

CLINICAL STUDY

The Protective Effect of Ebselen on Radiocontrast-Induced Nephrotoxicity

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Abstract

Aim: Radiocontrast-induced nephropathy has become one of the most important causes of renal acute failure. The most effective management of reducing the incidence of contrast nephropathy is to understand and prevent its causes. We aimed to investigate the protective role of ebselen against radiocontrast-induced nephrotoxicity in terms of tissue oxidant/antioxidant parameters and light microscopy in rats. **Methods:** Albino Wistar rats were randomly separated into four groups. The Group 1 rats were treated with sodium chloride as the control group, Group 2 with radiocontrast, Group 3 with radiocontrast plus ebselen, and Group 4 with ebselen alone. After 24 h, the animals over the experimental period were euthanized and blood samples were analyzed for blood urea nitrogen (BUN) and serum creatinine (Cr) levels. Kidney sections were analyzed for malondialdehyde (MDA) levels and superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities, as well as histopathological changes. **Results:** In the radiocontrast group, BUN, MDA, and GSH-Px levels increased while SOD activity decreased compared with the control group. These decays were improved by ebselen administration in the radiocontrast group. Significant histological deteriorations were observed in the radiocontrast group. We noted improvement in the histologic findings with ebselen administration. **Conclusion:** These results indicate that ebselen might produce a protective mechanism against radiocontrast-induced nephrotoxicity.

Keywords: radiocontrast, ebselen, nephrotoxicity, protective effect, rats

INTRODUCTION

Contrast nephropathy (CN) is defined as a decrease in renal function after iodinated radiocontrast media administration.¹ Renal medullar hypoxia and direct tubular toxicity by reactive oxygen species (ROS) are the proposed pathophysiologic mechanisms of CN.^{2,3} Many risk factors are defined, but diabetes mellitus and the amount of radiocontrast media are the most common risk factors of CN. CN is one of the most frequent causes of acute renal failure.⁴ After introducing ionic contrast media into the blood, ROS begin to generate as a result of partial decrease in oxygen concentration and toxic radicals such as superoxide anions, hydrogen peroxide and hydroxyl radicals increase in amount. The destruction of basal membrane and mesenchyme cells causes the chemotaxis of neutrophils that play an important role in the

generation of ROS, which induce tubular injury.⁵ A variety of preventive studies have been conducted to reduce the risk of CN, but an optimal solution is not clear yet.⁶

Ebselen (2-phenyl-1,2-benzioselenazol-3(2H)-one) is a nontoxic seleno organic compound showing peroxidase-like activity and is a synthetic antioxidant that has been studied extensively. The main cytoprotective role of ebselen is related to its glutathione peroxidase (GSH-Px)-like activity that inhibits the effects of membrane hydroperoxides.^{7–9} Ebselen has also been reported to be renoprotective against ischemic injury.¹⁰ Based on these findings, we planned this study to determine whether ebselen might prevent radiocontrast-induced nephropathy. The aims of this study were to determine the protective role of ebselen, administered immediately before CN, with the effect of this treatment for renal biochemical and pathologic evaluations.

