Protective effects of quercetin on thioacetamide-induced acute liver damage and its related biochemical and pathological alterations

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Background
Acute liver damage may be followed by biochemical, behavioral, and pathological alterations, which can end up in serious complications and even death.
Aim
The aim of this study was to determine whether quercetin, a flavonoid compound, which is also known to have cell-protective, antioxidant, and anti-inflammatory effects, has any protective impacts against thioacetamide (TAA)-induced liver damage in rats.
Methods
Thirty-six Sprague–Dawley rats were divided into three groups: group C1, normal rats; group C2, rats that received a single dose of TAA (350 mg/kg) intraperitoneally; and group E, rats that received a single dose of TAA (350 mg/kg)+300 mg/kg quercetin intraperitoneally. At the end, liver enzymes and plasma ammonia (NH_4) were measured, and pathological analysis of the liver carried out.
Results
The measured serological markers except for total bilirubin (alanine aminotransferase, aspartate aminotransferase, and NH_4) showed a significant decrease in group E compared with group C2. The quercetin-treated group showed a significantly lower clinical grade of encephalopathy. Pathological findings showed a significantly lower piecemeal necrosis in group E compared with group C2. Moreover, there was a nonsignificant decrease in focal necrosis, apoptosis, and focal inflammation in group E compared with group C2. Portal inflammation scores were lower in group E than in group C2. Therefore, quercetin significantly affected the grade of liver damage, as group E had lower grades compared with group C2 ($P<0.05$).
Conclusion
Overall, quercetin showed positive effects on both the liver injury and its related behavioral and biochemical changes.

Keywords:
acute liver damage, pathological change, quercetin, thioacetamide

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Introduction

Acute liver failure (ALF) is shown to be an outcome of various liver diseases [1–4]. Thioacetamide (TAA)-induced hepatic encephalopathy model is one of the most popular one for acute hepatic disorders [5]. TAA triggers hepatocellular necrosis, bridging necrosis, and lymphocytic infiltration [6–9].

Quercetin is a natural flavonoid compound of ordinary diet, which [10,11] is considered as an antioxidant and anti-inflammatory agent and acts by inhibiting the processing and formation of free radical agents [10,12].

In this study, we aimed to investigate whether quercetin can have any therapeutic effect on TAA-induced ALF in laboratory rats.

Methods

Animals and drug administrations

Thirty-six adult male healthy Sprague–Dawley rats, obtained from the Animal House Unit, Shiraz University of Medical Sciences, weighing 200±20 g were kept in standard cages at 25±3°C temperature, 40–50% humidity, 12-h light–dark cycle, and free access to standard diet and water *ad libitum* during the experiment for at least a week before the experiment. They were randomly distributed into

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three groups (n=12) as follows: the control group 1 (C1), which received 1 ml of normal saline intraperitoneally once a day from day 1; the control group 2 (C2), which received 1 ml of normal saline intraperitoneally daily initiated from 48 h before the first TAA injection; and the experimental group (E), which received 300 mg/kg (intraperitoneally) of quercetin once a day 48 h before the first TAA injection.

TAA (Sigma-Aldrich, St Louis, Missouri, USA) was dissolved in sterile normal saline solution and was injected intraperitoneally at a dose of 350 mg/kg on 2 consecutive days to all of the rats of groups C2 and E [13]; the next day the animals received 1 ml solution of 0.45% NaCl, 5% dextrose, and 0.2% KCl subcutaneously to prevent hypovolemia, hypokalemia, and hypoglycemia induced by TAA [14]. The last day of the study (day 10 here) was programmed as the day on which half or more than a half of the rats in the control group reached to grade IV of hepatic encephalopathy state according to the neurobehavioral test scores.

The experimental procedure was authorized by the Ethics Committee of Shiraz University of Medical Sciences and all criteria of taking care of laboratory animals in the 'Guide for the Care and Use of Laboratory Animals' were taken into account.

Clinical grading of encephalopathy state

Among the rats that received TAA, the behavioral symptoms of hepatic encephalopathy may modify through different stages. In this study, the scoring method showed in Table 1 was applied to compare the groups [15]. To exclude observation bias, the test was performed every other day by one observer who was blinded to the groups.

Biochemical assessment

To estimate the effect of quercetin on liver function and plasma ammonia, at the end of the study, blood samples were taken from the animals by means of cardiac puncture. The samples were then tested for the following markers: aspartate aminotransferase/alanine aminotransferase (AST/ALT), total bilirubin (TBili), alkaline phosphatase (AlkP), and level of plasma ammonia (NH_4) using clinical test kits (Randox; Randox Laboratories Ltd, London, UK).

