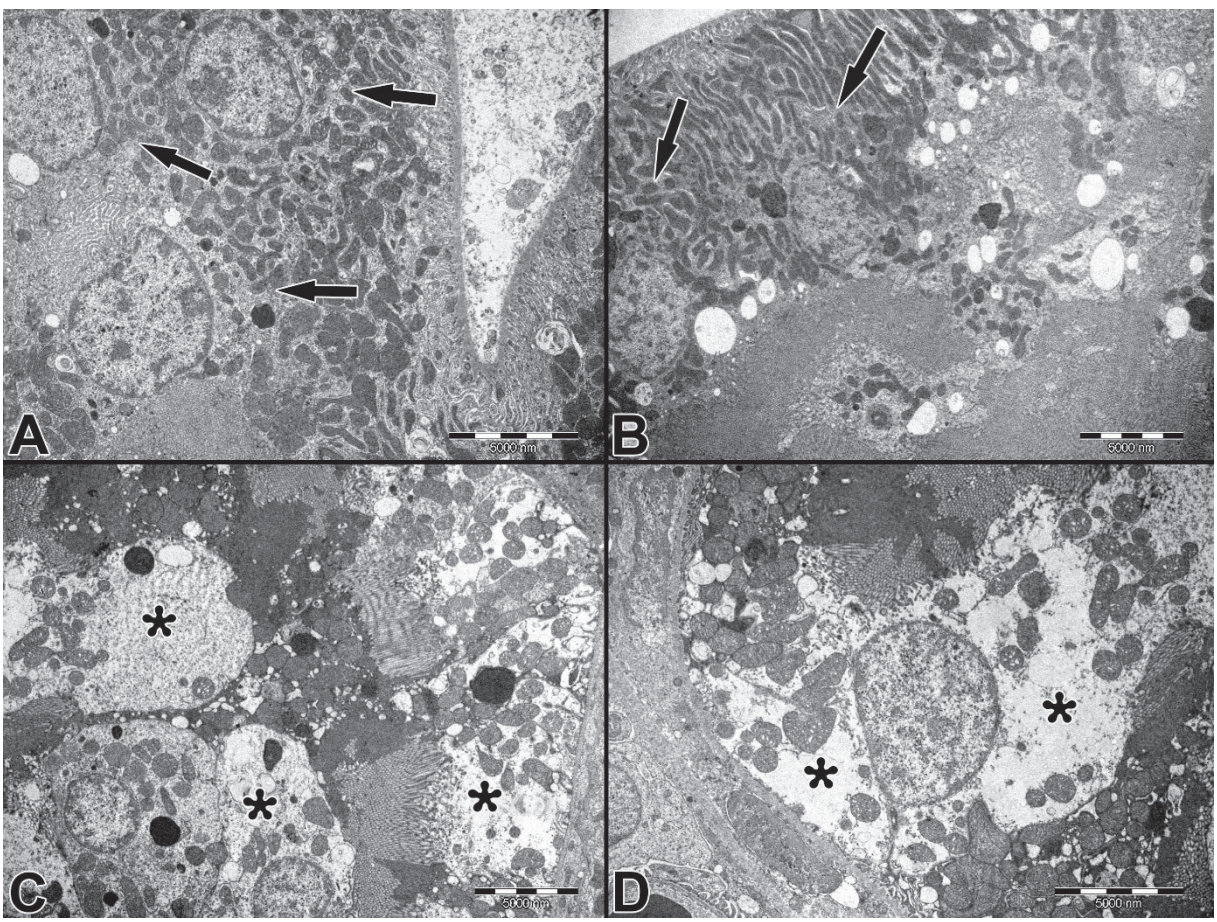

Figure 3. Pre-model control groups. A and B: Tubule epithelial cells with normal morphological features (thick arrow). C and D: Cellular enlargements due to intracellular edema in tubule epithelial cells (asterisk). A and D: X5000; B and C: X4000.