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MATERIALS AND METHODS

Animals and Experimental Procedures

Twenty-eight adult male Wistar albino rats, weighing 165–220 g, were used in experiments. The animals were housed in quiet rooms with 12-h light–dark cycles (7 am to 7 pm), and the experiments were performed in accordance with Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996). All rats were given unlimited access to rat chow (Ankara Yem Sanayi, Ankara, Turkey) and tap water. At the beginning of the study, rats were randomized into four groups: Group 1, controls; Group 2, contrast; Group 3, contrast plus ebselen; and Group 4, ebselen (Figure 1). Rats were weighed at the beginning of the study and after 24 h of water deprivation period. After the 24-h water deprivation period, ebselen was administered to Groups 3 and 4 at a dose of 5 mg/kg intraperitoneally. Ebselen was given to rats in various doses in the light of literature.^{11,12} Contrast medium (urograffin) was administered intraperitoneally at a dose of 10 mL/kg to Group 2, which is the dosage scheme reported to cause marked nephrotoxicity in rats.¹³ Group 3 was administered the same dose of contrast media after 3-h period of ebselen administration. Ebselen capsules were obtained from Sigma (St. Louis, MO, USA) and prepared in heated serum physiologic. Urograffin was obtained from Schering, Berlin, Germany, in a 50 mL of 76% solution. On the third day, the animals of all groups were anesthetized with ketamine hydrochloride (75 mg/kg) and xylazine (8 mg/kg), venous blood samples were taken, and serums were separated. Then, kidneys were rapidly excised and right kidneys were used for microscopic examination and left ones for biochemical analyses. The renal tissues and serum samples were stored at -70°C until biochemical analyses. The study protocol

was approved by the Local Ethical Committee of Laboratory Animals at Mustafa Kemal University.

Biochemistry

Oxidative parameters

The kidney tissue samples were stored at -70°C until assayed for kidney tissue malondialdehyde (MDA) levels, catalase (CAT), superoxide dismutase (SOD), and GSH-Px activities. Kidney tissues were homogenized (for 2 min at 5000 rpm) in four volumes of ice-cold Tris–HCl buffer (50 mmol, pH 7.4) using a glass Teflon homogenizer (Ultra Turrax IKA T10 Basic, Staufen, Germany). MDA and protein levels were measured in homogenate. And then, the homogenate was centrifuged at $5000 \times g$ for 60 min to remove debris. Supernatant fluids were collected, and analyses of CAT and GSH-Px activities as well as measurement of protein concentration were performed. The supernatant solutions were used for the assay. The supernatant solutions were mixed with an equal volume of an ethanol/chloroform mixture (5/3, volume per volume, v/v). After centrifugation at $5000 \times g$ for 30 min, the clear upper layer (the ethanol phase) was collected and used for the analysis of SOD activity and protein assays. All preparation procedures were carried out at $+4^{\circ}\text{C}$.

Determination of MDA levels

The thiobarbituric acid reactive substance (TBARS) level was determined by a method based on its reaction with thiobarbituric acid (TBA) at $90\text{--}100^{\circ}\text{C}$.¹⁴ In the TBA test reaction, MDA or MDA-like substances [i.e., the by-product of lipid peroxidation process of the polyunsaturated fatty acids (PUFAs)] and TBA react together to produce a pink pigment having an absorption maximum at 532 nm. The reaction was performed at pH 2–3 and 90°C for 15 min. The sample was mixed with two volumes of cold 10% (w/v) trichloroacetic acid to precipitate protein. The precipitate was pelleted by centrifugation, and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm (Ultra spec Plus, Pharmacia LKB Biochrom Ltd., UK). The results were expressed as nmol/g wet tissue, by reference to a standard curve prepared from measurements made with a standard solution (1,1,3,3-tetramethoxypropane).

Determination of CAT activity

CAT (EC 1.11.1.6) activity was measured according to the method of Aebi.¹⁵ The principle of the assay is based on the determination of the rate constant k (dimension: s^{-1} , k) of H_2O_2 decomposition. By measuring the absorbance changes per minute, the rate constant of the enzyme was determined. Activities were expressed as k (rate constant) per gram protein.

