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The protective effects of naringin against 5-fluorouracil-induced hepatotoxicity and nephrotoxicity in rats

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ABSTRACT

Objective(s): 5-fluorouracil-induced (5-FU), an anticarcinogenic agent, is reported to have side-effects that include hepatotoxicity and nephrotoxicity. The study objective was to investigate the protective effects of naringin on 5-FU-induced hepatotoxicity and nephrotoxicity.

Materials and Methods: Thirty rodents were assigned to three groups. The control group received 1 ml of intragastric distilled water for 14 days. The 5-FU group received 1 ml of distilled water for 14 days as a placebo. On day 9, this same group received a 20 mg/kg dose of 5-FU administered intraperitoneally(IP) for a further five days. The naringin+5-FU group received a 100 mg/kg dose of naringin (IP) for 14 days. On day 9, 20 mg/kg of 5-FU was administered (IP) to this group for a further five days. On day 15, the rats were decapitated, and blood and renal and hepatic tissues were taken.

Results: It was determined that serum creatinine, BUN, AST, ALT, ALP, and LDH levels, as well as cytokine levels in the liver and kidney tissues were significantly elevated in the 5-FU group, compared to the control group. The comparative values were similar in the control and naringin+5-FU groups. When the liver tissue was examined histopathologically, in the control group it was found to be normal in structure. However, necrosis was observed in the hepatocytes of the pericentric region in the 5-FU group. 8-OHdG cell density was significantly elevated in the 5-FU group, compared to the control and naringin+5-FU groups.

Conclusion: Naringin was observed to have a protective effect on 5-FU-induced liver and kidney damage.

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Introduction

5-fluorouracil (5-FU), a fluorinated pyrimidine, is classified as an antimetabolic agent and influences the synthesis of DNA and RNA in normal and tumor cells. The majority of 5-FU is abolished through liver metabolism and only a small portion is removed from the body via kidney excretion. 5-FU is widely used in chemotherapy for various cancers (1). As a fluoropyrimidine antimetabolite agent, it plays an important role in the treatment of colon, breast, gastrointestinal, head, neck, and pancreatic cancer (1). However, serious toxicity and unwanted side-effects occur following its use (2) and it is considered to be a nephrotoxic compound (3). In addition, it has hepatotoxic effects, with increased aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activity in tissue (4, 5).

A number of studies have been conducted on the use of natural therapies to avoid the side-effects of anticancer agents (6-10). Naringin is a flavonoid that is usually found in grapefruit, orange and cooked tomato paste (11). It has antioxidant, immunomodulatory and anti-inflammatory properties. Flavonoids may have a protective effect against disease through various mechanisms, i.e., by activating and protecting antioxidant enzymes in the cells (12) or reacting directly or indirectly to reactive oxygen species (ROS) via the transfer of hydrogen atoms (13). Naringin was reported in various studies to be protective against hepatotoxicity and nephrotoxicity (14, 15).

The objective of the current study was to evaluate the hepatoprotective and nephroprotective potential of naringin against 5-FU-induced liver and renal toxicity.

Materials and Methods

Thirty male adult Sprague-Dawley rats, weighing 220–250 g, were included in the study. The animals were housed under adequate moisture and light conditions, at a suitable room temperature, and were provided with sufficient water and food until the day of the experiment. The study was performed in accordance with the national guidelines on the use and care of laboratory animals and was approved by the Animal Experiments' Ethics Committee of Kafkas University.

The rats were divided into three groups (Table 1), consisting of a control and two experimental groups. The control group received 1 ml intragastric distilled water for 14 days. The 5-FU group received 1 ml of distilled water for 14 days as a placebo. On day 9, this same group received a 20 mg/kg dose of 5-FU that was administered (IP) for a further five days. The naringin +5-FU group received a 100 mg/kg dose of naringin (dissolved in water oil) for 14 days. On day 9, 20 mg/kg 5-FU was administered (IP) to this group for a further five days.

