International Journal of Surgery 53 (2018) 117–121

Contents lists available at ScienceDirect

International Journal of Surgery

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

Original Research

Quercetin dose affects the fate of hepatic ischemia and reperfusion injury in rats: An experimental research

Mustafa Ufuk Uylaş[a,*], Adnan Şahin[b], Varol Şahintürk[c], İbrahim Özkan Alataş[d]

[a] Department of General Surgery, Turgut Ozal Medical Center, Inonu University, Malatya, Turkey
[b] Department of General Surgery, Eskisehir Osmangazi University, Eskisehir, Turkey
[c] Department of Histology and Embryology, Eskisehir Osmangazi University, Eskisehir, Turkey
[d] Department of Medical Biochemistry, Eskisehir Osmangazi University, Eskisehir, Turkey

ARTICLE INFO

Keywords:
Ischemia
Reperfusion
Quercetin
Liver

ABSTRACT

BACKGROUND: Quercetin found in fruits and vegetables has an antioxidative effect. We aimed to investigate the protective effects of quercetin according to different doses on hepatic and ischemia-reperfusion (I/R) injury.
METHODS: Fifty mature male Sprague-Dawley rats were randomly divided into five groups (n = 10 for each). All the animal groups underwent laparotomy. Group 1 rats served as a sham-operated group. Groups 2-5 underwent 1 h hepatic ischemia and were followed by 2 h reperfusion. Group 3-5 animals received an additional intraperitoneal dose of 25, 50 or 100 mg/kg quercetin respectively before I/R operation. Blood samples were collected for determining serum aspartate transaminase (AST), alanine transaminase (ALT) and malondialdehyde (MDA) levels. Also, liver tissue samples were taken for measuring of liver MDA concentration and for histopathology assessment.
RESULTS: The highest levels of biochemical parameters were observed in group 2. In quercetin-treated groups, serum AST, ALT, MDA levels, and tissue MDA concentration were decreased as inversely with increasing quercetin dose. Microscopic evaluation revealed that most conspicuous histological improvement was observed in 50 mg/kg quercetin co-treated rats. 25 and 100 mg/kg quercetin co-treatment could not protect completely against hepatic I/R injury.
CONCLUSION: Quercetin can be effective in preventing of hepatic I/R injury when the correct dose was used.

1. Introduction

Ischemia is an insufficient supply of blood due to arterial occlusion resulting from any cause. Cell death and organ failure are the most commonly observed results of ischemia [1]. Interrupted blood flow and decreased transport of oxygen amount activate the anaerobic metabolism which leads to deposition of lactic acid and toxic metabolites in tissues, thus creating cellular injury [2]. Reperfusion is the restoration of blood flow in an organ or tissue following ischemia. For surviving of ischemic tissues, reperfusion is needed, but it causes additional damages called reperfusion injury [3]. The oxidative stress after ischemia mainly occurs in the extracellular space of the liver and not inside the hepatocytes. This leads to the activation of Kupffer cells, which cause a vascular oxidative stress during the early (≤1 h) reperfusion phase. Then, the complement fragments are activated and the production of reactive oxygen species (ROS) is enhanced. Besides, the neutrophils migrate into the liver, where they generate additional ROS, mainly hydrogen peroxide. However, an actual neutrophil-mediated oxidative stress occurs between 6 and 24 h after the beginning of reperfusion. Neutrophil-derived ROS diffuse into the hepatocytes and attack mitochondria. Ultimately, mitochondrial dysfunction occurs and hepatocytes go to the necrosis [4].

Quercetin is a flavonoid which is found in fruits and vegetables and exerts a strong antioxidative effect through scavenging of free radicals [5]. It was previously shown that quercetin has a protective effect on ischemia and reperfusion (I/R) injury in various organs [5–7] including liver. However, the dose of quercetin used in previous studies ranges widely and the effective dose of quercetin remains unclear [8,9].

In this study, we aimed to investigate the protective effect of quercetin on I/R induced hepatic injury and to compare the effectiveness of different quercetin doses in rats.

* Corresponding author.
E-mail addresses: ufukuylas@hotmail.com (M.U. Uylaş), asahin@ogu.edu.tr (A. Şahin), varols@ogu.edu.tr (V. Şahintürk), oalatas@ogu.edu.tr (İ.Ö. Alataş).

https://doi.org/10.1016/j.ijsu.2018.03.043
Received 13 December 2017; Received in revised form 25 January 2018; Accepted 14 March 2018
Available online 22 March 2018

