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Naringin attenuates oxidative stress, inflammation, apoptosis, and oxidative DNA damage in acrylamide-induced nephrotoxicity in rats

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ABSTRACT

Objective: To explore the possible effects of naringin on acrylamide-induced nephrotoxicity in rats.

Methods: Sprague-Dawley rats weighing 200-250 g were randomly divided into five groups. The control group was given intragastric (*i.g.*) saline (1 mL) for 10 d. The acrylamide group was given *i.g.* acrylamide in saline (38.27 mg/kg titrated to 1 mL) for 10 d. The treatment groups were administered with naringin in saline (50 and 100 mg/kg, respectively) for 10 d and given *i.g.* acrylamide (38.27 mg/kg) 1 h after naringin injection. The naringin group was given *i.g.* naringin (100 mg/kg) alone for 10 d. On day 11, intracardiac blood samples were obtained from the rats when they were under anesthesia, after which they were euthanized. Urea and creatinine concentrations of blood serum samples were analyzed with an autoanalyzer. Enzyme-linked immunosorbent assay was used to quantify malondialdehyde, superoxide dismutase, glutathione, glutathione peroxidase, catalase, tumor necrosis factor-α, nuclear factor-κB, interleukin (IL)-33, IL-6, IL-1β, cyclooxygenase-2, kidney injury molecule-1, mitogen-activated protein kinase-1, and caspase-3 in kidney tissues. Renal tissues were also evaluated by histopathological and immunohistochemical examinations for 8-OHdG and Bcl-2.

Results: Naringin attenuated acrylamide-induced nephrotoxicity by significantly decreasing serum urea and creatinine levels. Naringin increased superoxide dismutase, glutathione, glutathione peroxidase, and catalase activities and decreased malondialdehyde levels in kidney tissues. In addition, naringin reduced the levels of inflammatory and apoptotic parameters in kidney tissues. The histopathological assay showed that acrylamide caused histopathological changes and DNA damage, which were ameliorated by naringin.

Conclusions: Naringin attenuated inflammation, apoptosis, oxidative stress, and oxidative DNA damage in acrylamide-induced nephrotoxicity in rats.

KEYWORDS: Acrylamide; Nephrotoxicity; Apoptosis; Inflammation; Naringin; Oxidative stress; Oxidative DNA damage

1. Introduction

Acrylamide, one of the foremost wastes in the environment, is a chemical compound widely used in polyacrylamide and dye

Significance

Acrylamide is one of the most important wastes in the environment, and it has caused damage to kidney tissues in experimental studies. Although previous studies showed that some flavonoids can prevent the harmful effects of acrylamide on kidney tissue, the effect of naringin on acrylamide-induced nephrotoxicity is not investigated yet. In our study, naringin protected against acrylamide-induced kidney damage by inhibiting oxidative stress, inflammation, apoptosis, and oxidative DNA damage. It should be further explored as a possible treatment of nephrotoxicity.

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synthesis, textiles, paper manufacturing, and the cosmetic industry[1]. In addition, tobacco and cigarette smoke contain high concentrations of acrylamide[2]. Acrylamide has some systemic toxic effects as a result of either occupational or dietary exposure in humans[1]. Acrylamide is formed through operations such as frying or grilling at high temperatures, manufacturing crackers, and bread and roasting coffee[3]. Glycidamide is a metabolite of acrylamide, and it induces DNA damage. After inhalation or ingestion, acrylamide accumulates in the blood more than in tissues. Acrylamide causes deleterious effects, such as inflammatory cell infiltration and periglomerular edema, in renal tubular cells[4]. In addition, acrylamide has been reported to cause renal oxidative stress, inflammation, apoptosis, and DNA damage in rats and increases in serum urea and creatinine levels[5]. There are various studies in which some flavonoids found in plants are used for therapeutic or preventive purposes in drug-induced organ toxicity models in rats[6]. Naringin is a flavonoid compound used for this purpose[7]. Naringin is mostly found in tomato paste, citrus juices, and citrus fruits such as grapefruit and orange[7]. Naringin has antioxidant and anti-apoptotic properties[8] and displays radical scavenging activity[9]. Furthermore, naringin has anti-inflammatory effects by suppressing proinflammatory cytokines[10]. The effects of naringin in experimental organ toxicity models have been reported in many studies[7]. There is scarce study on the possible effects of naringin on acrylamide-induced renal injury in rats. In this study, we aimed to determine the possible protective effects of naringin on acrylamide-induced nephrotoxicity in rats.

2. Materials and methods

2.1. Chemicals

Acrylamide (≥ 99%) and naringin (CAS Number:10236-47-2) were supplied by Sigma Chemical Co. (St. Louis, MO). Malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-33 (IL-33), kidney injury molecule-1 (KIM-1), nuclear factor kappa-B (NF-κB), cyclooxygenase-2 (COX-2), mitogen-activated protein kinase-1 (MAPK-1), and caspase-3 enzyme-linked immunosorbent assay (ELISA) kits were purchased from YL Biont (Shangai, China). 8-Hydroxy-2'-deoxyguanosine (8-OHdG) and B-cell lymphoma 2 (Bcl-2) antibodies for immunohistochemical staining were purchased by Abcam (United Kingdom).

