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Relationship with nephrotoxicity of Abemaciclib in rats: Protective effect of Curcumin

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Abemaciclib (ABE) has been reported to cause gastrointestinal toxicity. Therefore, it is important to investigate the question of whether abemaciclib administration causes nephrotoxicity in the gastrointestinal tract and if so, what pathophysiological pathways it follows. This study investigated the relationship between ABE administration, nephrotoxicity, and Curcumin's protective effect (CMN). Forty albino female rats were equally divided into five groups. The sham group was fed with standard pellet food. Dimethyl sulfoxide (DMSO) group: 150 µL of DMSO was administered to each rat once a day for 28 days.CMN group: 30 mg/kg/day of CMN was administered to each rat for 28 days. ABE group:26 mg/kg/day of ABE was administered to each rat at a dose once a day for 28 days. ABE+CMN group: 26 mg/kg/day ABE and 30 mg/kg/day CMN were administered to each rat dose for 28 days. Aquaporin (AQP) 1-7, TNF-α, IL-1β, intercellular adhesion molecule (ICAM)-1, IL-10 and IL-37 levels in serum and kidney tissue homogenates were measured by ELISA. In addition, Urea and Creatinine were measured in serum samples. Furthermore, histopathological examination was performed in kidney tissues and Bax, Caspase-3 and Bcl-2 expression levels were determined immunohistochemically. The levels of AQP1-7 and IL-10 in the ABE group were partially lower than in the other groups, while the ratio of TNF-α, IL-1β, MDA, caspase-3 and Bax/Bcl2 were high. In addition, kidney tissue was examined histopathologically. However, AQP1 and AQP7 levels in the ABE+CMN group were higher than in the ABE group, while TNF-α, IL-1β, MDA, Caspase-3 levels and Bax/Bcl2 ratio were low. In addition, the poor histopathological changes in the ABE group were mainly restored in the ABE+CMN. The data presented that ABE in rats can adversely affect functions and histology of kidneys through the increase in oxidative stress, pro-inflammatory cytokines and apoptosis, but CMN therapy may be protective against the nephrotoxic effects of ABE.

Keywords: Abemaciclib, Apoptosis, Aquaporin, Curcumin, Nephrotoxicity

Cancer refers to a pathological condition characterised by excessive division of cells[1]. In a report published in 2020, the World Health Organization defined cancer as a serious health problem in all populations, regardless of wealth or social status. In this report, it was reported that 18.1 million people worldwide had cancer in 2018 and 9.6 million people died from this disease, these figures will almost double by 2040. In addition, this report declared that one out of every five people worldwide will face a cancer diagnosis during their lifetime by 2020[2]. For this reason, it has become important to better understand the cancer pathogenesis and the role of cell cycle regulators and to develop effective therapeutic agents. In particular, cyclin-dependent kinases (CDK), which regulate the progression of the cell cycle throughout the phases and have a role in the pathogenesis of many cancers, have been identified as important targets for therapeutic strategy. The FDA's designation of drugs targeting CDK inhibition as "Breakthrough therapy" also demonstrated the importance of this strategy[3]. In the past few decades, numerous CDK inhibitors with different molecular formulas have been designed and developed. Abemaciclib (ABE) is one of the newest members of the CDK inhibitor family, which has emerged for the treatment of patients with metastatic breast cancer and has Human Epidermal Growth Factor Receptor-2 negative and hormone receptor-positive[4]. ABE is a potent, selective, small-molecule pyrimidine-benzimidazole inhibitor for oral administration. ABE prevents phosphorylating the Rb tumour suppressor protein by CDK 4/6. Thus, it causes the cell division process to be kept in the G1 phase and eliminates cell growth[5]. ABE provides progression-free survival in the treatment of advanced

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breast cancer. In addition, it is actively used for treatment in preclinical and clinical trials in many cancer types such as colon cancer, glioblastoma, malignant melanoma, non-small cell lung cancer and oesophageal adenocarcinoma, and positive feedback has been received[6].

However, ABE has undesirable side effects as with many drugs used in therapy[7]. In phase studies conducted before the drug was approved by the US Food and Drug Administration (FDA), the most common side effects were reported to be diarrhoea, neutropenia, fatigue, abdominal pain, decreased appetite, and highly increased serum creatinine levels[4]. In one of the studies conducted to elucidate the mechanism of diarrhoea, one of these side effects was that ABE caused gastrointestinal toxicity[8]. Therefore, it is important to investigate the question of whether the application of ABE causes nephrotoxicity in the gastrointestinal tract, and if so, by which pathophysiological pathways.

Curcumin (CMN) has been evaluated as one of the natural antioxidant substances that can be used to prevent drug-induced nephrotoxicity[9]. It was reported that CMN was a natural antioxidant[10] with many beneficial properties such as anti-inflammatory[11], renoprotective[12], accelerating wound healing[13], anticancer[14,15], immunomodulatory[16] and anti-lipidemic[17]. Many experimental studies were conducted to reveal the protective effects of CMN on the kidney. In these studies, the protective effect of CMN on nephrotoxicity caused by drugs such as doxorubicin, gentamicin, cisplatin, and cyclosporine was demonstrated[18-21]. In addition, the curative effects of CMN were demonstrated against nephrotoxicity caused by different pathways such as ischemia/reperfusion injury, kidney damage caused by chemicals, and kidney damage caused by diabetes[9]. Therefore, evaluation of the protective effects of CMN against possible nephrotoxicity induced by ABE will provide new information to the literature. This study aimed to investigate the relationship of ABE with nephrotoxicity and the protective effect of CMN.

Materials and Methods

Chemicals

Abemaciclib (Verzenio®, Eli Lilly and Company, USA) was purchased commercially. Curcumin was obtained from Sigma-Aldrich Company (Sigma Aldrich, USA, Catalog number: BD9137). ELISA kits (Bioassay Technology Laboratory) for the biochemical parameters measured in the serum and kidney tissue homogenates were purchased from Aktif Diagnostics.

Experimental study

The effective dose and duration of ABE were applied by considering the effective dose and duration previously used by Kosovec *et al.*[22]. The effective dose amounts previously used by Dacosta *et al.*[23] and Liu*et al.*[24] were applied for effective doses of CMN. Before starting the experiment, the weights of the rats were measured separately and randomly distributed so that the weighted average of each group was close to each other, and the 26 mg/kg/day of ABE and 30 mg/kg/dayof CMN to be administered were determined according to the literature[22-24]. Dimethyl sulfoxide (DMSO) was used as a solvent for ABE and CMN to be applied in the experiment. The groups were created as follows;

Sham Group (n=8): Oral gavage was applied once a day for 28 days and fed with standard pellet rat food throughout the study.