On day 15, the rats were anesthetized, intracardiac

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Table 1. All groups of study and animal protocols

Groups	Treatment	number of animals in groups
Control	Control (distilled water)	10
5-FU	5-FU (20 mg/kg IP)	10
Naringin+ 5-FU	100 mg/kg naringin + 5-FU (20 mg/kg IP)	10

blood samples were taken and the animals were sacrificed. Blood, kidney, and liver tissue samples were collected for biochemical analysis to determine the cytokine parameters (interleukin [IL]-1α, tumor necrosis factor-alpha [TNF-α], and IL-6). A histopathological examination was conducted.

Blood sample collection

Five days after taking the 5-FU treatment (i.e., on day 15), blood samples were separately collected from the liver and kidneys of each rat. Thereafter, the rodents were euthanized via cervical dislocation. The blood samples were centrifuged at 1,500 g for 12 min within 1 hr of collection to obtain serum samples, which were immediately analyzed.

Oxidative parameters

The hepatic and renal tissues were homogenized using TissueLyzer II® (Qiagen, Germantown, USA). The homogenates were centrifuged at 10,000 g for 20 min at 4 ˚C and supernatants were obtained. Superoxide dismutase (SOD) activity was assessed and thiobarbituric acid reactive substance and glutathione (GSH) levels were determined (16).

Biochemical cytokine analysis

Rat-specific cytokine levels were established for IL-6, TNF-α, and IL-1α using an immunoassay kit (Elabscience Biotechnology Co., Ltd, USA), according to manufacturer's instructions. The results were expressed as mean±standard deviation (SD) (pg/ml or ng/ml).

Histopathological and immunohistochemical study

The liver and kidney tissue samples were collected, placed in a 10% formalin solution for 48 hr and then washed under running tap water for histopathological evaluation. The tissue was routinely processed and was then buried in blocks of paraffin. Tissue sections cut to 4 μm thickness were taken from each block, placed on the slides, stained and examined under a Leica DM 1000 Laboratory Microscope (Leica Microsystems, Buffalo Grove, USA) to perform an accurate assessment of the hematoxylin-eosin and fibrous tissue staining and adhesion. 8-hydroxy-2' -deoxyguanosine (8-OhdG) staining was conducted with respect to hepatic and renal immunohistochemistry. 8-OhdG-positive cell intensity was scored as none = -; weak = +; moderate = ++ and strong = +++.

Statistical analysis

All data were statistically evaluated by one-way ANOVA using SPSS 20.00, followed by Duncan *post hoc* test. The data were expressed as mean ± SD. $P<0.05$ was considered statistically significant.

Results

Effect of naringin on liver and renal weight in g/kg body weight

The weight of the liver and kidneys (g/kg of body weight) in the 5-FU-treated animals was shown to be significantly increased compared to the control group ($P<0.050$). By contrast, that in the 100-mg naringin-treated group was observed to be significantly decreased when compared to the 5-FU group ($P<0.050$). The liver and kidney weight measurements, expressed as g/kg of body weight, are outlined in Table 2.

Table 2. Weight of organ in g/kg body weight in the experimental groups ($P<0.05$, n=10) the results are expressed as mean±SD

	weight of liver in g/kg body weight	renal weight in g/kg body weight
Control	$0.44^{a} \pm 0.01$	$0.08^{a} \pm 0.003$
5-FU	$0.52^{b} \pm 0.02$	$0.11^{b} \pm 0.008$
Naringin+5-FU	$0.39^{a} \pm 0.03$	$0.07^{a} \pm 0.002$