Pathological investigation of the livers

After killing the rats, livers were removed and directly placed in 10% neutral-buffered formalin. Tissues were then embedded in paraffin, and 10-μm-thick sections were prepared, dehydrated, and stained with hematoxylin and eosin. The Ishak scoring system [16] was applied for reporting the results. Periportal or periseptal interface hepatitis (piecemeal necrosis), confluent necrosis, focal (spotty) lytic necrosis, apoptosis and focal inflammation, portal inflammation, and grade of the liver damage were scored. Existence of intraparenchymal hemorrhage (IPH) and liver congestion were also reported.

Data analysis

All data were presented as mean±SD. Statistical analyses were performed using the Mann–Whitney U-test for comparison between the groups. All data were analyzed using SPSS software (17.0; IBM, New York, USA) program and P-value of 0.05 or less was considered as statistically significant.

Results

Biochemical study

Baseline levels of the measured serological markers (C1) are shown in Table 2. There was a significant decrease in ALT, AST, and NH_4 in group E compared with group C2 ($P<0.05$) (Table 2). Plasma levels of NH_4 were significantly lower in group E (679.24±83.61) compared with group C2 (1222.85±83.61) ($P<0.05$). ALT levels were significantly decreased in group E (1734±85.97) compared with group C2 (778.33±136.78) and nonsignificantly higher compared with group C1 (129±58.97) – that is, ALT plasma concentrations approximately reached the baseline level (P=0.347).

Table 1 Clinical grading scores of the animals' behavior

Clinical grades	Definitions
0	Normal behavior
1	Mild lethargy
2	Decreased motor activity, poor gesture control, diminished pain perception
3	Severe ataxia, no spontaneous righting reflex
4	No righting reflex, no reaction to pain stimuli

Table 2 Comparison between the control and experimental groups as regards the studied parameters (mean±SD)

Groups	TBili (mg/dl)	AST (IU/l)	ALT (IU/l)	AlkP (IU/l)	NH_4 (μg/dl)
C1	1.17±0.5	109.8±53.21	129±58.97	51.2±30.74	198.98±59.097
C2	0.20±0.84	1262.67±83.04[b]	778.33±136.78[b]	898.5±256.49[b]	1222.85±83.61[b]
E	0.95±0.25[a]	330.4±80.93[a,b]	173.4±85.97[a]	371.8±81.87[a,b]	679.24±185.88[a,b]

AlkP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBili, total bilirubin. [a]$P\leq0.05$ vs. C2 group. [b]$P\leq0.05$ vs. C1 group.

AST levels were significantly decreased in group E (330.4±80.93) compared with group C2 (1262.67±83.04) ($P<0.05$). Moreover, there was a significant decrease in AlkP in group E (371.8±81.87) compared with group C2 (898.57±256.49) ($P<0.05$). In contrast, there was a significant elevation of total bilirubin in group E compared with group C2 ($P<0.05$) and a nonsignificant difference when compared with group C1 ($P>0.05$) (Table 2).

Clinical grading of encephalopathy state

As it is shown in Table 3, group E (the quercetin-treated group), despite showing an increase in clinical grades compared with C1, manifested to have significantly lower clinical grade in comparison with C2 ($P=0.037$).

Pathological analyses

Pathological findings (Table 4) demonstrated that in group E piecemeal necrosis was significantly decreased in comparison with group C2 ($P=0.012$). Although the confluent necrosis was decreased in group E, there was a statistically nonsignificant difference compared with group C2 ($P>0.05$). Focal necrosis, apoptosis, and focal inflammation were also shown to decrease nonsignificantly in group E compared with group C2 ($P>0.05$). Portal inflammation scores in group E were also manifested to be lower than that in group C2 ($P=0.03$). It was also found that group E has significantly lower grades of liver damage compared with group C2 ($P=0.003$; Table 4). Table 4 also illustrates the positive effect of quercetin on IPH and liver congestion as these were presented in group C2 in a higher percentage in comparison with group E.

Discussion

The scarcity of ALF and its unpredictable and harsh course make it a challenging entity as a subject for prospective studies. As ALF is difficult to diagnose in its early stages, the initiation of treatment frequently is delayed. Thoughtful decision making is often difficult [17,18]. Considering clinically emergent condition of these patients besides several complications of transplantation makes ALF a challenge, demanding more investigations to achieve a fruitful therapeutic method. By this way, the patients will survive until an appropriate transplant or other possible treatments can be performed for them.

According to the literature findings, apoptosis may play a significant role in the pathogenesis of TAA-induced liver injury through increased oxidative stress (for example, apoptosis mediated by H_2O_2) and an inequality of proapoptotic and antiapoptotic proteins in Bcl-2 family [19]. TAA as a hepatotoxic and a beneficial substance in ALF model also causes an elevation of oxidative stress, increasing free radical-mediated damage to proteins, lipids, and DNA [20,21].

