Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2017.06.013

The protective effect of rutin and quercetin on 5-FU-induced hepatotoxicity in rats

Volkan Gelen[1*], Emin Şengül[2], Semin Gedikli[3], Gözde Atila[1], Hamit Uslu[4], Mustafa Makav[1]

[1]*Department of Physiology, Veterinary Faculty, Kafkas University, Kars, Turkey*

[2]*Department of Physiology, Veterinary Faculty, Atatürk University, Erzurum, Turkey*

[3]*Department of Histology-Embryology, Veterinary Faculty, Atatürk University, Erzurum, Turkey*

[4]*Department of Health Care Services, Atatürk Health Vocational School, Kafkas University, Kars, Turkey*

ARTICLE INFO

Article history:
Received 12 Jun 2017
Received in revised form 16 Jun 2017
Accepted 20 Jun 2017
Available online 30 Jun 2017

Keywords:
Rat
5-Fluorouracil
Quercetin
Rutin
Hepatotoxicity

ABSTRACT

Objective: To investigate the effects of quercetin (Q) and rutin on 5-fluorouracil (5-FU)-induced hepatotoxicity.

Methods: The control group was corn oil. The 5-FU group rats were corn oil and injected intraperitoneal 5-FU 50 mg/kg. Groups rutin 50 + 5-FU and rutin 100 + 5-FU were respectively 50 mg/kg and 100 mg/kg rutin. These groups were given 5-FU (50 mg/kg) in the 18th day. The group rutin 100 was rutin (100 mg/kg *i.g.*). Groups Q50 + 5-FU and Q100 + 5-FU were respectively 50 mg/kg and 100 mg/kg quercetin. These groups were given 5-FU (50 mg/kg) in the 18th day of quercetin application. The group Q100 was quercetin (100 mg/kg *i.g.*). In the end of experimental applications, blood was collected from anesthetized rats.

Results: The MDA level was significantly higher in the 5-FU group compared with control group, and determined to be decreased in other groups. GPx and GSH levels were significantly decreased in the 5-FU group compared to the control, rutin 100 + 5-FU and Q100 + 5-FU groups. AST, ALT, LDH and ALP levels in the serum were significantly increased in the 5-FU group compared with the other groups. The results from this analysis show that while the caspase-3 level increases in the 5-FU group, it decreases in the Q50 + 5-FU, Q100 + 5-FU, rutin 50 + 5-FU and rutin 100 + 5-FU groups. Bcl-2 level decreased in the 5-FU group compared to the control group, but increased in the rutin 100 + 5-FU, Q50 + 5-FU and Q100 + 5-FU groups.

Conclusions: In this study it was determined that the rutin and Q have protective effects on 5-FU-induced hepatotoxicity.

1. Introduction

Chemotherapy is widely used to treat various types of cancers [1]. However, chemotherapeutic drug use can result in unwanted side effects and toxicity in various organs and tissues [2]. 5-Fluorouracil (5-FU) is a chemotherapeutic agent that functions throughout the S phase of the cell cycle. 5-FU, thymidine phosphorylase activates thromidylate synthase-inhibiting fluorodeoxyuridine, thus preventing DNA synthesis. This leads to cell growth and ultimately cell death [3]. In addition, 5-FU is metabolized to 5-fluorouridine monophosphate (5-FUMP), which degrades its function by binding to RNA. 5-FU is metabolized by the liver and has a half-life of 10 min [4]. It has previously been determined that 5-FU causes liver damage [5]. 5-FU is primarily eliminated via liver metabolism; only a small portion is removed by the kidney. Dihydropyrimidine dehydrogenase (DPD), which is found in the liver during 5-FU catabolism, is enzymatically effective. A variety of studies have shown that 5-FU causes damage to the liver. Like other chemotherapeutic agents, 5-FU creates over-reactive oxygen species (ROS) and suppresses the antioxidant defense mechanism. Therefore, much attention has been paid to the potential role of antioxidants in protecting against chemotherapy-induced hepatotoxicity [6].

*Corresponding author. Volkan Gelen, Department of Physiology, Veterinary Faculty, Kafkas University, Kars, Turkey.

E-mail: gelen_volkan@hotmail.com (V. Gelen).

Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by the local animal-care committee of the Local Ethics Committee of Ataturk University for Animal Experiments.

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

Quercetin (Q) and rutin are natural flavonoids found in many fruits and vegetables. Several experimental studies have reported that these molecules have many effective qualities; they are anti-ischemic, hypolipidemic, cytoprotective, anti-angiogenic, anti-spasmitic, anti-mutagenic, antiplatelet, antihypertensive, antioxidant, anti-inflammatory, anti-thrombotic, anti-cancer, anti-proliferative, and anti-viral [7–11]. In studies performed in several cancer toxicity models, Q and rutin were reported to prevent toxicity and tissue damage induced by anti-cancer agents [12,13]. The aim of this study was to investigate the hepatoprotective effects of Q and rutin, which have shown strong antioxidant properties in experimental hepatotoxicity-induced rats treated with 5-FU.

