CARDIOPROTECTIVE ACTIVITIES OF QUERCETIN AND RUTIN IN SPRAGUE DAWLEY RATS TREATED WITH 5-FLUOROURACIL

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ABSTRACT

In this study, the protective effects of Quercetin (Q) and Rutin in 5-fluorouracil (5-FU)-induced cardiotoxicity in rats were investigated and used eighty-piece Sprague Dawley male rats. Rats were divided randomly to eight groups; control, 5-FU, Q50+5-FU, Q100+5-FU, Q100, Rutin50+5-FU, Rutin100+5-FU, Rutin100. The control group was given intragastric (ig) corn oil for 14 days. 5-FU group was injected intraperitoneal (ip) with a single dose of 50 mg/kg. The doses of Q (50 and 100 mg/kg) and Rutin (50 and 100 mg/kg) were administered for 14 days. Rat of the 5-FU-administered groups were sacrificed under anesthesia 72 hours after 5-FU administration. The other groups were sacrificed on the day 15 after the administration. The blood samples were collected from anesthetized rats and sera were isolated from the clotted blood. The cardiac tissues were used for biochemical, histopathological and immunohistochemical analysis. Cardiac enzyme levels were significantly increased in the 5-FU group. Malondialdehyde (MDA) level was significantly higher than control in the 5-FU group and decreased in the Q100+5-FU and Rutin100+5-FU. Superoxide dismutase (SOD) and glutathione (GSH) activities were significantly decreased in the 5-FU group compared to the control, Q100+5-FU and Rutin100+5-FU. 5-FU groups had degeneration of cardiac cells and myofibril. The intensity of β -MHC positivity was higher in the 5-FU group compared to the other groups. In conclusion, this study demonstrates that Q (50 and 100 mg/kg) and Rutin (50 and 100 mg/kg) have protective effects on the 5-FU-induced cardiotoxicity in rats.

Keywords: 5-fluorouracil, Cardiotoxicity, Quercetin, Rutin: Rat.

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INTRODUCTION

The 5-fluorouracil (5-FU) is an agent with fluoropyrimidine antimetabolite, which is widely used in pancreatic and skin cancer treatment (Malet-Martino *et al.*, 2002). 5-FU is converted to fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP) by a dihydropyrimidine dehydrogenase (DPD) enzyme, and these compounds are active metabolites of 5-FU (Miura *et al.*, 2010). FdUMP and FdUTP cause degradation of DNA synthesis and repair, resulting in DNA damage (Miura *et al.*, 2010; Nagasaki *et al.*, 2010). Hepatotoxicity (Gelen *et al.*, 2018a; Gelen *et al.*, 2017; Ali, 2012), nephrotoxicity (Gelen *et al.*, 2018a; Ali, 2012), gastrointestinal and hematologic toxicities (Gelen and Sengul, 2018b; Lévy *et al.*, 1998) are some of the most common side effects of 5-FU.

Cardiotoxicity, which is well recognized for other antineoplastic agents, is a major complication of 5-FU, and the cardiotoxicity resulting from the use of 5-FU is characterized by angina, pulmonary edema, congestive heart failure, myocardial infarction, ventricular arrhythmias, myocardial ischemia and sudden death

(Canale *et al.*, 2006; Jensen and Sørensen, 2012; Polk *et al.*, 2014; Sorrentino *et al.*, 2012; Yuan *et al.*, 2019). The symptom of initiation in 5-FU induced cardiotoxicity appear nearly 12 hrs following the infusion (Becker *et al.*, 1999). Past studies have reported that cardiotoxicity cases have increased due to 5-FU (Polk *et al.*, 2014). The 5-FU's themselves, or its metabolites, are toxic for myocytes (Ensley *et al.*, 1986) and 5-FU has direct toxic effects on vascular endothelium that have been associated with a decrease of endothelial nitric oxide (NO) synthase, and the reduction of NO synthase leads to coronary spasms and endothelium-independent vasoconstriction via protein kinase C (Chong and Ghosh., 2019; Çalık *et al.*, 2012; Dechant *et al.*, 2012; Tsibiribi *et al.*, 2006). It has been reported that cardiotoxicity is induced the cell damage and apoptosis occur due to increased oxidative stress in cardiomyocytes (Rashid *et al.*, 2014).