DMSO group (n=8): 150 µL of DMSO was administered to each rat once a day by oral gavage for 28 days and fed with standard pellet rat chow throughout the study.

ABE group (n=8): For each rat, ABE was administered at a dose of 26 mg/kg/day dissolved in 150 µL DMSO once a day by oral gavage method for 28 days and fed with standard pellet rat food throughout the study.

CMN group (n=8): For each rat, 30 mg/kg/day of CMN dissolved in 150 µL DMSO was administered once a day by oral gavage for 28 days and fed with standard pellet rat chow throughout the study.

ABE+CMN group (n=8): 26 mg/kg/day ABE and 30 mg/kg/day CMN dissolved in 150 µL DMSO was administered orally by gavage once a day for 28 days. The rats were fed with standard pellet rat food throughout the study.

Termination of the study and obtaining serum samples

On the last three days of the study, animals were sequentially placed in metabolic cages, and 24 h urine samples were collected. After the urine specimens were centrifuged at 3000 × g for 5 min, the upper phase was taken into Eppendorf and stored at –80°C until the study day. At the end of the 28th day, the animals were weighed separately for the second time, and their weights were noted. Then, the abdominal

regions of the rats were opened under anaesthesia with 50 mg/kg ketamine + 20 mg/kg xylazine, and their blood was taken intracardially. The serum samples were obtained by centrifuging 4 mL of blood samples in a dry biochemistry tube for 10 min at 3500 × g and were stored at −80°C until the study day.

Homogenisation of kidney tissues and production of supernatant

After washing the right kidney tissues with 0.9% NaCl, they were frozen at −80°C and stored for biochemical parameter measurements. On the study day, 2.25 mL of 50 mM phosphate buffer (pH 7.4) was added to 250 mg of kidney tissue (1:10 wt-vol), and the mixture was homogenized using a homogeniser (Ultra Turrax-T25). The samples were centrifuged at 3000 × g and 4°C for 20 min, and the supernatant remaining in the upper phase was transferred to a new tube. All kidney homogenates were stored at −80°C until analysis.

Quantitative protein determination

Protein amounts in kidney tissue homogenates were measured by the Bradford method[25]. Ten µL samples were added onto 4990 µL Coomassie-Brillant Blue solution and incubated at room temperature and in the dark for 10 min. Reading was then taken at 595 nm in the spectrophotometer (ShimadzuUV Mini 1240 from Japan). A mixture of 0.1 mL of distilled water and 4900 mL of Coomassie-Brillant Blue solution was used as the blank.Protein amounts were calculated by comparing the obtained absorbance values with the standard curve[26].

Measurement of cytokines in serumand tissue homogenates

The levels of Aquaporin 1 (Bioassay Technology Laboratory Cat.No: E0565Ra), Aquaporin 7 (Bioassay Technology Laboratory Cat.No: E1421Ra), TNF-α (Bioassay Technology Laboratory Cat.No: E0764Ra), ICAM-1 (Bioassay Technology) in serum and kidney tissue Laboratory Cat.No: E0623Ra), IL-37 (Bioassay Technology Laboratory Cat.No: E2576Ra), IL-10 (Bioassay Technology Laboratory Cat.No: E0108Ra), IL-1β (Bioassay Technology Laboratory Cat.No: E0119Ra) were measured on the BioTek EL&800 instrument at 450 nm with commercial ELISA kits following the instructions in the kit procedure. Quantitative values were calculated by comparing the obtained absorbance values with the standard curve values.

Measurements of urea and creatinine in the serum, protein in the urine

Serum urea and creatinine were measured spectrophotometrically, and urine protein levels were measured by a turbidimetric method based on the denaturation of protein with benzethonium chloride and its measurement at 404 nm in a biochemical autoanalyser (Abbott Architect c16000, U.S.A).

Measurement of malondialdehyde (MDA) in the serum and kidney tissue by high-pressure liquid chromatography (HPLC)

MDA was measured according to the method of Khoschsorur *et al.*[27]. 750 µL of phosphoric acid (H3PO4, 0.44 M), 250 µL of thiobarbituric acid (TBA, 42 mM) and 450 µL of distilled water were added to 50 µL of serum or kidney supernatant. Tubes were tightly capped and incubated in a boiling water bath for 60 min. After cooling in tap water, alkaline methanol (50 mL methanol + 4.5 mL 1 M NaOH) was added to the samples in a 1:1 ratio (*i.e* 1.5 mL each). The mixture was centrifuged at 2500 × g for 3 min. 200 µL of the supernatant in the upper phase was taken and placed in a vial.

For MDA standards, 10µmol of 1,1,3,3 tetra-ethoxy-propane were dissolved in 50 mL of distilled water. Standards at 100, 50, 25, 12.5 and 6.25 µM concentrations were prepared by diluting the stock standard solution with distilled water at the ratios of 1/2, 1/4, 1/8, 1/16 and 1/32.The device's flow rate was 8 mL/min, and the injection volume was determined as 20 µL. The samples were read against the standards at 527 nm excitation and 551 nm emission wavelengths in the HPLC device.

An RP18 column (150 × 4.6 mm length and 5 µM particle size) was used as the measuring column for HPLC. The mobile phase consisted of 400 mL of 50 mM phosphate buffer (pH 6.8) and 600 mL of pure methanol. For phosphate buffer, 3.56 g of $Na_2HPO_4.2H_2O$ was weighed and dissolved in approximately 0.5 L of distilled water. The pH was adjusted to 6.8 with 1N HCl acid solution. The final volume was made up to 400 mL with distilled water.

Histopathological examination of kidney tissue

Left kidneys were placed in 10% formaldehyde solution and fixed for histopathological and immunohistochemical examinations. After, the samples were embedded in paraffin blocks following routine histological steps. Then, 4 µM thick sections taken with a microtome were stained with

Hematoxylin-Eosin and examined under a light microscope (Nikon Y-IM 7551012, Japan). In the histopathological evaluation, a mean of 15 fields for each rat in the groups was evaluated by random sampling. The findings were evaluated semiquantitatively according to the number of lesions observed in the examined regions. Accordingly, it was classified as: - (no lesions), mild: + (1-4 lesions), moderate: ++ (5-8 lesions), severe: +++ (9 or more lesions).