2. Material and methods

2.1. Animals

Our study protocol was approved by local animal ethics committee of the university (Protocol No:28.12.2012/311) and was compatible with the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences (Institute of Laboratory Animal Resources, 1996). Fifty adult Spraque-Dawley male rats (weighing 250–300 g) were used in this study. Rats were housed in standard laboratory conditions (50% humidity, 25 ± 2 °C temperature and a 12-h light/12-h dark cycle) and standard industrial food and tap water were provided ad libitum. Rats were assigned randomly into five groups (n = 10 in each). Group 1 rats served as a sham-operated group. Group 2-5 were applied 1 h hepatic ischemia and followed by 2 h reperfusion. Group 3-5 animals received additionally an intraperitoneal (i.p.) dose of 25, 50 or 100 mg/kg quercetin respectively 30 min before I/R operation. Quercetin doses were selected according to previous studies [10,11]. All rats fasted for 8 h before they underwent laparotomy under general anesthesia with 50 mg/kg ketamine and 20 mg/kg xylazine via i.p. injection. All sections of this report adhere to the ARRIVE Guidelines for reporting animal research http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0106108 [12].

2.2. Biochemical analysis

Immediately after the ending of reperfusion time, blood samples were collected into silicon tubes using cardiac puncture and then centrifuged for separating sera which were subsequently stored at −20 °C for measuring of serum aspartate transaminase (AST), alanine aminotransferase (ALT) and malondialdehyde (MDA) levels. All the measurements including tissue MDA concentration were performed by using commercial assay kits.

2.3. Histopathological analysis

After collecting bloods of rats, tissue samples were taken from different parts of the liver and fixed in 10% formaldehyde, dehydrated in graded ethanol, immersed into xylol and embedded in paraffin. Serial sections of 4–5 μm thickness were cut by microtome, stained with the hematoxylin-eosin (H&E) method and evaluated under a binocular light microscope. Histopathological assessment was performed in a blind manner by an experienced histologist with regards to the presence of inflammatory cell infiltration, congestion in hepatic vessels, hemorrhage and degeneration of hepatocytes and scored between 1 and 5 for extensity (%) of lesions as follows: 1*: < 10%*, 2:*11-25%*, 3:*26-50%*, 4:*51-75%*, and 5*: > 75%*. Finally, total scores were calculated for each rat [13–15]. Microscopic images were obtained using a digital camera.

2.4. Statistical analysis

All statistical analyses were performed by using SPSS 20.0 software. Shapiro-Wilk test was used to assess the distribution of values. Because the variables did not fit to the normal distribution, non-parametric comparisons were performed by Kruskal-Wallis test. Then each group was compared pairwise with other groups using Tukey test. The significance level was set at $p < 0.05$. The data were presented as median (min-max) because they did not distribute normally.

3. Results

3.1. Biochemical results

The comparison of serum ALT, AST, MDA and tissue MDA levels of the groups were shown in Table 1. Serum AST, ALT, and MDA levels were significantly different between the groups ($p < 0.05$). Maximal AST, ALT and MDA values were seen in Group 2. With increasing dose of quercetin AST, ALT and MDA levels were decreased in an inversely proportional manner. Tissue MDA levels were significantly different between the groups ($p < 0.05$). Maximal tissue MDA value was observed in Group 2. With increasing dose of quercetin tissue, MDA levels showed an inversely proportional decrease.

Table 1
Comparison of serum ALT, AST, MDA and liver tissue MDA levels between the rat groups. All the values showed as median (min-max).

Groups	AST (U/L)	ALT (U/L)	MDA (U/L)	Liver MDA (nmol/g)
Control	**393** (125-1206)	**67** (35-835)	**6.60** (4.99–9.90)	**7.46** (4.59–9.66)
I/R	**3918 a** (1525-12120)	**3318 a, c** (1027-11823)	**16.25 a** (14.81–18.45)	**9.97 b, c** (8.2511.85)
I/R + 25Q	**1208** (252-2801)	**1158** (100-2084)	**15.87 a** (13.79–17.26)	**5.63** (4.78–7.31)
I/R + 50Q	**1178** (328-1720)	**433** (93-958)	**13.20** (11.21–14.56)	**2.89 a** (2.35–3.52)
I/R + 100Q	**1095** (164-4625)	**342** (134-878)	**12.74** (10.79–15.23)	**3.01 a** (2.47–5.44)

Control: Sham operation.
I/R: One hour ischemia and 2 h reperfusion.
I/R + 25Q: 25 mg/kg quercetin pretreatment + 1 h ischemia and 2 h reperfusion.
I/R + 50Q: 50 mg/kg quercetin pretreatment + 1 h ischemia and 2 h reperfusion.
I/R + 100Q: 100 mg/kg quercetin pretreatment + 1 h ischemia and 2 h reperfusion.
a: significantly different from control group ($p < 0.05$).
b: significantly different from I/R + 50Q group ($p < 0.05$).
c: significantly different from I/R + 100Q group ($p < 0.05$).

