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RESEARCH ARTICLE

Myrtus Communis (Myrtle Tree: leaf and body) extract in Rat Renal Ischemia/Reperfusion injury

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ABSTRACT

Aim: The aim of this study was to investigate the protective effect of myrtus communis extract (MCE) against renal I/R injury.

Materials and methods: 36 adult males Wistar albino rats, weighing 250-300 g, were divided into six groups of six rats each. Group 1 was designated as the sham group. Group 2 was designated as the control one. MCE was administered via gastric gavage over a period of 10 days, dosed at 0.1 mL/kg/day in the third group, 0.2 mL/kg/day in the fourth group, 0.3 mL/kg/day in the fifth group, and 0.5 mL/kg/day in the sixth group. On the 10th day, the I/R procedure was performed.

Results: The pathological examination showed that the formation of hyaline cylinders in the tubular lumen was significantly higher in the control group when compared to the others ($p<0.05$). Glomerular Bowman space enlargement was statistically significantly lower in groups 5 and 6 compared with the controls ($p<0.05$). The levels of tumor necrosis factor-alpha (TNF-α), interleukin 1-beta (IL-1β), IL-6, matrix metalloproteinase-8 (MMP-8), hydroxyproline, myeloperoxidase (MPO), and total oxidant status (TOS) were found to be increased in the control group when compared with the others ($p<0.05$), while a statistically significant reduction was seen in the levels of total antioxidant capacity (TAC) and superoxide dismutase (SOD) ($p<0.05$).

Conclusions: MCE protected kidney tissue against I/R injury. This result was most likely due to the antioxidant properties of MCE. MCE is a promising natural substance for preventing I/R damage.

Keywords: myrtus communis, rat, renal ischemia/reperfusion injury, acute kidney injury

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INTRODUCTION

Ischemia/reperfusion injury (I/R) is tissue damage that transpires during the reduction of arterial nutrition and oxygenation of an organ or tissue for various reasons, and after an improvement in arterial flow [1, 2]. Renal ischemia not only causes acute kidney damage, leading to mortality and morbidity, but also exacerbates chronic kidney damage [3]. Renal ischemia is seen in various clinical conditions such as kidney transplantation, partial nephrectomy, cardiopulmonary bypass, myocardial infarction, and sepsis [2, 4, 5]. Acute kidney injury (AKI) that develops after ischemia is distinguished by a decline in glomerular filtration rate, tubular necrosis, and a rise in resistance in renal vessels [4]. AKI that develops due to I/R injury significantly increases the mortality and morbidity of patients [6].

The pathophysiology of I/R injury is quite complex. ATP production decreases and the intracellular Ca^{2+} concentration increases with the disruption of mitochondrial oxidative phosphorylation during ischemia and reperfusion. Also, excessive numbers of free oxygen radicals (ROS) are formed and cause oxidative stress due to protease and phosphatase activation, resulting in degradation of cytoskeleton and membrane phospholipids [7-9]. The free radicals formed react with almost all biomolecules in the structure of living

organisms, exerting reversible or irreversible effects on them [10, 11]. Although the number of free radicals formed during ischemia is small, a higher number of free radicals are formed after tissue re-oxygenation during the reperfusion period, causing further damage via lipid peroxidation [8, 11]. The main goal of studies conducted to date was to suppress the inflammatory cascade that occurred during I/R injury, thus protecting kidney tissue [2, 12].

In previous studies, many substances such as melatonin [13], having prominent antioxidant properties, ulinastatin [14], a potent protease inhibitor, and propofol [15, 16], and N-acetylcysteine [17], having antioxidant properties, were used to prevent I/R damage. Besides these substances, some plants such as garlic extract [18], baicalin [19] and curcumin [20], which were used in traditional medicine and displayed antioxidant properties, were also used in I/R damage studies.

Some aromatic and medicinal plants have antioxidant properties due to the polyphenols and essential oils in them. One of these plants is myrtus communis L. belonging to the myrtaceae family. This plant, also known as blueberry, is a woody plant that grows in different geographical regions. To date, different parts (fruits, branches, and leaves) of M. communis have been used in traditional medicine, especially for various purposes such as blood sugar regulation, wound healing, and treating infectious diseases and lung diseases [21, 22]. Laboratory studies have proved the insecticidal, antioxidant, anti-inflammatory, and antimicrobial activities of the essential oils of blueberry [22, 23]. The drugs and substances used so far have not been highly effective in treating I/R injury. Further, no study in the literature used MCE in the renal I/R model. The aim was to reveal if MCE, which might serve as an alternative to the conventional treatment methods, had a shielding effect in renal I/R injury model, using blood biochemistry and histopathological examination of tissue samples.

