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ARAŞTIRMA YAZISI / RESEARCH ARTICLE

KONTRASTLA İNDÜKLENEN NEFROPATİNİN TAVŞAN MODELİNDE CURCUMİNİN KORUYUCU ETKİSİNİN ARAŞTIRILMASI

INVESTIGATION OF THE PROTECTIVE EFFECT OF CURCUMIN IN A RABBIT MODEL OF CONTRAST - INDUCED NEPHROPATHY

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ÖZET

AMAÇ: Tavşanlarda curcuminin kontrast nefropatisi üzerine etkilerinin araştırılması.

GEREÇ VE YÖNTEM: Bu çalışmada 14 yetişkin, 2,5-3 kg beyaz erkek Yeni Zelanda tavşanı rastgele 3 gruba ayrıldı. Gruplar kontrol grubu (n=2), kontrastla indüklenen nefropati grubu (n=6) ve Curcumin grubundan (n=6) oluşturuldu. Curcumin grubunda kontrast madde verilmesinden bir gün önce ve kontrast maddenin verildiği gün 500 mg/kg Curcumin gastrik gavaj ile uygulandı. İopromid kontrast nefropatisini oluşturmak için 30 dakikalık süre boyunca Vena auricularis marginalise bir kateter yerleştirilerek 8 g/kg dozda intravenöz olarak enjekte edildi.

BULGULAR: Kontrastla indüklenen nefropati grubunda Miyeloperoksidaz düzeyi 0. Saatte 4,899±0,424ng/ml bulunurken 48 saat sonra anlamlı bir artış (7,467±0.353 ng/ml) gözlendi (p=0,002). Kontrastla indüklenen nefropati grubunda glomerüllerin vakuolizasyonu, tübüler epitel hücrelerinin vakuoler dejenerasyonu, hiyalin silindirleri ve tübül lümeninde tübüler nekroz Curcumin grubuna göre istatistiksel anlamlı olarak yüksekti (P=0,000).

SONUÇ: Bu sonuçlara dayanarak, güçlü bir antioksidan olan Curcuminin 24 ve 48 saat sonra kontrastla indüklenen nefropatiye karşı önemli bir koruyucu etkiye sahip olduğu sonucuna varıldı. Bu nedenle kontrast maddelerin kullanılmasından önce Curcumin uygulanması, seçilmiş vakalarda kontrastla indüklenen nefropatiyi önlemek için yararlı olabilir.

ANAHTAR KELİMELER: Kontrast nefropatisi, Curcumin, Iopramid, Tavşan.

ABSTRACT

OBJECTIVE: To evaluate the effects of curcumin on contrast nephropathy in rabbits.

MATERIAL AND METHODS: In this study, 14 adult, 2.5-3 kg white male New Zealand rabbits were randomly divided into 3 groups. Goups consisted of the control group (n=2) consisted of the contrast-induced nephropathy group (n=6) and the Curcumin group (n=6). In the curcumin group, curcumin was administered via gastric gavage at a dose of 500 mg/kg one day before and on the day of contrast agent administration. Iopromide was injected intravenously at a dose of 8 g/kg via a catheter in the V. auricularis marginalis over a period of 30 minutes at a slow rate to induce contrast nephropathy.

RESULTS: Myeloperoxidase was 4,899 ± 0,424 ng/ml at hour 0 in the contrast-induced nephropathy group and a significant increase was observed after 48 hours (7.467 ± 0.353 ng/mL) (p=0.002). In the contrast-induced nephropathy group, vacuolization of the glomeruli, vacuolar degeneration of the tubular epithelial cells, hyaline casts, necrotic tubular epithelial cells in the tubules was statistically higher compared to the curcumin groups (P=0.000).

CONCLUSIONS: Based upon these results, it was concluded that curcumin, which is a strong antioxidant, had a significant protective effect against contrast-induced nephropathy after 24 and 48 hours. Therefore, the administration of curcumin before the contrast material administration may be beneficial to prevent nephropathy in selected cases.

KEYWORDS: Contrast induced nephropathy, Curcumin, Iopromid, Rabbit.

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INTRODUCTION

With the development of modern medicine, imaging methods have been widely used for diagnostic and therapeutic purposes. To enhance imaging quality, contrast materials are utilized. In addition to the positive properties of iodinated contrast materials, allergic hypersensitivity reactions and side effects related to cardiac (hypertension, tachycardia, arrhythmia), circulatory (platelet aggregation, vascular wall contraction, thrombosis) and renal issues have been reported in relation to their use. The contrast material is filtered freely from the kidney glomeruli without release or absorption by the tubules. Renal clearance of contrast material in humans is similar to creatinine clearance with a half-life of 30 to 60 minutes. Because of the increase in the number of elderly patients and patients with comorbidities in the last 30 years, contrast materials have rapidly increased to the top among toxic substances causing kidney damage. Contrast materials are reported to be responsible for 5 % to 30 % of acute kidney injury cases in hospitalized patients (1).

The morphologic changes in the kidney during contrast-induced nephropathy (CIN) have not been adequately investigated. While biopsy results showed that 20 % of patients had vacuoles in the cytoplasm of the proximal tubules, it has been reported that renal tissue does not show significant morphologic changes (2).

