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Vitamin A could be a Therapeutic Agent in Ischemia/ Reperfusion Induced Kidney Injury.

La vitamina A podría ser un agente terapéutico en la lesión renal inducida por isquemia/reperfusión

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RESUMEN

Introducción: La isquemia renal (I) puede desarrollarse debido a la disminución o interrupción del flujo sanguíneo al riñón en algunas condiciones clínicas como shock, sepsis y trasplante renal. El reabastecimiento de sangre al riñón se denomina reperfusión (R). Tanto la isquemia como los períodos de reperfusión pueden causar graves daños renales. **Objetivos:** Cuando examinamos la progresión molecular I/R, las moléculas antioxidantes como la vitamina A parecen agentes de tratamiento prometedores. El objetivo de este estudio fue investigar los efectos de la vitamina A sobre la lesión renal I/R. **Material y Métodos:** En el estudio, 40 ratas macho Sprague-Dawley se dividieron en 5 grupos (n=8) como: control, solo I/R, I/R+1000, I/R+3000 e I/R+9000 UI/kg de la Vitamina A. La vitamina A se administró a cada grupo durante 7 días por vía oral forzada. Al final del experimento se recolectaron muestras de sangre y tejido del riñón. A partir de muestras de sangre se determinaron los niveles de superóxido dismutasa (SOD), malondialdehído (MDA), catalasa (CAT), nitrógeno ureico en sangre (BUN) y creatinina (Cr). Las muestras de tejido se tiñeron con hematoxilina/eosina y los cambios en la histología renal se examinaron histopatológicamente y estereológicamente al microscopio de luz. **Resultados:** Los cambios histopatológicos causados por I/R disminuyeron con la administración de la vitamina A de manera dependiente de la dosis ($p<0,05$). La administración de la vitamina A disminuyó los niveles de MDA, aumentó las actividades de SOD y CAT ($p<0,05$). La dosis más eficaz entre los grupos del tratamiento fue de 9000 UI/kg. No hubo una diferencia significativa entre el grupo control y todos los demás grupos

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con respecto a las concentraciones de BUN y Cr. **Conclusiones:** Consiguientemente, la administración de la vitamina A, después de I/R renal, redujo el daño histológico y mejoró el estado antioxidante. Estos resultados mostraron que la vitamina A puede ser un agente promisorio en el tratamiento de la lesión renal aguda (LRA) inducida por I/R.

PALABRAS CLAVES: Lesión por isquemia/reperfusión renal; riñón; vitamina A; lesión renal aguda; insuficiencia renal aguda.

ABSTRACT

Introduction: Renal ischemia (I) could develop due to decreased or ceased blood flow to the kidney in some clinical conditions such as shock, sepsis, and kidney transplantation. The re-supply of blood to the kidney is called reperfusion (R). Ischemia and reperfusion periods can cause severe kidney damage. **Objectives:** When we examined the I/R molecular progression, antioxidant molecules such as vitamin A seem promising treatment agents. This study aimed to investigate the effects of vitamin A on renal I/R injury. **Material and Methods:** In the study, 40 Sprague-Dawley male rats were divided into five groups (n=8): the control group, only I/R, I/R+1000, I/R+3000, and I/R+9000 IU/kg of Vitamin A groups. Vitamin A was administrated to each group for seven days via oral gavage. Blood and kidney tissue samples were collected at the end of the experiment. We took blood samples for Superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), blood urea nitrogen (BUN), and creatinine (Cr) levels, and determined their values. The tissue samples were stained with hematoxylin/eosin to examine the renal changes histopathologically and stereologically under a light microscope. **Results:** Histopathological changes caused by I/R were decreased with vitamin A administration in a dose-dependent manner ($p<0.05$). Vitamin A administration decreased MDA levels and increased SOD and CAT activities ($p<0.05$). The most effective dose among treatment groups was 9000 IU/kg. There was no significant difference between the controls and all other groups regarding BUN and Cr concentrations. **Conclusions:** Consequently, administration of vitamin A after renal I/R reduced the histological damage and ameliorated the antioxidant state. These results showed that vitamin A could be a promising agent in treating I/R-induced acute kidney injury.

KEYWORDS: Renal ischemia/reperfusion injury; kidney; vitamin A; acute kidney injury; acute renal failure.

INTRODUCTION

Renal diseases are one of the most important reasons to reduce the quality of human life [1]. Despite the great improvements made in medicine over the past 30 years, the mortality rates of hospitalized patients due to acute renal failure (ARF) range from 30 to 70% [2,4]. Several studies revealed that acute ischemic injury [1,5] is one of the most common causes of ARF.

In Particular, renal dysfunction induced by ischemia/reperfusion (I/R) is still one of the most challenging conditions encountered in the clinic [6]. Ischemia is caused by sudden decreases in the blood amount entering, either in part or in the whole organ, or by the complete cessation of blood flow. Following ischemia, the re-supply of blood flow to the organ is called reperfusion. After the completion of ischemia and reperfusion periods, a condition called ischemia-reperfusion injury (I/R injury) occurs in the organ. I/R injury in the kidney can also be observed in cardiac arrest, shock, and nephrectomy [7].