To prevent or treat the toxic effects of anticancer agents such as 5-FU, antioxidant compounds are commonly used (Gedikli and Şengül., 2019; Gelen and Şengül., 2020). Antioxidant compounds prevents oxidative stress and oxidative stress mediated tissue damage (Sengul *et al.*, 2019; Rahal *et al.*, 2014a). Quercetin (Q) and Rutin are natural flavonoids and have

strong antioxidant effects; they are available in many fruits (Raw apricot %2.54, Apple % 0.17, Weet cherry % 0.18), vegetables (Raw broccoli %3.22, Red pepper %0.17, Red hot chillis pepper %0.22) and herbs (Raw plums % 1.21, Green tea % 10.18, Grape seeds % 10.16) (Gibellini *et al.*, 2011; Manach *et al.*, 1997; Rahal *et al.*, 2014b; Atanassova and Bagdassaria., 2009; Muhammad *et al.*, 2018). Absorption of both Q and rutin from the small intestine of the rat is very good. However, in previous studies, it is stated that rutin is absorbed slower than Q (Carbonaro and Grant., 2005). Several experimental studies have reported that these molecules have anti-ischemic, hypolipidemic, cytoprotective, anti-mutagenic, antioxidants, anti-inflammatories, anti-cancer, anti-proliferative and anti-viral effects (Guo *et al.*, 2007; Jeong *et al.*, 2009; Kelly, 2011; Russo *et al.*, 2012; Yang *et al.*, 2008). It has been suggested that Q and Rutin have prevented toxicity and tissue damage induced by some anticancer agents (Gelen *et al.*, 2017; Şengül *et al.*, 2017). The aim of the present study, in the context of 5-FU-induced cardiotoxicity in rats, was to investigate the possible cardioprotective effects of Q and Rutin.

MATERIALS AND METHODS

Chemicals: 5-FU, Q and Rutin were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO). 5-FU, Q and Rutin doses were determined base on previous studie (Gelen *et al.*, 2017).

Animals: In the study, 80 adult male Sprague Dawley rats were used, whose weights were between 220-250 g. Rats were provided from the Animal Laboratory at the Experimental Research Centre of Ataturk University, Erzurum, Turkey. All the animals were housed under standard environmental conditions and were allowed access to standard diet and ad libitum drinking water. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by the Local Ethics Committee of Ataturk University for Animal Experiments (Protocol no: 2017/72).

Experimental Protocol: The rats were randomly divided into eight groups, and there were ten rats in each group. The eight groups are described below.

- a. Control: The rats were given only intragastric (i.g.) solvent (corn oil) for 14 days.
- b. 5-FU: The rats were given corn oil for 14 days and single-dose intraperitoneal (i.p.) 5-FU (50 mg/kg) were injected on the 11th day of the study.
- c. Q50+5-FU: The rats were administered 50 mg/kg doses of Q dissolved in corn oil for 14 days and injected the single dose (50 mg/kg) of 5-FU on the 11th day.
- d. Q100+5-FU: The rats were given i.g. 100 mg/kg doses of Q dissolved in corn oil for 14 days and

injected with a single dose (50 mg/kg, i.p.) of 5-FU on the 11th day.

- e. Q100: The rats were given Q (100 mg/kg, i.g.) for 14 days.
- f. Rutin50+5-FU: The rats were given i.g. doses of 50 mg/kg of Rutin dissolved in corn oil for 14 days, and a single dose (50 mg/kg, i.p.) of 5-FU was administered on the 11th day.
- g. Rutin100+5-FU: The rats were given i.g. doses of 100 mg/kg of Rutin dissolved in corn oil for 14 days, and a single dose (50 mg/kg, i.p.) of 5-FU was administered on the 11th day.
- h. Rutin100: The rats were administered Rutin (100 mg/kg, i.g.) for 14 days.