3.2. Histopathological results

Liver histology of control (Group 1) was normal. In Group 2, it was observed that hepatocyte nuclei lost their normal shape and staining characteristics and became small, dense and irregular. Vacuolization was remarkable in the cytoplasm of the hepatocytes. Sinusoids were seen enlarged and hepatocyte cordons were disorganized (Fig. 1). In Group 3, hepatocyte nuclei partially lost their normal shape and

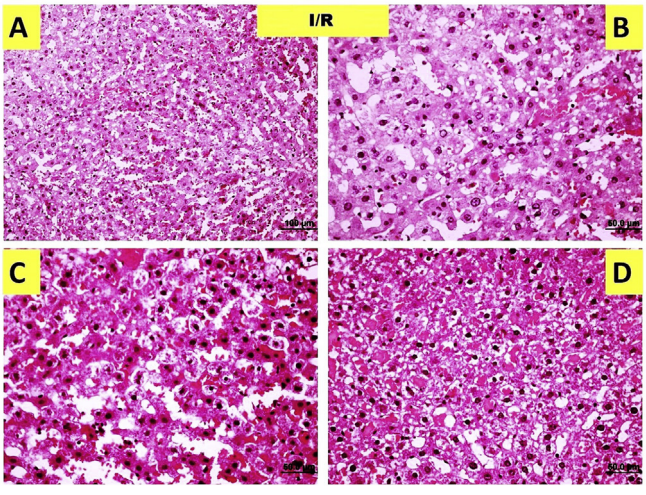

Fig. 1. Micrographs of only ischemia and reperfusion (I/R) injured liver sections of rats. Note that hepatocytes are disarranged and degenerated; their nuclei are generally pycnotic, condensed and irregular in shape. Hepatocytes have highly vacuolated cytoplasm. Oedematous fields are present between hepatocytes and vessels and sinusoids are congested. Sections are stained with H&E; scale bars are 100 μm in A, and 50 μm in B, C and D.

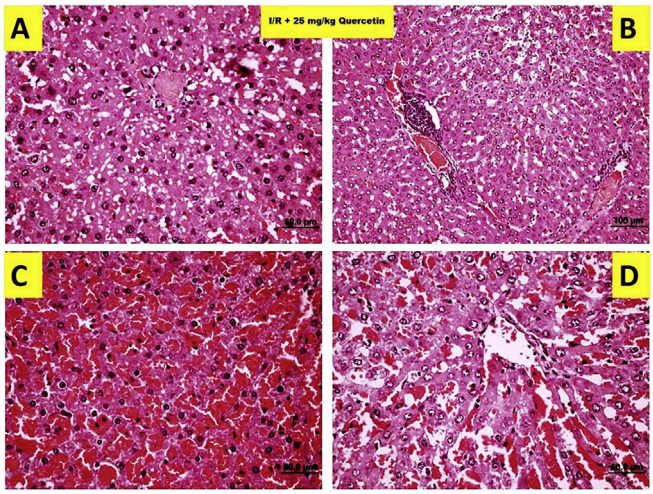

Fig. 2. Micrographs of liver sections of ischemia and reperfusion (I/R) injured and 25 mg/kg quercetin pretreated rats. There are fewer hepatocytes with degeneration signs (**A** and **B**) compared with I/R group. However, congestion of vessels is still present (**C** and **D**). Note that accumulation of inflammatory cells around vessels (**B**). Sections are stained with H&E; scale bars are 100 μm in B, and 50 μm in A, C and D.

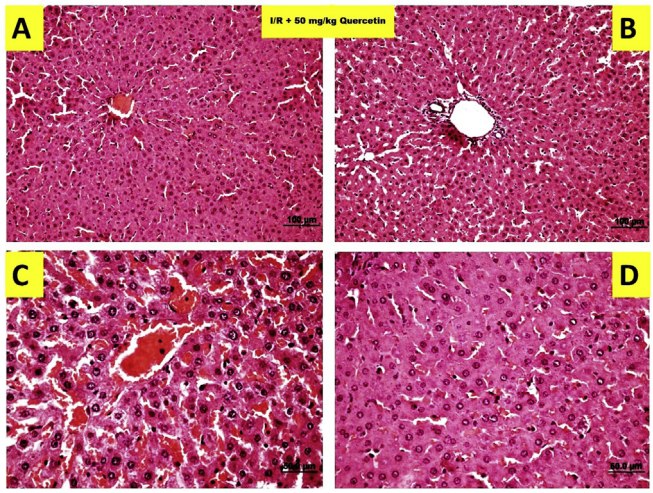

Fig. 3. Micrographs of liver sections of ischemia and reperfusion (I/R) injured and 50 mg/kg quercetin pretreated rats. There are well preserved hepatocytes and liver histology compared with other groups. Congestion of vessels is rare (**C**). Sections are stained with H&E; scale bars are 100 μm in A, B and 50 μm in C and D.