2.2. Animals

In our study, 50 male Sprague-Dawley rats approximately weighing 200-250 g were obtained from Atatürk University Medical Experimental Application and Research Center and were housed at a room temperature of 25 ℃ with a standard 12-h light-dark cycle, ample ventilation, and *ad libitum* access to standard diet (Bayramoglu Feed and Flour Industry Trade A.Ş., Erzurum) and water.

2.3. Experimental design and treatment protocol

The rats were weighed and their weights were standardized. The rats were randomly divided into five groups and given different treatments. Rats in the control group were given intragastric (*i.g.*) saline (1 mL) daily for 10 d[11]. Rats in the acrylamide group were given acrylamide (38.27 mg/kg, *i.g.*)[11] in 1 mL of saline daily for 10 d. Rats in the treatment groups were given naringin (50 mg/kg or 100 mg/kg, *i.g.*)[12] for 10 d. Acrylamide (38.27 mg/kg, *i.g.*) was then administered to rats 1 h after naringin treatment. Rats in the naringin group were given naringin (100 mg/kg, *i.g.*) alone for 10 d.

2.4. Determination of the ratio of kidney weight to body weight of rats and blood samples

Rats were weighed on the 11th day of experimental administrations, and blood samples were taken while the rats were under anesthesia. The rats were decapitated after being euthanized. The kidneys were extracted from the rats in the experimental groups and weighed. The left kidney of each rat was washed and immediately placed in formaldehyde for histopathological examination. The other kidney was washed and then frozen and stored at −20 ℃.

2.5. Analysis of renal function markers

The blood samples obtained were placed in serum tubes and centrifuged for 10 min at 4 ℃. Urea and creatinine parameters were determined with the help of a modular auto-analyzer.

2.6. Analysis of renal oxidative stress indicators

MDA level[13], GSH[14], CAT[15], SOD[16], and GPx[17] activity in the kidney tissues of rats were measured using the corresponding anti-rat ELISA kits (YL Biotech, Shanghai, China) according to the manufacturer's instructions.

2.7. Determination of cytokines in renal tissues

Kidney tissues obtained from the experimental groups were homogenized under appropriate conditions, then the levels of NF-κB, TNF-α, IL-6, IL-33, IL-1β, and COX-2 were measured using anti-rat ELISA kits (YL Biont, Shanghai, China) according to the manufacturer's instructions.

2.8. Analysis of KIM-1, MAPK-1, and caspase-3 levels

According to the manufacturer's instructions, the levels of KIM-1, caspase-3 and MAPK-1 were measured using anti-rat ELISA kits (YL Biont, Shanghai, China).

2.9. ELISA study protocol

Before starting the study, the samples and the kit were brought at room temperature: 24 ng/mL for SOD, 320 ng/mL for CAT, 2 000 ng/mL for GSH, 12.8 mmol/mL for MDA, 12.8 ng/mL for KIM-1, 1 280 ng/L for COX-2, 48 ng/L for IL-6, 240 ng/mL for GPx, 480 ng/mL for IL-33, 9 600 pg/mL for IL-1β, 80 ng/mL for MAPK-1, 1 280 ng/L for NF-κB, 1 280 ng/L for caspase-3, and 1 280 ng/L for TNF-α were obtained by serial dilutions of stock standards. Then, 50 μL of each standard was pipetted. After pipetting 40 μL of the sample (supernatant, serum, or perfusate) into the sample wells of the antibody-coated microplate, 10 μL of the relevant antibody was added for each parameter examined. Then 50 μL of HRP-conjugate (horseradish peroxidase) was added to all wells. The microplate was incubated for 1 h at 37 ℃. After incubation, the 30× washing solution in the kit was prepared with distilled water and washed 4 times (350 μL) with a microplate ELISA washer. Subsequently, 50 μL of solution A and solution B were pipetted into all wells, respectively. The microplate was then incubated at 37 ℃ for 15 min without light. Finally, 50 μL of stop solution was pipetted into all wells and the microplate was read in a plate reader at 450 nm absorbance.

2.10. Histopathological analysis

After autopsies of the rats in the experimental groups, the left kidney tissues were fixed in 10% formalin solution for 48 h. Samples were embedded in paraffin blocks after tissue follow-up procedures. Afterward, sections were taken from each block with a thickness of 4 μm. Tissue sections were stained with hematoxylin-eosin and examined under a light microscope (Leica DM1000, Germany) at ×40 magnification. The relative severity of congestion in the vessels, necrosis of the tubular epithelium, and degeneration of the tubular epithelium was scored in a semiquantitative manner, which ranged from 0 (normal), 1-10 (mild), 11-20 (moderate), and 21-30 (severe).

2.11. Immunohistochemical analysis

The expression of 8-OHdG and Bcl-2 in kidney tissues was determined by specific monoclonal antibodies in kidney sections taken from rats. Diaminobenzidine was used as a chromogen and the slides were counterstained with hematoxylin, dried, and covered with coverslips. Immunohistochemical staining of 8-OhdG and Bcl-2 was performed using an anti-8-OHdG antibody (Cat no: sc-66036, dilution 1:50; Santa Cruz) with a Novolink Polymer Detection Kit (Leica Microsystems Pte Ltd., Taipei, Taiwan), as well as an anti-Bcl-2 antibody (Cat no: sc-509, dilution 1:50; Santa Cruz), respectively, according to the manufacturer's instructions. At least ten high-power fields (×200) were taken per section and the number of positive cells in each high-power field was counted (light microscope at ×40 magnification).