The mechanisms leading to CIN are not fully known. Studies have shown that renal medullary hypoxia and direct cellular toxicity are two important mechanisms that become prominent in the development of nephrotoxicity. Contrast material initially causes vasodilatation in the kidney; thereafter, vasoconstriction causes a decrease in renal blood flow and glomerular filtration. The levels of endothelin, angiotensin, and vasopressin, which are vasoconstrictor-effective hormones, were found to be high after contrast intake; and inhibition was found in the synthesis of the vasodilators prostaglandin and nitric oxide. It is known that as a result of contrast media intake, increased blood viscosity and osmotic load in the distal tubules, and an impaired tubuloglomerular "feedback" mechanism, contribute to the development of hypoxia (3, 4).

In recent years, antioxidant agents have become prominent in the prevention of CIN. Due to its antioxidant effect, N-acetylcysteine can prevent CIN by inhibiting renal hemodynamics and direct oxidative damage (5). This effect is considered to be generated through the prevention of renal vasoconstriction by increasing nitric oxide production (6). Ascorbic acid, an antioxidant agent, has been reported to have a protective effect against CIN (7, 8). Studies performed with theophylline and aminophylline, which belong to the adenosine antagonist group, also showed promising results (9, 10).

Curcuma longa, a plant belonging to the Zingiberaceae family, is commonly found in India and China. CUR (diferuloylmethane), the active substance of turmeric obtained from the roots of this plant, is the main component of curry spice. In addition to the antioxidant properties of CUR, its anti-inflammatory, immunomodulatory, anti-tumoral, and anti-psoriatic efficacy has also been demonstrated (11). CUR exhibits antioxidant activity by inhibiting the conversion of xanthine dehydrogenase to xanthine oxidase, as well as lipid peroxidation, and aggregating the reactive oxygen species in the ischemic environment (12, 13). By increasing the activity of enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX), CUR reduces peroxidation of lipids found in the cell membrane (14). Studies have shown that curcumin inhibits nitric oxide synthesis (12, 15). This study aimed to determine whether single dose curcumin had a positive effect in a rabbit model of CIN or not. For this purpose, biochemical, electrolyte, and antioxidant parameters were measured, and histopathologic examination of serum and tissue samples was performed.

MATERIAL AND METHODS

Animals

All animals were given human care in accordance with the criteria of the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health. In this study, 14 adult male white New Zealand rabbits weighing 2.5 kg to 3 kg were used. The rabbits were individually housed with standard rabbit feed and water ad libitum in standard climate conditions for the species. After 5 days of acclimatiza-

tion to the laboratory environment, the rabbits were randomly divided into 3 groups, identified as the control (n=2), contrast-induced nephropathy (n=6), and the curcumin (n=6) groups.

Experimental Design

Control Group n=2: Rabbits in this group were not given curcumin and contrast material throughout the study.

CIN Group, n=6: On day 0, rabbits in this group were sedated by intramuscular injection of Xylazine HCl (Rompun, 23.32 mg/ml, Bayer, Germany) at a dose of 3 to 5 mg/kg. Iopromide (Ultravist®, Bayer, Berlin, Germany) was injected intravenously at a dose of 8 g/kg via a catheter in the V. auricularis marginalis over a period of 30 minutes at a slow rate. Rabbits were euthanized after 48 hours with a high dose of thiopental sodium.

CUR Group, n=6: The rabbits in this group were treated with curcumin (Sigma Life Science, Lot: SLBN7214V, MO, USA) (dissolved in ethanol by gastric gavage at a dose of 500 mg/kg which is covered with 2 ml physiological saline solution, once on the day before CIN (-1 day) and on day 0. Iopromide also given to this group at day 0. After 48 hours, rabbits in all group were euthanized with a high dose of thiopental sodium.

Right and left kidneys were excised at necropsy for histopathologic examination.

Evaluation Of Renal Function

Serum urea and creatinine values were measured in venous blood samples as a marker of the glomerular functions at hour 0 (just before contrast material administration) and after 2, 12, 24 and 48 hours using an autoanalyzer (Human Humastar-180).

Ischemia Modified Albumin (IMA) levels in rabbits were measured by using an ELISA kit (Rabbit IMA ELISA Kit Cat No. YLA0164RB- Shanghai YL Biotech Co. Ltd., China).

Malondialdehyde (MDA) in blood samples, as well as the oxidative stress parameters Nitric Oxide (NO), Superoxide Dismutase (SOD), and Antioxidant Activity (AOA), were measured in tissue and blood samples. Antioxidant activity were measured by commercial Elisa kits with an MVGt Lambda Scan 200 (Bio-Tek Instrument, Winooski, VT, USA).

Myeloperoxidase (MPO) activity, used as an indicator of neutrophil accumulation in the tissue, was measured using a Rabbit MPO Elisa Kit (Sunred Rabbit MPO ELISA Kit Cat. No. YLA0057RB-Shanghai YL Biotech Co. Ltd., China), (16, 17).

Biochemical Measurements

Serum MPO (Sunred Rabbit MPO ELISA Kit Cat. No. YLA0057RB-Shanghai YL Biotech Co. Ltd., China), AOA (Cayman chemical campany, ELISA Kit Cat. No. 709001), and MDA levels in the samples were measured by the Elisa method(18). SOD efficacy was determined according to the method reported by (19). For the measurement of nitric oxide in serum samples, the modified method reported by (20) was used where nitrite + nitrate level was used as an indicator of nitric oxide. Blood gases and potassium, calcium, sodium, chloride levels in venous blood samples taken from all groups at hours 0, 2, 12, 24, and 48 were measured on a blood gas analyzer (Radiometer, ABL 9, Copenhagen, Denmark). Hemogram measurement was performed on the blood samples from all groups using a Cell Counter (Huma Cell Count 80 TS).