staining characteristics and became relatively small, dense and irregular. Similar to Group 2, vacuolization was remarkable in the cytoplasm of the hepatocytes. Sinusoids were seen enlarged and filled with erythrocytes. Also, inflammatory cell accumulation was marked (Fig. 2). In Group 4, both hepatocyte nuclei and cytoplasm maintained their normal shapes and staining characteristics. Occasionally, sinusoidal congestion sites were seen (Fig. 3). Quercetin at 100 mg/kg dose showed adverse effects in the protection of liver (Fig. 4). In Group 5, although normal histological features were observed in some areas, most hepatocyte nuclei lost their normal shape and staining characteristics and became small, dense and irregular. Inflammatory cell accumulation and congestion in sinusoids were observed in several areas (Fig. 4).

The histopathological scores were presented in Table 2. According to the scoring results; I/R ($p < 0.001$), I/R + 25Q ($p < 0.001$) and I/R + 100Q ($p < 0.01$) groups were showed significant differences when

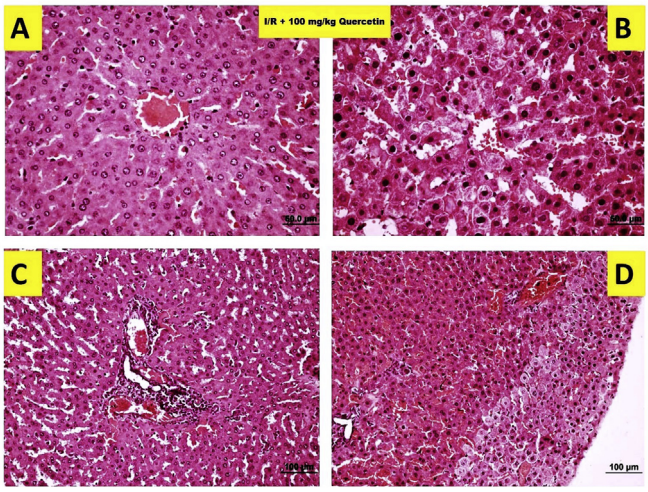

Fig. 4. Micrographs of liver sections of ischemia and reperfusion (I/R) injured and 100 mg/kg quercetin pretreated rats. There are normal (**A**) and degenerated areas (**B**) together. Note that accumulation of inflammatory cells around vessels (**C**). Hepatocytes in some areas show different staining patterns, which point abnormal histology (**D**). Sections are stained with H&E; scale bars are 100 μm in C, D and 50 μm in A and B.

Table 2
Comparison of semi-quantitative histopathological scores between the rat groups. All the values showed as median (min-max).

Groups	Median (min-max)
Control	1.00 (1.00–2.00)
I/R	5.00 (4.00–5.00) **a**
I/R + 25Q	4.00 (3.00–5.00) **a**
I/R + 50Q	3.00 (2.00–4.00) **b**
I/R + 100Q	3.50 (3.00–5.00) **a**

Control: Sham operation.
I/R: One hour ischemia and 2 h reperfusion.
I/R + 25Q: 25 mg/kg quercetin pretreatment + 1 h ischemia and 2 h reperfusion.
I/R + 50Q: 50 mg/kg quercetin pretreatment + 1 h ischemia and 2 h reperfusion.
I/R + 100Q: 100 mg/kg quercetin pretreatment + 1 h ischemia and 2 h reperfusion.
a: significantly different from control group; for I/R group ($p < 0.001$), for I/R + 25Q group ($p < 0.001$), and for I/R + 100Q group ($p < 0.01$).
b: significantly different from I/R group ($p < 0.01$).

compared to the control group. On the other hand, IR + 50Q group was not significantly different from the control group, but it was significantly different when compared to the I/R group ($p < 0.01$).

4. Discussion

Since the blood loss is important for mortality and morbidity in major hepatic surgery, it should be minimized by vascular isolation of the liver. However, vascular isolation can also cause I/R injury and increases the damage. Free oxygen-derived radicals have been suggested as the major source of ischemic tissue injury. Cell and organelle membranes, DNA and enzymes are mainly influenced. The importance of free radicals oriented the researchers to investigate the metabolism of free radicals in ischemic tissue [16]. Enzymatic and non-enzymatic defense systems including superoxide dismutase, glutathione peroxidase, and CAT decrease the detrimental effects of free radicals in the organism under normal conditions. The organism is not affected negatively when occurrence and inhibition rates of free radicals are equal.

However, a harmful effect can arise if this balance breaks down [17].