The ischemic condition in the kidney results in kidney damage and necrosis if not treated [8,9]. Studies have also shown that re-vascularization during the reperfusion period causes some additional kidney damage [7]. Mechanisms underlying kidney damage are still not fully resolved. However, reactive oxygen species (ROS), neutrophil infiltration, vasoactive peptides, and ATP deficiency are known to contribute to I/R-induced acute kidney injury (AKI) [10]. The time duration of ischemia is crucial in terms of damage. Depending on the time, the organ may heal completely, or if the critical time for ischemia is exceeded, the cellular damage may be permanent. Critical ischemia time in humans varies depending on the organs, tissues, and cells (e.g., a few minutes for the brain and 30 minutes for the kidney) [11,12].

I/R injury in the kidney causes AKI, which is

characterized by sudden renal dysfunction, and high mortality rates [13,14]. Studies have shown that in addition to the prolonged treatment process of the patients, AKI causes chronic kidney diseases and therefore brings patients rapidly to the final stage of kidney disease [15,16]. No therapy proved to be successful in preventing effectively or treating ischemic AKI [16]. New treatment agents must be developed to prevent or reduce I/R injury effectively.

Under normal conditions, aerobic metabolism forms oxidants that have to be removed by many antioxidant defense systems in the body.

Either increased production of oxidants or inadequate quantity of antioxidant molecules, caused by some pathophysiological cases, shifts the balance of the oxidant-antioxidant system, which results in cellular damage [17]. This condition is known as oxidative stress, induced by excessive ROS and free superoxide radicals [18]. ROS plays a critical role in cellular damage by oxidizing several cellular molecules, such as lipids, proteins, and nucleic acids, which results in cellular damage [18,19]. Endogenous antioxidant defense systems of the body prevent the formation of free radicals and reduce their amount. Exogenous antioxidants, including vitamins and antioxidants, are often used in medicine. Antioxidants for the prevention and treatment of I/R damage have been used for many years [20]. Among the antioxidants used in the treatment, vitamins are essentially significant. Not all biological activities of vitamin A, known to play a crucial role in maintaining growth, regulating the differentiation and proliferation of epithelial tissues, and performing visual and reproductive functions, have yet to be identified [21,22].

Studies conducted in the middle of the 20th century have shown that vitamin A inhibits lipid peroxidation, and subsequent studies have demonstrated the antioxidant actions of vitamin A [23,24]. Contrary to these arguments, some studies in recent years have claimed that vitamin A has a pro-oxidant effect, especially in chronic administration [25].

In our study, therapeutic doses of vitamin A (Low- 1000 IU/kg/day, Moderate- 3000 IU/kg/day, High- 9000 IU/kg/day) was administered for seven days, as indicated in the literature, against the I/R induced kidney damage in rats [26].

The effects of various concentrations of vitamin A administration on renal histology, antioxidant markers, blood urea nitrogen (BUN), and creatinine (Cr) concentration, were investigated in renal I/R injury.

MATERIAL AND METHODS

Our study has been approved (10.10.2018-687) by the "Eskisehir Osmangazi University (ESOGU)- Animal Experiments Local Ethics Committee" and conducted by following the ethical principles of "Guide for the Care and Use of Laboratory Animals" (8th ed., 2011). The animals were provided by the "Medical and Surgical Research and Practicing Center" of ESOGU. The entire experiment phases were carried out in the same center. Vitamin A used in our study (Merck®) contains 200.000 IU Vitamin A palmitate per milliliter.

Animals and Groups

Since there were many studies on this species before, 40 Sprague-Dawley male rats, eight weeks old and 250-300 gr, were preferred in the experiment. Rats housed in polycarbonate cages had cycles of 12 h light/12 h dark the settings of ambient temperature were 22 ± 2° C and humidity from 50 ± 5%. Rats were fed ad libitum with laboratory pellet chows and tap water.

During the procedures, we observed strict compliance with the Guidelines and the Helsinki Declaration for the Care and Use of Laboratory Animals.

Animals were randomly divided into five groups, containing eight individuals each. One group was the control group, whereas, in the four remaining groups, I/R was developed. Three I/R groups received low, medium, and high doses of vitamin A, which were previously stated [26].

Control: No administration was performed to the control group to obtain the normal values.

I/R: After the I/R operation, only sunflower oil was administered, by the oral gavage method, for seven days.

I/R+1000 IU/kg of Vitamin A: After the I/R

operation, 1000 IU/kg of vitamin A was given, and mixed with sunflower oil by the oral gavage method for seven days.

I/R+3000 IU/kg of Vitamin A: After the I/R operation, 3000 IU/kg of vitamin A was administered with sunflower oil by the oral gavage method for seven days.