On the 15th day of the experiment, the intracardiac blood samples were taken under sevoflurane (Sevorane liquid 100%, Abbott Laboratory, Istanbul, Turkey) anesthesia from rats, and all the animals were sacrificed.

Sample Collection: The blood samples were transferred into test tubes without an anticoagulant and allowed to clot at room temperature for 15 min. Afterwards, the bloods were centrifuged at 3000 rpm for 10 min at 4°C, and serum was obtained for the analysis of the cardiac enzymes. The whole hearts were isolated immediately from the animals. The hearts were washed with cold physiological saline and stored at -20°C.

Cardiac Enzymes Analysis: The sera samples were immediately processed using an auto-analyzer to determine cardiac troponin I (cTnI), lactate dehydrogenase (LDH), alanine transaminase (ALT), aspartate transaminase (AST), creatine kinase (CK) and creatine kinase-MB (CK-MB) parameters.

Analysis of Oxidant and Antioxidants: The cardiac tissues were homogenized in a Teflon-glass homogenizer using a buffer of 1.15% KCl to obtain a 1:10 (w/v) homogenate. The malondialdehyde (MDA) levels in the heart homogenate were measured using the thiobarbituric acid reaction (Placer *et al.*, 1966). The MDA levels were expressed as µmol/g of tissue. The generation of superoxide radicals produced by xanthine and xanthine oxidase, following the reaction of nitro blue tetrazolium and the formation of formazan dye, was used to measure superoxide dismutase (SOD) activity (Sun *et al.*, 1988). SOD activity was measured as the level of inhibition of absorbance at 560 nm and is expressed as U/mg protein. The cardiac glutathione (GSH) content was measured at 412 nm (Sedlak and Lindsay, 1968) and is expressed as µmol/g.

Histopathological Analysis: At the end of the experiment, all rats were sacrificed, and their hearts were removed. After processing the samples with the rutin histological method, heart tissue sections stained with Crossman's modified Mallory triple staining were

examined under a light microscope. For myocardial immunohistochemistry, β -myosin heavy chain (β -MHC) staining was performed. β -MHC positivity was scored as follows: none = -; weak = +; moderate = ++; strong = +++; very strong = ++++.

Statistical Analysis: The all data were analyzed with one-way ANOVA using the SPSS package program. Post-hoc Tukey's test was used to compare of the parameters among the groups and data were expressed as the mean $\pm$ standard deviation (SD). P value less than 0.05 was considered as significant.

RESULTS AND DISCUSSION

Cardiotoxicity Indices: As compared to the other groups, the serum activities of LDH, AST, ALT, CK, CK-MB and cTnI were significantly increased in the 5-FU group (Table 1, ^{a,b, b,c}; $p < 0.01$; ^{a,c}; $p < 0.05$ $n=10$). The treatment with both doses of Q and Rutin prevented to increase in the serum activities of these enzymes (Table 1, ^{a,b; b,c}; $p < 0.01$; ^{a,c}; $p < 0.05$; $n=10$).

Table 1. The cardiac enzyme parameters in the all groups (n=10).