Hepatic I/R consists of two phases. In the early phase, ischemia gives harm to the organ in minutes. After reperfusion was initiated, its damaging effect lasts till 6 h. The initial phase is characterized by Kupffer cell-mediated oxidative stress during the first 2 h of reperfusion and free radicals activate Kupffer cells [18]. During the second phase, activated neutrophils adhere to endothelial cells and release free radicals and proteases such as myeloperoxidase, elastase and collagenase. Dawson et al. suggested that oxygen-derived free radicals stimulate the neutrophil activation leading to hepatocyte death [19]. It was shown that the prevention of leukocyte adhesion can block I/R injury [20]. Forming of free radicals in hepatic I/R models were reported in several animal studies [4]. The most important factor of action mechanism of free radicals is cellular membrane damage via lipid peroxidation. Also, free radicals activate some inflammatory mediators, which cause to tissue damage [21–23]. It was demonstrated that oxidative stress is effective especially in the early stage of hepatic I/R and leads to necrosis or apoptosis [24]. Antioxidants scavenge free radicals and save oxidant-antioxidant balance in the liver. They are critical to restoring the disrupted balance. Quercetin improves superoxide dismutase, catalase, AST, and ALT levels [17]. Numerous hepatocyte protective agents such as allopurinol, roscovitine, α-tocopherol, mannitol, dopamine, prostaglandins, activated carbon, glucagon, melatonin, carnitine, chlorpromazine, aprotinin, methylprednisolone, desferrioxamine, cyclosporine, catalase, aspartic acid, ubiquinone, platelet activating factor antagonists, ATP, verapamil, nifedipine and superoxide dismutase were previously described in experimental I/R models [25–28]. Hepatic I/R injury has a complex mechanism for which oxygen-derived free radicals are mainly responsible [29].

Surgical operations for liver should be performed very carefully in order to provide minimum damage and functional loss because the liver is a vital organ. In our study, we investigated the hepatic responses to I/R injury and the effects of different quercetin doses and detected that quercetin in a dose of 50 mg/kg was effective in preventing hepatic I/R injury.

Various markers have been used to evaluate the hepatic functions following the I/R injury. Most commonly used markers are ALT and AST activity. It is known that the activity of these enzymes increases in various hepatic damages. In hepatic I/R injury, serum ALT and AST levels, tissue myeloperoxidase activity and MDA levels increase while glutathione levels decrease [24]. Several studies demonstrated that reoxygenation enhances the production of detrimental oxygen radicals and therefore destroys the innate antioxidant mechanisms and increases the oxidative load in the whole body, especially in the reperfused organs. Previous studies reported that ALT and AST levels increased following the hepatic I/R [30–32]. Yabe et al. reported that the increased ALT and AST levels may result from free radical-induced tissue damage following the hepatic I/R [33]. It was reported that serum ALT and AST levels significantly increased in rats following hepatic I/R and 50 mg/kg oral quercetin pretreatment decreased AST and ALT levels significantly, indicating the hepatoprotective effect of quercetin [6]. In other studies, pretreatment with quercetin of 100 mg/kg 24 h before hepatic I/R [13] and 20 mg/kg [9] improves AST and ALT levels in rats. Additionally, it was suggested that treatment with 500 mg/kg quercetin improves methotrexate-induced AST and ALT levels [8]. In the present study, different quercetin doses caused a significant decrease in AST and ALT levels compared with I/R alone group and our results were similar to above-mentioned studies.

In the present study, we also measured both serum and tissue MDA levels which is an indicator of oxidative stress. Pretreatment of 20 [9], 40 [34] and 50 mg/kg [6] quercetin before hepatic I/R significantly lowers MDA levels in rats. In our study, pretreatment with 50 or 100 mg/kg quercetin was effective in decreasing serum and tissue MDA levels while 25 mg/kg quercetin was ineffective. Decreased MDA levels in our quercetin pretreated groups support the other studies regarding the antioxidant properties of quercetin.

I/R causes serious histopathological alterations in the liver. Several studies reported sinusoidal congestion and enlargement, cytoplasmic vacuolization, hepatocellular necrosis, neutrophil infiltration and hemorrhage in rats which underwent hepatic I/R [30–32]. Pretreatment of rats with quercetin in 20 [9], 50 [6] and 100 mg/kg [13] doses in hepatic I/R prevents the histopathological alterations in various degrees. In the study of Knudsen et al. who used a stereological method following hepatic I/R, found a close relationship between hepatocellular necrosis and ALT levels [35]. Our study results were generally similar to the above-mentioned studies. Although only a partial, but statistically insignificant histological improvement was observed in rats pretreated with 25 and 100 mg/kg doses of quercetin, 50 mg/kg dose provided a sufficient and significant protection for liver histology in our study. Therefore, we concluded that high-dose quercetin may be toxic to the liver. However, this result should be confirmed with further studies by which more reliable therapeutic dose of quercetin against hepatic I/R injury can be determined.