MATERIALS AND METHODS

Afyon Kocatepe University Experimental Animals Application and Research Center (AKUHADYEK) granted permission for this study, with reference number 266-20, dated 17/12/2020. All animals received humane care following the criteria mentioned in "guidelines for the care and use of laboratory animals" published by the US National Institutes of Health.

Preparation of the M. Communis Extract

The leaves and stems of M. communis (myrtle tree) were boiled in 6 g/L at 100 °C for 15 min. The water of the extracts was roughly evaporated in an evaporator device. A lyophilize device was used to completely evaporate the remaining water in the extract. Then, 150 µg/mL solutions were prepared from each of the samples and sterilized in an autoclave at 120 °C for 1 h. Also, 50 vials were put into tubes and stored at 4 °C to be used in experiments. The alcohol extracts were prepared with a Soxhlet device. Alcohol was evaporated with the evaporator device [24]. Myrtle tree extract was provided by Ars Arthro Biotechnology A.S. (Ankara, Turkey).

Note that systemic toxicity studies of myrtle tree extract were carried out following International Organization for Standardization (ISO) 0993-11 standards, and no side effects were observed. In addition, the skin, eye irritation, and sensitization test showed that the plant extracts did not cause irritation and sensitization, especially on the skin (SANTEZ-0352.STZ.2013-2 project final report).

STUDY DESIGN

For this study, 36 adult males Wistar albino rats that weighed 250-300 grams were given standard rat pellet feed and water ad libitum and kept in individual cages under standard climatic conditions. They were allowed to get used to the laboratory environment for five days. The rats were separated into six groups at random. All manipulations of the animals were carried out in accordance with the guidelines for the care and use of laboratory animals as published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Anesthesia

All rats were premedicated with 13 mg/kg intramuscular (i.m.) administration of xylazine HCl, and general anesthesia was achieved by i.m. administration of 87 mg/kg ketamine HCl.

Surgical Procedure

The abdomen of the experimental animals was anesthetized under sterile conditions, and median laparotomy was performed.

I/R Application

The renal artery and pedicle of the animals who underwent median laparotomy under general anesthesia were exposed by dissection and a bulldog clamp was used for occlusion over a 30-minute period. The clamp was taken off, and reperfusion was achieved for 1 h. While the abdomen was closed, saline at a volume of 5% of the animals' body weight was administered intraperitoneally (i.p.).

Groups

Group 1 (sham group) (G1) (n=6)

Only median laparotomy was performed on the animals in G1, and then the abdomen was closed under sterile conditions following the technique.

Group 2 (control group) (G2) (n=6)

After median laparotomy was applied to the animals in G2 under general anesthesia, the renal artery and pedicle were exposed by dissection and occluded with a bulldog clamp for 30 minutes. At the conclusion this duration, reperfusion was achieved for one hour by removing the clamp. While the abdomen was closed, saline at a volume of 5% of the animals' body weight was administered i.p.