I/R+9000 IU/kg of Vitamin A: After the I/R operation, 9000 IU/kg of vitamin A was administered with sunflower oil by the oral gavage method for seven days.

Three groups of rats receiving 1000 IU group (I/R + 1000 IU Vitamin A/kg/d for seven days), 3000 IU group (I/R + 3000 IU Vitamin A/kg/d for seven days), 9000 IU group (I/R + 9000 IU Vitamin A/kg/d for seven days) respectively, were designed to demonstrate the effects of different doses of vitamin A administration on I/R injury.

Surgical Procedures and Administration of Vitamin A

All groups had performed a left renal I/R operation, excluding the control group. Rats were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) by intramuscular injection. Animals under deep anesthesia were confirmed by checking the cornea and nail reflexes every 2 minutes. Rats fixed on their back in the surgery area received a 2 cm incision on the median line (linea alba), first across the skin and subcutaneous tissues, and finally on the muscle layer, to reach the abdominal cavity. Then, the left kidney was visualized by removing the stomach, bowels, and adjacent left-side structures. The left renal pedicle was detected and occluded with an atraumatic clamp (small bulldog 1", for single use) for 45 minutes.

After 45 minutes, by lifting the clamp, the kidney was reperfused. When the I/R process was complete, the skin and muscle layers of the rats were closed with surgical sutures. After the operation, rats were placed in separate cages and observed for their vital parameters for 24 hours.

After the 24-hour recovery period, sunflower oil was administered to the I/R group by oral gavage method, while different doses of vitamin A were given in vitamin A groups, with the sunflower oil as a vehicle.

After 1 week of vitamin A administration, intracardiac blood samples were collected from the rats under anesthesia.

Then, the rats were sacrificed by excessive bleeding and perfused. The left kidneys were taken for histological examination.

Histopathologic Processes, Examination, and Scoring

The removed kidney samples were embedded in paraffin after being processed in a 10% formalin solution as standard procedure and were taken several 5 μm thickness sections from the tissue, then stained with hematoxylin and eosin (H&E). The histopathological examinations were performed with a light microscope (OLYMPUS, Japan), and all histopathological samples were examined by the same histologist, blinded to the groups and tissue samples. All samples were examined for tubular damage (epithelial cell desquamation), tubular dilatation, cellular infiltration, and glomerular damage.

While observing the morphological changes, at least twenty sample slides for each kidney were examined at 40x magnification. The scores for morphological changes and damage were "0: none; 1: mild; 2: medium; 3: severe" [27].

Stereological Analysis

Sections taken from the kidney tissue at equal intervals were examined with unbiased stereological methods.

The mean diameters of renal corpuscles and distal and proximal tubules were measured in all sections using an unbiased counting frame.

As previously explained in the literature, the mean diameter in stereological analyzes is calculated with a formula using the lengths of the longer axis (a), drawn between the two farthest ends of the sample, and the minor axis (b) by cutting the longer axis vertically [28].

For each animal, a few hundred interest structures were sampled and then used the formula a.b, to calculate the mean diameters. The calculated mean tubular diameter was effectuated by averaging the diameter of an equal number of distal and proximal tubules.

Also, the volume fraction (Vv) of Bowman's space in the glomeruli was calculated using the "point count" analysis in the renal corpuscles at the light microscopic level. All these calculations have been accomplished with Stereo Investigator

10® (SI-MBF Bioscience) software.

Biochemical Analysis

In our study, malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) activity levels have been determined biochemically. BUN and Cr levels were also measured, to analyze renal function. Blood samples were taken into EDTA-containing tubes and centrifuged at 1500 g for 5 min. Pellets containing erythrocytes were saline-washed three times. The remaining pellets needed distilled water added, as much as the volume of the remaining pellets, and were centrifuged at 5000 rpm for 15 minutes at +4°C.

The supernatant was taken and maintained at -80°C until MDA, SOD, and CAT measurements were performed.

Based on the homogenate preparation method, specified in the CAYMAN enzyme determination kit, homogenates to be used in CAT measurement were prepared (CAYMAN CAT Assay Kit No: 707002- 1180 E. Ellsworth Rd, Ann Arbor, MI, USA). CAT levels calculated were expressed as kilounits/milliliter (kU/ml).

SOD activity was determined with the Sigma SOD Determination Kit (Cat. No. 19160- Sigma-Aldrich, Germany) based on the WST (water-soluble tetrazolium salt) reaction. The SOD activities calculated were expressed as inhibition% (inh%) [29].

It is known that small molecular weight alcohols serve as electron donors for the peroxidative activity of CAT. CAT activity was determined by the ''CAYMAN CAT Assay Kit (707002)", based on the enzyme reaction with methanol at the appropriate hydrogen peroxide (H2O2) concentration.