2. Materials and methods

The study involved the use of 80 adult male Sprague Dawley rats, whose weights were 220–250 g. The animals were provided with proper moisture, light, and room temperature, as well as free water and food, until the day of the experiment. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by the local animal-care committee of the Local Ethics Committee of Ataturk University for Animal Experiments. The rats were divided into eight groups, consisting of a control and 7 experimental groups, respectively. Control group was given only intragastric (*i.g.*) solvent (corn oil) for 21 days. Group 5-FU was given *i.g.* corn oil for 21 days as a placebo, and single-dose intraperitoneal (*i.p.*) 5-FU (50 mg/kg) was given on the 9th day of the study. Groups rutin 50 + 5-FU and rutin 100 + 5-FU, respectively, were administered *i.g.* doses of 50 and 100 mg/kg of rutin dissolved in corn oil for 21 days, and single injection of *i.p.* 5-FU (50 mg/kg) was administered on the 18th day. In Group rutin 100, rutin's 100 mg/kg dose was given to rats *i.g.* for 21 days. Groups Q50 + 5-FU and Q100 + 5-FU, respectively, were administered *i.g.* doses of 50 and 100 mg/kg of quercetin dissolved in corn oil for 21 days, and single injection of *i.p.* 5-FU (50 mg/kg) was administered on the 18th day. In Group Q100, quercetin's 100 mg/kg dose was given to rats *i.g.* for 21 days (Table 1). On the 22th day of the experiment, the rats in all groups were anesthetized, intracardiac blood samples were taken, and all animals were sacrificed. The blood and liver tissue samples were collected for biochemical analysis, oxidative stress (MDA, GPx, and GSH), immun-histochemical and histopathological examination.

Table 1
All groups of study and animal protocols.

Groups	Treatment	No. of rats in groups
Control	Control (corn oil *i.g.*)	10
5-FU	5-Fluorouracil (50 mg/kg *i.p.*)	10
Rutin 50 + 5-FU	50 mg/kg Rutin (*i.g.*) + 5-fluorouracil (50 mg/kg *i.p.*)	10
Rutin 100 + 5-FU	100 mg/kg Rutin (*i.g.*) + 5-fluorouracil (50 mg/kg *i.p.*)	10
Rutin 100	100 mg/kg rutin (*i.g.*)	10
Q50 + 5-FU	50 mg/kg Q (*i.g.*) + 5-fluorouracil (50 mg/kg *i.p.*)	10
Q100 + 5-FU	100 mg/kg Q (*i.g.*) + 5-fluorouracil (50 mg/kg *i.p.*)	10
Q100	100 mg/kg Q (*i.g.*)	10

2.1. Blood sample collection

Three days after from the 5-FU treatment or in other words 15th day of the experiment, rats were anesthetized with ketamine hydrochloride (*i.p.*, 75 mg/kg) (Ketalar, Pfizer, Turkey)-xylazine (15 mg/kg) (Rompun, Bayer, Turkey) and blood samples were separately collected from the liver of each rat. After rats were euthanized with cervical dislocation. The blood samples were centrifuged at 1 500 ×*g* for 12 min within 1 h after collection to obtain serum samples. The serum samples were immediately studied with an autoanalyzer.

2.2. Biochemical analysis

The liver tissues were homogenized by a tissue homogenizer. The homogenates were centrifuged at 10 000 ×*g* for 20 min at 4 °C, and the supernatants were obtained. The malondialdehyde (MDA), glutathione (GSH) and glutathione peroxidase (GPx) levels were measured by respectively the methods of Placer *et al.* [14], Sedlak and Lindsay [15] and Matkovics *et al.* [16].

2.3. Histopathological and immuno-histochemical analysis

At the end of the experiment, all rats were sacrificed and their livers were removed. The livers were fixed in 10% formalin, dehydrated in ascending grades of ethyl alcohol, cleared in xylol series and embedded in paraffin blocks. The blocks were cut at 5-μm thickness by using microtome and liver tissue sections stained with Crossman's modified Mallory triple staining were evaluated for any structural changes under a light microscope. For hepatic immunohistochemistry, Bcl-2 and Caspase-3 staining were performed. Also, the Bcl-2 positive cell intensity and Caspase-3 positivity were scored as follows: none = −; weak = +; moderate = ++; strong = +++; very strong = ++++.

2.4. Statistical analysis

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

3. Results

3.1. Biochemical findings

The animals' values of the liver enzymes (AST, LDH, ALT, ALP) in experimental groups on 21th day are shown in Table 2.