Flavonoids have anti-inflammatory effects including the inhibition of xanthine oxidase, phospholipase-A2, cyclooxygenase and lipoxygenase and reduction of leucocyte adhesion. Quercetin which is suggested as a potent flavonoid has positive effects on I/R injury [21,22]. It prevents the I/R injury by scavenging OH• radicals [36,37] and its precursor O_2^- radicals [38] and terminating the lipid peroxidation chain reaction via breaking lipid peroxyl radical and chelating Fe and Cu by Fenton's reaction [2,37]. Several studies showed that flavonoids decrease MDA levels [2,21,22,39,40]. In our study, serum MDA levels decreased inversely proportional with ascending quercetin dose. However, 25 mg/kg quercetin was ineffective in decreasing serum and tissue MDA levels, which indicates the importance of quercetin dose. In another study, 10, 50 and 250 μM quercetin was tested in human hepatocyte cultures, which showed that the daily dose was not very effective, but additional doses had significant effects on gene expression of hepatocytes. This points to the importance of quercetin dose [41]. We found similar results with respect to tissue MDA levels. Tokyol et al. reported that single or combined quercetin and desferrioxamine treatment decreased lipid destruction and improved histopathology results in a rat hepatic/IR model [15]. Besides, the daily administration of 10 and 20 mg/kg quercetin for one week prior to sodium fluoride intoxication provided protection in the rat liver [42].

Altogether, it is apparent that quercetin has antioxidant, anti-inflammatory, anti-apoptotic and hepatoprotective effects. Its major effect seems to be the strong antioxidant effect which was demonstrated with different doses. Quercetin exerts its beneficial effect through oxidative stress, inflammation and cell death mechanisms in hepatic disorders. It is very difficult to determine the most effective hepatoprotective dose of quercetin because there are not sufficient clinical studies on humans [5].

Nevertheless, the current study had some limitations, which should be mentioned. We performed only a one-time-point experiment in this study. It could be better to try different time points after hepatic I/R. Moreover, other serum parameters such as total oxidant state (TOS), total antioxidant capacity (TAC) and oxidative stress index (OSI) might be measured.

In conclusion, in the present study, we showed that 50 mg/kg quercetin had protective effects in rat hepatic I/R injury. However, quercetin at 25 mg/kg dose was insufficient to protect the liver, while quercetin at 100 mg/kg dose showed adverse effects in the protection of liver. Based on our results, we suggest that pretreatment of quercetin in a reliable dose may be effective to prevent the hepatic I/R injury during the hepatic vascular surgery or liver transplantation operations in clinical practice. We believe that further studies are needed to determine the proper quercetin dose for the prevention or treatment of hepatic I/R injuries.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest

There is no conflict of interest.

Ethical approval

Our study protocol was approved by local animal ethics committee of the university (Eskisehir Osmangazi University) and was compatible with the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences (Institute of Laboratory Animal Resources, 1996).

Author contribution

1-Mustafa Ufuk Uylaş (Correspond author) Data collection, conception and writing the article.

2- Adnan Şahin Conception, design and critical revision of the article.

3- Varol Şahintürk Conception, design, analysis and interpretation.

4- İbrahim Özkan Alataş Conception, design, analysis and interpretation.

Guarantor

Dr. Uylaş accept full responsibility for the manuscript.