2.12. Statistical analysis

The data were analyzed by one-way ANOVA followed by *t* test using SPSS 20.00 statistical data program. All values were expressed as mean ± standard error mean (SEM) and $P<0.05$ was considered significant.

For immunohistochemical examination, to determine the intensity of positive staining from the obtained images, 5 random areas were selected from each image. As a result of the antibody staining used for the evaluation process, the positive/total area was calculated by measuring with the ZEISS Zen Imaging Software program. Data were presented as mean ± standard error mean (mean ± SEM) for % area. As a result of the test, a *P* value of <0.05 was considered significant.

2.13. Ethical statement

The protocol of this study was approved by the Atatürk University Rectorate Animal Experiments Local Ethics Committee (HADYEK 2020/205).

3. Results

3.1. Effect of naringin on the ratio of kidney weight to body weight

Acrylamide-treated rats showed significantly decreased body weight when compared with the control and naringin alone groups ($P<0.05$). Although kidney weight was decreased in the acrylamide-treated groups, there was no significant difference compared with the control group. In addition, the ratio of kidney weight to body

Table 1. Body weights, kidney weights, kidney/body weight ratio, serum urea, and creatinine levels of rats.

Group	Initial body weights (g)	Final body weights (g)	Absolute kidneys weights (g)	Kidney/body weight ratio (×1 000)	Urea (mg/dL)	Creatinine (mg/dL)
Control	220.30±10.50[a]	274.60±14.91[a]	1.18±0.08[a]	4.29±0.41[a]	34.32±3.17[a]	0.61±0.08[a]
ACR	221.00±12.62[a]	189.50±21.43[b]	1.05±0.12[a]	5.54±0.49[b]	55.44±5.19[b]	0.84±0.07[b]
NA50+ACR	207.60±16.25[a]	205.40±15.12[b]	1.03±0.08[a]	5.01±0.65[ab]	43.46±5.48[c]	0.71±0.03[a]
NA100+ACR	211.00±17.45[a]	213.80±14.05[b]	1.09±0.12[a]	5.09±0.40[ab]	39.25±2.65[ac]	0.69±0.03[a]
NA100	213.20±16.78[a]	246.20±16.08[a]	1.09±0.14[a]	4.42±0.39[a]	35.31±2.28[a]	0.66±0.06[a]

Values are given as mean ± SEM (n=10) and analyzed by one-way ANOVA followed by *t*-test. Different letters indicate statistically significant differences, $P<0.05$. ACR: acrylamide; NA: naringin.

weight was significantly increased in the acrylamide group compared with the control group (P<0.05). Moreover, 50 and 100 mg/kg of naringin decreased the ratio of kidney weight to body weight with no significant difference compared with the acrylamide group (Table 1).

3.2. Effects of naringin on renal injury markers

Acrylamide significantly increased serum urea levels (P<0.05). Naringin at 50 and 100 mg/kg prominently diminished the

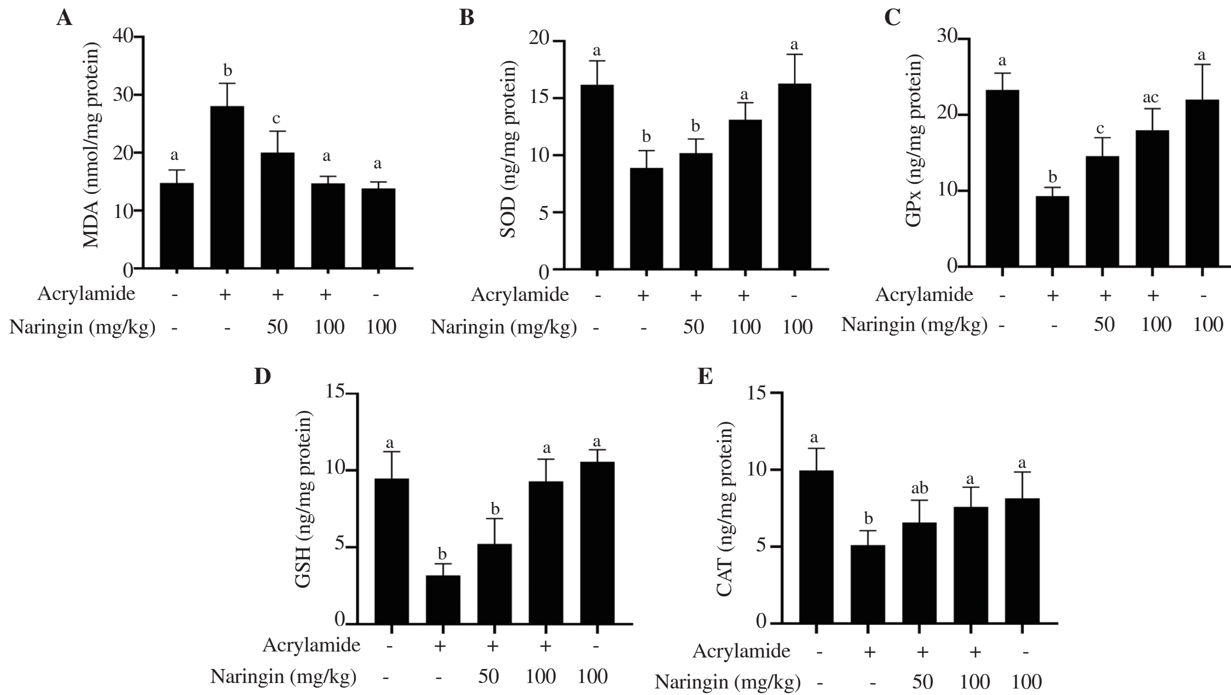

Figure 1. Effects of naringin on renal oxidant and antioxidant parameters in acrylamide-induced nephrotoxicity in rats. Values are given as mean ± SEM (n=10) and analyzed by one-way ANOVA followed by t-test. Different letters indicate statistically significant differences, P<0.05. MDA: malondialdehyde; GSH: glutathione; SOD: superoxide dismutase; GPx: glutathione peroxidase; CAT: catalase.