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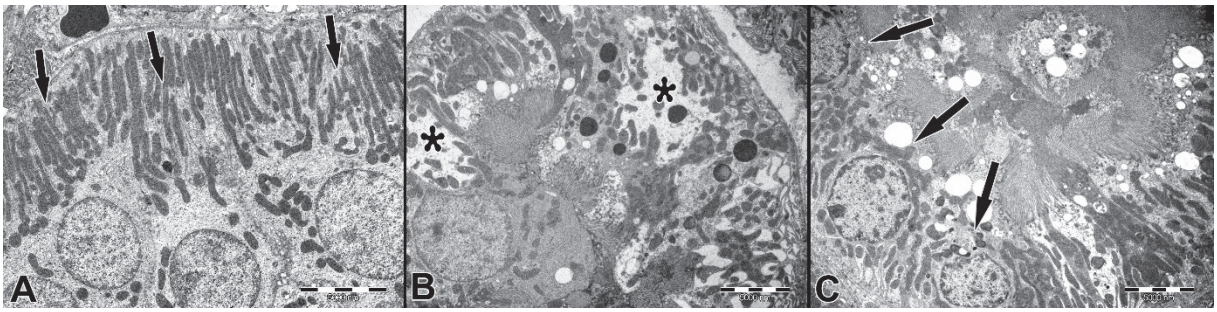

Figure 4. Pre-model treatment groups. A and B: Pre-model 40 mg group. C: Pre-model 80 mg group. A and C: tubule epithelial cells with normal morphological features (thick arrow). B: Cellular enlargements due to intracellular edema in tubule epithelial cells (asterisk). A: X5000; B and C: X4000.

lumen of the tubule was filled with an electron-dense material (Fig. 5B, C and D), and degenerated cell residues were found in some tubule lumens (Fig. 5E). In addition, there were degenerative changes, intracellular edema (Fig. 5D), mitochondrial damage (Fig. 5D and E) and localized loss in tubule epithelial cells (Fig. 5B).

In the post-model 40 mg treatment group, tubules generally displayed normal ultrastructural features (Fig. 6A and B). Moreover, in the post-model 80 mg treatment group, while some tubules had normal morphological features (Fig. 6C), there were vacuoles in the cytoplasm of the tubule epithelial cell lining and marked degenerative changes in some epithelial cells; it was also observed that some tubule lumens were filled with an electron dense material (Fig. 6D and E).

3.3. Plant extract analysis

On the basis of the results of GC/MS analysis, the *Ferula elaeochytris* extract contains a high rate of Thiirane and 1H-benzotriazole (Table 4).

4. Conclusion

This study was performed according to the Mersin University Animal Experiments Local Ethics Committee (Decision of the Board dated 10/04/2017, meeting number 05 and number 09) approval. In the present study, a rat mARF model was established after receiving glycerol, and biochemical parameters, as well as oxidative and nitrosative stress markers, were investigated. Given the lack of relevant experimental investigations of the effect of *Ferula elaeochytris* on any kidney failure model, there are no available reference studies to compare our findings.

The accumulation of nitrogen waste products in ARF, which is defined by the deterioration of kidney functions within hours or a few days, was anticipated. In our study, the blood and urine levels were measured once, 24 hours after the establishment of the mARF model. Therefore, when serum creatinine levels were evaluated based on RIFLE criteria, an accurate assessment could not be made, although there was an increase in the groups given saline and *F. elaeochytris* extract doses before the creation of the mARF model. After the mARF model was generated, the groups given saline and extract doses could be evaluated as "Failure". This suggests that the fluids given before the mARF model are effective for creatinine level homeostasis.

It is known that serum BUN levels increase faster than serum creatinine levels in prerenal acute kidney diseases [5]. Accordingly, in our study, serum BUN levels increased rapidly in direct proportion to serum creatinine levels. However, in the 4th group, serum creatinine level increased, while serum BUN level decreased.

In general, the increase in urea and creatinine levels, which are indicators of glomerular dysfunction, is consistent with the results of studies conducted in the same animal model. However, the absence or steady levels of urea and creatinine in the pre-model groups lead to the suggestion that the protective effect of the plant extract in the mARF model surpasses the curative effect, although it appears to have a higher therapeutic effect due to the increase in BUN and creatinine levels [25-27].