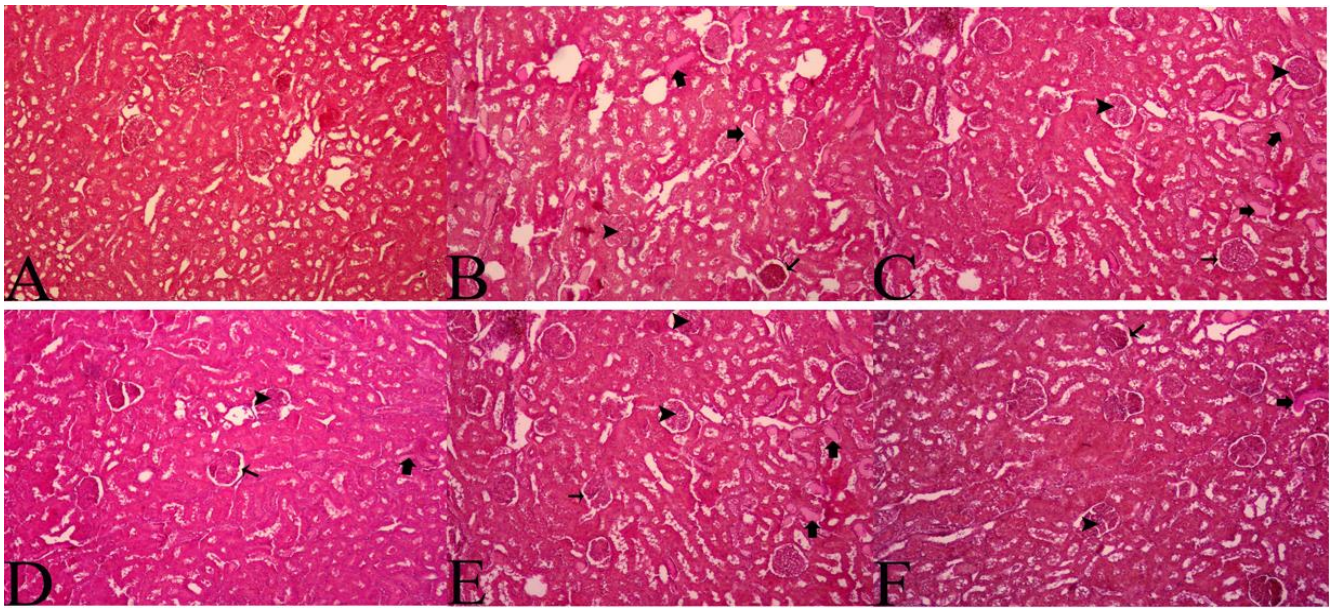

Figure 1. Histopathological examination with HE stain (A: Group 1; B: Group 2; C: Group 3; D: Group 4; E: Group 5; F: Group 6; Thick arrow: Hyaline cylinder formation in the tubular lumen; Thin arrow: Enlargement of the glomerulus in the Bowman space; & Arrowhead: Vacuolization formation in the glomerular capillary)

Group 3 (G3) (n=6)

The animals in this group were administered the *M. communis* extract at a dose of 0.1 mL/(kg×day) by gastric gavage for 10 days. The I/R procedure was performed on the 10th day, and the animals were sacrificed at the 24th hour after reperfusion.

Group 4 (G4) (n=6)

The animals in this group were administered the *M. communis* extract at a dose of 0.2 mL/(kg×day) by gastric gavage for 10 days. The I/R procedure was performed on the 10th day, and the animals were sacrificed at the 24th hour after reperfusion.

Group 5 (G5) (n=6)

The animals in this group were administered the *M. communis* extract at a dose of 0.3 mL/(kg×day) by gastric gavage for 10 days. The I/R procedure was performed on the 10th day, and the animals were sacrificed at the 24th hour after reperfusion.

Group 6 (G6) (n=6)

The animals in this group were administered the *M. communis* extract at a dose of 0.5 mL/(kg×day) by gastric gavage for 10 days. The I/R procedure was performed on the 10th day, and the animals were sacrificed at the 24th hour after reperfusion.

Histopathological Examination

The animals were opened with relaparotomy under anesthesia as in the I/R procedure, and right and left nephrectomies were performed. The renal capsule was incised. The kidneys taken from the animals were fixed with 10% neutral buffered formaldehyde solution in the pathology laboratory. The samples were taken from all detected kidneys. After the routine follow-up, the tissues were blocked in paraffin. Sections of size 4–6 µm were prepared from the blocks. They were colored with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) staining techniques for histopathological evaluation. From all HE- and PAS-stained sections, 10 randomly selected areas were examined at ×20 magnification (**Figure 1** and **Figure 2**, respectively).

The lesions seen in the samples included congestion, vacuolar degeneration, interstitial inflammation, accumulation of proteinaceous substance accumulation in the lumen, and tubular separation. Necrosis was scored by giving values between 0 and 4 (0=normal, 1=mild lesions affecting 25% of kidney samples, and 2=lesions affecting 25%-50%% of kidney samples, 3=lesions affecting 50%-75%, and 4=lesions affecting more than 75%).

Biochemical Examination

Two ml of blood samples were extracted from the rats in all groups on the first day, before I/R, and before being sacrificed by an intracardiac puncture. The following analyses and measurements were performed using the serum/plasma samples: blood urea, creatinine, calcium, phosphorus (P), magnesium (Mg), sodium (Na), chlorine (Cl), bicarbonate (HCO_3), potassium (K), total protein, tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), interleukin-6 (IL-6), hydroxyproline (Hyp), superoxide dismutase (SOD), myeloperoxidase (MPO), matrix metalloproteinase-8 (MMP-8), total antioxidant capacity (TAC), total oxidant status (TOS).

Evaluation of kidney functions

An autoanalyzer was used to measure the serum, urea, and creatine values as the indicators of a decrease in