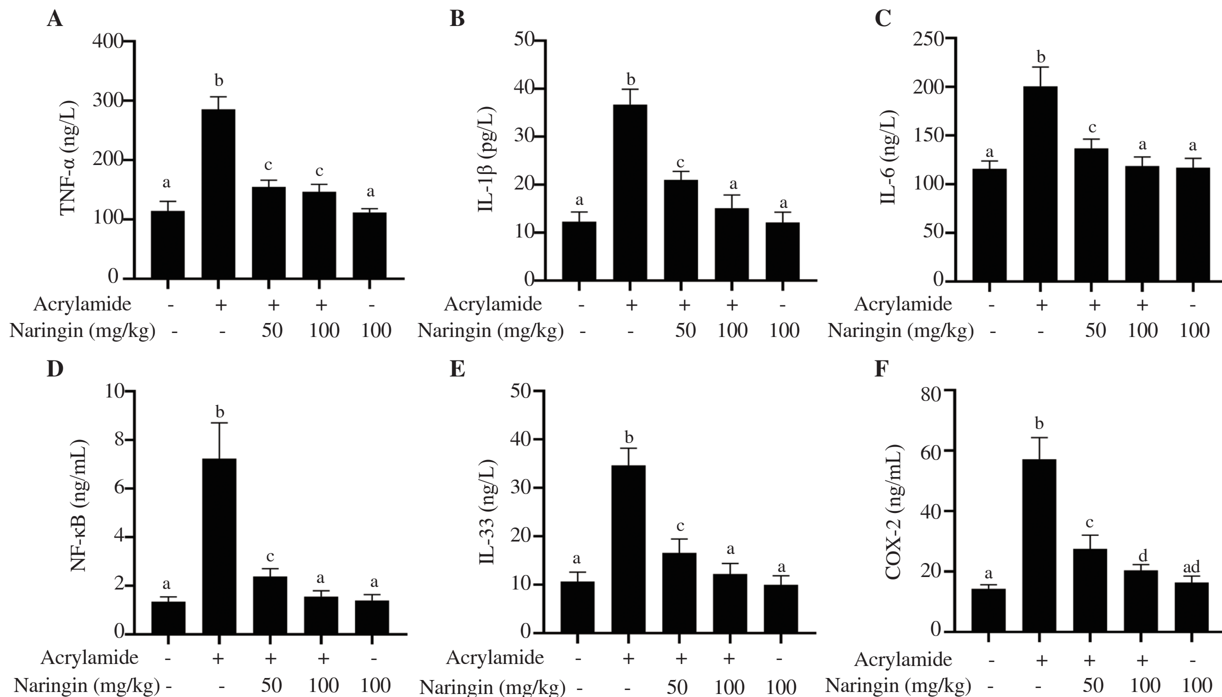

Figure 2. Effects of naringin on inflammatory parameters in acrylamide-induced nephrotoxicity in rats. Values are given as mean ± SEM (n=10) and analyzed by one-way ANOVA followed by t-test. Different letters indicate statistically significant differences, P<0.05.

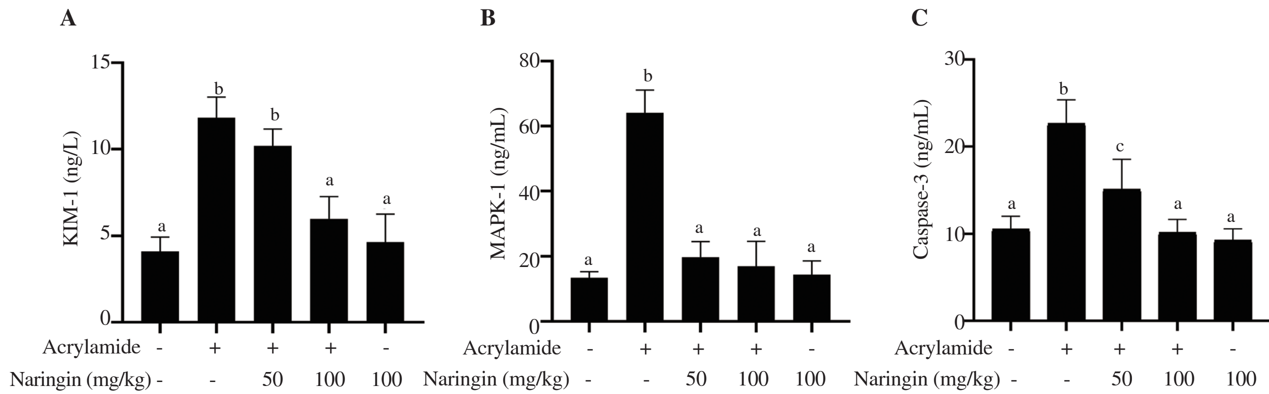

Figure 3. Effects of naringin on KIM-1, MAPK-1, and caspase-3 levels in kidney tissues of rats. Values are given as mean ± SEM (n=10) and analyzed by one-way ANOVA followed by t-test. Different letters indicate statistically significant differences, P<0.05. KIM-1: kidney injury molecule-1, MAPK-1: mitogen-activated protein kinase-1.