Groups	LDH (U/L) Mean $\pm$ SD	AST (U/L) Mean $\pm$ SD	ALT (U/L) Mean $\pm$ SD	CK-MB (U/L) Mean $\pm$ SD	CK (U/L) Mean $\pm$ SD	cTnI (ng/ml) Mean $\pm$ SD
Control	771 $\pm$ 126 ^a	144 $\pm$ 45 ^a	41 $\pm$ 13 ^a	749 $\pm$ 104 ^a	820 $\pm$ 270 ^a	5 $\pm$ 0.28 ^a
5-FU	2688 $\pm$ 104 ^b	242 $\pm$ 38 ^b	70 $\pm$ 12 ^b	1650 $\pm$ 119 ^b	2443 $\pm$ 315 ^b	8 $\pm$ 0.41 ^b
Q50+5-FU	970 $\pm$ 113 ^a	146 $\pm$ 38 ^a	32 $\pm$ 17 ^a	949 $\pm$ 206 ^a	1341 $\pm$ 164 ^c	5 $\pm$ 0.1 ^a
Q100+5-FU	771 $\pm$ 154 ^a	140 $\pm$ 42 ^a	33 $\pm$ 4 ^a	755 $\pm$ 218 ^a	940 $\pm$ 14 ^a	4 $\pm$ 0.34 ^c
Q100	785 $\pm$ 85 ^a	155 $\pm$ 43 ^a	46 $\pm$ 11 ^a	627 $\pm$ 219 ^a	749 $\pm$ 145 ^a	5 $\pm$ 0.15 ^a
Rutin50+5-FU	921 $\pm$ 125 ^a	175 $\pm$ 25 ^a	34 $\pm$ 9 ^a	1103 $\pm$ 104 ^a	1428 $\pm$ 114 ^c	7 $\pm$ 0.63 ^b
Rutin100+5-FU	896 $\pm$ 86 ^a	158 $\pm$ 33 ^a	37 $\pm$ 10 ^a	864 $\pm$ 98 ^a	1388 $\pm$ 96 ^c	4 $\pm$ 0.21 ^c
Rutin100	807 $\pm$ 104 ^a	156 $\pm$ 32 ^a	44 $\pm$ 14 ^a	658 $\pm$ 101 ^a	869 $\pm$ 108 ^a	5 $\pm$ 0.22 ^a

There is a statistical difference between values that are expressed in different letters on the same column.

^{a,b; b,c}; $p < 0.01$ ^{a,c}; $p < 0.05$

Oxidative Stress Findings: To assess the effects of Q and Rutin on 5-FU-induced oxidative stress, we analyzed MDA levels, SOD and GSH activities (Figure 1 and 2). In the 5-FU group, MDA levels were higher than all the other groups. The 100 mg/kg dose of the Q and Rutin significantly prevented the increase in MDA, whereas the 50 mg/kg dose of the Q and Rutin was unable to significantly prevent the increase in MDA level (Figure 1A and 2A). The cardiac SOD activity of the 5-FU treated rats was significantly lower than in the control, Q100+5-FU, Q100, Rutin100+5-FU and Rutin100 groups. The SOD activity in the Q50+5-FU and Rutin50+5-FU groups was lower in comparison with the control (Figure 1B and 2B). The GSH levels in the 5-FU group were significantly lower than the control. In the Q100+5-FU and Rutin100+5-FU groups, GSH levels were like the control (Figure 1C and 2C).

Histopathological and Immunohistochemical Examination: The myocardial tissues of the control group displayed normal cardiac muscle bundles, without any infarction and degeneration (Figure 3A), whereas the heart tissue of the 5-FU treatment group had significantly degenerated cardiac myofibril and abundant fibrous tissue deposition (Figure 3B). The Rutin100 and Q100 groups had similar histological structures and morphology as compared to the control group (Figure 3C and 3D). For the Rutin50+5-FU and Q50+5-FU treatments in the

cardiac myofibers degeneration and collagen fibers deposition was reduced (Figure 3E and 3F). In the Rutin100+5-FU group tissues, a small number of degenerative myofibers were seen, and these tissues exhibited a nearly normal, non-inflamed structure according to the Q100+5-FU group tissues (Figure 3G and 3H).

The intensity of β -MHC positivity was higher in the 5-FU group sections as compared to the control group sections (Table 2, Figure 4A, 4C and 4D). Furthermore, immunopositivity of β -MHC was significantly decreased in the Rutin100+5-FU groups as compared with the Rutin50+5-FU, Q50+5-FU and Q100+5-FU groups (Table 2, Figure 4G, 4H, 4I, 4J).