Immunohistochemical examination of kidney tissue

After the tissues were fixed in 10% buffered formaldehyde, they were embedded in paraffin blocks following routine histological steps. Sections of 4 µM thickness were taken from paraffin blocks to lysine slides with a microtome. Sections taken were deparaffinized and rehydrated. The samples were incubated in 3% hydrogen peroxide (H_2O_2) for 10 min to prevent endogenous peroxidase enzyme activity. They were heated in antigen retrieval (citrate buffer, pH 6.1) solution in a microwave oven 2 times to prevent antigen masking in the nucleus. Then, to prevent nonspecific binding, the protein block was dropped and incubated for 10 min. Caspase-3 (Santa Cruz Biotechnology, sc-7272, dilution: 1/50), Bax (Santa Cruz Biotechnology, sc-20067, dilution: 1/50), and Bcl-2 (Santa Cruz Biotechnology, sc-7382, dilution: 1/50) antibodies were used as primary antibodies. After adding the antibody, the samples were incubated in a humidity chamber at +4°C overnight. After washing with PBS, Biotinylated Goat Anti-Polyvalent and Streptavidin–peroxidase conjugate were dropped and incubated for 10 min, respectively. After the application of Diaminobenzidine (DAB), which is used as a chromogen, the samples were stained with Mayer's hematoxylin. In the immunohistochemical evaluation,10 fields were randomly selected for each group of rats and examined under a light microscope (Nikon Y-IM 7551012, Japan). In selected areas for each animal randomly in the groups, cells were counted as negative, low positive, moderately positive and high positive cells according to their immunoreactivity and evaluated with an H-score[28,29].

Statistical analysis

SPSS package program version 21 was used for statistical analysis. Biochemical results were expressed as mean ± standard deviation. Kolmogorov-Smirnov test was used to determine whether the data were normally distributed. For biochemical analysis, One Way ANOVA test was performed because the data were normally distributed and then Duncan's multiple range test were performed. The Kruskal-Wallis test was used to analyze whether the data from the immunohistochemical examination differed between groups. Mann-Whitney U test was used to compare groups in pairs. Results with $P \leq 0.05$ were considered significant.

Results

Before the study, the mean weights of all the groups were similar and there was no significant difference between them ($P > 0.05$). After the experiment, there was a difference between the weight averages of some groups ($P = 0.001$). Accordingly, the two groups with the lowest mean weight were the ABE and ABE+CMN groups. The weights of the ham, DMSO and CMN groups were similar.

Results of biochemical parameters in the serum samples

When the AQP1 values in serum samples were analysed, the AQP1 values of the ABE and ABE+CMN groups were considerably lower than those of the Sham, DMSO and CMN groups ($P = 0.001$). The AQP1 value of the ABE+CMN group was higher than the ABE group, but there was no significant difference between them ($P > 0.05$). (Fig. 1A)

In serum samples, TNF-α levels of the ABE group were higher than the TNF-α levels of the Sham, CMN and ABE+CMN groups. However, TNF-α values in the ABE+CMN group were lower than in the sham, DMSO and CMN groups ($P = 0.001$). (Fig. 1C)

When IL-1β values in serum samples were analyzed, it was observed that IL-1β levels in the ABE group were quite high compared to other groups ($P = 0.001$). In addition, serum IL-1β levels in the Sham group were higher than in the DMSO group ($P = 0.001$). Furthermore, serum IL-1β levels in the ABE+CMN group were lower than in the ABE and DMSO groups ($P = 0.001$) and were similar to the sham group values ($P > 0.05$). (Fig. 1E)

In serum samples, IL-10 values in the DMSO group were found to be higher than in the sham group ($P = 0.018$), while IL-10 levels in the ABE+CMN group were higher than in the ABE and sham groups ($P = 0.018$). In addition, serum IL-10 levels did not change much in the ABE and CMN groups ($P > 0.05$) and were similar to the sham values. (Fig. 1F)

When IL-37 values in serum samples were examined, IL-37 levels in the CMN group were lower than in the