We used the method based on the color reaction of MDA, one of the lipid peroxidation products, with thiobarbituric acid (TBA). 0.1 mL of homogenate, 3 mL of 1% phosphoric acid, 0.5 mL of distilled water, and 1.0 mL of 0.6% 2-TBA were mixed and boiled for 45 min., and measured homogenate, and hemolysate MDA levels after the addition of 4.0 mL of n-butanol/pyridine, spectrophotometrically at 532 nm [30].

Kidney function was determined by analyzing serum levels of BUN and Cr. Analyzes with Roche COBAS C501 auto-analyzer (Roche Diagnostics GmbH, Mannheim, Germany) were done. Results have been presented as mg/dl. We measured blood parameters instead of tissue parameters to observe the effects of I/R and vitamin A on systemic conditions.

Statistical Analysis

The suitability of the distribution of continuous variables obtained in the study to the normal distribution, was determined by using Kolmogorov- Smirnov. A one-way analysis of variance (ANOVA) was used for comparing normally distributed variables between groups, and the Kruskal- Wallis test was used for variables that did not show normal distribution. Descriptive statistics of the variables were given as mean ± standard deviation.

All analyzes were done in package programs. The statistical significance level was determined as $p < 0.050$.

RESULTS

Histopathological and Damage Scoring Results

As a result of the examinations performed under the light microscope, the control group had normal kidney histology. On the contrary, abnormal findings such as desquamation in tubular epithelial cells, tubular dilatation, glomerular degeneration, and cellular infiltration were present in the kidneys of the I/R group. Intense tubular damage, tubular dilatation, and cellular infiltration were still observed in the kidneys of the 1000 IU group.

While in the kidneys of the 3000 IU group, the abnormal findings were rarely observed, the histological appearance of the kidneys of the 9000 IU group was very similar to the healthy kidney histological appearance (**Fig. 1**).

In scoring damage, the evaluation was on tubular epithelial cell desquamation, tubular dilatation, glomerular degeneration, and cellular infiltration.

There was a significant increase in the whole types of histopathological damage scores of the I/R group when compared to those in the control group. It was observed that the mean scores in the treatment groups decreased significantly depending on the dose (**Fig. 2**).

The most effective dosing was determined to be 9000 IU/kg of vitamin A (**Table 1**).

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Figure 1. The H&E staining of the rats kidney sections: Normal renal histologic structure was observed in the control group (A). Tubular epithelial cell desquamation (arrow), tubular dilatation (star), glomerular degeneration in renal corpuscle (arrowhead), and intersititial cellular infiltration (inf) were observed in the kidneys of the rats in the I/R group (B). Intense tubular damage and tubular dilatation (star), and cellular infiltration (inf) were still observed in the kidneys of the rats in the 1000 IU group (C). Tubular epithelial cell desquamation and dilatation, and cellular infiltration were rarely observed in the kidneys of the rats in the 3000 IU group (D). Distal tubules and proximal tubules and renal corpuscle looked like in their normal structure in the kidneys of the rats in the 9f000 IU group (E). The scale bar is 50.0 μm in all the figures.