Histopathologic Examination

The kidneys of the necropsied animals were fixed in 10% buffered neutral formalin solution. After 48 hours, the specimens were trimmed and placed into cassettes for follow-up tissue evaluation. Tissues were run through an alcohol and xylene series and then were blocked in paraffin. Blocks were cut at a thickness of 4-5 microns with a microtome and transferred to microscope slides. Sections stained with hematoxylin-eosin (HE) were examined under a light microscope.

Statistical Analysis

Data were presented as mean ± standard deviation (SD). Non-parametric ANOVA was used to determine the alterations in biochemical, electrolyte and oxidative stress parameters. In addition, a Tukey test was used for significant data. The histopathological data evaluations were analyzed using one-way analysis of variance (ANOVA), followed by Duncan post-hoc tests. Data were analyzed using Statistical Package for Social Sciences software (SPSS, 18.0, USA). $p<0.05$ was considered significant.

Ethical Committee:

The study started with the approval given by the Local Ethics Committee for Animal Experiments at Afyon Kocatepe University (approved on 24/10/2017 and number of AKÜ HADYEK-283-17).

RESULTS

There was a no statistically significant increase in serum creatinine (sCr) levels at 24 and 48 hours in the CIN Group (p>0.05). However, a statistically insignificant increase in sCr levels was observed at 24 and 48 hours in the CUR group. There was no statistically significant change in serum urea value in both groups and at all times. **Table 1** shows the findings regarding serum urea and creatinine values.

Table 1: Serum Urea and creatinine values in the CIN and CUR groups

Parameter/Group		Hour 0	Hour 2	Hour 12	Hour 24	Hour 48	p
Urea (mg/dl)	CIN	33.300±1.811	33.883±3.365	38.150±1.980	37.533±1.488	36.046±1.001	**0.411**
	CUR	40.333±9.732	43.766±9.261	46.800±9.426	39.617±11.771	36.233±6.984	**0.947**
P		**0.472**	**0.342**	**0.462**	**0.853**	**0.876**	
Creatinine (mg/dl)	CIN	0.4135±0.171	1.216±0.145	0.860±0.206	1.218±0.166	1.075±0.127	**0.460**
	CUR	0.933±0.077	0.805±0.120	0.9150±0.061	0.9133±0.045	0.948±0.060	**0.712**
P		**0.206**	**0.793**	**0.238**	**0.402**	**0.991S**	

Values with p <0.05 were considered significant. Values having different letters (a, b, ab and c) (p<0.05) on the same row at in-group measurement times were considered to be significantly different.

While there was a statistically significant decrease in serum ionized calcium level at 48th hour in the CIN group (p<0.05), no statistically significant change was observed in the CUR group at all measurement times. A statistically significant increase was observed in the serum Chlorine level in the CIN group at the 12th hour. A statistically significant decrease was observed at the 24th hour compared to the 12th hour. In the 48th hour, it decreased to the baseline level. Chlorine value in the CUR group remained within the reference range at all times. A statistically significant increase was observed in the sodium level in the CIN group at the 12th, 24th and 48th hours (p<0.001). Although a statistically significant increase was observed in the sodium level in the CUR group compared to the baseline value at all measurement times, the change in the measurement values was within the reference range. The findings regarding serum electrolyte values are shown in **Table 2**.

Table 2: Serum electrolyte levels in groups

	P	**0.632**	**0.326**	**0.531**		**0.435**	
K^+ (mmol/L)	CIN	4.933±0.547	4.080±0.110	4.195±0.198	4.615±0.257	3.921±0.130	**0.128**
	CUR	4.692±0.143	4.143±0.064	4.378±0.227	4.413±0.132	4.510±0.297	**0.472**
	P	**0.582**	**0.651**	**0.495**	**0.477**	**0.034**	
Na^+ (mmol/L)	CIN	135.166±0.600b	135.500±0.763b	138.500±0.500ba	136.333±0.494b	139.000±1.032 a	**0.001**
	CUR	135.333±3.565b	141.666±1.38a2	143.000±1.125a	143.666±1.145a	141.333±1.054 a	**0.038**
	P	**0.966**	**0.003**	**0.008**	**0.006**	**0.122**	
Ca^{++} (mmol/L)	CIN	1.465±.0628a	1.548±.0164a	1.576±.0226a	1.485±.0256a	1.230±0.089b	**0.001**
	CUR	1.637±0.030	1.603±0.053	1.555±0.102	1.533±0.056	1.415±0.068	**0.269**
	P	**0.083**	**0.396**	**0.859**	**0.395**	**0.160**	
Cl^- (mmol/L)	CIN	97.500±1.335b	99.000±1.483b	103.166±0.703a	100.833±1.492ab	97.000±0.856b	0.009
	CUR	107.000±1.632	108.833±1.558	109.666±1.498	109.000±1.143	107.166±1.222	0.677
	P	**0.000**	**0.003**	**0.009**		**0.000**	

Values with p <0.05 were considered significant. Values having different letters (a, b, ab and c) (p<0.05) on the same row at in-group measurement times were considered to be significantly different

A statistically significant increase in White blood cell (WBC) value was observed in the CIN group 24 hours after contrast agent administration (p<0.05). Although there was a slight increase in the CUR group, it was not statistically significant. Similarly, there was a statistically significant decrease in Hemoglobin (Hb) after 24 hours in the CIN group (P<0.05), while the decrease in the CUR group was not statistically significant (P>0.05). Whole blood results from the study are shown in **Table 3**.