5-FU has been commonly used as a chemotherapeutic in the treatment of gastrointestinal malignancies for years (Jia-Cheng *et al.*, 2016). Unfortunately, as well as its beneficial antitumor effects, 5-FU also has some toxicities, and cardiotoxicity is one of these toxicities (Kosmas *et al.*, 2008). Some flavonoids are widely used in research to prevent or treat the possible side effects of anticancer agents (Gelen *et al.*, 2017; Razavi-Azarkhiavi *et al.*, 2016; Şengül *et al.*, 2017). The Q and Rutin compounds used for this purpose are found in the structure of many fruits and plants (Gibellini *et al.*, 2011; Manach *et al.*, 1997; Rahal *et al.*, 2014b). In this study, the 5-FU-induced cardiotoxicity

model in rats determined the possible protective effects of Q and Rutin.

Figure 1. Effects of Q on the oxidative stress parameters in cardiac tissues of the experimental groups. (MDA (A), SOD (B) and GSH (C), n=10, ***: $p<0.001$, **: $p<0.01$, *: $p<0.05$).

Figure 2. Effects of Rutin on the oxidative stress parameters in cardiac tissues of the experimental groups. (MDA (A), SOD (B) and GSH (C), n=10, ***: $p<0.001$, **: $p<0.01$, *: $p<0.05$).

Figure 3. Micrograph of myocardial section of the Control (A), 5 FU (B), Rutin 100 (C), Q100 (D), Rutin 50+5-FU (E), Q50+5-FU (F), Rutin 100+5-FU (G) and Q100+5-FU (H) groups. mf: cardiac myofiber; star: myofibrillar degeneration; arrows: fibrous tissue deposition in the heart. Crossman's modified Mallory triple staining.

Figure 4. Immunohistochemically staining for the β -MHC for the Control (A), Negative Control (B), 5-FU (C-D), Rutin100 (E), Q100 (F), Rutin50+5-FU (G), Q50+5-FU (H), Rutin100+5-FU (I) and Q100+5-FU (J) groups. Arrowheads show β -MHC immunopositivity in cardiac myofibrils; star: degenerative areas; Negative control; not primer antibody used sections of control and other groups. Streptavidin–biotin peroxidase staining.

Table 2. Immunohistochemical evaluation scoring of β -MHC antibody (n=10).

Experimental Groups	β -MHC
Control	+
5-FU	++++
Q50+5-FU	+++
Q100+5-FU	+
Q100	-
Rutin50+5-FU	++
Rutin100+5-FU	+
Rutin100	-

The positive cell intensity was scored as follows: none = -; weak = +; moderate = ++; strong = +++; very strong = ++++.

Sera LDH, CK, CK-MB, AST, ALT and cTnI levels are commonly used parameters in the evaluation of cardiotoxicity (Andreadou *et al.*, 2007; Cardinale *et al.*, 2010). These enzymes increase in the case of cardiotoxicity, cardiac injury, heart failure, myocarditis and myocardial infarction. LDH is a specific enzyme released into the blood and cytoplasm during cardiotoxic dysfunction. AST and ALT enzymes are critical transaminases that are released as a result of cardiac metabolism. cTnI, CK and CK-MB enzymes are specific biomarkers in cases of heart failure (Alhumaidhaa *et al.*, 2015; Benstoem *et al.*, 2015; Bertinchant *et al.*, 2003; Viswanatha Swamy *et al.*, 2013). In some studies, it has been reported that 5-FU infusion elevated cardiac enzyme levels (De Forni *et al.*, 1992; Robben *et al.*, 1993). In the isoproterenol-induced cardiac necrosis in rats, it has been reported that cardiac enzyme levels increases and Q administration have reduced these increases (Panda and Kar, 2015). Furthermore, in doxorubicin-induced cardiotoxicity, Rutin administration has significantly inhibited the DOX-induced increase of some cardiac levels (Hozayen and Seif, 2011). In our study, 5-FU administration significantly increased the LDH, CK, CK-MB, AST, ALT and cTnI levels. In the Q and Rutin-administered groups, the increase in the level of these enzymes was inhibited. This is thought to be due to the strong antioxidant properties of Q and Rutin, preventing 5-FU-induced damage in cardiac muscle cells. We are of the opinion that Q and Rutin achieved the lowering of the cardiac enzymes levels by preventing cardiotoxicity.