Table 2
The values of the liver enzymes in the experimental groups.

Experimental groups	AST	ALT	ALP	LDH
Control	124 ± 58	52 ± 9	316 ± 28	876 ± 105
5-FU	256 ± 38*	83 ± 14*	125 ± 19*	1 744 ± 124*
Rutin 50 + 5-FU	185 ± 25	44 ± 11	162 ± 22	1 635 ± 112
Rutin 100 + 5-FU	168 ± 43	48 ± 14	282 ± 17	916 ± 69
Rutin 100	116 ± 12	48 ± 17	293 ± 23	707 ± 98
Q50 + 5-FU	192 ± 18	47 ± 9	135 ± 11	840 ± 120
Q100 + 5-FU	152 ± 40	54 ± 12	205 ± 14	691 ± 134
Q100	126 ± 8	52 ± 15	285 ± 36	805 ± 75

Data are shown as mean ± SD, $n = 6$. *: $P < 0.05$.

According to these values, in 5-FU-treated rats, there was significant increase in the AST, LDH, ALT and ALP levels in serum as compared with other groups ($P < 0.05$), but no statistical difference was observed among the other groups ($P > 0.05$). In the 5-FU group, we found a significant increase in the MDA level compared with that in the control group ($P < 0.05$). The livers MDA levels were reversed to the control values by treated with rutin and quercetine (Figure 1B). The levels of liver GSH and GPx were significantly decreased in the 5-FU group compared with that in the control group ($P < 0.05$), and in all experimental groups compared with 5-FU group it was observed that there were significant increased GSH levels in the liver ($P < 0.05$). Similarly, the level of GPx in the liver was significantly increased in all experimental groups except rutin 50 + 5-FU compared with 5-FU group ($P < 0.05$) (Figure 1A–C).

3.2. Histopathological findings

The liver's histological structure (Figure 2A) was normal in the control group, whereas liver tissues of the 5-FU treatment

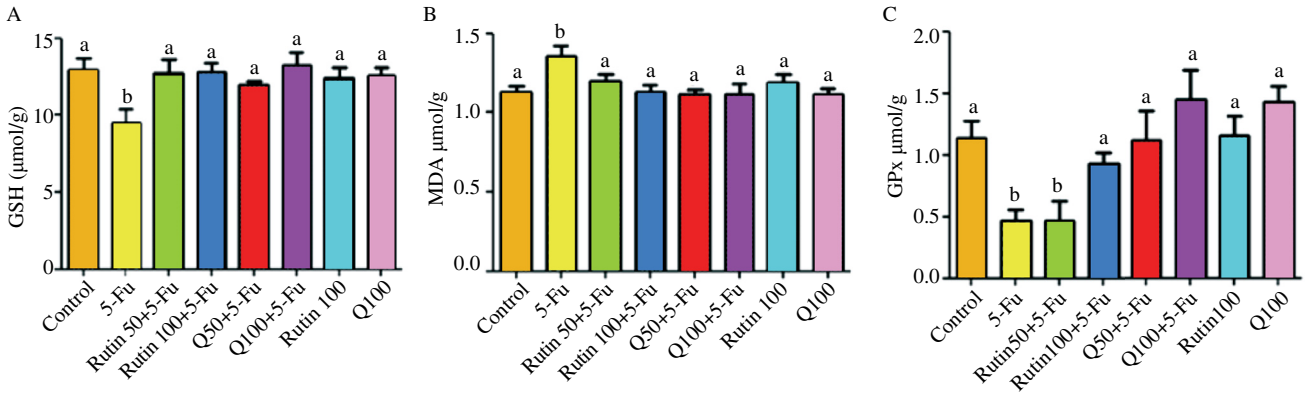

Figure 1. The liver GSH (A), MDA (B) and GPx (C) levels in experimental groups.
a,b: $P < 0.05$, $n = 6$. There is no statistical difference among the groups expressed the with same letters.