Table 3: Blood gas analysis results from the CIN and CUR groups

Parameter/Group		Hour 0	Hour 2	Hour 12	Hour 24	Hour 48	p
pH [$-\log_{10}H^+$]	CIN	7.461±0.026	7.450±0.017	7.436±0.013	7.470±0.022	7.448±0.030	**0.865**
	CUR	7.223±0.089b	7.426±0.015a	7.398±0.040a	7.406±0.021a	7.403±0.015a	**0.026**
p		**0.019**	**0.407**	**0.491**	**0.046**	**0.349**	
PCO_2 (mmHg)	CIN	30.850±2.497b	31.966±0.863b	29.266±1.139b	28.716±1.425b	44.556±3.018a	**0.000**
	CUR	32.950±3.243a	26.283±1.242b	27.683±1.494ab	28.366±0.605ab	34.150±2.769a	**0.056**
p		**0.508**	**0.012**	**0.538**	**0.837**	**0.078**	
PO_2 (mmHg)	CIN	80.833±4.742	70.666±1.605	80.666±6.765	80.333±12.459	63.500±7.776	**0.392**
	CUR	96.666±11.259	69.833±5.850	77.666±6.146	79.666±4.572	56.166±4.430	**0.007**
p		**0.171**	**0.912**	**0.805**	**0.962**	**0.472**	
Hct	CIN	34.333±1.429a	34.500±1.821a	32.333±1.563ab	27.833±1.740bc	24.500±2.446c	**0.002**
	CUR	39.333±3.179	40.000±1.632	39.666±1.837	37.166±2.773	37.666±1.909	**0.880**
p		**0.267**	**0.009**	**0.002**	**0.003**	**0.016**	
HCO_3 (mmol/L)	CIN	21.783±1.383b	22.266±0.946b	19.666±0.661b	20.966±1.386b	30.666±1.072a	**0.000**
	CUR	13.883±1.865b	17.333±1.125ab	17.166±1.251ab	17.966±1.045ab	21.300±1.490a	**0.018**
p		**0.001**	**0.025**	**0.213**	**0.141**	**0.001**	
AGAP (mmol/L)	CIN	15.933±1.533	14.250±0.625	15.316±0.555	14.633±0.760	11.466±1.697	**0.090**
	CUR	14.500±2.315	15.766±1.003	16.266±1.115	19.300±0.000	12.816±1.342	**0.388**

Values with p <0.05 were considered significant. Values having different letters (a, b, ab and c) (p<0.05) on the same row at in-group and same column at inter-groups measurement times were considered to be significantly different.

In our study, no statistically significant difference was observed in the venous blood gas parameters in both groups at the 24th and 48th hours (P>0.05). The results regarding the venous blood gas parameters are shown in **Table 4.** In this study, while the MDA level in the kidney tissue in the CIN group showed a statistically significant (p<0.05) increase at the 24th and 48th hours, no change was observed in the MDA level in the CUR group at all measurement times compared to the baseline value. The decrease in NO level at the second hour in the CUR group was statistically significant compared to the CIN group (P<0.032). An increase in SOD en-

zyme levels was observed in both CIN and CUR groups. However, the increase in SOD enzyme activity in the CUR group at 24 and 48 hours was statistically significant (p<0.05). In our study, a statistically insignificant mathematical increase in IMA level was recorded in the CUR group at the 48th hour measurement (P=0.052). MPO at 0 hour was 4.899 ± 0.424 in the CIN group; A significant increase was observed after 48 hours (7.467 ± 0.353) (p=0.002). Antioxidant activity (AOA) was found to be significantly higher in the CUR group after 48 hours compared to the CIN group (4.700 ± 0.446, 6.611 ± 0.391, respectively) (p=0682), **Table 5** shows the oxidant/antioxidant parameters measured from serum samples of the CIN and CUR groups.