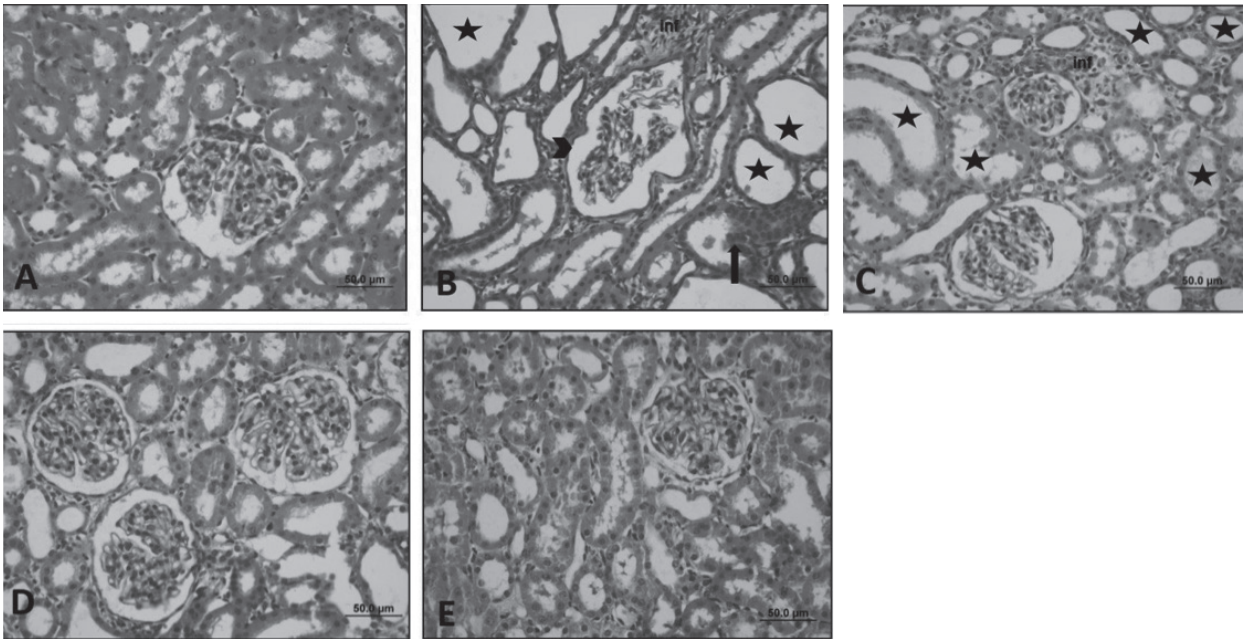


Figure 2. Mean histopathological scores of all groups. Data are shown as mean ± SD.

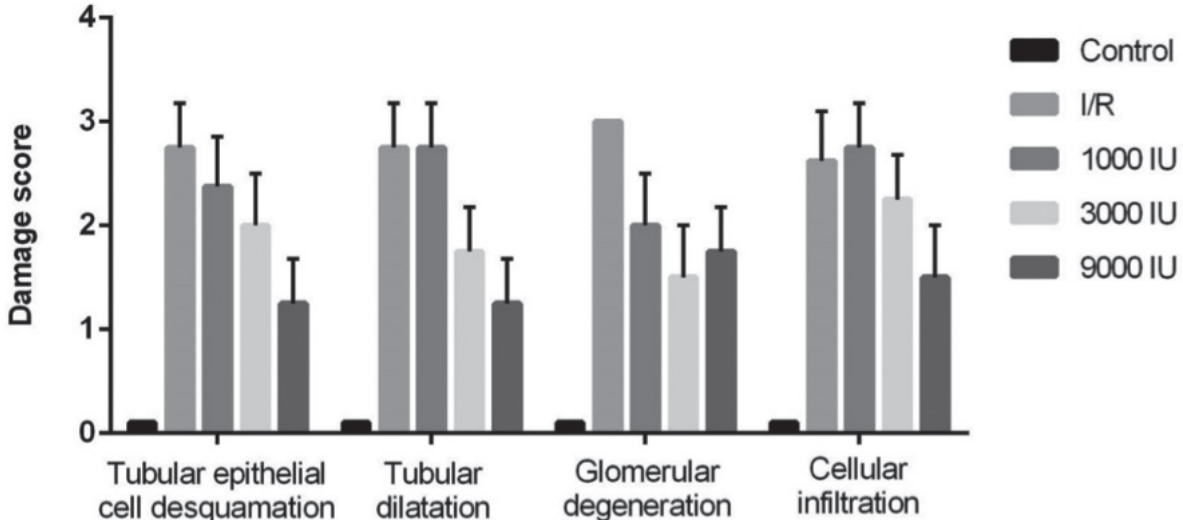


Table 1. Table showing the statistical significance of all histopathological scoring between groups.

Groups	Tubular epithelial cell desquamation	Tubular dilatation	Glomerular degeneration	Cellular infiltration
Control vs. I/R	***	***	***	***
Control vs. 1000 IU	***	***	**	***
Control vs. 3000 IU	**	ns	ns	**
Control vs. 9000 IU	ns	ns	*	ns
I/R vs. 1000 IU	ns	ns	ns	ns
I/R vs. 3000 IU	ns	ns	*	ns
I/R vs. 9000 IU	*	*	ns	ns
1000 vs. 3000 IU	ns	ns	ns	ns
1000 vs. 9000 IU	ns	*	ns	ns
3000 vs. 9000 IU	ns	ns	ns	ns

ns; no significance, *; p<0.05, **; p<0.01, ***; p<0,001

Results of Stereological Analysis

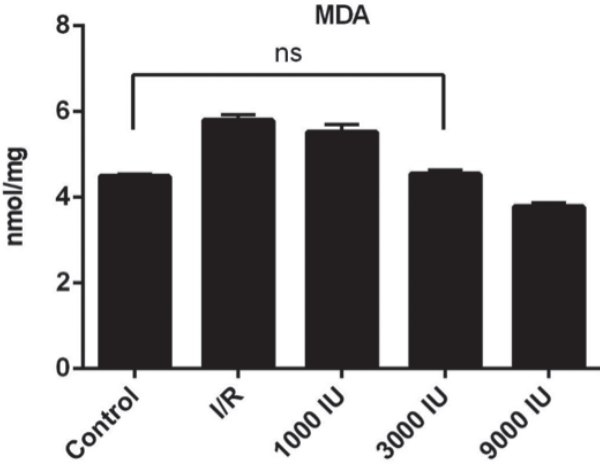
The Bowman's space volume fraction (Vv) in the glomeruli was significantly higher in the I/R group (38±2%) compared to the control group (25±3%). This value returned to normal levels in the groups treated with vitamin A, and there was no significant difference between the control group and any other treatment group. The mean diameters of distal and proximal tubules were significantly increased in the I/R group (33±3 μm) and 1000 IU group (34±1 μm) compared to the control group (23±1 μm).