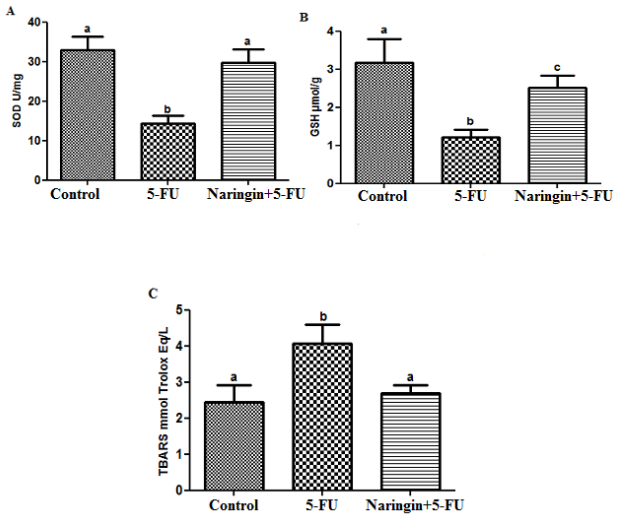

Figure 1. Illustration of levels of oxidative parameters (SOD, GSH, and TBARS) for all groups in the liver tissues. A; SOD activity, B; GSH activity, and C; TBARS levels, the letters indicate the statistical differences among groups ($P<0.05$, n=10), the results were expressed as mean±SD

Liver and renal tissues SOD activity, GSH and TBARS levels

The SOD activity and the GSH levels of the 5-FU-treated animals were markedly reduced compared with the control group ($P<0.050$). However, the GSH levels in the 100-mg naringin-treated group were significantly elevated compared to those in the 5-FU group ($P<0.050$). The GSH levels that were determined for all groups are shown in Figures 1 A and B and Figures 2 A and B.

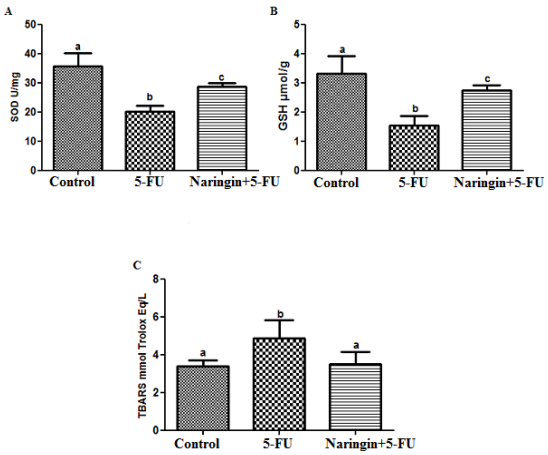

Figure 2. Illustration of levels of oxidative parameters (SOD, GSH, and TBARS) for all groups in the renal tissues. A; SOD activity, B; GSH activity, and C; TBARS levels, the letters indicate the statistical differences among groups (P<0.05, n=10), the results were expressed as mean±SD

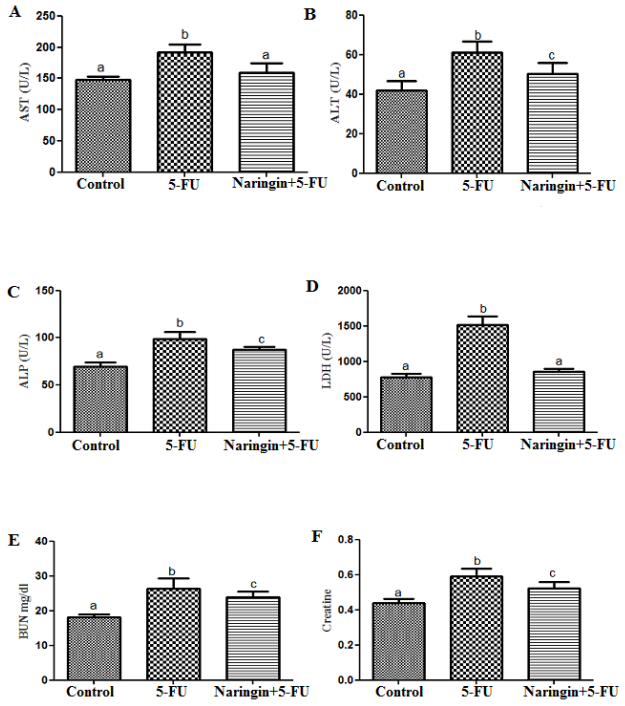

Figure 3. Illustration of serum liver and kidney parameters for all groups. A; AST, B; ALT; C; ALP, D; LDH, E; BUN, and F; Creatine, the letters indicate the statistical differences among groups (P<0.05, n=10), the results were expressed as mean±SD