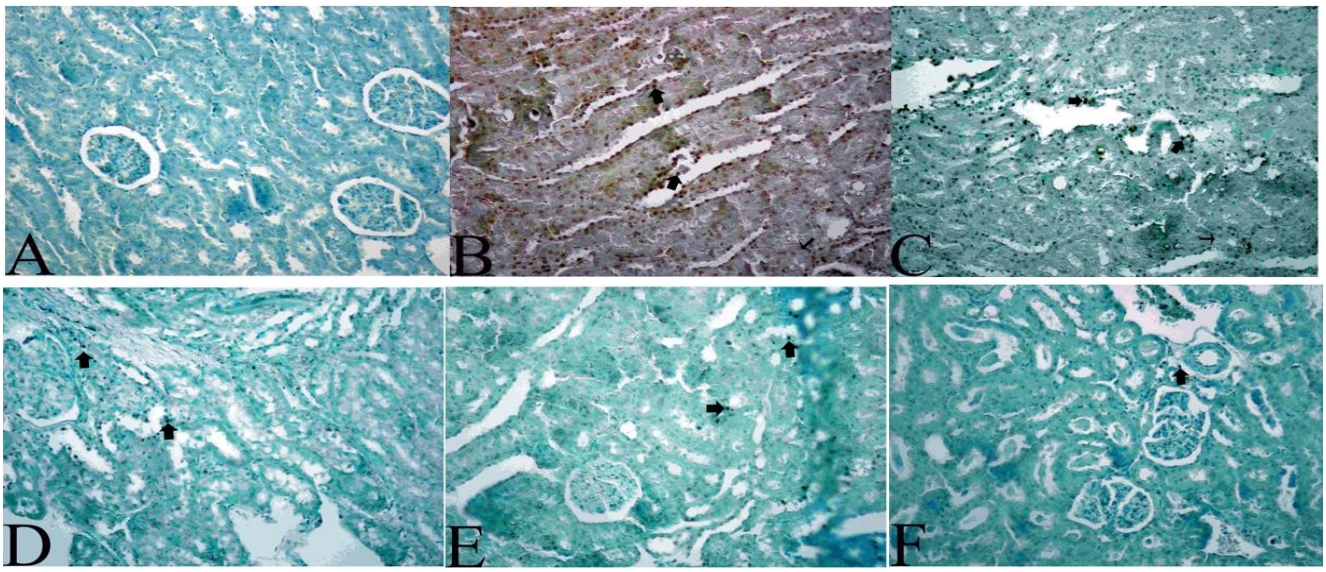

Figure 2. Histopathological examination with PAS stain (A: Group 1; B: Group 2; C: Group 3; D: Group 4; E: Group 5; F: Group 6; Thick arrow: Hyaline cylinder formation in the tubular lumen; Thin arrow: Enlargement of the glomerulus in the Bowman space; & Arrowhead: Vacuolization formation in the glomerular capillary)

glomerular function in blood samples obtained by an intracardiac puncture at the 24th hour.

Measurement of serum electrolyte levels

Serum levels of K^+, Ca^{++}, Na^+, Cl^-, and Mg^{++} were measured in an autoanalyzer (Roche Cobas 8000, Germany) in blood samples taken from all groups at the 24th hour.

Evaluation of neutrophil migration

Myeloperoxidase (MPO) activity was used to indicate neutrophil accumulation in tissues. Enzyme-linked immunosorbent assay (ELISA) was used to measure rat MPO.

Evaluation of proinflammatory cytokines and antioxidant status in serum

The serum MPO (Sunred Rabbit MPO ELISA Kit; Catalog. No: YLA0046RA; Shanghai YL Biotech Co., Ltd., China), IL-6 (Rat Interleukin-6 Catalog No: YLA 0031RA; Shanghai YL Biotech Co., Ltd.), IL-1β (Rat Interleukin-1β Catalog No: YLAE0119RA; Shanghai YL Biotech Co., Ltd.), TOS (Rat Total Oxidant Status; Catalog No: YLA E1512RA; Shanghai YL Biotech Co., Ltd.), TAC (Rat Total Antioxidant Capacity; Catalog No: YLA0311RA; Shanghai YL Biotech Co., Ltd.), MMP-8 (Rat Metalloproteinase-8; Catalog No: YLA 0648RA; Shanghai YL Biotech Co., Ltd), SOD (Rat Superoxide Dismutase; Catalog No: YLA0115RA; Shanghai YL Biotech Co., Ltd.) HYP (Rat Hydroxyproline; Catalog No: YLA0068RA; Shanghai YL Biotech Co., Ltd.), and tumor necrosis factor (TNF)-α (Rat Tumor Necrosis Alfa; ELISA Kit; YLA0118RA) were measured using commercial ELİSA kits with an MVGt Lambda Scan 200 (Bio-Tek Instrument, VT, USA).

STATISTICAL ANALYSIS

Mean±standard deviation was used to express the data. Variance analysis was performed as a nonparametric test to determine the changes in biochemical, electrolyte analysis, and oxidative stress parameters. In addition, the Tukey test was applied for meaningful data. Data analysis was carried out using the statistical package for social sciences (SPSS, 18.0 software, NY, USA. $p<0.05$ indicated a statistically significant difference.

RESULTS

The creatinine levels in groups 1 and 2 were higher than those in groups 3 and 4, in this study, though with no statistically significant differences ($p=0.052$). However, it was lower than that in groups 5 and 6 ($p=0.052$). The serum urea level increased statistically in groups 3, 5, and 6 compared with groups 1, 2, and 4 ($p=0.001$) (**Table 1**).