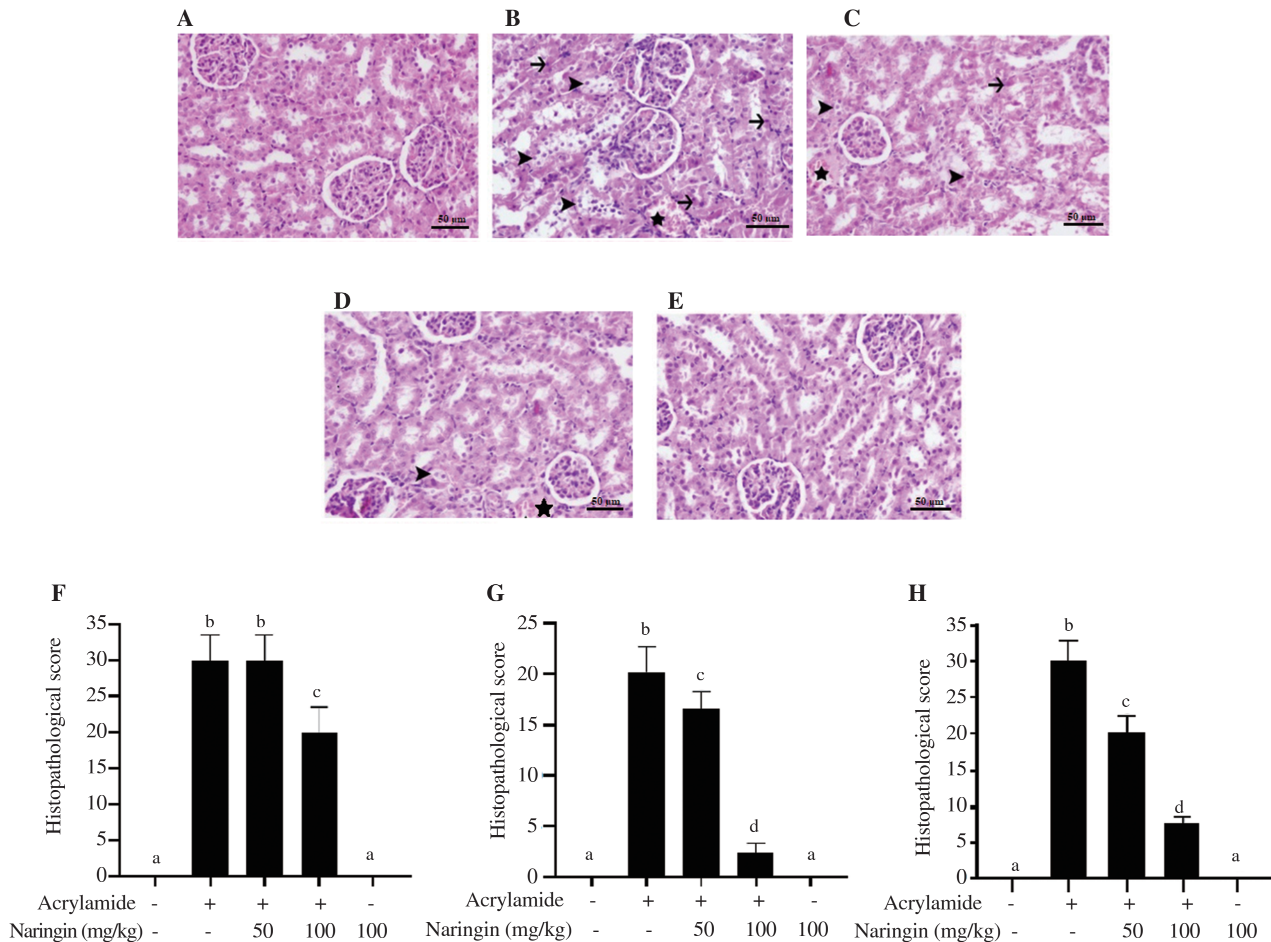

Figure 4. The histopathological findings of all experimental groups. (A) The control group shows normal histological appearance. (B) The acrylamide treated group shows severe degeneration of the renal tubular epithelium (arrowhead), necrosis (arrows), and hyperemia in vessels (star). (C) The group treated with acrylamide and 50 mg/kg naringin shows moderate degeneration of the renal tubular epithelium (arrowheads), moderate necrosis (arrow), and severe hyperemia (star). (D) The group treated with acrylamide and 100 mg/kg naringin shows mild degeneration of the renal tubular epithelium (arrowhead) and moderate hyperemia (star) in vessels. (E) The group treated with 100 mg/kg naringin alone shows a normal renal structure. H&E, Bar: 50 µm; Magnification: ×40. (F-H) Bar graphs show the results of congestion in the vessels, necrosis of the tubular epithelium, and degeneration of the tubular epithelium. The data are expressed as mean ± SEM (n=10) and analyzed by one-way ANOVA followed by t-test. Different letters indicate statistically significant differences, P<0.05.

acrylamide-induced serum urea level ($P<0.05$). A similar trend was also observed in serum creatinine level of the acrylamide group. Naringin reversed the acrylamide-induced changes in serum creatinine level (Table 1).