Thiobarbituric acid reactive substance (TBARS) levels were higher in the 5-FU group than in the other groups (P<0.050). However, treatment with a naringin dose of 100 mg greatly inhibited the increase in TBARS levels (P<0.050). The data collected on TBARS levels for all groups are presented in Figures 1 C and 2 C.

Effect of naringin on liver marker enzymes (ALT, AST, and ALP)

The liver enzyme activities in the 5-FU-treated animals were shown to be significantly increased when compared to the control group (P<0.050). An inverse result was found for the 100-mg naringin-treated group when compared to the 5-FU group (P<0.050). The liver enzyme activity in all groups is depicted in Figures 3 A, B, and C.

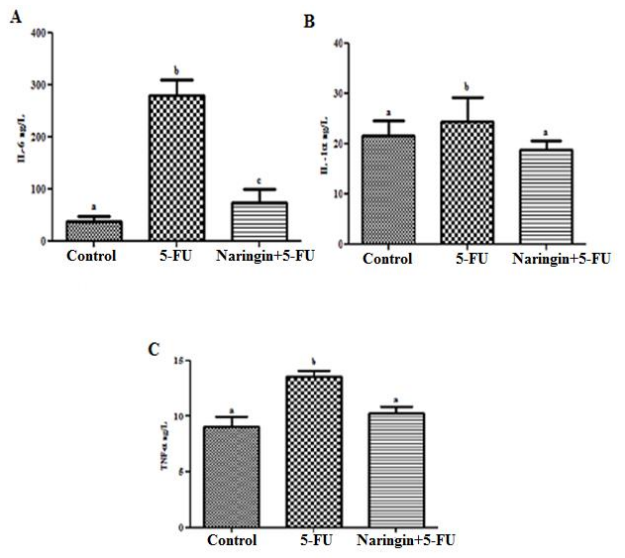

Figure 4. Biochemical cytokine levels in the liver tissues for all groups. A; IL-6, B; IL-1α; C; TNF-α, the letters indicate the statistical differences among groups (P<0.05, n=10), the results were expressed as mean±SD

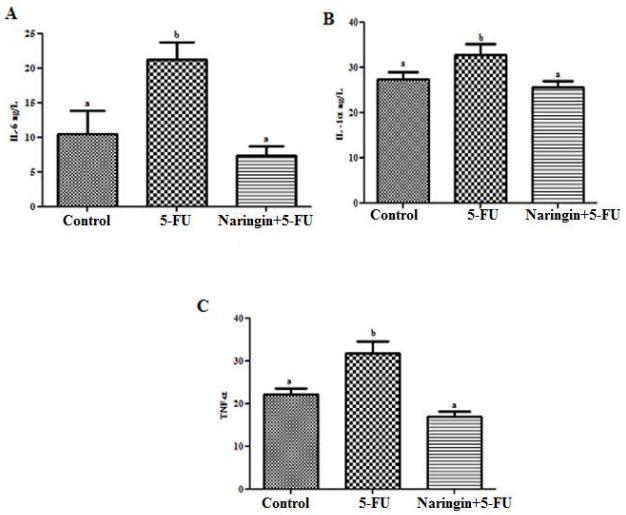

Figure 5. Biochemical cytokine levels in the kidney tissues for all groups. A; IL-6, B; IL-1α; C; TNF-α, the letters indicate the statistical differences among groups (P<0.05, n=10), the results were expressed as mean±SD

Effect of naringin on renal markers (LDH, BUN, and Creatinine)

The LDH, blood urea nitrogen (BUN), and creatinine levels of the 5-FU-treated animals were greatly elevated, compared to the control group (P<0.050). By contrast, the BUN and creatinine levels in the 100-mg naringin-treated group were significantly decreased in the 5-FU group (P<0.050). The data on the LDH, BUN, and creatinine levels for all the groups are shown in Figures 3 D, E, and F.