On the other hand, there was no significant difference observed in terms of mean tubular diameter in controls, 3000 IU (27±1 μm) and 9000 IU (22±0.5 μm) vitamin A groups compared to the control group. The mean diameters of renal corpuscles values in the I/R, 1000 IU, 3000 IU, and 9000 IU (84±3 μm, 91±2 μm, 90±3 μm, 89±2 μm respectively) were significantly decreased compared to those in the control group (114±4 μm).

Biochemical Results

When comparing the control group and I/R group's data, the SOD and CAT activities significantly decreased, while a significant increase was observed in the MDA levels in the I/R group. Vitamin A administration restored all analyzed parameters. In terms of MDA level, it has no significant difference between the control group and the 3000 IU group (**Fig. 3**).

Figure 3. MDA levels obtained by all groups.



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All groups are significantly different from each other ($p<0.05$) excluding the control and 3000 IU group. Data are shown as mean ± SD (ns; no significance).

Both SOD and CAT activities were at the lowest level in the I/R group, but they increased gradually with the administration of Vitamin A and approached the control group values. On the other hand, in the 9000 IU group, no significant difference was detected in the SOD activity value compared with those in controls, while the CAT activity value was higher than the control group value (**Fig. 4**).

As indicators of kidney function, BUN and Cr levels revealed no significant differences between the control group and experimental groups (**Fig. 5**).

Figure 4. Mean values antioxidant enzymes (SOD and CAT) data of all groups. Data are shown as mean ± SD (ns; no significance).

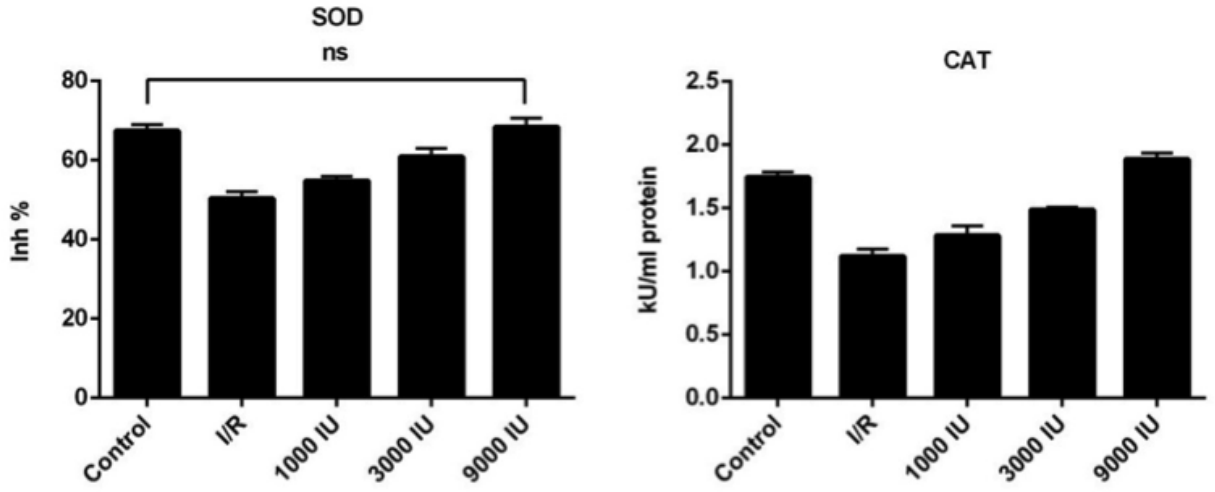
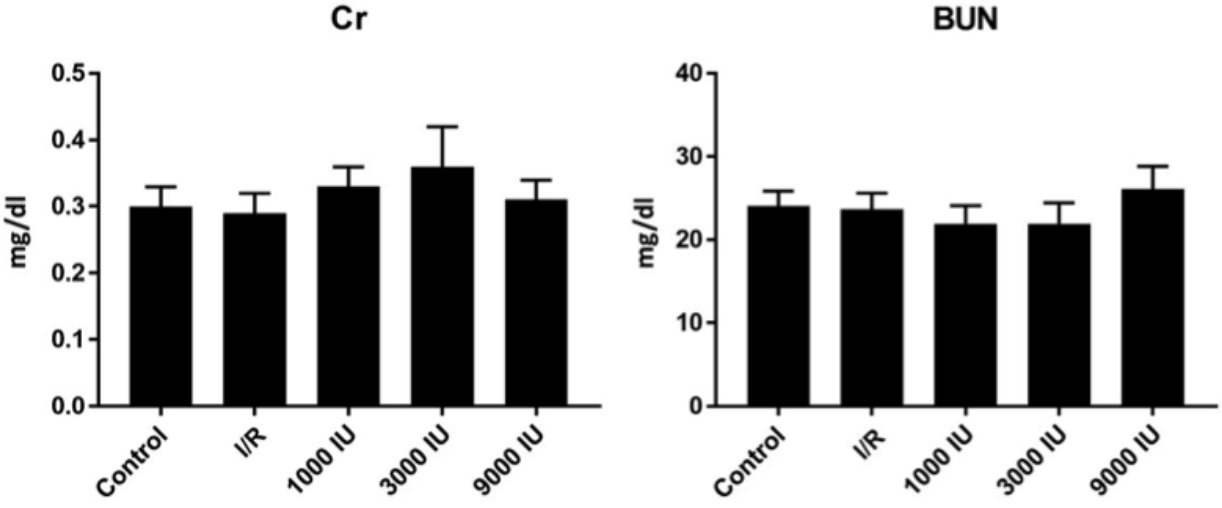