TNF-α levels were observed to be statistically significantly increased in the controls when compared with the other groups ($p<0.05$). This increase was inversely proportional to the MCE dose administered. The IL-1β level increased statistically significantly in the control group when compared with the other groups ($p<0.05$). The IL-1β level was found at the lowest level in group 5. The levels of IL-6, MMP-8, Hyp, MPO, and TOS were found to be higher in the control group compared with the other groups ($p<0.05$). The levels of TAC and SOD were found to be markedly higher in the other groups when compared with the controls ($p<0.05$) (**Table 2**).

In the pathological evaluation, the formation of hyaline cylinders in the tubular lumen was statistically significantly less in the other groups when compared with the controls ($p<0.05$). This ratio was inversely proportional to the MCE dose given. The enlargement of the glomerular Bowman space was statistically significantly lower in groups 5 and 6 compared with the control group ($p<0.05$). Vacuole

Table 1. Kidney Function Tests

	G1	G2	G3	G4	G5	G6	p-value
Cre	.293±.006^{ab}	.361±.0370^{a}	.321 ±.011^{ab}	.275±.0105^{b}	.371±.040^{a}	.374±.0326^{a}	0.052
Na^{+}	138.500±.718^{b}	141.833± 1.137^{a}	140.833±.792^{a}	141.000±.365^{a}	142.500±.341^{a}	142.000±.447^{a}	0.006
K^{+}	5.806±.222^{a}	4.831±.193^{b}	5.598±.299^{a}	5.238±.092^{ab}	5.766±.198^{a}	5.826±.338^{a}	0.027
Cl^{-}	99.000±.637^{ab}	98.766±.963^{ab}	98.400±.718^{b}	100.133±.244^{ab}	100.983±.765^{a}	100.500±780^{ab}	0.088
Ca^{+2}	9.903±.180^{a}	9.068±.151^{b}	9.165±.115^{b}	9.100±.068^{b}	9.310±.074^{b}	9.038±.186^{b}	0.001
Ure	39.683±1.517^{b}	46.783±4.216^{b}	61.716±6.144^{ab}	45.533±2.123^{b}	79.666±14.693^{a}	73.000±11.368^{a}	0.006
Mg^{+2}	2.411±.050^{cd}	2.435±.0631^{cd}	2.635±.075^{bc}	2.295±.032^{d}	2.818±.197^{ab}	2.962±.1143^{a}	0.001

Note. Cre: Creatinine; Na: Sodium; K: Potassium; Cl: Chlorine; Ca: Calcium; & Mg: Magnesium

Table 1. Prooxidant Cytokines and Antioxidant Status

	SG 1	CG 2	M1G 3	M2G 4	M3G 5	M4G 6	p-value
TNF (ng/L)	57.888± 3.695^{bc}	80.756±4.983^{a}	68.151±3.792^{b}	66.290±3.508^{b}	55.526±2.015^{c}	54.081±1.564^{c}	0.000
IL1B (ng/mL)	7.415±.234^{b}	10.933±1.003^{a}	7.591±.181^{b}	7.045±.331^{b}	6.903±.159^{b}	7.330±.418^{b}	0.000
IL-6 (ng/L)	3.058±.185^{b}	3.943±.285^{a}	3.123±.087^{b}	3.216±.159^{b}	3.531±.215^{ab}	3.200±.111^{b}	0.018
MMP-8 (nmol/mL)	14.743±.676^{c}	19.951±1.003^{a}	15.156±.634b^{c}	14.578±.836^{c}	16.136±.452^{bc}	17.011±.319^{b}	0.001
Hyp (ng/L)	286.983±17.70^{b}	441.558±61.21^{a}	253.041±10.02^{b}	226.880±17.12^{b}	253.833±9.001^{b}	242.156±14.78^{b}	0.000
MPO (ng/mL)	6.910±.309^{b}	8.458±.912^{a}	5.946±.085^{bc}	5.555±.157^{c}	6.415±.146^{bc}	5.900±.197^{bc}	0.000
TOS (U/mL)	5.566±.371^{c}	7.053±.289^{a}	6.611±.259^{ab}	6.653±.174^{ab}	6.035±.298^{bc}	6.616±.293^{ab}	0.015
TAC (U/mL)	2.913±0.144^{a}	1.957± 0.169^{b}	3.403± 0.143^{a}	3.068±.225^{a}	3.855± 0.670^{a}	3.84± 0.220^{a}	0.002
SOD (ng/mL)	1.626±.148^{ab}	1.239±.028^{c}	1.511±.097^{cb}	1.925±.140^{a}	1.682±.142^{ab}	1.868±.140^{ab}	0.006

Note. Hyp: Hydroxyproline; IL-1β: Interleukin 1β; IL-6: Interleukin-6, MMP-8: Myeloperoxidase-8; MPO: Myeloproxidase; SOD: Superoxide dismutase; TAC: Total antioxidant capacity; TNF: Tumor necrosis factor; TOS: Total oxidant status; S: Sham; C: Control; M: Myrtus; & G: Group

formation in glomerular capillary was reduced in other groups compared with control group, and most significant decrease was observed in group 6 ($p<0.05$) (**Table 3**).