Biochemical cytokine (IL-6, IL-1α, and TNF-α) levels in the liver and renal tissues

Following cytokine analysis, the IL-6 levels were shown to be greatly higher in the 5-FU group than in the other groups (P<0.050) (Figure 4 A). A statistically significant difference between the control and naringin +5-FU groups in this regard was also observed (P<0.050). A marked difference was also applicable to the IL-1α and TNF-α levels in the 5-FU group, when compared to the other groups (P<0.050). A statistically significant difference was not found between the control and the naringin+5-FU groups in this regard (P<0.050) (Figures 4 B and C).

Similarly, IL-6, IL-1α, and TNF-α levels were significantly higher in the 5-FU group than in the other groups (P<0.050). A statistically significant difference between the control and naringin+5-FU groups was reported (P<0.050) (Figures 5 A, B, and C).

Histopathological examination

Following liver and kidney tissue analysis, normal structure and histology were determined in the control group. Hydropic degeneration and coagulative necrosis were observed in the hepatocytes in the psoriasis region, and mononuclear cell infiltration in the portal region, in the 5-FU group, when the liver tissue was examined.

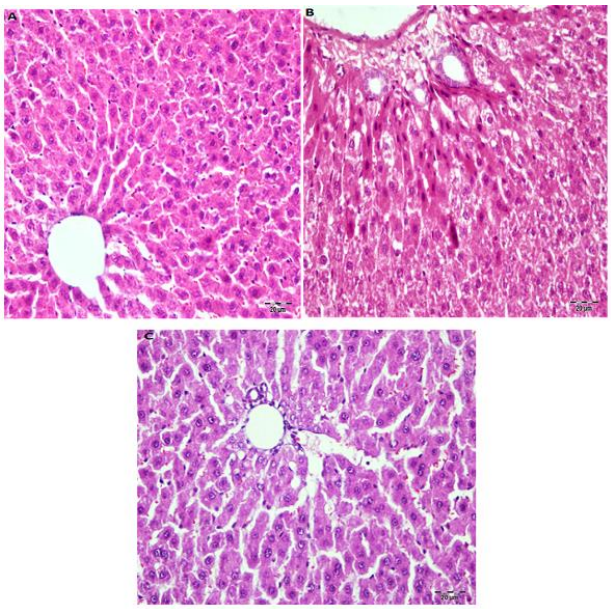

Figure 6. Histopathologic examinations of rat liver sections of control (A), 5-FU (B), naringin 100+ 5-FU (C), H&E, Bar:20 µm

Following a kidney tissue assessment, tubular dilation, glomerular atrophy, dilatation of the Bowman's capsule, and degeneration and/or necrosis of the renal tubular epithelial cells were identified in the 5-FU group. However, following liver tissue analysis, necrotic cells were not identified when hydropic degeneration of the hepatocytes was observed in the pericentric region in the naringin+5-FU group. Mild hydropic degeneration was detected in the tubular epithelium cells when the kidney tissue was examined in the naringin+5-FU group (Figures 6(A, B, C), 8(A, B, C) and Table 3, 4).