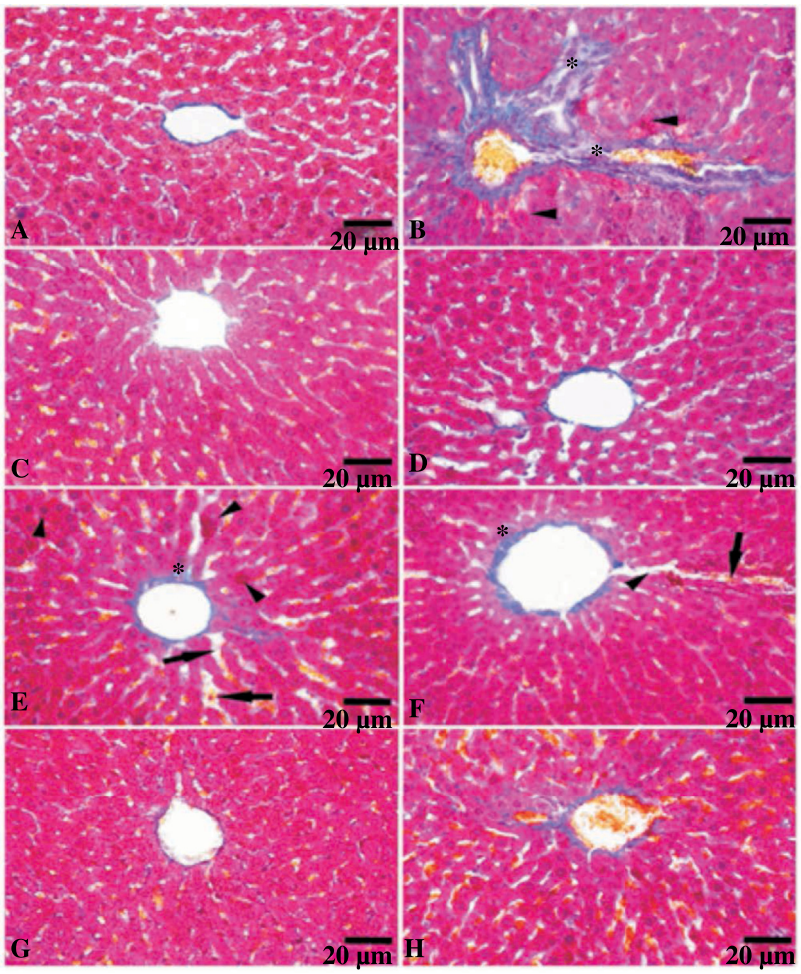

Figure 2. Histopathologic examinations of rat liver sections.
A: Control; B: 5-FU; C: Rutin 100; D: Quercetin 100; E: Rutin 50 + 5-FU; F: Q50 + 5-FU; G: Rutin 100 + 5-FU; H: Q100 + 5-FU. Asterisk: Connective tissue deposition in the rat liver; Arrows: Sinusoidal dilatation; Arrowheads: Degenerated hepatocytes with nuclear condensation. Crossman's modified Mallory triple staining.

group had significantly degenerated hepatocytes with nuclear condensation, and sinusoidal dilatation and an increase of connective tissue around the central vein and portal area were seen (Figure 2B). Rutin 100 and Q100 groups showed no hepatic abnormalities, and the arrangements of the hepatocytes in the liver were almost normal (Figure 2C and D). In the rutin 50 + 5-FU and Q50 + 5-FU treatment, hepatocyte degeneration and connective tissue deposition were reduced (Figure 2E and F). In the rutin 100 + 5-FU group tissues were seen nearly normal hepatic structure according to Q100 + 5-FU group tissues (Figure 2G and H).

3.3. Immuno-histochemical findings

In the present study, anti-apoptotic (Bcl-2) and pro-apoptotic (Caspase-3) immunopositive reactions in the liver sections of all groups were examined with Bcl-2 and Caspase-3 antibodies, respectively. In Bcl-2 cell density estimation, there was lower density in 5-FU group than control group (Figure 3A, C, D). Also, immunopositivity of Bcl-2 was significantly increased in Rutin 50 + 5-FU, Q50 + 5-FU, rutin 100 + 5-FU and Q100 + 5-FU groups compared with 5-FU group (Figure 3G–J).