In our study, there were no statistically significant differences in the serum Na^+ levels as compared to the control group, except in the case of the pre- and post-model 80 mg/kg groups. There was a significant

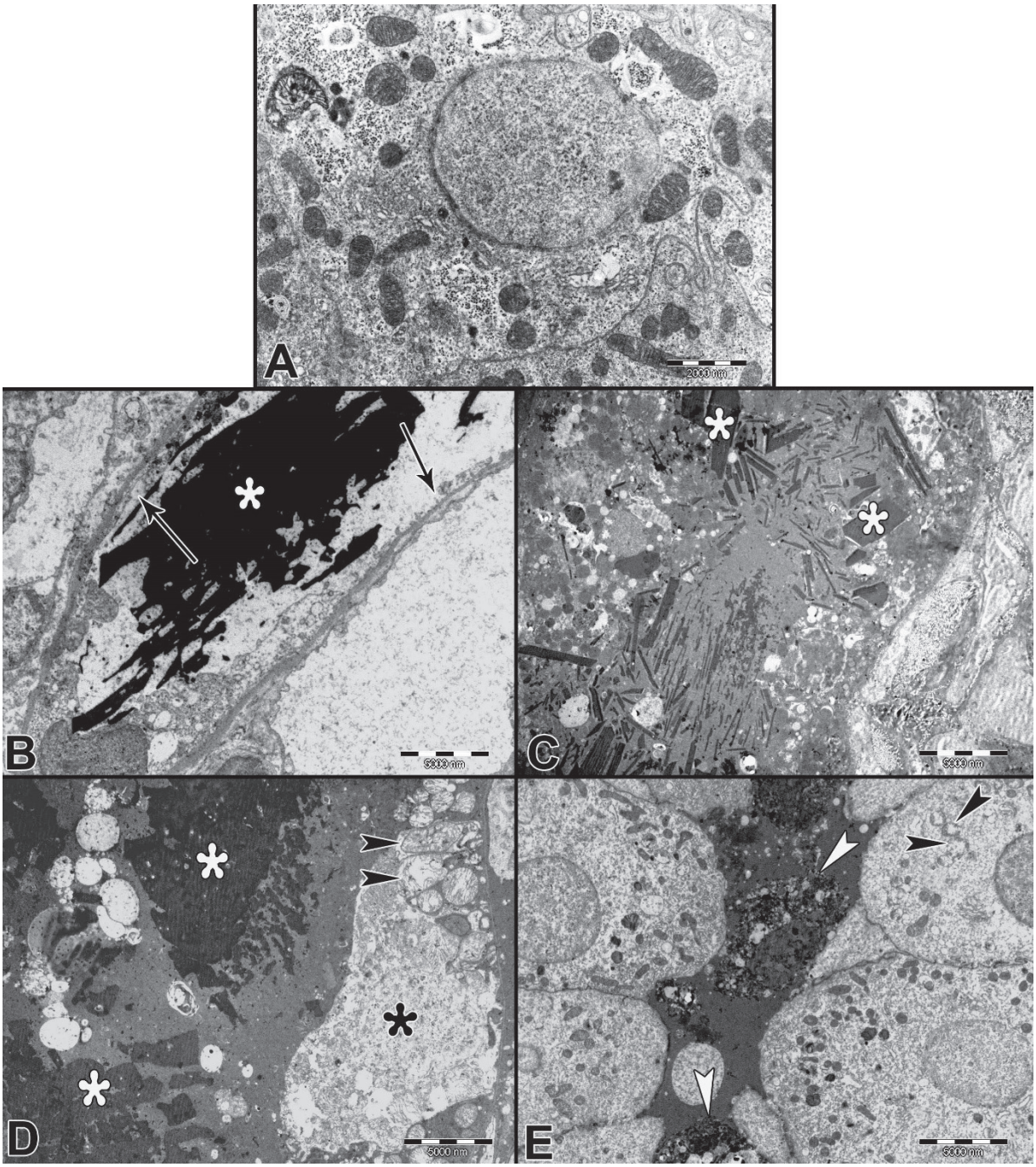

Figure 5. Post model control group. A. Tubule epithelial cells with normal morphological features. B, C, D and E: An electron-dense material in tubule lumens (white asterisk), degenerated cell debris in the tubule lumen (white arrowhead), intracellular edema (black asterisk) and damaged mitochondria in tubule epithelial cells (black arrowhead), loss of tubular epithelial cell (thin arrow). A: X10.000; B-E: X4000.

difference between the serum Na^+ level of the pre-model 80 mg/kg group and the 80 mg/kg post-model group compared to the corresponding saline group. There was no statistically significant difference in the serum K^+ levels in the pre- and post-model groups compared to the control groups. A significant difference was also observed in the serum K^+ levels of the pre-model 40 mg/kg and 80 mg/kg groups compared to the corresponding pre-model saline group. We suggest that the decrease in the serum Na^+ level