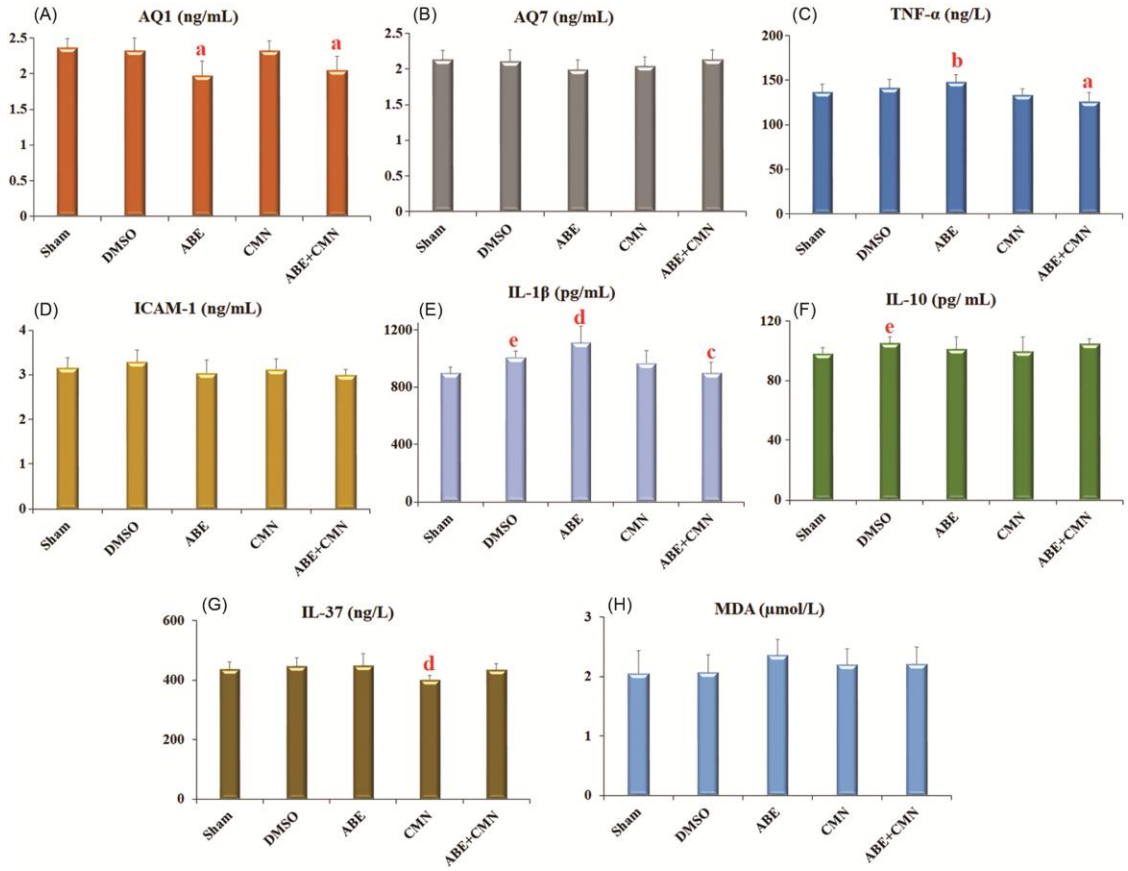

Fig. 1 — Comparison of aquaporins, MDA and cytokine levels in the serum samples. [a]p: It is significant according to the sham, DMSO and CMN groups (P <0.05), [b]p: It is significant according to the sham, CMN and ABE+CMN groups (P <0.05), [c]p: It is significant according to the DMSO group (P <0.05), [d]p: It is significant according to the other groups significant (P <0.05), [e]p: It is significant according to the sham group (P <0.05)

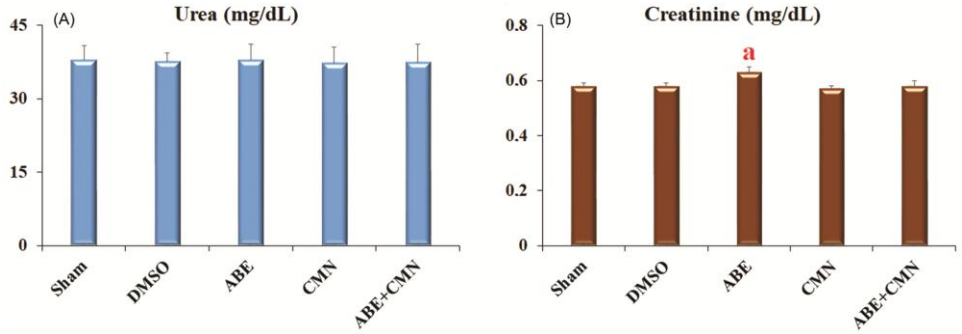

Fig. 2 — Comparison of urea (A) and creatinine; and (B) levels in serum samples. [a]p: It is significant according to the sham, DMSO and CMN groups (P <0.05)

other groups (P = 0.006). In addition, there was a non-significant increase in IL-37 levels in the ABE group compared to the sham group. However, there was a decrease in IL-37 levels in the ABE+CMN group compared to the ABE group, but their difference was not significant (P >0.05; Fig. 1G).When the AQP7, ICAM-1 and MDA values in serum samples were examined, no significant difference was found between the groups (P >0.05; Fig. 1B, D and H, respectively).

We also measured creatinine and urea levels in serum samples. Serum creatinine levels in the ABE group were significantly higher than in the Sham, DMSO and CMN groups (P = 0.021). The creatinine levels in the ABE+CMN group were lower than in the ABE group, but the difference was not significant (P >0.005). There was no significant difference between serum urea values (P >0.005) (Fig. 2).

Measurement of routine parameters in urine samples

Urine protein values of the ABE group increased dramatically compared to the other groups ($P \leq 0.001$). However, there was no significant difference between the other groups ($P > 0.05$). In addition, urine pH and density were similar in all groups, and there was no significant difference between them ($P > 0.05$ and Table 1).

Results of biochemical parameters in tissue samples

When the AQP1 values of the kidney tissue were compared, it was found that the AQP1 level of the ABE group was considerably lower than the other groups ($P = 0.001$). In addition, the AQP1 value of the CMN group was significantly higher than the other groups ($P = 0.001$). However, the AQP1 level of the ABE+CMN group was similar to the values of the sham group ($P > 0.05$; Fig. 3A). While the AQP7 level of the CMN group was higher than in the Sham, ABE and ABE+CMN groups, the AQP7 level of the ABE group was lower than in the DMSO group ($P = 0.007$). In addition, the AQP7 level of the ABE+CMN group was higher than in the ABE group,

Table 1 — Mean and standard deviation values of the biochemical parameters measured in the urine samples.

Groups	Sham	DMSO	ABE	CMN	ABE+CMN	P value
Protein (mg/dL)	7.70±2.01	7.64±1.53	50.52±23.21*	9.93±3.51	16.86±7.64	0.001
Urine Density	1025±8.1	1026±7.7	1037.4±17.5	1029.1±14.1	1039.8±10.1	0.09
Urine pH	7.14±0.48	7.07±0.79	6.71±0.57	7.57±0.73	7.75±0.85	0.085

*p: It is significant compared to the other groups ($P < 0.05$)