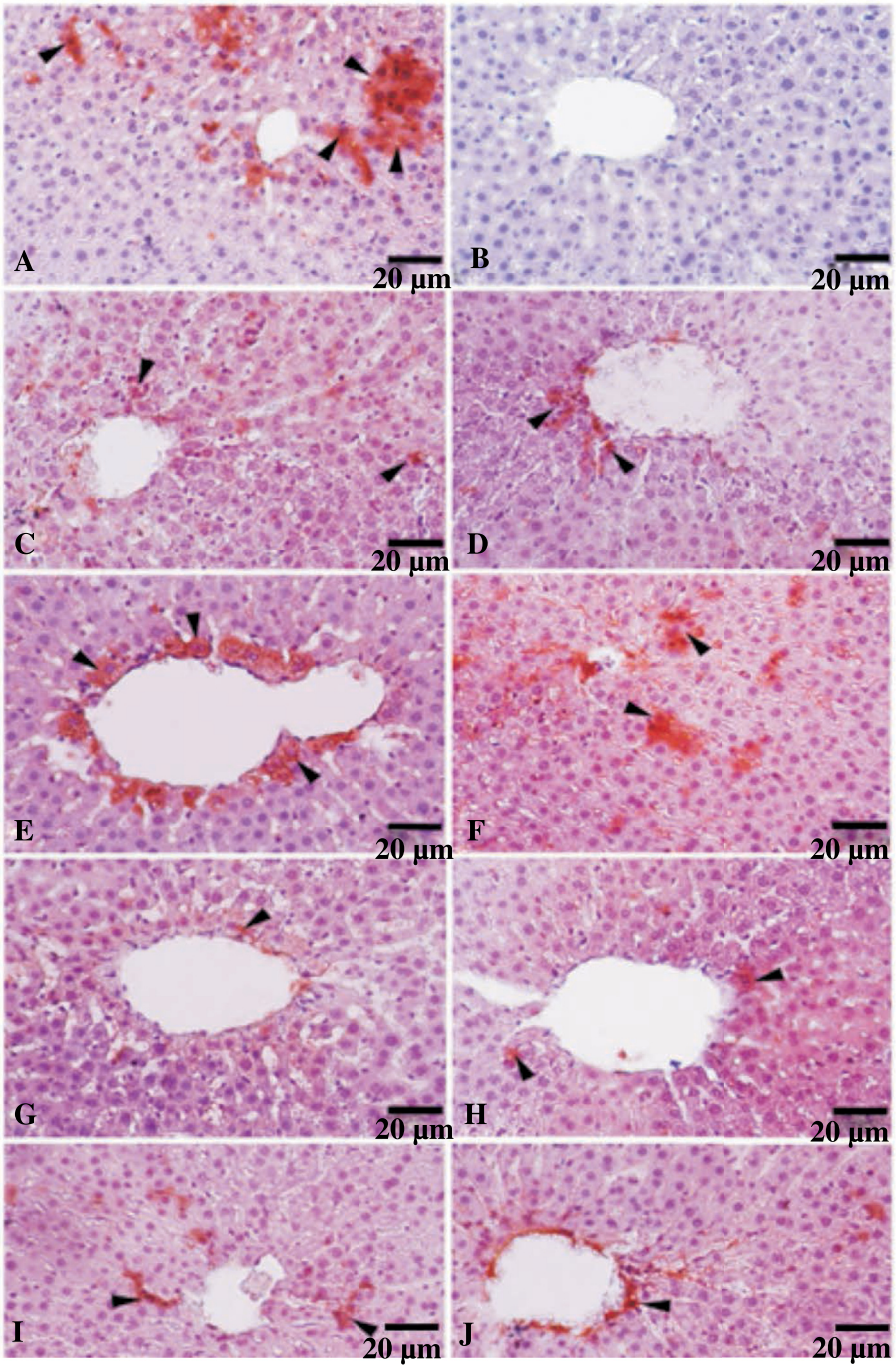

Figure 3. Immunohistochemical staining for the Bcl-2.
A: Control; B: Negative control; C–D: 5-FU; E: Rutin 100; F: Q100; G: Rutin 50 + 5-FU; H: Q50 + 5-FU; I: Rutin 100 + 5-FU; J: Q100 + 5-FU. Arrowheads show Bcl-2 positivity in cell in the liver sections. Negative control: Not primer antibody used sections of control and other groups. Streptavidin–biotin peroxidase staining.

Intensity of Caspase-3 positivity was higher in the 5-FU group sections compared to the control group sections (Figure 4A, C, D). Also, there was intense mononuclear cell inflammation in the 5-FU group (Figure 4D). Furthermore, immunopositivity of Caspase-3 was significantly decreased in rutin 100 + 5-FU group compared with rutin 50 + 5-FU, Q50 + 5-FU and Q100 + 5-FU groups (Figure 4G–J). The positive cell intensity of Bcl-2 and Caspase-3 in the groups is shown in Table 3.