Immuno-histochemical findings

8-OHdG immunopositive reactions, with the use of 8-OHdG antibodies, were assessed in the liver and

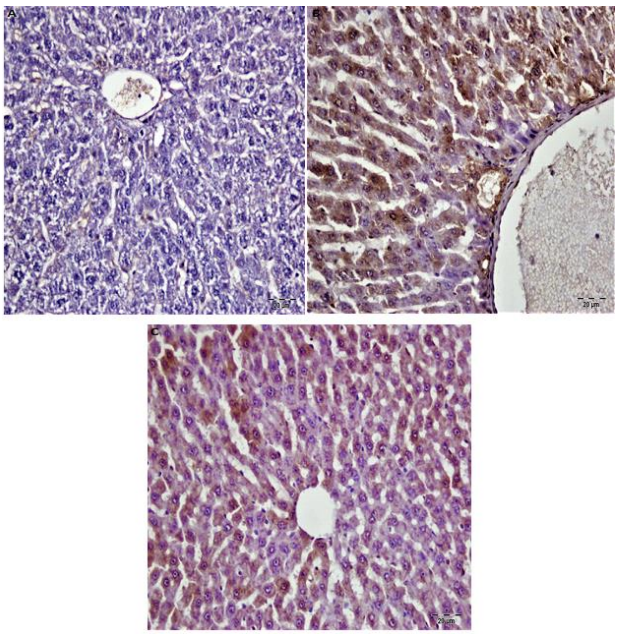

Figure 7. Immunohistochemical staining for 8-OHdG in the liver sections of control (A), 5-FU (B), and naringin +5-FU (C) groups, IHC, Bar: 20 µm

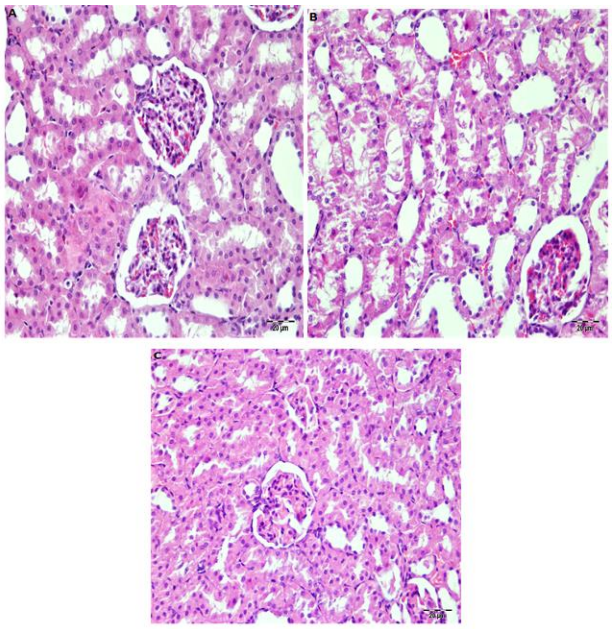

Figure 8. Histopathologic examinations of rat renal sections of control (A), 5-FU (B), naringin 100+ 5-FU (C), H&E, Bar:20 µm

kidneys in all groups. 8-OHdG cell density was significantly elevated in the 5-FU group when compared with the control and naringin+5-FU groups (Figures 7-9).

Discussion

A widely used chemotherapeutic agent, 5-FU, has proven efficacy in human malignancy. However, its clinical utility is inhibited by hepatotoxic and nephrotoxic side-effects (17,18). ROS are produced when electrons from different systems penetrate oxygen in a living organism. The cellular antioxidant enzymatic and nonenzymatic defense plays a crucial role in alleviating tissue damage caused by free radicals (19-21). ROS has a direct effect on various biological components. It causes cellular damage and necrosis in the liver, kidney and other tissues (22-25).