Table 4: Complete blood analysis results from the CIN and CUR groups

Parameter/Group		Hour 0	Hour 2	Hour 12	Hour 24	Hour 48	P
WBC 10^3/ µl	CIN	7.700±0.666b	7.9500±0.717b	7.5167±0.611b	10.1333±1.196 ab	13.3667±2.30 0a	**0.014**
	CUR	10.433±0.972	13.283±1.438	11.066±2.056	13.500±1.074	13.266±1.102	**0.385**
p		**0.094**	**0.007**	**0.186**	**0.053**	**0.971**	
LYM	CIN	4.816±0.514	4.783±0.397	3.750±0.323	4.366±0.437	7.8000±2.328	**0.125**
	CUR	5.433±1.166b	4.116±0.876ab	2.416±0.436a	6.400±0.725b	5.933±1.334b	**0.047**
P		**0.683**	**0.420**	**0.049**	**0.078**	**0.575**	
Mon	CIN	0.400±0.103b	0.316±0.047b	0.350±0.131b	0.883±0.245a	0.766±0.125a b	**0.029**
	CUR	0.366±0.098	0.433±0.088	0.483±0.094	0.533±0.196	0.916±0.212	**0.108**
p		**0.851**	**0.201**	**0.337**	**0.419**	**0.670**	
Gran	CIN	2.483±0.340	2.933±0.470	3.416±0.637	4.883±1.132	4.800±0.575	**0.066**
	CUR	4.633±1.354	8.733±1.748	8.166±1.908	6.566±1.361	6.416±1.256	**0.382**
p		**0.228**	**0.014**	**0.074**	**0.352**	**0.393**	
Len%	CIN	62.600±4.215	60.016±3.523	51.183±5.396	47.016±8.370	54.133±7.171	**0.370**
	CUR	53.500±11.715	34.050±8.290	25.117±5.925	49.350±7.060	44.933±8.973	**0.156**
P		**0.566**	**0.009**	**0.020**	**0.852**	**0.573**	
Mon%	CIN	5.566±1.228	4.200±0.808	6.500±1.114	7.833±1.693	6.500±1.499	**0.295**
	CUR	3.350±0.537	3.516±0.531	4.716±1.056	4.133±1.503	7.000±1.512	**0.171**
P		**0.187**	**0.336**	**0.880**	**0.261**	**0.868**	
Gran%	CIN	31.833±3.177	35.783±3.300	44.316±4.407	45.150±6.805	39.366±5.727	**0. 285**
	CUR	43.150±11.537	62.433±8.110	70.166±5.853	46.516±6.756	48.066±7.532	**0.123**
P		**0.454**	**0.011**	**0.017**	**0.897**	**0.515**	
RBC 10^6/ µl	CIN	5.705±0.283a	5.531±0.171ab	5.541±0.220ab	4.710±0.425b	3.573±0.249c	**0.000**
	CUR	6.675±0.407	6.295±0.331	5.766±0.627	6.060±0.330	5.101±0.280	**0.118**
P		**0.079**	**0.040**	**0.757**	**0.002**	**0.002**	
Hb g/dl	CIN	13.550±0.478a	12.733±0.560ab	13.166±0.531ab	11.466±0.998b	8.800±0.427c	**0.000**
	CUR	15.816±0.745	15.000±0.569	13.783±1.388	14.350±0.633	12.316±0.521	**0.068**
P		**0.023**	**0.03**	**0.707**	**0.017**	**0.002**	
HCT %	CIN	40.383±1.587a	37.850±1.639ab	39.166±1.803ab	33.433±3.102b	25.400±1.681c	**0.000**
	CUR	46.650±2.007a	43.783±1.555a	40.116±4.042ab	42.350±1.873a b	35.666±1.406 b	**0.037**
P		**0.026**	**0.003**	**0.847**	**0.018**	**0.002**	
MCV fl	CIN	71.066±0.823	70.683±0.925	70.733±0.912	71.033±0.494	71.383±0.974	**0.977**
	CUR	70.316±1.284	69.916±1.364	69.916±1.298	70.216±1.337	70.300±1.263	**0.999**
P		**0.675**	**0.685**	**0.649**	**0.631**	**0.544**	
PLT 10^3/ µl	CIN	616.00±46.81	668.83±94.29	618.00±75.58	494.16±52.09	434.66±56.93	**0.111**
	CUR	552.000±83.50 3	664.833±56.047	689.833±130.30 3	518.666±13.23 2	650.000±44.1 92	**0.444**
P		**0.602**	**0.978**	**0.689**	**0.705**	**0.058**	

Values with p <0.05 were considered significant. Values having different letters (a, b, ab and c) (p<0.05) on the same row at in-group measurement times were considered to be significantly different.

Table 5: Blood oxidant/antioxidant results of the CIN and CUR groups

Parameter/Group		Hour 0	Hour 2	Hour 12	Hour 24	Hour 48	*p*
MPO ng/ml	CIN	4.899±0.424b	5.133±0.365b	5.645±0.434b	5.898±0.542b	7.467±0.353a	0.002
	CUR	5.441±0.324	5.938±0.879	8.047±0.691	6.722±1.079	8.009±1.040	0.134
p		**0.116**	**0.516**	**0.188**	**0.602**	**0.701**	
IMA ng/ml	CIN	1.669±0.156	1.624±0.161	1.443±0.170	1.507±0.157	1.907±0.137	0.314
	CUR	1.429±0.115	1.768±0.154	1.531±0.186	1.408±0.115	1.748±0.145	0.266
p		**0.290**	**0.573**	**0.809**	**0.628**	**0.521**	
NO mcmol/L	CIN	3.982±0.446	3.561±0.417	3.248±0.929	2.769±0.308	2.157±0.329	0.072
	CUR	2.428±0.273	1.979±0.229	2.180±0.338	2.816±0.177	2.806±0.203	0.098
p		**0.067**	**0.033**	**0.434**	**0.833**	**0.180**	
MDA nmol/L	CIN	1.860±0.116c	2.056±0.064bc	2.195±0.146bc	2.386±0.151a b	2.721±0.115a	0.000
	CUR	1.901±0.079	1.925±0.085	2.081±0.081	2.060±0.152	2.306±0.146	0.124
p		**0.574**	**0.298**	**0.751**	**0.190**	**0.019**	
SOD U/ml	CIN	1.060±0.078b	1.086±0.041b	1.067±0.093b	1.135±0.035b	1.578±0.113a	0.000
	CUR	1.011±0.02b6	1.165±0.105b	1.223±0.077b	1.690±0.098a	1.741±0.148a	0.000
P		**0.636**	**0.598**	**0.580**	**0.007**	**0.529**	
AOA mmol/L	CIN	6.793±0.346a	6.556±0.218a	6.972±0.577a	5.760±0.373a b	4.700±0.446b	0.002
	CUR	6.676±0.130	6.655±0.273	7.133±0.219	6.660±0.338	6.611±0.391	0.682
p		**0.757**	**0.713**	**0.963**	**0.015**	**0.000**	