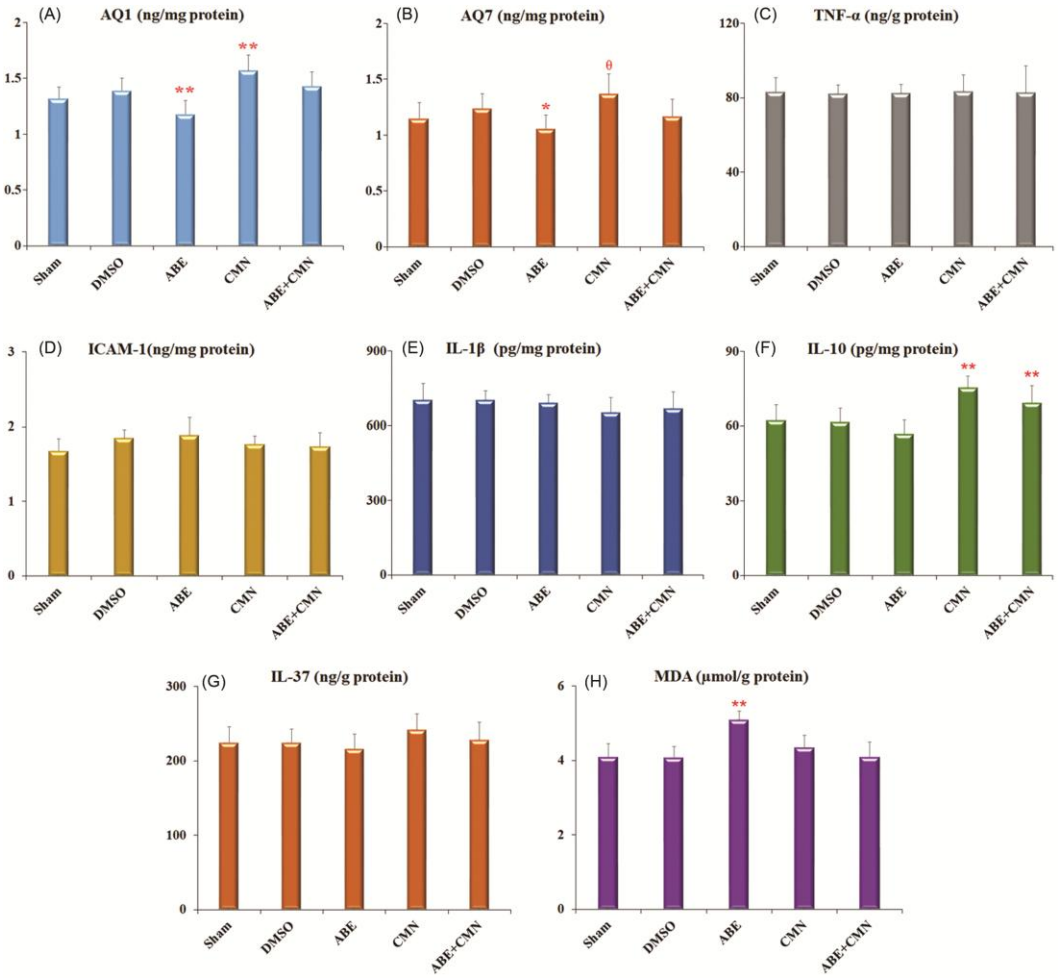

Fig. 3 — Comparison of aquaporins, MDA and cytokine levels in the kidney tissues. *p: It is significant according to the DMSO group ($P < 0.05$). **p: It is significant according to the other groups, ($P < 0.05$), θp: It is significant according to the sham, ABE and ABE+CMN groups ($P < 0.05$)

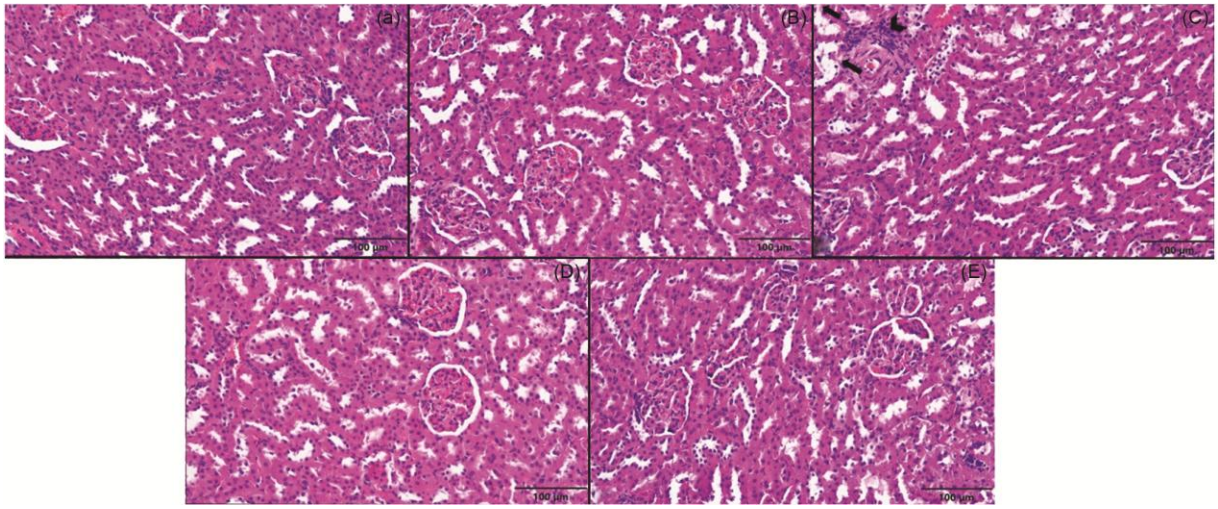

Fig. 4 — Light microscopy images of kidney tissue obtained in all the experimental groups. A and B: In the sham and DMSO groups, the standard histological structure of the kidney tissues is observed, respectively. C: It is observed that the glomerular and tubular integrity is impaired due to severe glomerular atrophy (thin arrow), moderate necrosis in the tubular epithelium (thick arrow) and mild interstitial leukocyte infiltration, in the kidney tissue of the rats in this group (arrowhead). D: In the CMN group, the normal histological structure of the kidney tissues is observed. E: Findings such as glomerular atrophy, necrosis of tubular epithelium and interstitial leukocyte infiltration were observed to improve significantly in the ABE+CMN group compared to the ABE group (H-E. Bar: 100 µM for all the images)

but the difference between them was not significant (P >0.05; Fig. 3B)

Tissue IL-10 levels were similar in the sham, DMSO and ABE groups, and the difference between them was not significant (P >0.05). The IL-10 level of the CMN group was considerably higher than in the other groups (P = 0.001). Also, IL-10 levels in the ABE+CMN group were significantly higher than in the sham, DMSO and ABE groups (P =0.001; Fig. 3F)

The MDA level of the ABE group was significantly higher than the other groups (P = 0.001). However, the MDA level of the ABE+CMN group was similar to that of the sham and DMSO groups (P >0.05; Fig. 3H). In addition, TNF-α, ICAM-1, IL-1β and IL-37 values of all groups were similar (P >0.05; Fig. 3C, D, E and G).

Results of biochemical parameters in urine samples

When the protein values of the urine samples were compared, it was determined that the protein level of the ABE group was relatively high compared to the other groups (P = 0.001). The protein level of the ABE+CMN group was similar to the values of the sham group, and there was no statistically significant difference between them (P >0.05). The urine density and pH values of all the groups were similar, and there was no significant difference between them (Table 1 and P >0.05).

Histopathological findings

It was observed that thekidney tissues of the sham (Fig. 4A), DMSO (Fig. 4B), and CMN (Fig. 4D) groups had typical histological structures. However, severe (+++) glomerular atrophy, moderate (++) tubular epithelial necrosis and mild (+) interstitial leukocyte infiltration were observed in the kidney tissue of rats in the ABE (Fig. 4C and Table 2) group. Based on these findings, it was observed that the integrity of the glomerulus and tubules was impaired. On the other hand, it was observed that these negative pathological findings improved significantly in the ABE+CMN (Fig. 4E and Table 2) group.