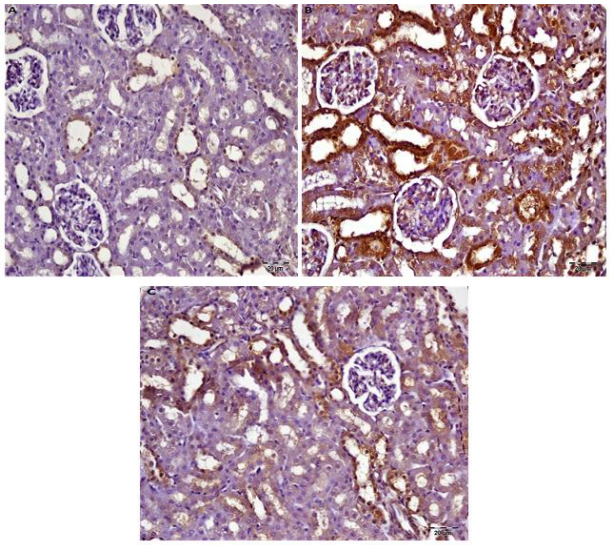

Figure 9. Immunohistochemical staining for 8-OHdG in the renal sections of control (A), 5-FU (B) and naringin +5-FU (C) groups, IHC, Bar: 20 µm

Table 3. Histopathological evaluation of liver tissues

	Control	5-FU	Naringin + 5-FU
Degeneration in hepatocytes	-	+++	++
Necrosis in hepatocytes	-	+++	+
Dilatation and hyperemia in sinusoids	-	++	+
8-OHdG	+	+++	++

Scored as: none = -; weak = +; moderate = ++; strong = +++

Table 4. Histopathological evaluation of renal tissue

	Control	5-FU	Naringin + 5-FU
Degeneration in tubul epithelium	-	+++	++
Necrosis in tubul epithelium	-	+++	+
Hyperemia	-	+++	+
8-OHdG	+	+++	++

Scored as follows: none = -; weak = +; moderate = ++; strong = +++

The antioxidant defense system is the primary protective mechanism used to prevent cell damage to ROS. ROS damage increases portal and systemic endotoxin levels and translocation to the liver, resulting in ingestion by neutrophils and the release of ROS at higher levels (26-28). The elimination of ROS in normal healthy cells is accomplished by a radical scavenging system, comprising catalase (CAT), superoxide dismutase (SOD), and reduced GSH (29). Oxidative stress can occur as a consequence of increased ROS production or a reduced antioxidant defense (30, 31).

An increased amount of ROS has been reported in liver hepatic and renal toxicity induced by 5-FU (32). It has been shown that antioxidants protect against 5-FU-induced hepatic and renal damage in rats. A reduction in the activity of significant antioxidant enzymes in the kidneys and liver, including SOD, CAT, and GSH, was also demonstrated following treatment with 5-FU (33), as was an increase in serum malondialdehyde levels due to 5-FU-induced hepatotoxicity and nephrotoxicity.

In the current study lipid peroxidation was determined by measuring TBARS levels in rodent liver and kidney tissues. TBARS levels were higher in the 5-FU group than in the other groups, thereby explaining accelerated peroxidation levels in the liver and kidneys. SOD activity and GSH concentrations were significantly decreased in the hepatic and renal tissue following treatment with 5-FU. In contrast, a marked increase in SOD activity and GSH levels were detected when naringin was given to this group. The findings suggest that 5-FU-induced hepatotoxicity and nephrotoxicity arises from ROS, which disrupts the antioxidant system. It is thought that naringin prevents damage due to its antioxidant properties. This result is consistent with that reported in a previous study (33).

Treatment with 5-FU resulted in a significant increase in serum ALT, AST, and ALP activities in the animals, in whom severe hepatotoxicity was established. These data are consistent with those reported elsewhere(34). Excessive oxidative production and the accumulation of oxidation products in the liver damaged the biological membranes and the endothelial lining of the liver in the current study. This was probably due to liver damage, causing elevated ALT, AST, and ALP concentrations in the blood. ALT, AST, and ALP are considered to be the most important biological markers of cellular damage and toxicity (35). A significant elevation in serum AST, ALT, and ALP activity has been used as an indicator of acute liver injury in other studies (36, 37).