MPO: Myeloperoxidase, IMA: Ischemic modified albumin, NO: Nitric oxide, MDA: Malondialdehyde, SOD: Superoxide dismutase, AOA: Antioxidant activity. Values with P<0.05 were considered significant. Values having different letters (a, b, ab and c) (p<0.05) on the same row at in-group measurement times were considered to be significantl different.

In the histopathological evaluation, vacuolization in glomeruli, vacuolar degeneration in tubular epithelial cells, hyaline cylinder formation and tubular necrosis were statistically higher in the CIN group than in the CUR group (P=0.000). Histopathological results are detailed in **Tables 6a and 6b.** Finally, **Figures 1, 2, 3, 4, 5, 6** also show representative histopathological results of tissue samples.

Table 6a: Histopathologic result in groups

Tissue	Histopathologic findings	CONTROL		POSITIVE CONTROL		CURCUMIN	
		Left Kidney	Right Kidney	Left Kidney	Right Kidney	Left Kidney	Right Kidney
Kidney	Vacuolization in glomeruli	-(6/6)	-(6/6)	+(2/6) ++(3/6) +++(1/6)	+(1/6) +++(5/6)	-(4/6) +(2/6)	-(5/6) +(1/6)
	Vacuolar degeneration areas in tubular epithelial cells	-(6/6)	-(6/6)	+(5/6) ++(1/6)	+(2/6) ++(4/6)	-(5/6) +(1/6)	-(4/6) +(2/6)
	Formations of hyaline cylinders in tubular lumens	-(6/6)	-(6/6)	+(3/6) ++(3/6)	-(3/6) +(3/6)	-(5/6) +(1/6)	-(5/6) ++(1/6)
	Tubular Necrosis	-(6/6)	-(6/6)	+(3/6) ++(3/6)	++(3/6) +++(3/6)	-(4/6) ++(2/6)	-(5/6) +(1/6)

-:absent, + light ,++:moderate, +++:severe

Table 6b: Histopathologic results in groups

GROUPS	Vacuolization in glomeruli	Vacuolar degeneration areas in tubular epithelial cells	Formations of hyaline cylinders in tubular lumens	Tubular Necrosis
CONTROL	0,00±0,00^b	0,00±0,00^b	0,00±0,00^b	0,00±0,00^b
LEFT KIDNEY				
POZİTİF KONTROL LEFT KIDNEY	1,83±0,75^a	1,17±0,41^a	0,67±0,52^a	1,51±0,55^a
CURCUMIN LEFT KIDNEY	0,00±0,00^b	0,00±0,00^b	0,17±0,41^b	0,67±1,03^b
LEFT KIDNEY "P" Value	0,000	0,000	0,022	0,006
CONTROL RIGHT KIDNEY	0,00±0,00^b	0,00±0,00^b	0,00±0,00^b	0,00±0,00^b
POZİTİF KONTROL RIGHT KIDNEY	2,67±0,82^a	1,17±0,52^a	1,51±0,55^a	2,51±0,55^a
CURCUMIN RIGHT KIDNEY	0,17±0,41^b	0,33±0,52^b	0,33±0,52^b	0,17±0,41^b
RIGHT KIDNEY "P" Value	0,000	0,000	0,001	0,000

Values with p <0.05 were considered significant. Values having different letters (a, b) (p <0.05) on the same row at in-group measurement times were considered to be significantly different.