Table 2 — Semiquantitative results of histopathological examination in the kidney tissues

Groups	Glomerular Atrophy	Necrosis of tubular Epithelium	Interstitial leukocyte Infiltration
Sham	-	-	-
DMSO	-	-	-
ABE	+++	++	+
CMN	-	-	-
ABE+CMN	+	+	-

"-": none, "+": low, "++": moderate, "+++": severe

Caspase-3 immunoreactivity results

Caspase-3 expression was not detected in the sham (Fig. 5A), DMSO (Fig. 5B) and CMN (Fig. 5D) groups. While intense Caspase-3 expression was observed in the ABE (Fig. 5C) group compared to the sham group, less Caspase-3 expression was detected in the ABE+CMN (Fig. 5E) compared to the ABE group (Fig. 5C and 6).

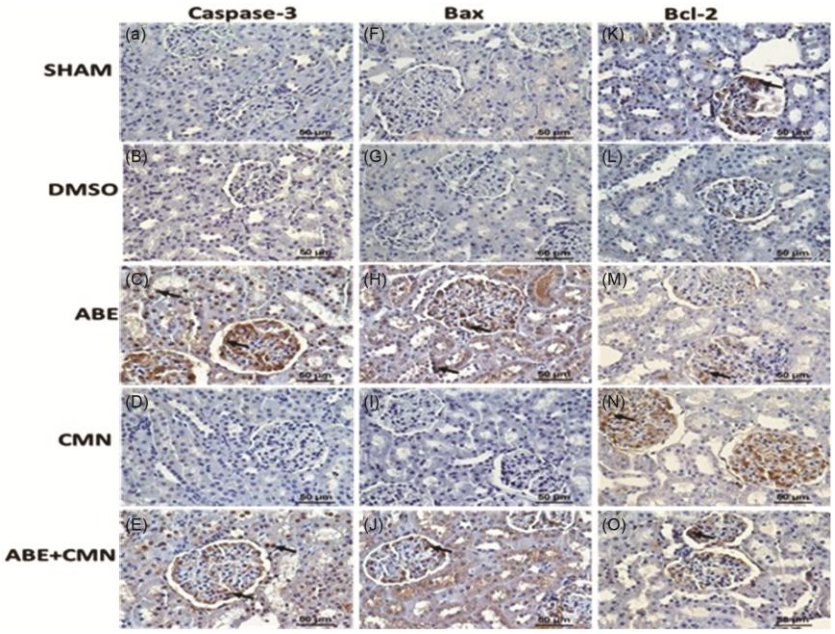

Fig. 5 — Immunohistochemical examination images of apoptosis factors in kidney tissue in all the experimental groups. A and B: In the sham and DMSO groups, Caspase-3 expression is not observed, respectively. C: Intense Caspase-3 expression is observed in the ABE group compared to the sham group. D: In the CMN group, Caspase-3 expression is not observed. E: Much less Caspase-3 expression was observed in the ABE+CMN group compared to the ABE group. F, G and I: In the sham, DMSO and CMN groups, Bax expression is not observed, respectively. H: Intense Bax expression is observed in the ABE group compared to the sham group. J: Less Bax expression is observed in the ABE+CMN group compared to the ABE group. K and L: In the sham, DMSO and ABE groups, low Bcl-2 expression is observed, respectively. N: In the CMN group, intense Bcl-2 expression is observed. O: In the ABE+CMN group, moderate Bcl-2 expression is observed (H-E. Bar: 50 μM for all the images)

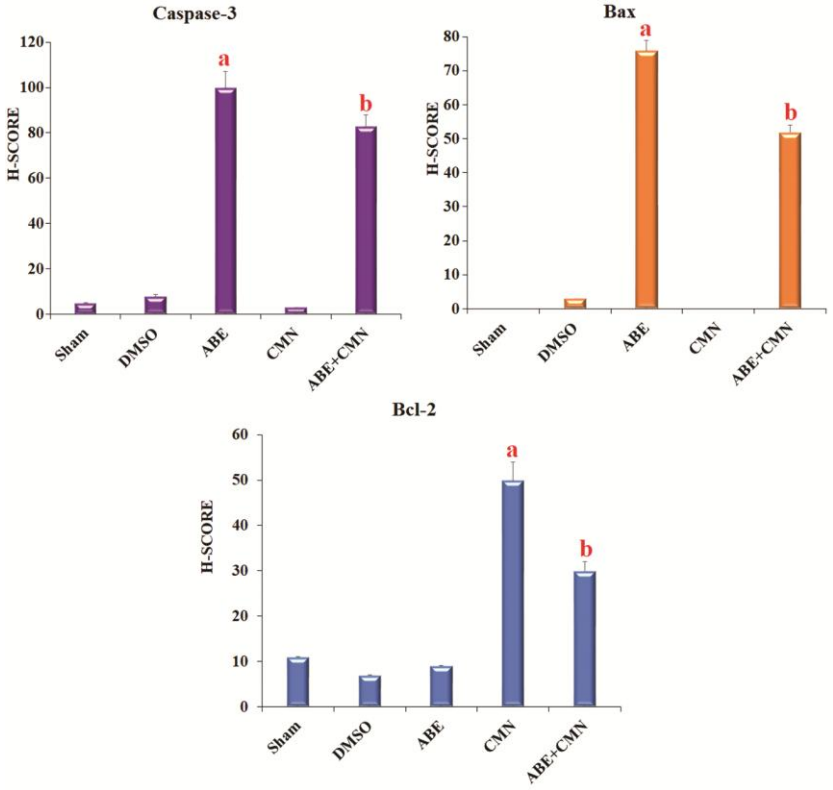

Fig. 6 — Comparison of Caspase-3, Bax and Bcl-2 immunoreactivities with H-Score in kidney tissues

Bax immunoreactivity results

While Bax expression was not found in the sham (Fig. 5F), DMSO (Fig. 5G) and CMN (Fig. 5I) groups, intense Bax expression was detected in ABE (Fig. 5H) group compared to the sham group. Also, less Bax expression was observed in the ABE+CMN (Fig. 5J) group compared to the ABE group (Fig. 5G and 6).