Figure 5. Mean values of kidney function markers (Cr and BUN) levels.



There is no significant difference between the groups. Data are shown as mean ± SD ($p>0.05$ for all groups in Cr and BUN analyzes).

The results of all statistical analysis of biochemical parameters are shown in **Table 2**.

Table 2. Table showing the statistical significance of all biochemical analysis between groups.

Groups (n=8)	SOD	MDA	CAT	BUN	Cr
Control vs. I/R	***	***	***	ns	ns
Control vs. 1000 IU	***	***	***	ns	ns
Control vs. 3000 IU	***	ns	ns	ns	ns
Control vs. 9000 IU	ns	***	***	ns	ns
I/R vs. 1000 IU	***	***	***	ns	ns
I/R vs. 3000 IU	***	***	***	ns	ns
I/R vs. 9000 IU	***	***	***	ns	ns
1000 vs. 3000 IU	***	***	***	ns	ns
1000 vs. 9000 IU	***	***	***	ns	ns
3000 vs. 9000 IU	***	***	***	ns	ns

Variables are normally distributed so ANOVA test was used to show significant differences.

DISCUSSION

In our study, we observed tubule cell desquamation, tubular dilatation, cellular infiltration, and glomerular damage in the kidney of the I/R groups. Similar pathological changes have also been observed in some other studies in the kidneys of I/R groups [31]. Besides these changes, pelvic inflammation, perirenal adipose infiltration, vascular congestion, and proteinaceous casts were also reported after renal I/R in the literature [32,33]

In the kidneys of the experimental group, the volume fraction (Vv) of Bowman's space within the renal corpuscle increased (25% in the control group and 38% in the I/R group). This increase in Bowman's space in the renal corpuscle occurs due to glomerular degeneration. Also, the mean tubule diameter increased in the I/R group of rats compared to control animals. However, the mean renal corpuscle diameter of the experimental rats decreased compared to the control rats. All these changes, seen in the kidney of the I/R group of rats, reflect glomerular damage, which causes some changes in kidney function.

In addition to these morphological changes, SOD and CAT activities of the I/R group decreased, while the MDA level, which indicates lipid peroxidation, was increased.

In our study for the first time, therapeutic doses of vitamin A were administered to rats after renal I/R injury. After administering vitamin A in different dosages, the kidney histology gradually normalized as they increased. Considering the scores of all histological changes, the most effective therapeutic vitamin A dose found was 9000 IU and was no significant difference between the renal histology of the 9000 IU group and the control group in terms of any pathological changes. In the literature, parallel to our results, many studies showed that antioxidant substances administered before or after renal I/R injury had a beneficial

effect on pathologic changes in renal histology [34,35]. Some studies have shown this property of Vitamin A and its derivatives in reducing acute kidney damage in different animals [36].

On the other hand, Cheng et al. (2020) administered vitamin A derivatives before kidney I/R injury and reported increased kidney damage. The same study revealed that the vitamin A derivative reduced histological damage after I/R in diabetic rats. This reducing effect is attributed to the increase in Nrf2 release, which is a known protective of the kidney from oxidative damage by regulating endogenous antioxidant defense [37]. The discrepancy motives between the findings may be due to the differences in the chemical form of the substance administered, the timing of administration, or the dosage differences. Studies focusing on the release of Nrf2 may clarify the effects of vitamin A on AKI.

In our study, the disrupted Bowman's space/ renal corpuscle ratio returned to control values after vitamin A treatment in all doses. On the other hand, dilated tubules returned to their normal values in only 3000 IU and 9000 IU groups. Vitamin A dosage failed to normalize the reduced renal corpuscle diameter after I/R. These differences may be due to the different properties of the structures, such as embryonic origin and tissue formation.

While there was no significant change in the mean diameter of the renal corpuscle after vitamin A treatment, the narrowing of the Bowman's space may be evidence that vitamin A has a reductive effect on glomerular degeneration. The data obtained from different studies by standard histological evaluation are compatible with the quantitative data obtained from our study [31,36].