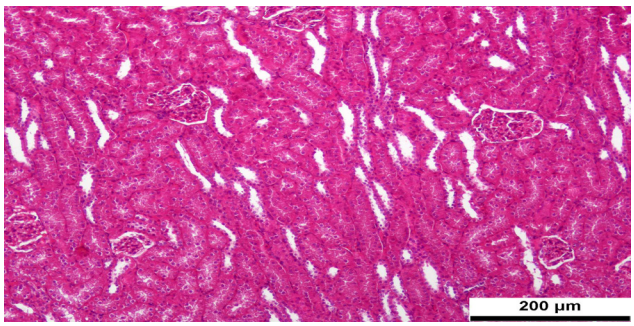

Figure 1: Left Kidney in Control Group

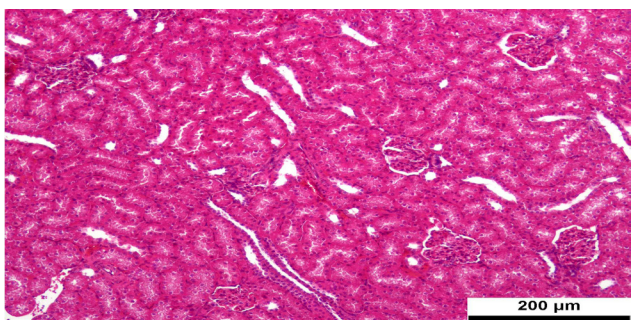

Figure 2: Right Kidney in Control Group

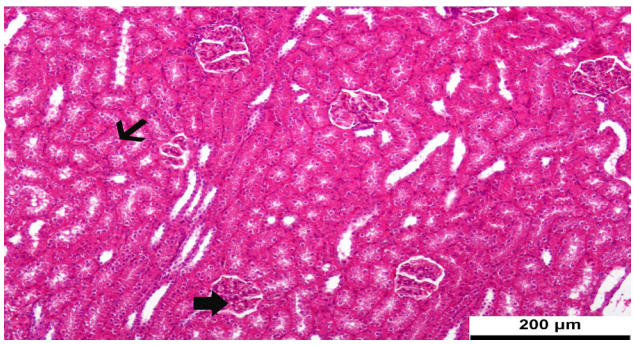

Figure 3: Left Kidney in CIN Group
THICK ARROW: Vacuolization formations in the glomerulus ball of capillaries
THIN ARROW: Vacuolar degeneration areas in tubular epithelial cells
ARROWHEAD: Formations of hyaline cylinders in tubular lumens

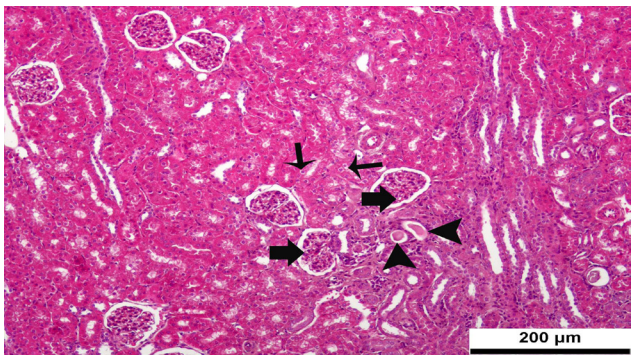

Figure 4: Right Kidney in CIN Group
THICK ARROW: Vacuolization formations in the glomerulus ball of capillaries
THIN ARROW: Vacuolar degeneration areas in tubular epithelial cells
CURVED ARROW: Tubular necrosis

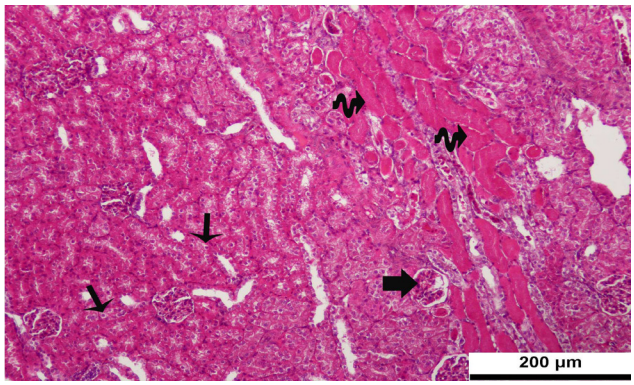

Figure 5: Left Kidney in CUR Group
THICK ARROW: Vacuolization formations in the glomerulus ball of capillaries
THIN ARROW: Vacuolar degeneration areas in tubular epithelial cells

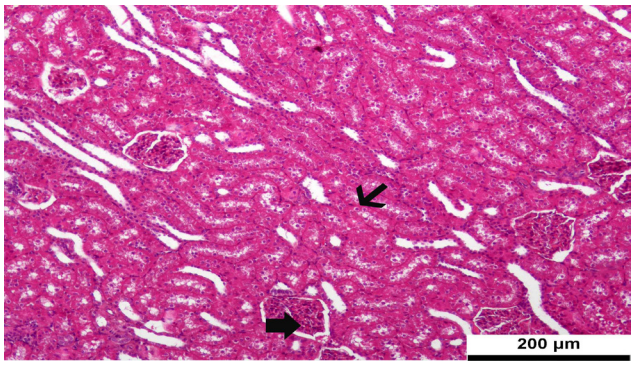

Figure 6: Right Kidney in CUR Group
THICK ARROW: Vacuolization formations in the glomerulus ball of capillaries
THIN ARROW: Vacuolar degeneration areas in tubular epithelial cells

DISCUSSION

The increased use of iodinated contrast agents in imaging and interventional procedures for diagnosis and treatment, and the increasing number of elderly and comorbid patients undergoing these procedures increase the incidence of CIN (21, 22). The increasing number of cases has resulted in many studies aiming to prevent CIN (23 - 26).

The pathophysiology of CIN is still unclear. However, some studies have shown that the administration of iodinated contrast materials increases the production of oxygen free radicals and causes toxic effects directly on renal tubular and glomerular cells (21, 27, 28). Substances with antioxidant properties may provide treatment options to prevent damage to kidney cells by these oxygen free radicals.

Curcumin is an herbal agent with anti-inflammatory, anticancer, and antioxidant properties. It has been shown to be protective against nephrotoxicity resulting from cisplatin (29), cyclosporin-A (30) and contrast materials (26).