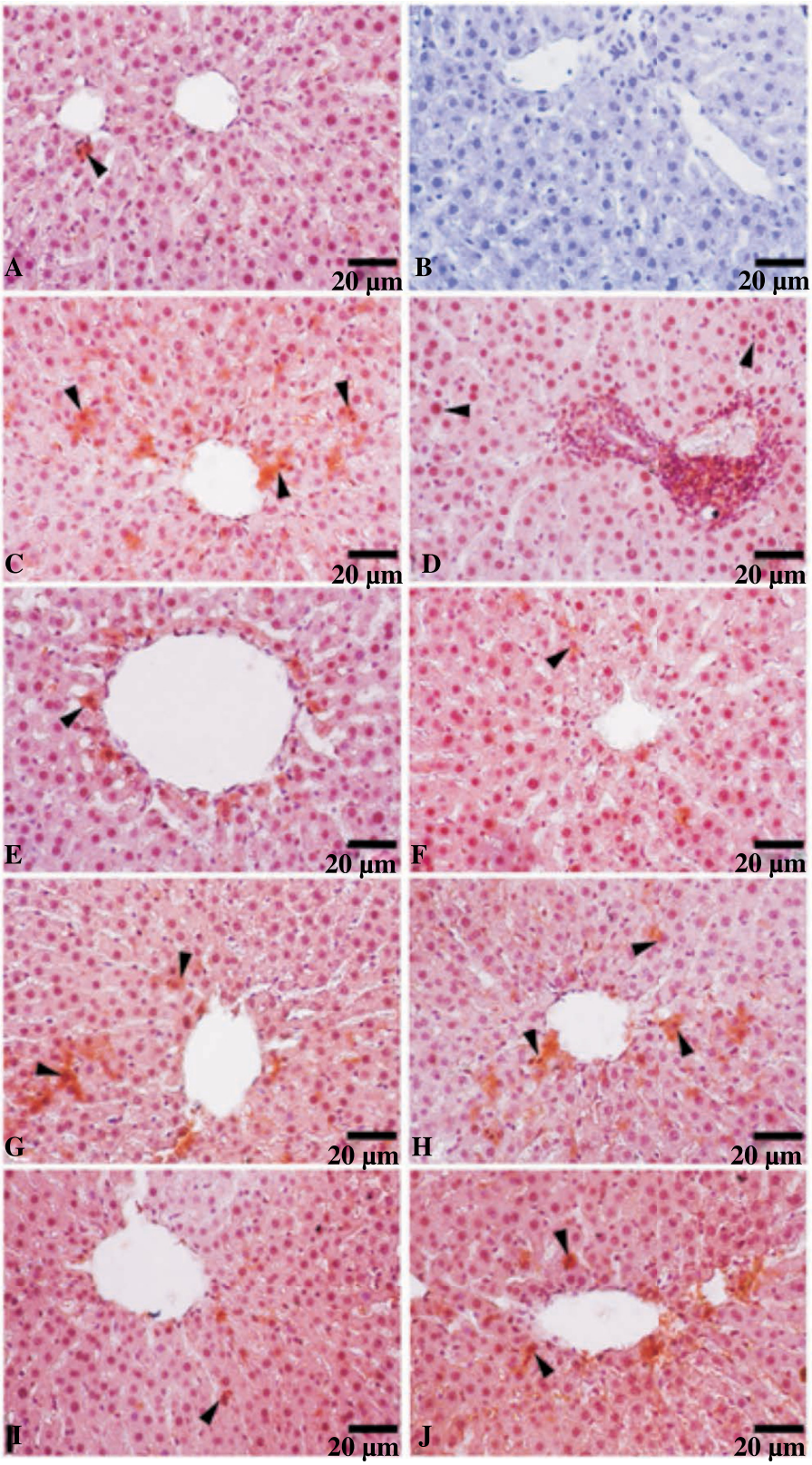

Figure 4. Immunohistochemical staining for the Caspase-3.
A: Control; B: Negative control; C–D: 5-FU; E: Rutin 100; F: Q100; G: Rutin 50 + 5-FU; H: Q50 + 5-FU; I: Rutin 100 + 5-FU; J: Q100 + 5-FU. Arrowheads show Caspase-3 positivity in cell in the liver sections. Asterisk: Mononuclear cell inflammation; Negative control: Not primer antibody used sections of control and other groups. Streptavidin–biotin peroxidase staining.

Table 3
The positive cell intensity.

Groups	Bcl-2	Caspase-3
Control	++++	–/+
5-FU	+	++++
Rutin 50 + 5-FU	+	++
Rutin 100 + 5-FU	++	–/+
Rutin 100	+++	–/+
Q50 + 5-FU	++	++
Q100 + 5-FU	+	+
Q100	++	–/+

None: –; Weak: +; Moderate: ++; Strong: +++; Very strong: ++++.

4. Discussion

Currently, chemotherapy is the main treatment option for cancer patients, but its therapeutic use is limited due to severe clinical side effects [17,18]. In this study, we investigated the effects of quercetin and rutin on 5-FU-induced oxidative stress and liver apoptosis. The protective effects of Q and rutin we observed may be related to lowered oxidative stress and apoptotic damage in the livers of 5-FU-treated rats.

Enzyme levels such as ALT, AST, and LDH are often used to assess hepatic damage. Liver injury causes membrane damage or necrosis, which allows intracellular enzymes to circulate and be detected in serum. Elevated AST levels indicate hepatic damage because the area is transformed by ALT-catalyzed reactions, and glutamate and pyruvate may be released. ALT is a more specific parameter than AST for determining liver damage. Higher concentrations of these enzymes in the serum indicate that the hepatic membrane's functional integrity has been lost. Serum, total protein, ALP, and total bilirubin levels are also associated with liver cell function. The rise in serum ALP is influenced by the increased bile pressure [19,20]. 5-FU administration caused a significant increase in enzyme levels including ALT, LDH, AST and ALP compared to the control. Quercetin and rutin administration significantly restored these parameters. This reversal in enzyme levels after Q and rutin treatment is probably due to membrane-stabilizing activities that inhibit intracellular enzyme leakage. This is consistent with the accepted view that liver cell regeneration, liver parenchyma recovery, and serum aminase levels return to normal [19–22].