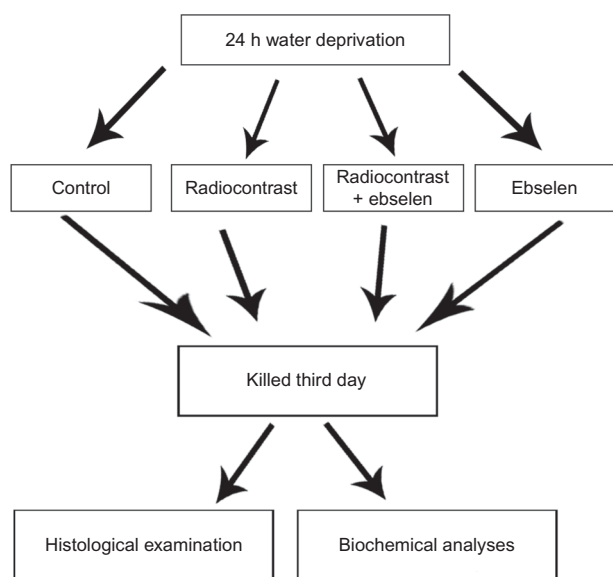


Figure 1. Experimental model of the study.

Determination of GSH-Px activity

GSH-Px (EC 1.6.4.2) activity was measured using the method of Paglia and Valentine.¹⁶ The enzymatic reaction was initiated by the addition of H₂O₂ to the reaction mixture containing GSH, nicotinamide adenine dinucleotide phosphate (NADPH), and glutathione reductase. The change in the absorbance at 340 nm was monitored by a spectrophotometer. Activity was expressed as U/g protein.

Determination of SOD activity

The principle of the total SOD (EC 1.15.1.1) activity method is based, briefly, on the inhibition of nitro blue tetrazolium (NBT) reduction by the generated xanthine/xanthine oxidase system.¹⁷ Activity was assessed in the ethanol phase of the serum after adding 1.0 mL ethanol/chloroform mixture (5/3, v/v) to the same volume of the serum and centrifuging. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. The SOD activity was expressed as U/g protein.

Serum creatinine and blood urea nitrogen

Renal impairment was assessed by blood urea nitrogen (BUN) and serum creatinine (Cr) levels, as well as by renal histology. The BUN and Cr levels were determined with an autoanalyzer (Syncron LX 20, Galway, Ireland) by using commercial Beckman Coulter diagnostic kits.

Pathology

For light microscopic examination, right kidneys of each rat were removed and tissue pieces were fixed in 10% neutral-buffered formalin solution, embedded in paraffin, whole surface of kidneys sectioned at 3 μ m thickness, and then stained with hematoxylin and eosin (H&E). All sections were examined for glomerular, tubular, interstitial, and vascular lesions. The slide was lined with a marker prior to examination of five standard areas from different parts. Approximately 100 tubules per group were evaluated for the toxic injury. For the grading of tubular injuries, the following grading system modified from Yenicierioglu et al.¹³ was used for all tubular cross-section images: mild, preserved brush borders, no evidence of necrosis; moderate, loss of brush borders, no evidence of necrosis; and severe, loss of brush borders with tubular necrosis identified by means of changes such as karyolysis, pyknosis, and/or tubulorrhexis. After scoring all tubules, the frequency of each score was calculated.

Statistical Analysis

Statistical evaluations were performed using the "SPSS 13.0 for Windows" packet program. In general, any significant differences between these groups were evaluated using the one-way analysis of variance (ANOVA) test. The post hoc least significant difference (LSD) test was used to compare the groups with each other. The results are presented as mean $\pm$ SD; $p < 0.05$ was regarded as

statistically significant and $p < 0.01$ was regarded as highly statistically significant.

RESULTS

Observation of Rats

Weight loss was observed in all rats during the water deprivation period, which was significant in the radiocontrast group than in the control group ($p < 0.01$). The weights of rats before and after the water deprivation period are shown in Table 1.

Biochemical Results

Cr, BUN values, and uric acid levels

There was no significant difference between the groups for Cr levels at the end of the study period ($p > 0.05$). Serum BUN levels increased significantly in the radiocontrast group compared with the control and ebselen groups ($p < 0.05$; Table 2). Additionally, the rats treated with ebselen and radiocontrast had decreased BUN levels compared with the group administered with radiocontrast alone ($p < 0.05$; Table 3).