DISCUSSION

The production of reactive oxygen species (ROS) in renal I/R injury increases during ischemia and especially during the reperfusion period. This increase triggers inflammation and cell death, leading to AKI. AKI due to renal I/R injury has a high risk of mortality and morbidity for patients. The goal of many studies involved in preventing I/R injury was to reduce ROS production and suppress inflammation. In this study, we aimed to show whether MCE, which has known antioxidant properties, has efficacy in the model of renal I/R injury.

Although the chemical composition of MCE shows regional differences depending on climatic conditions, it is known to have a strong antioxidant property [25, 26]. Studies examining the efficacy of MCE in I/R injury related to different organs have been published earlier. Our study was the first to examine the efficacy of MCE in renal I/R injury.

The inflammatory and proinflammatory cytokines have an important role in renal I/R injury. The cytokines most responsible for kidney damage are IL-6 and TNF-α [27]. Our study demonstrated that the TNF-α level decreased significantly with the increase in the amount of MCE applied. Similarly, a significant decrease in the IL-6 level was seen in other groups when compared with the controls.

In a study examining the effect of MCE on intestinal I/R injury in rats, SOR, H_2O_2 production, and neutrophil MPO production decreased with the in vitro administration of MCE. In addition, this study suggested that MCE prevented the depletion of antioxidant enzymes such as glutathione peroxidase, SOD, and catalase and exerted a protective effect against intestinal I/R damage [28]. Similarly, in our study, while the SOD level was lower in the control group in comparison to the other groups, the MPO level, which was an indicator of neutrophil migration to the kidney tissue, markedly lower in the other groups when compared with the controls. Our results revealed that MCE had a positive effect on antioxidant enzyme levels.

In a study examining the protective effect of MCE against liver toxicity and fibrosis in rats with bile duct ligation, the levels of TNF-α, IL-1β, and liver enzymes were discernibly lower in the MCE-treated group when compared with the non-MCE-treated group. In addition, MPO and hydroxyproline levels decreased markedly in the MCE-treated group [28]. Similarly, our study showed that MPO and hydroxyproline levels decreased dramatically in the MCE-treated groups when compared with the controls. In addition, it was observed that the serum creatinine level was lower in the MCE-treated groups than in the controls. This showed that MCE protected kidney tissue against I/R damage.

MCE was used in the liver I/R model in rats. It was concluded that it protected the liver against I/R damage. In addition, the high antioxidant efficacy of MCE was demonstrated in this study [29].

Table 3. Pathological Evaluation

Group	Hyaline cylinder formation in the tubular lumen	Enlargement of the glomerulus in the Bowman's space	Vacuolization formation in the glomerular capillary
G1	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{d}
G2	2.50±0.55^{a}	2.33±0.52^{a}	2.16±0.41^{a}
G3	2.33±0.52^{a}	2.16±0.41^{a}	1.83±0.41^{ab}
G4	2.16±0.41^{ab}	2.01±0.10^{a}	1.50±0.54^{bc}
G5	1.66±0.52^{bc}	1.50±0.55^{b}	1.33±0.52^{bc}
G6	1.33±0.51^{c}	1.16±0.41^{b}	1.01±0.63^{c}
p-value	0.000	0.000	0.000

Note. $^{a, b, c, \& d}$Histopathological values of kidneys with different letters in the same column are statistically significant ($p<0.05$).

When examined from a molecular point of view, our study indicated that MCE decreased the levels of TNF, IL-1β, IL-6, MMP-8, Hyp, MPO, and TOS, which were proinflammatory cytokines that exacerbated the damage in the kidney tissue after I/R. In addition, the increase in TAC and SOD activities was another positive result of our study. This study was important because it was novel in evaluating the effect of MCE on the cytokine response after renal I/R injury. Our study showed that MCE had a direct effect on the cytokine response in ischemic organs in renal I/R injury. This property of MCE was related to its antioxidant properties. However, a hitherto unknown mechanism that we could not explain might have been effective in our study.