References

[1] M. Aragno, J.C. Cutrin, R. Mastrocola, M.-G. Perrelli, F. Restivo, G. Poli, et al., Oxidative stress and kidney dysfunction due to ischemia/reperfusion in rat: attenuation by dehydroepiandrosterone, Kidney Int. 64 (3) (2003) 836–843.
[2] A. Kahraman, N. Erkasap, M. Serteser, T. Koken, Protective effect of quercetin on renal ischemia/reperfusion injury in rats, J. Nephrol. 16 (2) (2003) 219–224.
[3] P.K. Chatterjee, N.S. Patel, E.O. Kvale, S. Cuzzocrea, P.A. Brown, K.N. Stewart, et al., Inhibition of inducible nitric oxide synthase reduces renal ischemia/reperfusion injury, Kidney Int. 61 (3) (2002) 862–871.
[4] H. Jaeschke, B.L. Woolbright, Current strategies to minimize hepatic ischemia-reperfusion injury by targeting reactive oxygen species, Transplant. Rev. 26 (2) (2012) 103–114.
[5] S. Miltonprabu, M. Tomczyk, K. Skalicka-Woźniak, L. Rastrelli, M. Daglia, S.F. Nabavi, et al., Hepatoprotective effect of quercetin: from chemistry to medicine, Food Chem. Toxicol. 108 (Part B) (2016) 365–374.
[6] F.E. Ali, A.M. Abo-Youssef, B.A. Messiha, R.A. Hemeda, Protective Effects of quercetin and ursodeoxycholic acid on hepatic ischemia-reperfusion injury in rats, Clin. Pharmacol. Biopharm. 3 (2015) 128.
[7] B.L. Chen, L.T. Wang, K.H. Huang, C.C. Wang, C.K. Chiang, S.H. Liu, Quercetin attenuates renal ischemia/reperfusion injury via an activation of AMP-activated protein kinase-regulated autophagy pathway, J. Nutr. Biochem. 25 (11) (2014) 1226–1234.
[8] A.V.A. David, N. Satyanarayana, S. Parasuraman, S. Bharathi, R. Arulmoli, Ameliorative effect of quercetin on methotrexate induced toxicity in Sprague-Dawley rats: a histopathological study, Indian J Pharm. Educ. Res. 50 (3) (2016) 200–208.
[9] M. Abd-Elbaset, E.-S.A. Arafa, G.A. El Sherbiny, M.S. Abdel-Bakky, A.N.A.M. Elgendy, Quercetin modulates iNOS, eNOS and nostrin expressions and attenuates oxidative stress in warm hepatic ischemia-reperfusion injury in rats, BJBAS 4 (3) (2015) 246–255.
[10] M.F. Molina, I. Sanchez-Reus, I. Iglesias, J. Benedi, Quercetin, a flavonoid antioxidant, prevents and protects against ethanol-induced oxidative stress in mouse liver, Biol. Pharm. Bull. 26 (10) (2003) 1398–1402.
[11] M. Nassiri-Asl, S. Moghbelinejad, E. Abbasi, F. Yonesi, M.R. Haghighi, M. Lotfizadeh, et al., Effects of quercetin on oxidative stress and memory retrieval in kindled rats, Epilepsy Behav. 28 (2) (2013) 151–155.
[12] C. Kilkenny, W.J. Browne, I.C. Cuthill, M. Emerson, D.G. Altman, Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research, PLoS Biol. 8 (2010) e1000412.
[13] T. Shizuma, Relieving effect of quercetin on ischemia/reperfusion-induced liver damage in an animal model, J Nutr. Health Food Sci. 2 (1) (2014) 1–3.
[14] H. Elbe, M. Esrefoglu, N. Vardi, E. Taslidere, E. Ozerol, K. Tanbek, Melatonin, quercetin and resveratrol attenuates oxidative hepatocellular injury in streptozotocin-induced diabetic rats, Hum. Exp. Toxicol. 34 (9) (2015) 859–868.
[15] C. Tokyol, S. Yilmaz, A. Kahraman, H. Cakar, C. Polat, The effects of desferrioxamine and quercetin on liver injury induced by hepatic ischaemia-reperfusion in rats, Acta Chir. Belg. 106 (1) (2006) 68–72.
[16] S. Marubayashi, K. Dohi, K. Ochi, T. Kawasaki, Role of free radicals in ischemic rat liver cell injury: prevention of damage by alpha-tocopherol administration, Surgery 99 (2) (1986) 184–192.
[17] S. Casas-Grajales, P. Muriel, Antioxidants in liver health, World J. Gastrointest. Pharmacol. Therapeut 6 (3) (2015) 59–72.
[18] C. Nastos, K. Kalimeris, N. Papoutsidakis, M.K. Tasoulis, P.M. Lykoudis, K. Theodoraki, et al., Global consequences of liver ischemia/reperfusion injury, Oxid. Med. Cell. Longev. 2014 (2014) 906965.
[19] T.L. Dawson, G.J. Gores, A.L. Nieminen, B. Herman, J.J. Lemasters, Mitochondria as a source of reactive oxygen species during reductive stress in rat hepatocytes, Am. J. Physiol. 264 (4 Pt 1) (1993) C961–C967.
[20] D.J. Lefer, R. Scalia, B. Campbell, T. Nossuli, R. Hayward, M. Salamon, et al., Peroxynitrite inhibits leukocyte-endothelial cell interactions and protects against ischemia-reperfusion injury in rats, J. Clin. Invest. 99 (4) (1997) 684–691.
[21] R.J. Nijveldt, E. van Nood, D.E. van Hoorn, P.G. Boelens, K. van Norren, P.A. van Leeuwen, Flavonoids: a review of probable mechanisms of action and potential applications, Am. J. Clin. Nutr. 74 (4) (2001) 418–425.