Oxidants and antioxidants

The SOD, GSH, CAT, and MDA parameters are shown in Table 3. There was no significant increase in MDA activity in the radiocontrast group compared with the control group ($p > 0.05$). However, the MDA levels increased significantly in the radiocontrast group compared with the ebselen group, but decreased when compared with the ebselen plus radiocontrast group ($p < 0.05$). SOD levels decreased significantly in the radiocontrast group compared with the control and ebselen groups ($p < 0.05$). Besides, these SOD levels decreased in the radiocontrast plus ebselen group compared with

Table 1. Observation data of the weight change in rats before and after experiment.

	Before experiment Weight (g)	After experiment Weight (g)
Control	229 $\pm$ 48.6	213 $\pm$ 46.8
Ebselen	207 $\pm$ 17.3	188 $\pm$ 18.3
Radiocontrast	166 $\pm$ 32.2*	153 $\pm$ 31.0
Ebselen + radiocontrast	201 $\pm$ 30.6	184 $\pm$ 29.1

Note: * $p < 0.01$, radiocontrast group versus control group.

Table 2. BUN and Cr levels.

	BUN	Cr
I—Control	23 $\pm$ 1.85	0.43 $\pm$ 0.10
II—Ebselen	21 $\pm$ 2.99	0.41 $\pm$ 0.03
III—Radiocontrast	29 $\pm$ 2.21*	0.40 $\pm$ 0.00
IV—Ebselen + radiocontrast	23 $\pm$ 3.07	0.41 $\pm$ 0.43

Notes: BUN, blood urea nitrogen; Cr, serum creatinine.

* $p < 0.01$, Group 1 versus 3, Group 2 versus Group 3, and Group 3 versus Group 4.

Table 3. MDA, SOD, CAT, and GSH-Px values in the kidney of the four groups of rats ($n = 7$ for each group).

	MDA (nmol/g tissue)	SOD (U/g protein)	CAT (k/g protein)	GSH-Px (U/g protein)
I—Control	1.56 ± 0.60	0.32 ± 0.06**	0.40 ± 0.15***	1.83 ± 0.15*
II—Ebselen	1.03 ± 0.60****	0.28 ± 0.06****	0.49 ± 0.04*****	2.07 ± 0.15*****
III—Radiocontrast	1.72 ± 0.61	0.21 ± 0.02	0.46 ± 0.07	1.85 ± 0.32
IV—Ebselen + radiocontrast	1.12 ± 0.36*****	0.25 ± 0.03	0.15 ± 0.02*****	1.80 ± 0.17

Notes: The mean difference is significant at 0.05 level. The values are expressed as means ± SD. MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase.

* $p < 0.05$ versus Group 2, ** $p < 0.05$ versus Groups 3 and 4, *** $p < 0.05$ versus Group 4, **** $p < 0.05$ versus Group 3, ***** $p < 0.05$ versus Group 4; and ***** $p < 0.05$ versus Group 3.

the control group ($p < 0.01$). There was a significant decrease in CAT levels in the radiocontrast plus ebselen group compared with all other groups ($p < 0.001$). The rats exposed to ebselen administration had significantly higher GSH-Px levels compared with the control group ($p < 0.05$). There was a significant decrease in GSH-Px levels in the radiocontrast plus ebselen group compared with the ebselen-only group ($p < 0.05$).

Histopathological Results

The histopathologic scores are shown in Table 4. The control group revealed normal morphology in 75% of tubules while mild degenerative changes in 25% of tubules were seen (Figure 2). Ebselen demonstrated no significant change compared with the control group ($p < 0.01$) (Figure 3). In the contrast group, damaged tubules were more significant than in the control group and ebselen-only group. Edema, vacuolization, loss of brush border, and necrosis in the tubules were noted (Figures 4 and 5). Degenerative changes were more evident in proximal tubules. Ebselen significantly improved renal morphology in the contrast group compared with the contrast-only group ($p < 0.05$). Degenerative changes evidently diminished with ebselen treatment in the radiocontrast plus ebselen group, but some tubules with edema and vacuolization remained (Figure 6).