3.3. Effects of naringin on MDA level and antioxidants enzymes

Acrylamide markedly elevated the renal MDA level (Figure 1A) and decreased SOD (Figure 1B), GPx (Figure 1C), GSH (Figure 1D), and CAT (Figure 1E) activities ($P<0.05$). However, naringin reduced acrylamide-induced MDA level and increased SOD, GPx, GSH, and CAT activities, especially at a dose of 100 mg/kg ($P<0.05$).

3.4. Effects of naringin on inflammatory parameters

In the acrylamide group, significantly elevated levels of IL-1β, IL-6, TNF-α, NF-κB, IL-33, and COX-2 levels were observed ($P<0.05$).

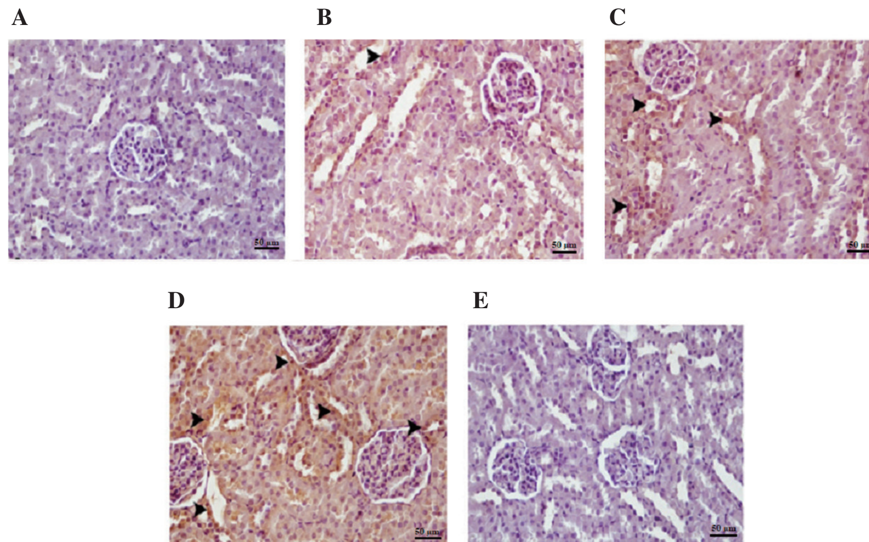

Figure 5. Bcl-2 expression in the kidney tissues of rats. (A) The Bcl-2 expression is negative in the control group. (B) Mild cytoplasmic Bcl-2 expression is observed in the tubular epithelium of acrylamide-treated rats (arrowheads). (C) Moderate cytoplasmic Bcl-2 expression is found in the group treated with acrylamide and 50 mg/kg of naringin (arrowheads). (D) The group treated with acrylamide and 100 mg/kg of naringin shows severe cytoplasmic Bcl-2 expression in the tubular epithelium (arrowheads). (E) Bcl-2 expression is negative in the group treated with 100 mg/kg of naringin alone. IHC-P, Bar: 50 μm; Magnification: ×40.

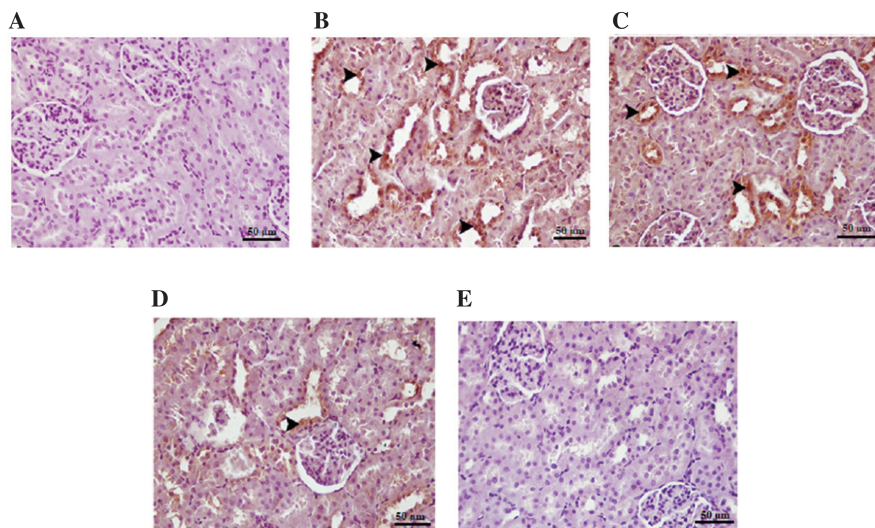

Figure 6. 8-OHdG expression in the kidney tissues of rats. (A) The 8-OHdG expression is negative in the control group. (B) Severe 8-OHdG expression in the tubular epithelium is found in the acrylamide group (arrowheads). (C) Moderate 8-OHdG expression is found in the group treated with acrylamide and 50 mg/kg of naringin (arrowheads). (D) Mild 8-OHdG expression in the tubular epithelium is observed in the group treated with acrylamide and 100 mg/kg of naringin (arrowheads). (E) The group treated with 100 mg/kg of naringin alone shows negative 8-OHdG expression. IHC-P, Bar: 50 μm; Magnification: ×40.