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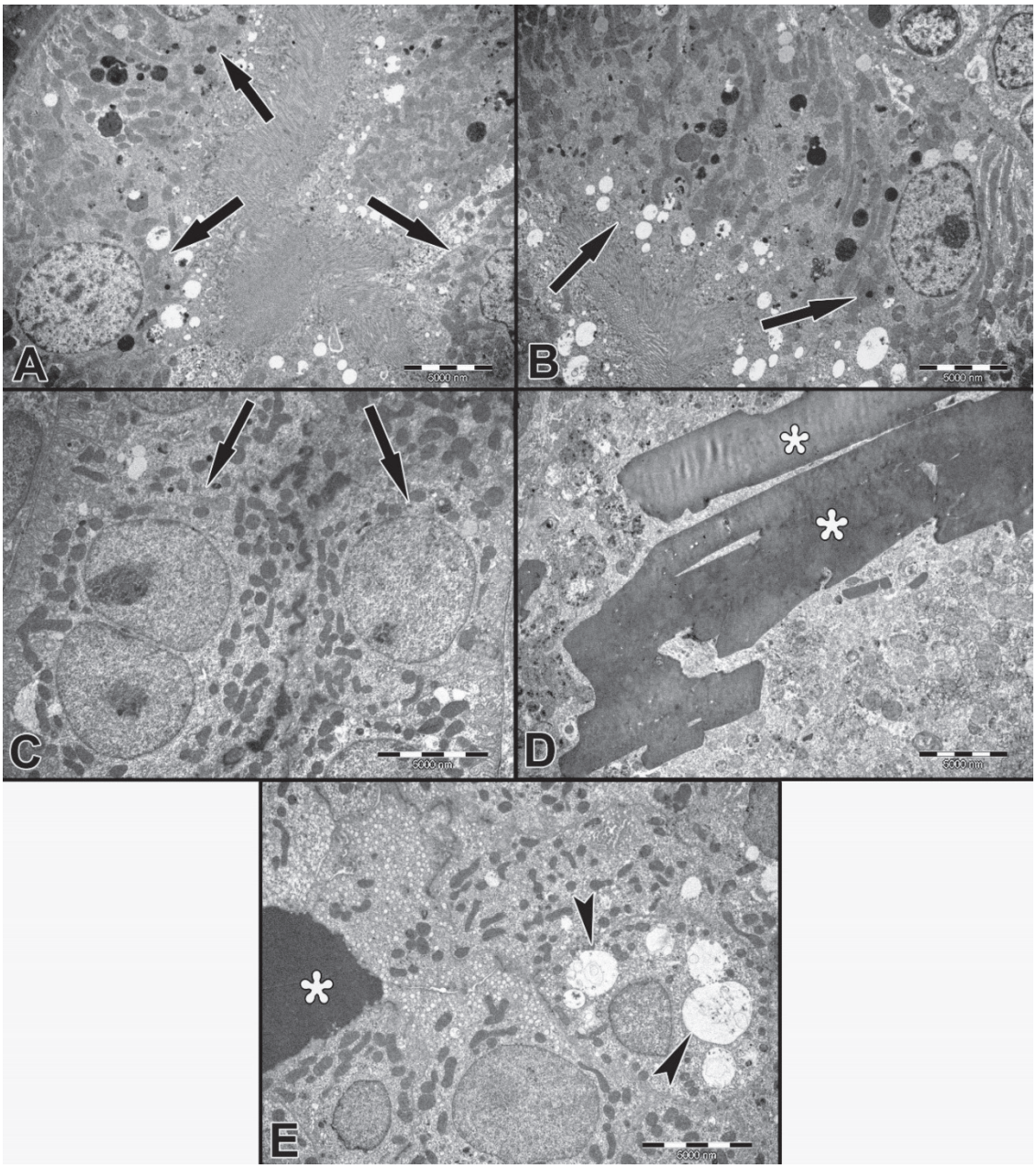

Figure 6. Post model treatment groups. A and B: Post model 40 mg. C, D and E: Post model 80 mg treatment group. A, B and C: tubule epithelial cells with normal morphological features (thick arrow). D and E: Vacuoles in the cytoplasm of the tubule epithelial cell (arrowhead), dense material in tubule lumens (white asterisk). A, B and D: X4000; C and E: X5000.

might be the result of deterioration of the tubular functions. We also presume that the K^+ level increase is attributed to the decrease in urinary excretion due to ARF, and the intracellular K^+ release is rather the result of intense muscle damage. These findings are consistent with previous studies [28-30].