[22] E. Middleton Jr., C. Kandaswami, T.C. Theoharides, The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer, Pharmacol. Rev. 52 (4) (2000) 673–751.
[23] P.A. Grace, Ischaemia-reperfusion injury, Br. J. Surg. 81 (5) (1994) 637–647.
[24] M. Elias-Miro, M.B. Jimenez-Castro, J. Rodes, C. Peralta, Current knowledge on oxidative stress in hepatic ischemia/reperfusion, Free Radic. Res. 47 (8) (2013) 555–568.
[25] N.C. Teoh, G.C. Farrell, Hepatic ischemia reperfusion injury: pathogenic mechanisms and basis for hepatoprotection, J. Gastroenterol. Hepatol. 18 (8) (2003) 891–902.
[26] N.E. Saxton, J.L. Barclay, A.D. Clouston, J. Fawcett, Cyclosporin A pretreatment in a rat model of warm ischaemia/reperfusion injury, J. Hepatol. 36 (2) (2002) 241–247.
[27] S. Topaloglu, O. Abbasoglu, A. Ayhan, C. Sokmensuer, K. Kilinc, Antiapoptotic and protective effects of roscovitine on ischemia-reperfusion injury of the rat liver, Liver Int. 23 (4) (2003) 300–307.
[28] J.M. Tredger, Ischaemia-reperfusion injury of the liver: treatment in theory and in practice, Biofactors 8 (1–2) (1998) 161–164.
[29] H. Jaeschke, Reactive oxygen and ischemia/reperfusion injury of the liver, Chem. Biol. Interact. 79 (2) (1991) 115–136.
[30] E.T. Crockett, J.J. Galligan, B.D. Uhal, J. Harkema, R. Roth, K. Pandya, Protection of early phase hepatic ischemia-reperfusion injury by cholinergic agonists, BMC Clin. Pathol. 6 (2006) 3.
[31] F. Serracino-Inglott, I.T. Virlos, N.A. Habib, R.C. Williamson, R.T. Mathie, Adenosine preconditioning attenuates hepatic reperfusion injury in the rat by preventing the down-regulation of endothelial nitric oxide synthase, BMC Gastroenterol. 2 (1) (2002) 22.
[32] C. Peralta, R. Bartrons, A. Serafin, C. Blazquez, M. Guzman, N. Prats, et al., Adenosine monophosphate-activated protein kinase mediates the protective effects of ischemic preconditioning on hepatic ischemia-reperfusion injury in the rat, Hepatology 34 (6) (2001) 1164–1173.
[33] Y. Yabe, N. Kobayashi, T. Nishihashi, R. Takahashi, M. Nishikawa, Y. Takakura, et al., Prevention of neutrophil-mediated hepatic ischemia/reperfusion injury by superoxide dismutase and catalase derivatives, J. Pharmacol. Exp. Therapeut. 298 (3) (2001) 894–899.
[34] J.F. Su, C.J. Guo, J.Y. Wei, J.J. Yang, Y.G. Jiang, Y.F. Li, Protection against hepatic ischemia-reperfusion injury in rats by oral pretreatment with quercetin, Biomed. Environ. Sci. 16 (1) (2003) 1–8.
[35] A.R. Knudsen, K.J. Andersen, S. Hamilton-Dutoit, J.R. Nyengaard, F.V. Mortensen, Correlation between liver cell necrosis and circulating alanine aminotransferase after ischaemia/reperfusion injuries in the rat liver, Int. J. Exp. Pathol. 97 (2) (2016) 133–138.
[36] Y. Hanasaki, S. Ogawa, S. Fukui, The correlation between active oxygens scavenging and antioxidative effects of flavonoids, Free Radic. Biol. Med. 16 (6) (1994) 845–850.
[37] H. Schroeter, J.P.E. Spencer, C. Rice-Evans, R.G. byl Cutler, H. Rodriguez (Eds.), Current Status of the Potential Role of Flavonoids in Neuroprotection. Critical Reviews of Oxidative Stress and Aging: Advances in Basic Science, Diagnostics and Intervention, Vol. I World Scientific Publishing, Singapore, 2003, p. 137.
[38] J. Peterson, J. Dwyer, Flavonoids: dietary occurrence and biochemical activity, Nutr. Res. 18 (12) (1998) 1995–2018.
[39] P. Devi, D. Shyamala, Protective effect of quercetin in cisplatin-induced cell injury in the rat kidney, Indian J. Pharmacol. 31 (6) (1999) 422–426.
[40] K. Nakagawa, M. Kawagoe, M. Yoshimura, H. Arata, T. Minamikawa, M. Nakamura, et al., Differential effects of flavonoid quercetin on oxidative damages induced by hydrophilic and lipophilic radical generators in hepatic lysosomal fractions of mice, J. Health Sci. 46 (6) (2000) 509–512.
[41] J. Waizenegger, D. Lenze, C. Luckert, A. Seidel, A. Lampen, S. Hessel, Dose-dependent induction of signaling pathways by the flavonoid quercetin in human primary hepatocytes: a transcriptomic study, Mol. Nutr. Food Res. 59 (6) (2015) 1117–1129.
[42] S.M. Nabavi, S.F. Nabavi, S. Eslami, A.H. Moghaddam, In vivo protective effects of quercetin against sodium fluoride-induced oxidative stress in the hepatic tissue, Food Chem. 132 (2) (2012) 931–935.