Acute Kidney Network consensus group criteria (absolute serum creatinine (sCr) increase ≥ 0,3 mg/dL and a 50 % increase in sCr or 1.5 times the basal level within 48 hours after exposure to the contrast agent) were used for the diagnosis of acute kidney injury, which is an important indicator of CIN in clinical practice. There was a significant increase in sCr levels in the CIN Group at hours 24 and 48 ($p=0.460$). An increase was observed in the sCr levels of the CUR group at hours 24 and 48, but this increase was insignificant. These results were similar to those obtained in previous studies (22, 24 - 26, 31).

On the other hand, there was a statistically insignificant increase in serum urea levels of both groups after 12 hours, and all measurements were in the reference range.

Serum ionized calcium levels of the CIN group significantly decreased after 48 hours ($p=0.001$), but no significant change was observed in any measurements for the CUR group.

No significant difference was observed in the groups' venous blood gas parameters at hours 24 and 48. A significant increase was observed in the CIN group's serum chlorine level at hour

12; however, it significantly decreased at hour 24 compared to the values at hour 12 and declined to the initial level at hour 48. In the CUR group, no significant change was observed at any time. The chloride value was within the reference range. There was a significant increase in the CIN group's sodium level at hours 12, 24, and 48 (p=0.001). In the CUR group, a significant increase in sodium levels was observed at all hours compared to the initial level, but the changes in measurement values were within the reference range.

An elevated MDA level in renal tissue is an indicator of an increase in lipid peroxidation due to nephrotoxicity. Similar to previous studies (24, 25, 32), in the present study, the MDA level of kidney tissue significantly increased in the CIN group (p=0.000) at hours 24 and 48, validating CIN, whereas there was no change in MDA at any time compared to the initial value in the CUR group.

The stable MDA levels in the CUR group suggest that curcumin protects the kidney against CIN due to its antioxidant properties.

Chronic inflammation and cytokines induce nitric oxide synthesis leading to DNA damage and cancer-causing peroxynitrite and nitrite formation. Curcumin has been shown to inhibit nitric oxide synthesis (33). In the present study, the comparison of the nitric oxide levels in the CIN and CUR groups at hour 2 indicated a significant decrease in the CUR group, corroborating previous study results.

Curcumin decreases the peroxidation of lipids in the cell membrane by increasing SOD enzyme activity (14). In the present study, SOD enzyme levels increased in both the CIN and the CUR groups. However, the increase in SOD enzyme activity in the CUR group was significant at hours 24 and 48 (p=0.000). This result supports the idea that CUR has a protective effect against CIN.

Curcumin has been reported to reduce oxidative stress and tissue destruction in the heart and brain as well as ischemia/reperfusion damage in the liver thanks to its antioxidant properties (13). It acts as an antioxidant by inhibiting the conversion of xanthine dehydrogenase to xanthine oxidase, lipid peroxidation, and reactive oxygen species in the ischemic environment. In addition, it reduces lipid peroxidation by increasing the activity of enzymes such as CAT, SOD, and GPX (14, 16, 17).

The decrease in the CIN group's AOA levels was significant at hours 24 and 48 (p=0.002); however, the CUR group's AOA levels were found to be significantly higher than those of the CIN group at hours 24 and 48 (p=0.682). These results are also considered evidence that curcumin has a positive effect in preventing CIN.

The activity of IMA, which is a marker of inflammatory diseases, increases due to oxidative stress and in most inflammatory diseases (34). The IMA level rises within minutes after ischemia, remains high for 6 to 12 hours, and declines to normal values within 24 hours (35). In the present study, despite being insignificant, an increase was recorded in the CUR group's IMA level at hour 48. Measurement of IMA levels is an important marker of renal ischemia-reperfusion injury (16, 17), but it may not be significant as a CIN marker.

In the present study, serum levels of MPO, which is an indicator of tissue neutrophil activity, increased in the CIN group and decreased in the CUR group. This finding may be an indication of the protective properties of curcumin against CIN.

A statistically significant increase was observed in the CIN group's WBC numbers 24 hours after contrast material administration (p=0.014). There was a slight but insignificant increase in the CUR group. Similarly, there was a significant decrease in the CIN group's Hb values after 24 hours, whereas the decrease in the CUR group was insignificant. These changes in the WBC and Hb values may be associated with the increased antioxidant capacity.

In the Vacuolization of the glomeruli, vacuolar degeneration of the tubular epithelial cells, hyaline cylinders, and tubular necrosis in the lumen of the tubules were examined during the pathologic evaluation of the kidney tissue. In the CIN group, vacuolization of the glomeruli, vacuolar degeneration of the tubular epithelial cells, hyaline cylinders, and tubular necrosis in the lumen of the tubules were statistically higher compared to the control and curcumin

groups. The statistical significance in favor of the CIN group compared to the control group is an expected result. These results showed that contrast material caused nephropathy in the CIN and the CUR Group. Histopathologic degeneration was statistically lower in the CUR group than in the CIN group especially in left kidney. Biochemical results also support the histopathological results.

In the present study, the lower creatinine value in the CUR group compared to the CIN group, the oxidant/antioxidant measurement results, and the pathologic findings of significantly higher tubular necrosis, tubular hyaline cylinder, and glomerular vacuolization formations in the CIN group compared to the CUR group support the study hypothesis.

In conclusion, in light of these findings, it was observed that the administration of curcumin before the iodinated contrast material significantly reduced the histopathologic renal findings related to CIN. This result can be attributed to the antioxidant properties of curcumin, which is therefore recommended in addition to the classical medication used to prevent CIN.

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