Kidney failure is one of the major causes of decreased creatinine excreted into the urine. In the case of normal kidney function, those factors that account for the increase of serum creatinine, are also responsible for the increase of urinary creatinine levels. In our study, changes in the serum BUN, urea and creatinine were directly proportional to changes in urine urea and creatinine. The increase of the corresponding values in saline groups before and after the model creation was more than 35%, indicating acute

Table 4. GC analysis results of *Ferula elaeochytris* extract.

	RT	%Area	Components
1	2.582	0.62	Ethyne, chloro
2	2,682	0.57	1,3-Butadiene, 2-methyl-isoprene
3	2,920	30.82	Thiirane, methyl-
4	3.597	11.63	Methane, trichloro
5	3.968	3.56	2-Methoxy-2-methylbut-3-ene Methyl 1,1-Dimethyl-2-propenyl ether
6	5.128	0.52	Butanoic acid, methyl ester
7	5.766	0.77	Methyl 3-Methyl-2-butenyl Ether 1-Methoxy-3-methyl-2-butene Methyl 1,1-Dimethyl-2-propenyl Ether
8	5.788	0.91	cis-1-Methoxy-3-methyl-1-butene 2H-Pyran, tetrahydro-2-methyl- (3E)-4-Methoxy-3-Buten-2-ene
9	6.360	1.10	1,4-Pentadiene, 2,3,3-trimethyl- 1,3-Butadiene, 2-methyl- 1,4-Pentadiene, 2,3,3-trimethyl
10	10.506	0.93	Hexanoic acid, methyl ester
11	12.193	0.17	Cyclohexane, methoxy-4-Heptanone
12	13.385	0.13	(+)-4-Carene Cyclohexene, 1-methyl-4-(1-methyle thylidene)
13	13.624	0.17	Benzene, methyl(1-methylethyl)- o-Cymene p-Cymene
14	14.693	0.18	1,4-Cyclohexadiene, 1-methyl-4-(1- methylethyl) .gamma.-Terpinene
15	15.455	3.18	2,5-Methanopentalen-1-ol, octahydr o-, (1.alpha.,2.alpha.,3a.beta.,5 alpha.,6a.beta.) Benzene, propyl-
16	15.862	0.19	.gamma.-Terpinene Tricyclo[2.2.1.02,6]heptane, 1,7, -trimethyl 3-Carene
17	16.884	0.36	Benzene, 1-ethenyl-3-methyl- Methylstyrene – sample
18	18.290	0.19	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)
19	18.572	0.11	Cyclododecane
20	20.060	2.97	3-Ethenylpyrrole-5-carboxaldehyde (R)-(-)-.alpha.-Methoxyphenylacetic acid 4-Ethyl-3-methylpyridine
21	20.186	0.16	2-Cyclohexen-1-one, 2-methyl-5-(1- methylethenyl)
22	21.340	0.16	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo) 2-Norbornanol, 1,3,3-trimethyl-, acetate, endo
23	22.099	0.14	1,7-Octadiyne 2,3-Heptadien-5-yne, 2,4-dimethyl
24	23.686	1.50	(2,6-Dimethylphenyl)Methanol 3-Pyridineacetonitrile .alpha.-Methyl-.alpha.-phenylsucci nimide
25	23.927	22.85	1H-Benzotriazole, 1-methyl-6-nitro Benzene, 1,2-dimethoxy-4-(2-propen yl) 3-Methyl-6-methoxychroman
26	23.999	0.40	2-Tetradecene 1-Tetradecene

tubular necrosis. There was a significant variation in the increased values in the saline groups. By examining the decreased creatinine rates in the groups after the addition of plant extract, the curative effect was quite pronounced.