Lipid peroxidation, one of the mechanisms involved in tissue damage through ROS formation, is measured using MDA. Significant increases in MDA levels in liver tissue were reported in rats treated with 5-FU [23]. Our results are consistent with previous findings. Q and rutin used as a prophylactic treatment significantly reduced MDA levels.

GSH is an antioxidant that defends against exogenous toxic injury, combatting ROS through the release of free radicals. GSH donates a direct hydrogen atom and neutralizes free radicals. Absence of GSH in tissues reduces the cell's defenses against oxidative stress. Our findings indicate that 5-FU precipitated GSH reservoirs, consistent with earlier findings [23]. However, the prophylactic treatment of Q and rutin significantly increased GSH levels.

GPx detoxifies H_2O_2 and the other ROS to H_2O_2, as well as H_2O and O_2 [24]. In this study, GPx activity was significantly lower in the 5-FU-treated rats than in the control group. The reduction in antioxidant enzyme activity (GPx and GSH) in the 5-FU-treated group suggests that oxidative stress interferes in the pathophysiology of 5-FU liver toxicity. Q and rutin application increased GPx and GSH activity by removing ROS such as peroxy radicals, peroxide, superoxide radicals, and oxygen. The activities of enzymatic and non-enzymatic antioxidants increased significantly after treatment with Q and rutin [25,26]. Our results are consistent with previous research, indicating that it is responsible for the protective effects of polyphenolic natural products.

5-FU-induced apoptosis forms in the cell [27]. One way to measure cell apoptosis is to measure the levels of the caspase-3 and Bcl-2 proteins. In this research, the levels of caspase-3 (a proapoptotic member of the Bcl-2 family) and Bcl-2 (an anti-apoptotic member) were examined using immuno-histochemical analysis. The results show that while the caspase-3 level increased in the 5-FU group, it decreased in the Q50 + 5-FU, Q100 + 5-FU, rutin 50 + 5-FU, and rutin 100 + 5-FU groups. The Bcl-2 level decreased in the 5-FU group compared to the control group, but increased in the rutin 100 + 5-FU, Q50 + 5-FU, and Q100 + 5-FU groups. Treatment with antioxidants has been reported to help regulate organ functions [28] and prevent apoptosis [29,30]. Our findings are consistent with this data.

In conclusion, Q and rutin treatment can mitigate liver damage after 5-FU-induced liver toxicity in rats. The protective roles of Q and rutin could improve restoration of biochemical oxidative enzymes and antiapoptotic and liver cells. This may occur due to the antioxidant effects of Q and rutin. Therefore, our experimental results suggest that Q and rutin might potentially be protective agents for 5-FU-induced liver toxicity. Further studies are necessary to investigate future clinical applications of Q and rutin.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Borek C. Antioxidants and radiation therapy. *J Nutr* 2004; **134**(11): 3207S-9S.
[2] Minami M, Matsumoto S, Horiuchi H. Cardiovascular sideeffects of modern cancer therapy. *Circ J* 2010; **9**: 1779-86.
[3] Shoemaker LK, Arora U, Rocha Lima CM. 5-Fluorouracil-induced coronary vasospasm. *Cancer Contr* 2001; **11**: 146-9.
[4] Sobrero AF, Aschele C, Bertino JR. Fluorouracil in colorectal cancer-a tale of two drugs: implications for biochemical modulation. *J Clin Oncol* 1997; **15**: 368-81.
[5] Ray S, Roy K, Sengupta C. *In vitro* evaluation of protective effects of ascorbic acid and water extract of *Spirulina plantesis* (blue green algae) on 5-fluorouracil-induced lipid peroxidation. *Acta Pol Pharm* 2007; **69**: 335-44.
[6] Behling EB, Sendao MC, Francescato HD, Antunes LM, Costa RS, Bianchi-Mde L. Comparative study of multiple dosage of quercetin against cisplatin-induced nephrotoxicity and oxidative stress in rat kidneys. *Pharmacol Rep* 2006; **58**: 526-32.
[7] Kahraman A, Erkasap N, Sertesex M, Koken T. Protective effect of quercetin on renal ischemia/reperfusion injury in rats. *J Nephrol* 2003; **16**: 219-24.
[8] Kelly GS. Quercetin monograph. *Altern Med Rev* 2011; **16**: 172-94.
[9] Russo M, Spagnuolo C, Tedesco I, Bilotto S, Russo GL. The flavonoid quercetin in disease prevention and therapy: facts and fancies. *Biochem Pharmacol* 2012; **83**: 6-15.
[10] Guo R, Wei P, Liu W. Combined antioxidant effect of rutin and vitamin C in Triton X 100 micelles. *J Pharm Biochem Anal* 2007; **43**: 1580-6.
[11] Yang J, Guo J, Yuan J. *In vitro* antioxidant properties of rutin. *LTW* 2008; **41**: 1060-6.
[12] Jeong JH, An JY, Kwon YT, Rhee JG, Lee YJ. Effects of low dose quercetin: cancer cell-specific inhibition of cell cycle progression. *J Cell Biochem* 2009; **106**: 73-82.