Bcl-2 immunoreactivity results

A low level of Bcl-2 expression was detected in the Sham (Fig. 5K), DMSO (Fig. 5L) and ABE (Fig. 5M) groups, while moderate Bcl-2 expression was observed in the ABE+CMN (Fig. 5O) group. In addition, intense Bcl-2 expression was determined in the CMN group (Fig. 5N and 6).

Discussion

This study provided important clues that ABE used in the treatment of breast cancer may have a toxic effect on kidney tissue, and the combined application of ABE and CMN may be protective. In previous studies, Zhou *et al.*[30] found that ABE application induced apoptosis by causing an increase in Caspase-3 expression in their flow cytometric analysis of human cardiomyocyte cell lines. Thibault *et al.*[8], on the other hand, found poorly differentiated degenerated enterocytes with proliferation, loss of goblet cells, loss of microvilli and inflammation in the crypt cells in their histopathological examination in the jejunum of rats treated with ABE. These reports revealed that ABE administration could exert intestinal toxicity-producing effects in rats. In the literature, we could not find any studies examining the possible nephrotoxic effects of ABE administration on aquaporins, inflammatory cytokines, apoptosis factors and histopathology. However, Bae *et al.*[31] found that aquaporin (AQP) 1 and 3 levels, which have an important role in the reabsorption of water from the filtrate in the kidney, were low in the nephrotoxicity model due to another anti-cancer drug, cisplatin. Lim *et al.*[32] found that cyclosporine, another nephrotoxic drug, decreased the amount of AQP 1 and 4 in rats. However, Foxley *et al.*[33] found that CMN increased AQP4 levels associated with increased atrophy and edemain lupus model mice. Our study determined that ABE significantly reduced AQP1 levels in kidney tissue and serum compared to other groups. In addition, AQP7 levels were lower in the ABE group's kidney tissue than in the other groups. However, combined administration of ABE and CMN partially restored AQP1 andAQP7 levels compared to the ABE group. These results showed that ABE may cause a decrease in kidney AQP1 and AQP7 levels in rats, resulting in impaired reabsorption of water in the kidneys and adversely affecting renal function. In addition, the fact that CMN causes a slight increase in AQP1 and AQP7 levels indicated that CMN can prevent the negative effect of ABE on AQP1 and AQP7.

The cytokine response characterized by the activation of chemokines and pro-inflammatory cytokines has an important role in cisplatin nephrotoxicity. It has been reported that an increase in TNF-α levels plays a central role in the activation of this cytokine response and the pathogenesis of cisplatin-induced kidney damage[34]. In this study, we found that serum TNF-α levels increased dramatically in the ABE administered group compared to the sham group. Similarly, Goel *et al.*[35] showed in a study that ABE application could induce TNF-α production in human breast cancer cell lines. Sahebkar *et al.*[36] stated that CMN had an anti-inflammatory effect by suppressing TNF-α levels. This result coincides with the significantly lower TNF-α values in the ABE+CMN group compared to the ABE group in our study. The fact that no difference was detected between the levels of TNF-α measured in the kidney tissue indicated that the pro-inflammatory effect mediated by the increased TNF-α due to ABE may have originated from non-renal tissues.

IL-1β, which is a pro-inflammatory cytokine released from macrophages, B-lymphocytes, monocytes, natural killers and dendritic cells, is frequently used in studies for the detection of drug-induced nephrotoxicity[37,38]. In our study, serum IL-1β levels were found to be higher in the ABE group compared to the other groups. We could not find a study in the literature examining the effect of ABE on IL-1β in kidney tissue. However, the FDA issued a recent warning that the patients treated with CDK4/6 inhibitors such as ABE, palbociclib, and ribociclib may develop a rare (1-3%) but life-threatening inflammation, namely interstitial lung disease[39]. This development suggests that the relationship between CDK inhibitors and inflammation should be examined more closely. In our study, serum IL-1β levels in the ABE+CMN group were significantly lower than in the ABE group and were similar to the values of the sham group. This was consistent with previous studies that CMN had an anti-inflammatory effect by

suppressing IL-1β levels[40,41]. The fact that no difference was detected between IL-1β levels measured in kidney tissue suggests that the pro-inflammatory effect of ABE through increased IL-1β may have originated from non-renal tissues. In addition, in our study, it was observed that IL-1β values in the DMSO group were higher than in the sham group. This indicated that DMSO may cause an inducing effect on IL-1β levels. DMSO is a good solvent frequently used in animal experiments. For this reason, DMSO, which is one of the solvents that can best dissolve CMN, was used in this study. On the other hand, Xiang *et al.*[42] stated that DMSO administration might cause increased IL-1β. This finding is consistent with our research.

IL-10 is a anti-inflammatory cytokine that strongly inhibits the inflammatory and cytotoxic pathways that cause acute kidney injury[43]. In this study, IL-10 values of the ABE group were partially lower than in the sham and DMSO groups in kidney tissue. However, the IL-10 values of the CMN group were significantly higher than the other groups. This result is consistent with previous studies in which CMN showed anti-inflammatory effects by increasing IL-10 levels[44,45]. Also, in kidney tissue, IL-10 levels of the ABE+CMN group were significantly lower than those of the CMN group. Zhang *et al.*[46] found that ABE suppressed IL-10 levels in B lymphocytes in patients with ovarian cancer. The results obtained from our study also revealed that ABE administration might have a suppressive effect on IL-10 levels in kidney tissue. This indicates that ABE may show inflammation-enhancing effects.

It was reported that IL-37 was expressed in various autoimmune disorders, cancers and chronic inflammatory and exhibited anti-inflammatory effects[47]. In our study, IL-37 levels in serum and kidney tissue of the CMN group were lower than in all the other groups. In addition, there was a partial increase in IL-37 levels in the ABE group compared to the sham group. These results showed that ABE could increase IL-37 levels, and CMN could have a suppressive effect on IL-37 levels. ABE's inducing effects on IL-1β, TNF-α and IL-37 levels and suppressive effects on IL-10 levels indicated that it might mediate pro-inflammatory effects. However, although the increasing impact of ABE on IL-37 levels is interesting, this may have resulted from the physiological response to the increase in IL-1β and TNF-α.