In addition to tubular, glomerular, vascular, and interstitial damage, which are intrarenal pathologies, oxidative stress and inflammatory response also play an important role in the progression of AKI [38]. Studies reveal that the damage caused by free oxygen radicals on many molecules, such as membrane lipids, DNA, and proteins, plays a prominent role in renal I/R injury [39,41]. Beta carotene, a precursor of Vitamin A, acts as a free radical scavenger contributing to reducing antioxidant damage [42].

In the present study, as a consequence of the administration of different doses of vitamin A, SOD, CAT, and MDA levels were restored, and no change has been observed in the renal function parameters BUN and Cr values. In a study conducted by Erkasap et al. (2004), MDA levels showed a significant increase after I/R injury of the kidney [43]. Many studies have suggested that the increased MDA levels observed after I/R injury decreases with pre or post-treatment of antioxidants [44,45]. In a study conducted by Hosseini et al. in 2010, protective administration of beta carotene (Vitamin A precursor) decreased the MDA level after I/R-induced AKI [46]. The underlying mechanisms for lipid peroxidation under I/R injury are needed to be studied more to resolve such differences.

With the elimination of these incompatibilities and risks, vitamin A use as a supplement or a therapeutic agent may become more strongly. While the MDA level of the 9000 IU group was significantly lower than the control group, the MDA level of the 1000 IU group was significantly higher than the control group. Considering only MDA levels found, 3000 IU/kg/d was to be the most effective therapeutic dose of vitamin A against I/R-induced AKI.

Park et al. (2019) have suggested that renal I/R injury decreased the SOD level, as detected in our study [47]. Several studies, including our study, revealed that decreased SOD activity after I/R injury was restored by antioxidant substances administration [47,49]. In the present study, considering the effects on only SOD levels, the most effective dose of vitamin A on I/R-induced AKI was determined to be 9000 IU/kg/d.

Studies have shown that CAT activity values decrease in rats with kidney I/R damage, and the change returns to normal in those groups administered with antioxidants [50,51]. In our study, similar to previous studies, CAT activity decreased in the I/R group, and decreased values increased in the vitamin A-treated groups. The similarities of studies related to restoring CAT activity support that vitamin A has an antioxidant and therapeutic effect.

When evaluating CAT activities, significant differences have been found between all vitamin A treatment and control values. Data obtained suggest that a dose between 3000 and 9000 IU/ kg/d may be more effective in restoring CAT levels in I/R-induced AKI.

On the other hand, other studies have shown some different results in rats administered with

Vitamin A. Studies conducted by Oliviera et al. showed that chronic vitamin A supplementation in healthy rats increased lipid peroxidation and had a different effect on SOD and CAT activities depending on the duration of administration [26,52]. These results are inconsistent with our study. The discrepancies between the results of these studies and our study may be due to methodological differences. In our study, vitamin A therapeutic effects were investigated, on kidney morphology and function after the production of renal I/R injury. The studies conducted by Oliviera et al. investigated the results of acute and chronic administration of vitamin A to healthy animals and its effects on brain tissue. Another factor that causes inconsistencies between results may be sampling. While blood samples were collected to see the systemic effects of I/R damage in our study, organ (brain) samples were collected to reveal tissue damage in the previous studies [26, 52].

In the present study, as indicators of kidney function BUN and Cr levels in all groups did not show any differences. Similar results were also found by Williams et al, 1997 [53].

According to their study, both BUN and Cr levels increased in the kidney, and those increases continued until the 24th hour, and then returned to normal levels on the 7th day [53]. As our blood samples were taken from rats seven days after renal I/R injury, it is possible to say that each dose of Vitamin A used in our study has not had any harmful effect on kidney function.

Our study also has some limitations: the antioxidant parameters were determined in the blood. Determination of these values in tissue could result differently. In addition, since we took blood samples once (before sacrification), it was not possible to show the effects after I/R over time. Vitamin A is known to exert antioxidant effects as a free radical scavenger. However, since this study conducted a morphological examination level rather than a molecular level, it could not reveal a mechanism for how vitamin A improves antioxidant parameters, kidney function, and cellular architecture.

CONCLUSION

The results obtained in our study shed light on the antioxidant properties of vitamin A and its dose-dependent effects. Accordingly, vitamin A, especially at the dose of 9000 IU/kg/d, may be a promising therapeutic agent to treat I/R-induced AKI. Studies in the future should focus on finding the mechanisms underlying these therapeutic properties of vitamin A, the most effective dosage, and the duration of administration.

These effects should be detailed, as should the mechanisms underlying in future studies.

Compliance with ethical standards

No compliance with ethical standards. Our study has been approved (10.10.2018- 687) by the "Eskisehir Osmangazi University (ESOGU)-Animal Experiments Local Ethics Committee" and conducted by following the ethical principles of "Guide for the Care and Use of Laboratory Animals" (8th ed., 2011).

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