The production of the oxidant agents is balanced by the antioxidant system. The imbalance between the antioxidant and oxidant system is called oxidative stress. MDA and antioxidant system parameters as GSH and SOD are commonly used parameters for determining oxidative damage (Kara *et al.*, 2016; Preiser, 2012). In this study, the cardiac tissue samples showed decreased SOD and GSH activity and increased MDA level, suggesting oxidative stress was induced by 5-FU. These findings are related to 5-FU-induced toxicity as described in previous study (Rashid *et al.*, 2014). Q and Rutin modifications of these parameters originating from 5-FU are due to the possible antioxidant and anti-inflammatory effects of Q and Rutin. These parameters are an

indication that Q and Rutin are protective effects on cardiotoxicity induced by 5-FU, especially in high dose Q and Rutin administrated groups. The effects of Q and Rutin may be due to antioxidant and anti-inflammatory effects of these compounds.

The pathologies (as thickening of the left ventricular wall, myocardial effusion, fibrillation in cardiac myocytes and interstitium) are the histopathologic changes that are seen in cardiotoxicity (Dhesi *et al.*, 2013). The 5-FU single dose demonstrated that massive hemorrhagic myocardial infarction associated with coronary spasms. Besides, the 5-FU treatment caused some changes, such as interstitial hemorrhages, multifocal myofibril necrosis and pericarditis in the heart (Tsibiribi *et al.*, 2006). Our histopathological examinations revealed that the cardiac tissue samples obtained from 5-FU treated rats were degenerated cardiac myofibril and fibrosis free. It was determined that these changes were reduced, especially in the heart tissues obtained from rats in the group of Rutin100+5-FU.

Myocardial hypertrophy and disorganization of cardiac myocytes are generally attributed to an imbalance in the expression of several hypertrophic genes, such as myosin heavy chains (MHC) (Barry *et al.*, 2008). β -MHC is an isoform of MHC that is commonly expressed in ventricles (Liu *et al.*, 2015). β -MHC levels are increased in patients with cardiac injury, left ventricular dysfunction and heart failure (Henderson *et al.*, 2009; Van der Meer *et al.*, 2005). In cyclophosphamide and doxorubicin-induced cardiotoxicity, it was reported that β -MHC mRNA expression has increased cardiac tissue (Liu *et al.*, 2015; Richard *et al.*, 2011). In the Carfilzomib-induced cardiotoxicity, it was determined that the Rutin treatment significantly reduced the β -MHC expression (Imam *et al.*, 2017). Also, in the rats with aortic constriction, the Q administration reduced the cardiac β -MHC expression level (Jalili *et al.*, 2006). In the present study, the β -MHC positivity was higher in the 5-FU-induced cardiotoxicity group sections compared to the control group. Also, immunopositivity of β -MHC was significantly decreased in the Q100+5-FU and Rutin100+5-FU groups. In this way, it was determined that our findings are consistent with the literature.

Conclusion: This study determined that 5-FU induced cardiac injury and Q and Rutin have protective effects against 5-FU-induced cardiotoxicity. The protective effects of Q and Rutin in the 5-FU-induced cardiotoxic rat model have been studied for the first time by us. This study will overcome this gap in the literature.

Conflict of Interest: The authors declared that there no conflict of interest.

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