Increased oxidative stress is an important molecular mechanism of drug-induced nephrotoxicity[48]. MDA is the end product of lipid peroxidation and is widely used to detect lipid damage induced by reactive oxygen species (ROS). In this study, in serum and kidney tissue, the MDA level of the ABE group was higher than the other groups. These results indicate that the administration of ABE in rats may increase MDA levels by causing lipid peroxidation and thus show nephrotoxic effects. We could not find any study in the literature showing the impact of ABE administration on MDA levels. However, Franco *et al.*[49] explored in a survey that ABE caused an increase in ROS by changing the oxidative metabolism in a pancreatic ductal adenocarcinoma cell line. In addition, Klein *et al*. stated that inhibition of CDK6 in T-cell acute lymphoblastic leukaemia may deplete antioxidants by changing cellular metabolism and induce apoptosis by increasing ROS[50]. In our study, the MDA level of the ABE+CMN group was similar to that of the sham and DMSO groups. This result indicates that ABE increased MDA levels in the serum and kidneys of rats, but CMN may have a therapeutic effect on kidney damage caused by oxidative stress. This finding that CMN exerts a renoprotective influence thanks to its antioxidant property is consistent with previous studies[51,52].

Creatinine is formed by the dehydration of creatine, which is in the creatine phosphate structure in muscle tissue and is one of the most frequently used tests to evaluate kidney functions. In this study, serum creatinine values in the ABE group were significantly higher than in the sham, DMSO, and CMN groups. This finding is consistent with the results of elevated creatinine in phase studies conducted before the introduction of ABE[4]. This result showed that ABE might adversely affect kidney functions. In our research, creatinine values in the ABE+CMN group were partially lower than in the ABE group. Another test frequently used in the routine monitoring of kidney functions is a measurement of serum urea levels. However, there was no significant difference between serum urea values in this study.

Proteinuria is one of the critical indicators of drug-induced nephrotoxicity[53]. In our study, when the protein values of the urine samples were compared, the protein level of the ABE group was higher than the other groups. A clinical study, found that ABE administration combined with Pembrolizumab caused

proteinuria in 33% of patients[54]. In addition, Omarini *et al*. reported that proteinuria developed in a patient receiving ABE treatment, but this could be due to Henoch Schönlein's purpura[55]. In this study, the urine protein level of the ABE+CMN group was similar to the values of the excretion group. In a review where they described the renoprotective properties of CMN, Trujillo *et al*. stated that CMN reduces proteinuria[12]. These results suggest that ABE administration in rats may cause proteinuria by negatively affecting kidney functions, and CMN can repair this.

An important subcellular mechanism of drug-induced nephrotoxicity is increased apoptosis[56]. Caspase-3 is the most studied member of the caspase family and one of the critical drivers of apoptosis[57]. Caspase-3 expression was not observed in this study. Intense Caspase-3 expression was detected in the ABE group compared to the sham group. This result showed that ABE increased caspase-3 expression levels and induced apoptosis. This finding is also consistent with the data of a recent study by Zhou *et al.* examining the apoptotic effects of ABE on cardiomyocytes[30]. In this study, less caspase-3 expression was found in the ABE+CMN group compared to the ABE group. This indicated that CMN exerted an anti-apoptotic effect by suppressing Caspase-3 expression, and this result was consistent with previous studies[58,59].

Antiapoptotic Bcl-2 and proapoptotic Bax are two critical molecules involved in cell death, and the Bax / Bcl-2 ratio is an important indicator that reflects whether cells will enter apoptosis[60]. In this study, Bax expression was not observed in the Sham, DMSO and CMN groups. While intense Bax expression was detected in the ABE group compared to the sham group, less Bax expression was seen in the ABE+CMN group compared to the ABE group. In addition, Bcl-2 expression levels measured in the sham, DMSO and ABE groups were similar and low, whereas moderate Bcl-2 expression was observed in the ABE+CMN group and intense in the CMN group. These findings showed that ABE administration in rats could induce apoptosis in kidney tissue by increasing the Bax/Bcl2 ratio. Still, CMN administration could reverse this situation with its antiapoptotic effect and reveal a nephroprotective effect. Consistent with our findings, Cao *et al.* found that ABE induced apoptosis by increasing the Bax/Bcl2 ratio in human glioma cell lines[61]. However, we could not find any study in the literature on how the condition may be shaped in kidney tissue. Zhao *et al*. revealed that CMN had an antiapoptotic effect by reducing the Bax/Bcl2 ratio in the testicles of diabetic rats exposed to streptozocin[60]. This result was consistent with our finding that CMN suppressed apoptosis by reducing Bax/Bcl2 ratios in kidney tissue.

In tissue toxicity studies, it is also important to evaluate whether the tissue is in a normal structure[50,51]. In our study, the kidney tissues of the sham, DMSO and CMN groups were observed to have a normal histological structure, while severe glomerular atrophy, moderate necrosis in the tubular epithelium, and mild interstitial leukocyte infiltration were observed in the kidney tissue of the rats in the ABE group. Accordingly, it was determined that the integrity of the glomerulus and tubules was impaired. These histopathological changes were consistent with the results of the study performed by Sepehri *et al.* in which they examined the histopathological change in the kidneys caused by nephrotoxicity formed by gentamicin[61]. Sepehri *et al*. stated that severe kidney damage, characterized by significant acute tubular necrosis, glomerular atrophy, infiltration of lymphocytic inflammatory cells in the cortex, and interstitial nephritis, occurred in the gentamicin given rat group compared to the control group. In addition, in our study, it was observed that the negative pathological findings observed in the ABE+CMN group were significantly reduced compared to the ABE group. This finding is consistent with the results of Mahmoud *et al*.'s study in which they investigated whether gentamicin-induced nephrotoxicity could be treated with CMN[62]. These results showed that ABE may have adverse effects on the histological structure of the kidney and CMN could significantly repair these negative effects and had a renoprotective effect thanks to its antioxidant, anti-inflammatory and anti-apoptotic properties.

Conclusion

This study showed that ABE could lead to the induction of apoptosis with an increase in pro-inflammatory cytokines and oxidative stress in rats and that it could adversely affect kidney functions and morphology in this way. However, it showed that CMN therapy in combination with ABE may be protective against the nephrotoxic effects of ABE. However, studies investigating different oxidative stress indicators, inflammatory cytokines and apoptosis markers at the molecular level are needed to

better understand the effects of ABE on nephrotoxicity and to investigate the possible protective effects of CMN therapy in combination with ABE. In addition, it is recommended to conduct new research at the cell culture and molecular level to understand the mechanisms by which CMN administered in combination with ABE and ABE exhibit its effects on the changing parameters in this study.

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Conflicts of interest

All authors declare no conflict of interest.

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