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Oxidative stress and inflammation are common in patients with ARF, and they provide the basis of atherosclerosis. Deferoxamine, which chelates free iron and plays an important role in the formation of hydroxyl radicals (·OH), has been shown to exert a protective effect in the glycerol model [31-32]. It has been demonstrated that the amount of glutathione, which protects the cell against oxidant damage by ROM, decreases in the glycerol model, and kidney damage is prevented upon glutathione replacement [33-34]. All of the above data underscore ROM's important role in the pathogenesis of ARF due to rhabdomyolysis.

Renal NO level is a major regulator of tubular transport and glomerular hemodynamics in the tubuloglomerular feedback response [35]. It also inhibits antidiuretic hormone (ADH)-dependent osmotic water permeability in the cortical collecting duct [36]. The reduced bioavailability of NO in ARF causes endothelial dysfunction [35]. Similar to other forms of ARF, rhabdomyolysis-induced ARF is associated with vasoconstriction as a result of decreased NO produced from the endothelium. In addition, increased NO production by iNOS exacerbates the deleterious effects of endotoxin and ischemia [37].

Since 3-NT is the stable end product of peroxynitrite oxidation, it has been reported to constitute a useful marker for detecting NO-dependent *in vivo* damage [38]. While 3-NT levels are undetectable in the plasma and tissues of normal individuals, they increase significantly in conditions associated with elevated NO· production and oxidative stress, such as inflammatory and degenerative processes [39].

Therefore, for the aforementioned reasons, antioxidants and the effectiveness of antioxidant substances are emerging as key players in managing ARF. Herein, we used *Ferula elaeochytris* extract, which is known to have an antioxidant effect and is frequently used by health professionals for the treatment of kidney failure, but its potential effect on the renal failure has not been explored yet.

In our study, the MDA levels were estimated and were found to be statistically significantly higher in all treatment groups compared to the control group. In previous studies, it was reported that the level of MDA, the end product of lipid peroxidation, which is an indicator of damage caused by free radicals, increased significantly in the kidneys of rats in which mARF was created by giving glycerol [40-43]. Herein, the most pronounced difference in the MDA levels was observed between the pre-model 40 mg/kg group and the pre-model saline group.

There was also a statistically significant decrease in the CAT and SOD enzyme activities in all treatment groups compared to the control group. Although no statistically significant difference was detected within the groups, an increase was observed in the groups to which the plant extract was added compared to the group given saline before the generation of the model. This finding leads to the suggestion that the effect of the antioxidant property of *F. elaeochytris* could not be detected during the 24-hour period due to absorption.

Furthermore, the GPx activity was found to be statistically significantly higher in all groups as compared to the control group. It is known that GPx is the most effective antioxidant against oxidant stress in erythrocytes [44]. In the mARF model created with glycerol, increased myoglobin level in the blood caused an increase in the GPx activity.

Regarding the NO activities, they were found to be statistically significantly higher in all treatment groups, except the 80 mg/kg group, after model creation, as compared to the control group. The 3-NT activity was shown to be statistically significantly lower in the saline group than the control group only after the model was generated. In addition, the 3-NT estimated activity increased statistically significantly in the post-model 40 mg/kg group compared to the post-model saline group, whereas it decreased significantly in the post-model 80 mg/kg group versus the post-model 40 mg/kg group.

In the present study, it was demonstrated histologically and biochemically that the mARF model was induced experimentally, by comparing the mARF pre-model and post-model groups with the control group. Of note, the electron microscopy observations corroborate the results of the biochemical parameters.

Elucidating the mechanisms of action of *Ferula elaeochytris* and demonstrating its effectiveness could provide more relevant information which could be taken into consideration in the clinical setting for the treatment of kidney. It has been deduced from the findings of this study that the formation of free radicals and the decrease in the NO levels play an important role in the mechanisms underlying the onset and etiology of mARF.

In conclusion, the observations and results of the present study provide preliminary evidence for the protective and curative effects of *Ferula elaeochytris*. In view of these findings, we suggest that in order to gain a deeper understanding of the protective and curative effect of *F. elaeochytris* on mARF, more comprehensive studies should be conducted by properly adjusting the application times after determining the effective doses in the mARF model.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Statement of Contribution of Researchers

Concept, Design - M.G., S.Y.; Experiments - M.G., E.B., G.B., F.Y., M.Y.; Analysis, Interpretation - M.G., S.Y., E.B., G.T.; Literature search, Writing - M.G., S.Y., A.E.Y.; Critical Review - A.P., A.G.

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