Renal I/R injury causes necrosis in epithelial cells and impairs the function and structure of tubular epithelial cells. In addition, ischemia causes interstitial inflammation and damage to the interstitial microvascular structure. Microvascular injury is characterized by swelling and occlusion of endothelial cells. This obstruction prevents reperfusion after ischemia. Interstitial inflammation and microvascular damage are important for kidney repair after ischemia [30, 31]. In this study, the histopathological examination revealed that the positive data from the molecular point of view were not accidental. The pathological evaluation showed that the indicators of renal I/R injury such as hyaline cylinder formation in the tubular lumen, enlargement of the glomerular Bowman space, and vacuole formation in the glomerular capillary bundle were significantly less in the MCE-treated groups. The renal tissue damage decreased with an increasing dose of MCE.

In our study, a statistically insignificant increase in the creatinine level was found after I/R. The histopathological evaluation revealed the damage in the kidney tissue. This result showed once again that the renal function tests alone were not reliable when evaluating AKI.

In conclusion, this study revealed biochemically that MCE decreased the levels of proinflammatory cytokines and increased anti-inflammatory response in renal I/R injury. Histopathologically, MCE has been proven to protect against ischemia. It may potentially serve as a protective agent in I/R injury due to its positive effects on oxidative stress and inflammation. In the lights of our study findings, it seems the better MCE Dose than in all study groups was 0.2 mL/kg/day in the fourth group.

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Ethics committee approval: The authors state that the study was approved by the Afyon Kocatepe University Experimental Animals Application and Research Center (AKUHADYEK) on 17 December, 2020 (reference number 266-20). All animals received humane care following the criteria mentioned in "guidelines for the care and use of laboratory animals" published by the US National Institutes of Health.

Declaration of interest: No conflict of interest is declared by authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

REFERENCES

1. Malek M, Nematbakhsh M. Renal ischemia/reperfusion injury; from pathophysiology to treatment. J Ren Inj Prev. 2015;4(2):20-7. doi:10.12861/jrip.2015.06
2. Wu MY, Yiang GT, Liao WT, et al. Current mechanistic concepts in ischemia and reperfusion injury. Cell Physiol Biochem. 2018;46(4):1650-67. doi:10.1159/000489241 PMid:29694958
3. Shiva N, Sharma N, Kulkarni YA, et al. Renal ischemia/reperfusion injury: An insight on in vitro and in vivo models. Life Sci. 2020;256:117860. doi:10.1016/j.lfs.2020.117860 PMid:32534037
4. Gaut JP, Liapis H. Acute kidney injury pathology and pathophysiology: A retrospective review. Clin Kidney J. 2021;14(2):526-36. doi:10.1093/ckj/sfaa142 PMid: 33623675 PMCid:PMC7886540
5. Sharfuddin AA, Molitoris BA. Pathophysiology of ischemic acute kidney injury. Nat Rev Nephrol. 2011;7(4):189-200. doi:10.1038/nrneph.2011.16 PMid: 21364518
6. Pefanis A, Ierino FL, Murphy JM, et al. Regulated necrosis in kidney ischemia-reperfusion injury. Kidney Int. 2019;96(2):291-301. doi:10.1016/j.kint.2019.02.009 PMid:31005270