[13] Sengül E, Gelen V, Gedikli S, Ozkanlar S, Gür C, Çelebi F, et al. The protective effect of quercetin on cyclophosphamide-induced lung toxicity in rats. *Biomed Pharmacother* 2017; **92**: 303-7.
[14] Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Anal Biochem* 1996; **16**: 359-64.
[15] Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* 1968; **25**: 192-205.
[16] Matkovics B, Szabo L, Varga I. [Determination of enzyme activities in lipid peroxidation and glutathione pathways]. *Lab Diag* 1988; **15**: 248-50. Hungarian.
[17] Ramadori G, Cameron S. Effects of systemic chemotherapy on the liver. *Ann Hepatol* 2010; **9**: 133-43.
[18] Naidu MU, Ramana GV, Rani PU, Mohan IK, Suman A, Roy P. Chemotherapy-induced and/or radiation therapy-induced oral mucositis – complicating the treatment of cancer. *Neoplasia* 2009; **6**: 423-31.
[19] Shehab NG, Abu-Gharbieh E, Bayoumi FA. Impact of phenolic composition on hepatoprotective and antioxidant effects of four desert medicinal plants. *BMC Complement Altern Med* 2015; **15**: 401.
[20] Bagban IM, Roy SP, Chaudhary A, Bhandari KK. Hepatoprotective activity of the methanolic extract of *Fagonia indica* Burm in carbon tetra chloride induced hepatotoxicity in albino rats. *Asian Pac J Trop Biomed* 2012; **2**: S1457-60.
[21] Zhao B, Hu M. Gallic acid reduces cell viability, proliferation, invasion and angiogenesis in human cervical cancer cells. *Oncol Lett* 2013; **6**: 1749-55.
[22] Sajid M, Khan MR, Shah NA, Shah SA, Ismail H, Younis T, et al. Phytochemical, antioxidant and hepatoprotective effects of *Alnus nitida* bark in carbon tetrachloride challenged Sprague Dawley rats. *BMC Complement Altern Med* 2016; **16**: 268.
[23] Al-Asmari AK, Khan AQ, Al-Masri N. Mitigation of 5-fluorouracil-induced liver damage in rats by vitamin C via targeting redox-sensitive transcription factors. *Hum Exp Toxicol* 2016; **35**: 1203-13.
[24] Rashid S, Ali N, Nafees S, Ahmad ST, Arjumand W, Hasan SK, et al. Alleviation of doxorubicin induced nephrotoxicity and hepatotoxicity by chrysin in wistar rats. *Toxicol Mech Methods* 2013; **23**: 337-45.
[25] Janbaz KH, Gilani AH. Evaluation of protective potential of *Artemisia maritima* extract on acetaminophen and CCl_4 induced liver damage. *J Ethnopharmacol* 1955; **47**: 43-7.
[26] Chandan BK, Sharma AK, Anand KK. *Boerhaavia diffusa*: a study of its hepatoprotective activity. *J Ethnopharmacol* 1991; **31**: 299-307.
[27] Rashid S, Ali N, Nafees S, Hasan SK, Sultana S. Mitigation of 5-fluorouracil induced renal toxicity by chrysin via targeting oxidative stress and apoptosis in wistar rats. *Food Chem Toxicol* 2014; **66**: 185-93.
[28] Lee JY, Lott JA, Kauffman EK, Sharma HM. Effect of herbal mixture MAK-4 on organ functions in WHHL rabbits. *Biochem Arch* 1997; **13**: 285-96.
[29] Kadkhodaee M, Gobé GC, Willgoss DA, Endre ZH. DNA fragmentation reduced antioxidants following ischemia-reperfusion in the isolated perfused rat kidney. *Nephrology* 1998; **4**(3): 163-75.
[30] Witenberg B, Kalir HH, Raviv Z, Kletter Y, Kravtsov V, Fabian I. Inhibition by ascorbic acid of apoptosis induced by oxidative stress in HL-60 myeloid leukemia cells. *Biochem Pharmacol* 1999; **57**: 823-32.