Glomerular infiltration was not observed in the sections of all groups. Scanty lymphocytic infiltration and

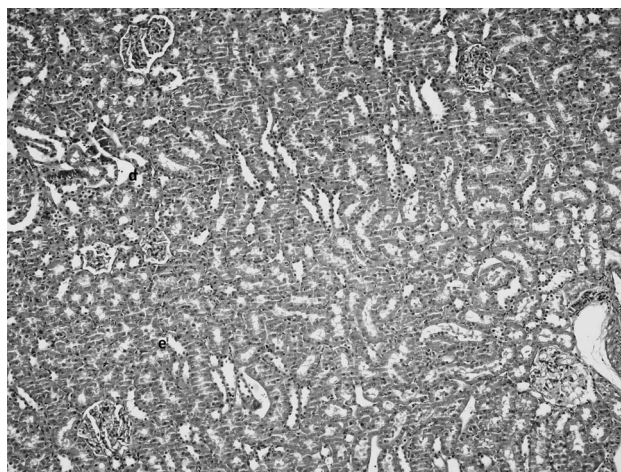


Figure 2. Control group with mild edema (e) and dilatation (d) (HEX100).

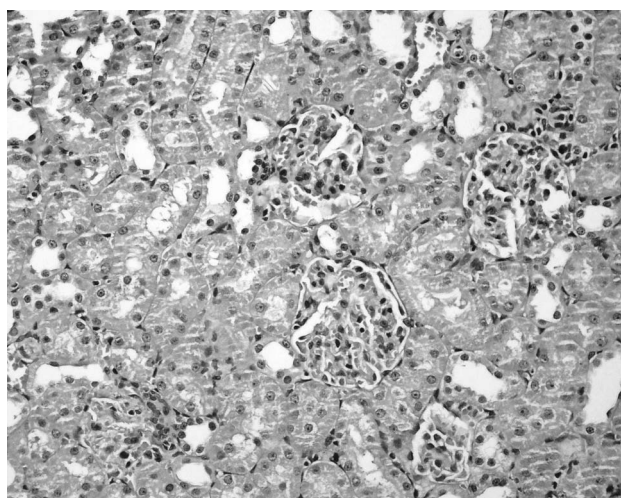


Figure 3. Preserved architecture in the ebselen group (HEX200).

Table 4. Histopathological changes with grade.

Grade	Histopathological changes
Grade 0	No pathological changes
Grade I	Mild, preserved brush borders, no evidence of necrosis
Grade II	Moderate, loss of brush borders, no evidence of necrosis
Grade III	Severe, loss of brush borders with tubular necrosis

Between-groups comparisons (p -values)

$p < (I \text{ vs. II})$	0.001
$p < (I \text{ vs. III})$	0.011
$p < (II \text{ vs. III})$	0.004
$p < (I \text{ vs. IV})$	ns
$p < (II \text{ vs. IV})$	0.001
$p < (III \text{ vs. IV})$	0.028

Note: ns, nonsignificant.

congestion were observed in the interstitium of all groups, but these findings were evident in the contrast plus ebselen group and ebselen-only group (Figure 7). To a certain extent, acute tubular necrosis developed frequently in the radiocontrast group (62.5%) compared with the radiocontrast plus ebselen group (25%) (Figure 8).