7. Baud L, Ardaillou R. Reactive oxygen species: production and role in the kidney. Am J Physiol-Renal Physiol. 1986;251(5):F765-76. doi:10.1152/ajprenal.1986.251.5.F765 PMid:3022602
8. Irazabal MV, Torres VE. Reactive oxygen species and redox signaling in chronic kidney disease. Cell. 2020;9(6):1342. doi:10.3390/cells9061342 PMid:32481548 PMCid:PMC7349188
9. Ishimoto Y, Tanaka T, Yoshida Y, et al. Physiological and pathophysiological role of reactive oxygen species and reactive nitrogen species in the kidney. Clin Exp Pharmacol. 2018;45(11):1097-105. doi:10.1111/1440-1681.13018 PMid:30051924 PMCid:PMC6221034
10. Kaushal GP, Chandrashekar K, Juncos LA. Molecular interactions between reactive oxygen species and autophagy in kidney disease. Int J Mol Sci. 2019;20(15):3791. doi:10.3390/ijms20153791 PMid:31382550 PMCid:PMC6696055
11. Nath KA, Norby SM. Reactive oxygen species and acute renal failure. Am J Med. 2000;109(8):665-78. doi:10.1016/S0002-9343(00)00612-4
12. Chung AC, Lan HY. Chemokines in renal injury. J Am Soc Nephrol. 2011;22(5):802-9. doi:10.1681/ASN.2010050510 PMid:21474561
13. Sahna E, Olmez E, Acet A. Effects of physiological and pharmacological concentrations of melatonin on ischemia–reperfusion arrhythmias in rats: can the incidence of sudden cardiac death be reduced? J Pineal Res. 2002;32(3):194-8. doi:10.1034/j.1600-079x.2002.1o853.x PMid:12074104
14. Wang Y, Peng C, Zhang Z, et al. Intravenous infusion of ulinastatin attenuates acute kidney injury after cold ischemia/reperfusion. Int Urol Nephrol. 2019;51:1873-81. doi:10.1007/s11255-019-02204-3 PMid:31332701
15. Yang S, Chou WP, Pei L. Effects of propofol on renal ischemia/reperfusion injury in rats. Exp Ther Med. 2013;6(5):1177-83. doi:10.3892/etm.2013.1305 PMid:24223641 PMCid:PMC3820756
16. Li Y, Zhong D, Lei L, et al. Propofol prevents renal ischemia-reperfusion injury via inhibiting the oxidative stress pathways. Cell Physiol Biochem. 2015;37(1):14-26. doi:10.1159/000430329 PMid:26277932
17. Kizilgun M, Poyrazoglu Y, Oztas Y. et al. Beneficial effects of N-acetylcysteine and ebselen on renal ischemia/reperfusion injury. Ren Failure. 2011;33(5):512-7. doi:10.3109/0886022X.2011.574767 PMid:21545313
18. Kabasakal L, Sehirli O, Cetinel S, et al. Protective effect of aqueous garlic extract against renal ischemia/reperfusion injury in rats. J Med Food. 2005;8(3):319-26. doi:10.1089/jmf.2005.8.319 PMid:16176141
19. Lin M, Li L, Li L, et al. The protective effect of baicalin against renal ischemia-reperfusion injury through inhibition of inflammation and apoptosis. BMC Complement Altern Med. 2014;14(1):1-9. doi:10.1186/1472-6882-14-19 PMid:24417870 PMCid:PMC3893527
20. Zhang J, Tang L, Li GS, et al. The anti-inflammatory effects of curcumin on renal ischemia-reperfusion injury in rats. Ren Failure. 2018;40(1):680-6. doi:10.1080/0886022X.2018.1544565 PMid:30741618 PMCid:PMC6282432
21. Hennia A, Miguel MG, Nemmiche S. Antioxidant activity of myrtus communis L. and myrtus nivellei Batt. & Trab. extracts: A brief review. Medicines. 2018;5(3):89. doi:10.3390/medicines5030089 PMid:30103510 PMCid:PMC6165143
22. Giampieri F, Cianciosi D, Forbes-Hernández TY. Myrtle (myrtus communis L.) berries, seeds, leaves, and essential oils: New undiscovered sources of natural compounds with promising health benefits. Food Front. 2020;1(3):276-95. doi:10.1002/fft2.37
23. Hennia A, Nemmiche S, Dandlen S, et al. MG. Myrtus communis essential oils: Insecticidal, antioxidant and antimicrobial activities: A review. J Essent Oil Res. 2019;31(6):487-545. doi:10.1080/10412905.2019.1611672
24. Aksay S. Total phenolic content and antioxidant properties of various extracts of myrtle (myrtus communis L.) berries. Cukurova J Agric Food Sci. 2016;31(2):43-50
25. Messaoud C, Boussaid M. Myrtus communis berry color morphs: A comparative analysis of essential oils, fatty acids, phenolic compounds, and antioxidant activities. Chem Biodivers. 2011;8(2):300-10. doi:10.1002/cbdv.201000088 PMid:21337502
26. de Peredo AVG, Espinosa MV, Bellido EE, et al. Alternative ultrasound-assisted method for the extraction of the bioactive compounds present in myrtle (myrtus communis L.). Molecules. 2019;24(5):882. doi:10.3390/molecules24050882 PMid:30832328 PMCid:PMC6429515
27. Thurman JM. Triggers of inflammation after renal ischemia/reperfusion. Clin Immunol. 2007;123(1):7-13. doi:10.1016/j.clim.2006.09.008 PMid:17064966 PMCid:PMC1888143
28. Jabri MA, Hajaji S, Marzouki L, et al. Human neutrophils ROS inhibition and protective effects of myrtus communis leaves essential oils against intestinal ischemia/reperfusion injury. RSC Adv. 2016;6(20):16645-55. doi:10.1039/C5RA26085J
29. Ferchichi H, Salouage I, Bacha S, et al. Effect of myrtus communis L. extracts on attenuation of liver normothermic ischemia-reperfusion injury. J Transplant Technol Res S. 2011;S3:001. doi:10.4172/2161-0991.S3-001

30. Patschan D, Patschan S, Müller G. Inflammation and microvasculopathy in renal ischemia reperfusion injury. J Transplant. 2012;2012. doi:10.1155/2012/764154 PMid:22530109 PMCid:PMC3316981

31. Jun W, Benjanuwattra J, Chattipakorn SC, et al. Necroptosis in renal ischemia/reperfusion injury: A major mode of cell death? Arch Biochem Biophys. 2020;689:108433. doi:10.1016/j.abb.2020.108433 PMid: 32470461