Naringin at both doses significantly decreased the acrylamide-induced levels of these inflammatory parameters (P<0.05). More importantly, 100 mg/kg of naringin could normalize the levels of inflammatory parameters to those of the control group (Figure 2). Moreover, treatment with naringin alone did not affect the levels of inflammatory parameters.

3.5. Effects of naringin on renal KIM–1, MAPK–1, and caspase–3 level

Acrylamide markedly increased the levels of KIM-1, MAPK-1, and caspase-3 (P<0.05). Naringin at 50 mg/kg prominently decreased the acrylamide-induced MAPK-1 and caspase-3 levels (P<0.05) with no significant effect on KIM-1 level. At a higher dose, naringin dramatically lowered the levels of KIM-1, MAPK-1, and caspase-3 (P<0.05) (Figure 3).

3.6. Histopathological findings

The renal tissue of the control group showed a normal histological appearance (Figure 4A). Severe degeneration and necrosis in the tubular epithelium and hyperemia in interstitial vessels were detected in renal tissues of the acrylamide group, especially in the cortex (Figure 4B). Moderate degeneration and necrosis in the renal tubular epithelium, and severe hyperemia in glomerular and interstitial vessels were observed in the acrylamide group treated with 50 mg/kg naringin (Figure 4C). In addition, mild degeneration of the tubular epithelium and moderate hyperemia of the vessels were observed in the renal cortex of the acrylamide group treated with 100 mg/kg naringin (Figure 4D). The group treated with naringin alone showed a normal histological structure (Figure 4E).

3.7. Immunohistochemical findings

The significantly higher Bcl-2 and 8-OHdG expressions were observed in the acrylamide group compared with the control group. In comparison with the acrylamide group, the groups treated with acrylamide and naringin (50 and 100 mg/kg) showed upregulated Bcl-2 expression and downregulated 8-OHdG expression. The Bcl-2, and 8-OHdG expressions were not affected in the group treated with naringin alone (Figures 5-6, Table 2).

Table 2. Immunohistochemical evaluation of renal tissues in rats.

Group	Bcl-2	8-OHdG
Control	19.20±5.20^{a}	22.21±4.52^{a}
ACR	41.33±7.34^{b}	57.23±7.21^{b}
NA50+ACR	47.25±7.42^{bc}	46.22±7.73^{bc}
NA100+ACR	58.13±5.72^{c}	39.13±4.45^{c}
NA100	20.55±3.22^{a}	21.97±4.43^{a}

The data are presented as mean ± SEM and analyzed by using the one-way ANOVA followed by t test. Different letters indicate statistically significant differences, P<0.05.

4. Discussion

Our results showed that the administration of acrylamide significantly induced oxidative stress, inflammatory response, oxidative DNA damage, and apoptosis, and reduced the activity of antioxidant enzymes in kidney tissues. On the other hand, we have observed that naringin significantly improved the inflammatory response and oxidative damage caused by acrylamide exposure, increased antioxidant enzyme activity, and inhibited acrylamide-induced apoptosis. In particular, a high dose of naringin (100 mg/kg) was more effective for ameliorating acrylamide-induced kidney damage than a low dose (50 mg/kg).

We have found that acrylamide application prevents body weight gain and this finding is consistent with our previous data[5]. This is probably due to the harmful effect of acrylamide on various tissues and organs. On the other hand, we observed that the administration of naringin caused weight gain, but this increase was not significant compared with the acrylamide group. We also found that the application of acrylamide and naringin did not affect kidney weights compared with the control group.

Serum urea and creatinine levels are measured routinely for the evaluation of renal function in clinical experiments because increases in their levels are highly indicative of renal dysfunction[18]. A study found that serum urea and creatinine levels were increased significantly in acrylamide-induced nephrotoxicity compared with controls[19]. In our study, serum urea and creatinine levels were significantly increased in the acrylamide group compared with the control group. It was notable that a high dose of naringin had protective effects against acrylamide-induced nephrotoxicity and that urea and creatinine levels were significantly lower than those in the acrylamide group and similar to those of the control group. These results are consistent with several previous studies[20,21]. Moreover, naringin normalized the serum urea and creatinine levels by preventing kidney tissue injury and renal dysfunction through antioxidant and anti-inflammatory effects.

Reactive oxygen species (ROS) is a broad term that includes many different types of oxygen-containing chemicals, such as superoxide, peroxides, and hydroxyl radicals[22]. It is previously known that ROS has important roles in hemostasis and intercellular signaling. ROS is produced in mammals as a product of cell metabolism[23]. However, ROS production can cause significant damage to cellular structures[24,25]. Thus, ROS-mediated oxidative stress can occur, and depending on this, various abnormal conditions may occur in the cell[26]. This may cause oxidation of amino acids, damage to DNA, oxidative deactivation of some enzymes, and oxidation of fatty acids[27]. In addition, it can affect the antioxidant defense system and prepare the ground for various diseases[28]. There are many studies in which some substances with antioxidant properties are used to prevent the oxidative stress caused by acrylamide in the kidney tissue[29]. Acrylamide caused significant decreases in GSH, GPx, SOD, and CAT activities, and significant increases in MDA

concentration in kidney tissues. Our results were consistent with the findings of many other studies[20,21]. Previous studies have shown that naringin protected various tissues against oxidative stress due to its strong antioxidant and anti-inflammatory properties[7,30]. In our study, we found that naringin (100 mg/kg) prevented acrylamide-induced kidney damage, reduced oxidative stress markers, and increased antioxidant enzyme activity. These findings are in line with previous studies.