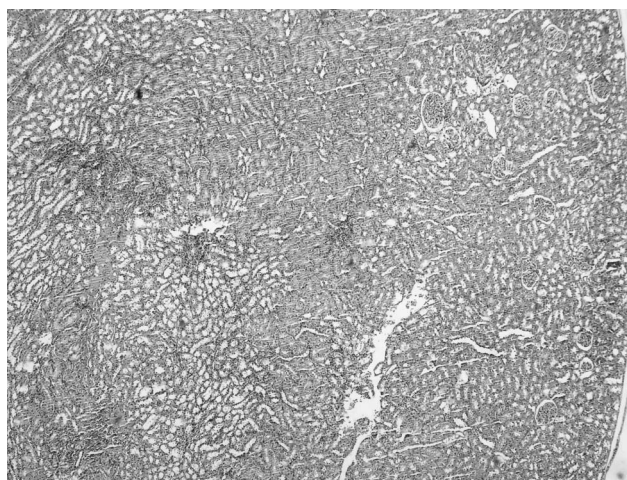


Figure 4. The extensive degeneration through the cortex of renal parenchyma in the radiocontrast-treated group (HEX40).

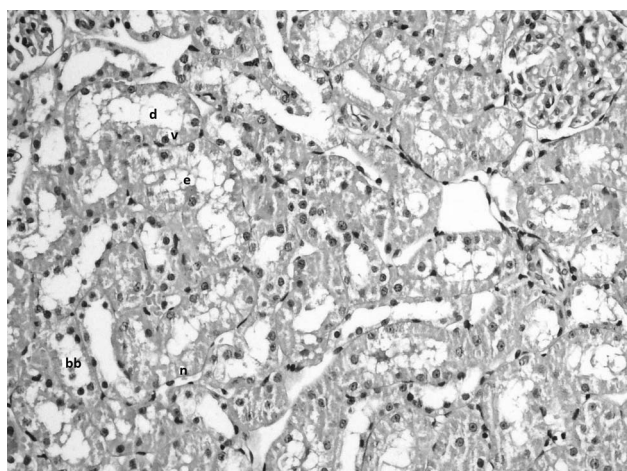


Figure 5. Degenerative changes with edema (e), vacuolization (v), tubular dilatation (d), loss of brush borders (bb), and necrosis (n) in the radiocontrast group (HEX200).

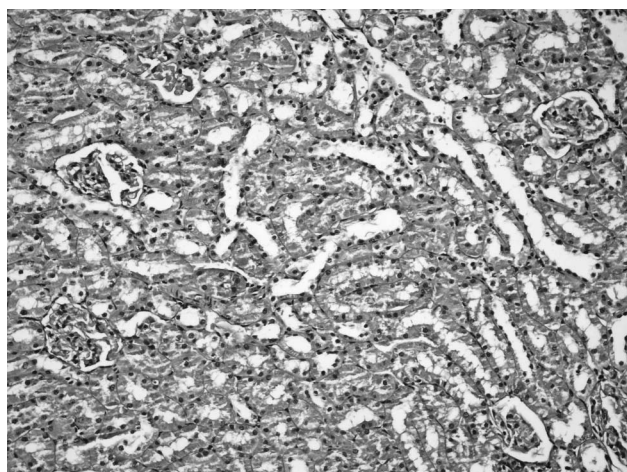


Figure 6. Improved renal morphology by ebselen (HEX100).

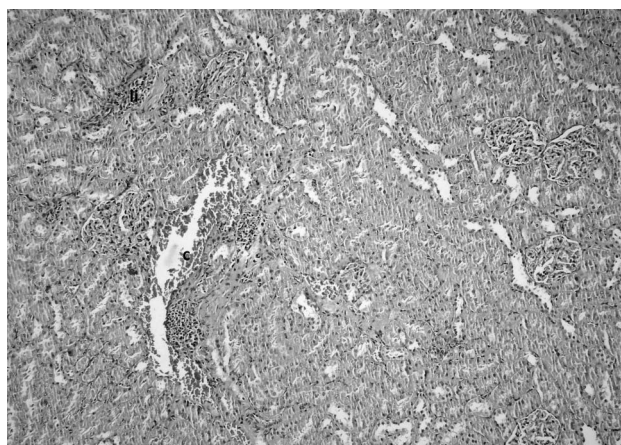


Figure 7. Scanty congestion (c) and lymphositic infiltration (li) in renal parenchyma (HEX100).