ALT is a cytosolic enzyme that targets the liver rather than other tissues, and AST is found in the mitochondria and targets the liver, skeletal muscles, and kidneys. ALP activity increases due to obstruction and inflammation of the biliary tract. To a great extent, its increase in the blood occurs when ALT and AST infiltrate the hepatocytes. This is considered to be a marker of liver damage or dysfunction. The administration of naringin resulted in the considerable recovery of and a significant reduction in the activity of these enzymes, suggestive of hepatotoxicity in animals exposed to 5-FU.

LDH, BUN, and creatinine are commonly used as markers in the analysis of kidney injury (38). High BUN and serum creatinine levels indicate 5-FU-induced kidney damage. The results of the current study were consistent with the findings of previous studies (32). Lower LDH, BUN, and serum creatinine levels, when compared with the 5-FU treated group, were found in rats to whom naringin had been administered. A reduction in LDH, BUN, and creatinine levels is possibly caused by the nephroprotective efficacy of naringin. A significant increase in LDH was seen in the 5-FU group, compared to the other groups (32). However, the present findings revealed that naringin treatment significantly attenuated LDH, demonstrating that it has the potential to prevent renal damage.

The role of proinflammatory cytokines in the pathogenesis of hepatic and renal toxicity in relation to the cellular signaling pathways is still being researched. Cytokine secretion is the mediator of inflammation and contributes to the pathogenesis of tissue injury (39, 40). It has been reported that proinflammatory cytokines are associated with a significant increase in serum IL-1ß, TNF-α, and IL-6 levels following 5-FU treatment in rats (41).

The inhibitory effect of flavonoids in the release of chemical mediators, including IL-1α, TNF-α, and IL-6, was also reported (42). Previous studies reported the inhibitory effect of naringin on IL-1α, TNF-α, and IL-6 (43). In the current study, 5-FU treatment markedly increased IL-1α, TNF-α and IL-6 levels. Conversely, naringin treatment caused a significant reduction in IL-1α, TNF-α, and IL-6 levels in 5-FU-induced experimental rats. This is possibly owing to its anti-inflammatory properties. In the light of these findings, it can be deduced that naringin prevents kidney and liver inflammation caused by 5-FU.

Histopathological examination of liver and renal tissues in the 5-FU- treated group suggested cell injuries and necrosis in the liver and renal tissues. In previous studies degeneration and coagulative necrosis were observed in the hepatocytes and mononuclear cell infiltration in the portal region (4, 44). Kidney tissue assessment, degeneration, and/or necrosis of the renal tubular epithelial cells were identified due to 5-FU administration (44). Thus, the results of the present

study revealed that naringin treatment resulted in minimal liver and renal damage and no necrosis in the liver and kidneys of 5-FU-induced rats.

8-OHdG is considered to be the most important indicator of DNA damage (45, 46). Hydroxyl radicals destroy the hydrogenation of nucleic acid or react with double bonds, leading to 8-OHdG (47). El-Sayyad *et al.* suggested that DNA damage intensified in rat testes following the administration of 5-FU (48). 8-OHdG was shown to be a good indicator of tissue damage following 5-FU-induced nephrotoxicity in experimental rats in the current study. It has been reported elsewhere that DNA damage was diminished following the administration of antioxidants (49). This supports the current study finding that naringin had an antioxidant effect, thereby reducing ROS-mediated 8-OHdG levels and preventing oxidative DNA damage. This suggests that naringin treatment has an important restorative effect on renal and hepatic injury induced by 5-FU.

Conclusion

Naringin treatment can mitigate renal and hepatic damage caused by 5-FU-induced renal and hepatic toxicity in rats. Naringin also enhances the restoration of biochemical oxidative enzymes as it has anti-inflammatory, antioxidant, and DNA-protective properties. It was found in the current study that naringin was protective against 5-FU-induced renal and hepatic toxicity. Further studies are warranted to investigate its future clinical applications.

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Conflicts of interest

The authors declare no conflicts of interest.

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