NF-κB is one of the important markers that are extremely sensitive to oxidative stress. NF-κB activation causes an increase in the level of proinflammatory cytokines such as TNF-α, IL-1β, and inflammatory response markers such as COX-2[31]. In addition, TNF-α plays an important role in inflammatory cytokines and many inflammatory diseases. In several previous studies, acrylamide administration has been reported to cause NF-κB activation and thus an increase in cytokines, causing subsequent inflammatory reactions responsible for kidney damage[5,32]. Therefore, inhibition of NF-κB can be beneficial in reducing nephropathy. Based on this information, we observed in our study that there was a marked increase in NF-κB levels in the kidney tissues of acrylamide treated rats, which was dramatically reduced by naringin. COX-2 promotes the production of prostaglandins[33] and the overproduction of COX-2 has already been determined in kidney damage caused by acrylamide[5]. Prostaglandin E_2, which is formed as a result of the increase in COX-2, causes the production of IL-6 and TNF-α, which are important agents of inflammatory disorders[12]. In the present study, acrylamide increased IL-6 and TNF-α levels in kidney tissues. In some previous studies, it has been reported that naringin administration is protective in kidney tissues by causing a decrease in cytokine levels in kidney damages experimentally created with various substances[7,34]. In this study, the findings showed that the administration of naringin diminished the levels of proinflammatory cytokines and prevented acrylamide-induced inflammation in the kidney.

The KIM-1 is a normal transmembrane glycoprotein that is not in the healthy kidney and is found in proximal tubule cells after kidney injury as a result of various toxic agent applications in animal models[35]. Some studies have shown that the level of KIM-1 increases in the tissue and urine due to the application of toxic agents[36]. In this study, we found that acrylamide application increased the level of KIM-1 in kidney tissues, and these data are in line with previous studies. However, the level of KIM-1 was decreased by treatment with naringin, suggesting that the administration of naringin protects against acrylamide-induced kidney damages.

Apoptosis is a cell suicide program that is extremely important for maintaining tissue homeostasis in multicellular organisms[37]. The cell has two main apoptotic signaling pathways, the death receptor-mediated extrinsic pathway and the mitochondria-mediated intrinsic pathway[38]. The proteins of the Bcl-2 family are key regulators of the mitochondrial pathway, which can regulate the activation of caspases that break down several cellular proteins such as caspase-3[39]. Several recent studies have shown that in liver and kidney tissues, acrylamide caused a decrease in Bcl-2 level and an increase in caspase-3 level, which is an indicator of apoptosis[7,40]. Our findings showed that acrylamide administration significantly increased caspase-3 and Bcl-2 expression levels. However, caspase-3 level was significantly decreased and Bcl-2 expression was markedly increased in the kidney tissues of naringin-treated rats, suggesting that naringin alleviated acrylamide-induced kidney tissue apoptosis.

It has been reported in previous studies that p38 mitogen-activated protein kinase (MAPK)/NF-κB marked activation occurs in many kidney diseases such as glomerulonephritis, diabetic nephropathy, and acute renal failure. Thus, it has been determined that MAPK-1 has an important role in kidney damages[41]. In this study, we observed that the expression levels of NF-κB and MAPK-1 were increased in the kidney tissues of rats in the acrylamide-treated group, indicating that acrylamide triggered inflammation and apoptosis in kidney tissue by stimulating NF-κB and MAPK-1. On the other hand, it was seen that the expression levels of NF-κB and MAPK-1 in the kidney tissues of rats in the naringin group were significantly reduced. This suggests that naringin administration protects kidney tissue against acrylamide-induced renal inflammation and apoptosis.

It has been reported that the application of acrylamide causes severe degeneration and necrosis in the tubular epithelium of the kidney tissue, and hyperemia in the interstitial vessels[42]. It has been stated that several applied agents with antioxidant and anti-inflammatory effects prevent the damage caused by acrylamide in the kidney tissue[5,42]. The histopathological study showed that acrylamide application caused severe necrosis and degeneration in the tubular epithelium, and hyperemia in interstitial vessels while naringin treatment significantly alleviated the acrylamide-induced kidney damages. Studies have reported that oxidative stress can cause amino acid oxidation and subsequent DNA damage[25]. One of the biomarkers used to determine DNA damage resulting from increased oxidative stress is 8-OHdG[43]. The previous study has shown that acrylamide application caused DNA damage by increasing oxidative stress and resulted in an increase in 8-OHdG levels[44]. In our study, we found that acrylamide application increased the expression of 8-OHdG in kidney tissues, which was reversed by naringin treatment.

In conclusion, we found that exposure to acrylamide in rats caused cellular damage, oxidative stress, inflammation, apoptosis, and DNA damage in kidney tissues and that naringin treatment protected kidney tissue against these acrylamide-induced changes. However, new studies are needed to understand the molecular mechanism of these protective effects. Our findings will shed light on future studies.

Conflict of interest statement

The authors declare no competing interests.

Authors' contributions

VG, SY, EŞ, AÇ, FÇ, MK, and MG contributed to experimental design and experiment implementation. VG and EŞ performed biochemical analyses and evaluation. SY performed the histopathological and immunohistochemical examination. VG, EŞ, and SY contributed to the writing and editing of the article and read and approved the final text.

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