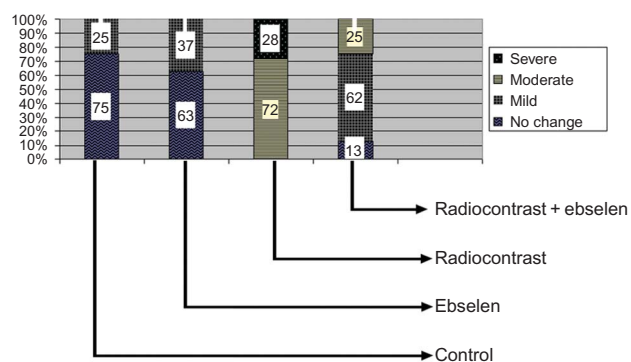


Figure 8. The frequency of tubular lesions.

DISCUSSION

Many experimental studies showed that iodinated contrast media administration induced nephropathy by means of ROS formation and hypoxia within the kidney.^{3,18–20} An imbalance between oxidants and antioxidants in favor of oxidants causes the damage.^{21,22} Some studies have been conducted to prevent CN with the administration of some agents such as N-acetyl cysteine, bicarbonate, and erythropoietin by reducing radiocontrast media-related tubular cell injury. This mechanism should be related to the increase in antioxidant systems.^{23–25}

The toxic effects of radiocontrast media in renal tissue have been reported in several studies.^{13,18,19} Acute tubular injury with edema, vacuolization, loss of brush borders, and necrosis was evaluated.^{13,26} Billings et al.²⁶ assessed proximal tubular necrosis, apoptosis, and cortical tubule vacuolization similarly to our study and found significant results with alpha-2 adrenergic agonist treatment against CN. The effect of sodium bicarbonate on CN was investigated by Barlak et al.²⁷ with the presence of interstitial nephritis and tubular necrosis. Histological

examination demonstrated an intermediate–severe injury in 71% of the radiocontrast group, consistent with our results of 72%. In our study, rats were deprived of water for 24 h and treated with iodinated radiocontrast media to create an efficient acute renal failure model, as supported by the literature.^{13,28,29} Acute tubular necrosis developed in 28% of the radiocontrast group, but in none of the control group.

Increased serum BUN and Cr levels, markers of renal functional impairment, were previously reported in several radiocontrast media-associated studies.^{13,26,27,30} Ma et al.³⁰ reported significant increases in BUN and Cr levels in the radiocontrast group as compared with the control. Similarly, Yenicerioglu et al.¹³ showed that the treatment of rats with radiocontrast media produced a significant increase in Cr levels. We also found that serum BUN levels increased significantly in the radiocontrast group compared with the control group, supporting the nephrotoxicity of radiocontrast media, but Cr levels were similar between all the groups at the end of the experiment ($p > 0.05$). This finding was similar to those of the study of Barlak et al.²⁷ Thus, creatinine clearance and uric acid evaluations together with BUN and Cr would be better methods to evaluate renal function than Cr.

Lipid peroxidation is initiated as a result of the ROS-induced abstraction of hydrogen from PUFAs of cellular membranes, which results in the formation of relatively stable compounds such as MDA.^{3,13,18,19} Therefore, MDA is a lipid peroxidation product that shows the oxidant stress. Barlak et al.²⁷ reported no significant increase in the levels of MDA in renal tissue in their study of the efficacy of bicarbonate in preventing CN. Similar to their study, MDA levels did not increase to statistically significant levels in the radiocontrast group compared with the control group, which can be related to insufficient dose of radiocontrast media. But the mean values increased in the radiocontrast group compared with the control group which could show that the radiocontrast media might cause lipid peroxidation in renal tissue, leading to oxidative damage.

The production of free radicals is blocked by endogenous antioxidant systems. SOD and GSH-Px are the enzymes that protect against the oxidant effects of ROS in cells.²⁷ In this study, we evaluated SOD and GSH-Px levels as antioxidants in the kidney tissue, and we found a significant deceleration in SOD levels in the radiocontrast group compared with the control and ebselen groups, supporting the nephrotoxicity of radiocontrast media. GSH-Px activity after radiocontrast administration was investigated in a study performed by Barlak et al.²⁷ and the total GSH level in the radiocontrast group was higher than in the radiocontrast plus bicarbonate group. Differently, we found a significant decrease in GSH-Px levels in radiocontrast plus ebselen-treated group compared with the ebselen-only group. Besides this, there was no difference in SOD and GSH-Px levels when comparing the radiocontrast group with the

radiocontrast plus ebselen group. This might be related to the oxidant species generation induced by toxic radiocontrast media.

The degradation of hydrogen peroxide is catalyzed by the CAT enzyme. There has been no report on the CAT activities of radiocontrast media-induced nephrotoxicity in animals. Yesildag et al.³¹ reported no significant change in the levels of CAT in hepatic tissue in a study where they investigated the protective effect of erdosteine on radiocontrast-induced hepatotoxicity in rats. In our study, there was a significant decrease in CAT levels in the radiocontrast plus ebselen group compared with the control, ebselen, and radiocontrast groups. The decrease in CAT levels in radiocontrast plus ebselen group might be related to diminishing demand for endogenous antioxidants, because the treatment with exogenous antioxidant ebselen is supported by the decrease in MDA levels.

Ebselen is widely studied because of its multifunctional antioxidant effects.^{32–35} Yoshida et al.¹¹ showed that ebselen-attenuated cisplatin induced renal injury histologically. With some differences, our study also revealed that ebselen significantly regressed radiocontrast-induced nephrotoxicity, to some extent. Morphologically, we found a significant improvement in the radiocontrast plus ebselen group compared with the radiocontrast group, which supports the renoprotective effects of ebselen against radiocontrast media. The frequency of moderate injury in tubules decreases (72–28%), which is similar to the findings of Yenicerioglu et al.¹³ However, we found the distributions of mild injury scores are more frequent in the control group compared with the ebselen group, which supports the protective effects of ebselen against renal damage. This finding could be related to the dehydration of the rats at the beginning of the study period.

In this study, ebselen significantly elevated GSH-Px levels compared with the control group, proving that this compound mimics the glutathione activity in cells. However, ebselen could not reverse the depletion of GSH-Px in the radiocontrast group compared with the control group. In contrast to our study, Husain et al.³⁶ showed the protection of cisplatin-treated rats with an ebselen dose of 16 mg/kg, which is a higher route of ebselen administration.

Furthermore, MDA levels were reversed via the administration of ebselen in this study compared with the radiocontrast-only group. Kizilgun et al. investigated ebselen and N-acetylcysteine (NAC) with respect to renal ischemia/reperfusion injury. Similarly, ebselen alone and combined with NAC decreased MDA levels significantly.³⁷ These findings suggested better protective results in combined regimens with ebselen.

There was a significant decrease in BUN levels in the ebselen plus radiocontrast group compared with the radiocontrast group, showing the improving effects of ebselen in kidney function tests. Similar studies have reported the protective role of ebselen in nephrotoxicity induced by various toxic agents other than from radiocontrast media.^{11,12,32}

This is the first study reporting that ebselen prevents radiocontrast-induced nephrotoxicity, as shown by our biochemical and histological findings, suggesting that radiocontrast-induced nephrotoxicity may be secondary to free oxygen radicals and the ebselen might play a protective role with its antioxidative and radical-scavenging effects.

Declaration of interest: We 'all authors' state that there are *not* any conflicts of interest regarding the submission and publication of the manuscript and its potential implications.

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