Several studies suggest that antioxidants protect the liver against TAA-induced injury and could be valuable and practical [9,22,23].

Quercetin, which is a typical flavonoid constituent [24], is present in many herbal drugs and foods (fruits and vegetables) [25], showing wide biopharmacological features [26]. As mentioned before, several in-vitro studies have shown different biological effects of quercetin, such as antioxidant, anti-inflammatory, antithrombotic and vasodilatory actions, apoptosis induction, etc. [11]. Quercetin intake is therefore proposed to be helpful for human health and its antioxidant activity should lead to such a variety of biological effects.

It has been known that quercetin is mainly obtained from sources such as tea, red wine, fruits, and vegetables [27].

Table 3 Comparison between the control and experimental groups as regards clinical grades (mean±SD)

Groups	Clinical grade
C1 group	0
C2 group	3.5±0.57
E group	2.5±0.54[a]

[a]$P\leq0.05$ vs. C2 and C1 groups.

Table 4 Comparison between the control and experimental groups as regards the pathological changes in the liver (mean±SD) according to Ishak *et al* [16]

Group	Grade				Grade (0–6)	IPH (%)	Congestion (%)
	Portal inflammation (0–4)	Focal lytic necrosis, apoptosis and focal inflammation (0–4)	Confluent necrosis (0–6)	Periportal/periseptal interface hepatitis (piecemeal necrosis) (0–4)			
C1 group	0	0	0	0	0	0	0
C2 group	2.6±0.89	2.6±0.89	4.6±1.67	3±0.71	5±0.7	60%	60%
E group	1.33±0.52[a]	2±0.21	3±0	1.83±0.41[a]	3±1.14[b]	46%	0%

IPH, intraparenchymal hemorrhage. [a]$P\leq0.05$ vs. group C2. [b]$P<0.01$ vs. group C2.

This flavonoid universally is present in (edible) plants, and owns the most potent antioxidant effect among flavonoids of plant origin [28]. However, intervention with quercetin in human trials has revealed inconclusive and even conflicting outcomes, so far [29].

The hepatoprotective effect of quercetin may be mediated through a combination of several mechanisms, such as antioxidant defense, free radical scavenging (highly reactive species implicated in the peroxidation process such as $O_2^{\circ-}$ and OH^- radicals), anti-inflammatory, calcium channel blocking, microsomal enzyme inhibitory action, inhibition of nitric oxide production, and prevention of collagen accumulation in hepatocytes [30,31]. Quercetin also could show a potential therapeutic effect on liver toxicity by inhibiting the oxidative stress and/or directly intervening in apoptotic pathways [19].

Domitrovic and colleagues demonstrated that the increased oxidative stress and its related pERK1/2 activation plus an inequality of proapoptotic and antiapoptotic agents in the Bcl-2 family all are involved in high hepatocyte apoptosis that may contribute to the pathogenesis of TAA-induced liver damage. As confirmed in mentioned study, quercetin could characterize a potential effective therapeutic agent for liver toxicity by inhibiting the oxidative stress and directly intervening in apoptotic mechanisms [32]. Thus, using these mechanisms, quercetin could promote its effects against TAA-induced injury.

The current study evaluated the effects of quercetin administration on acute liver damage induced by TAA administration. As it was demonstrated in this study, consumption of quercetin led to a decrease in plasma ammonia level. According to Kosenko *et al.* [4], ammonia induces NO production, leading to an increased formation of superoxides, and, according to another theory, ammonia induces alterations in neurotransmission even after the metabolism of this toxin in the astrocytes [33], causing functional alterations in this cell, leading to a series of neurochemical events such as astrocyte swelling [34]. The concentrations of liver enzymes (ALT, AST, and AlkP) as indicators of liver damage were reduced in the quercetin-treated groups significantly according to the reported results in this study. Pathological analyses also approved the hepatoprotective effect of quercetin through the reduction of the portal inflammation and periportal necrosis, focal necrosis, apoptosis, and inflammation. IPH and liver congestion were also ameliorated by quercetin to some extent. Similar to these results, previous research studies demonstrated that quercetin possesses hepatoprotective effect against acrylonitrile-induced hepatotoxicity through its antioxidant activity [35]. Ji *et al.* [36] also revealed that quercetin can prevent acute liver injury caused by clivorine through apoptotic cell death inhibition and weakening of oxidative stress damage through the enhancement of the body defense capacity. Another study combined with liver histopathology showed that quercetin had a potential protective effect against perfluorooctanoic acid-induced liver injury through mechanisms such as the attenuation of oxidative stress, decreasing inflammation and inhibition of hepatocellular apoptosis [37]. The antioxidant defense of quercetin against oxidative stress action [38,39] is also the major mechanism of its protective effect on liver injury induced by ethanol [40,41]. Chen [42] indicated that the serum levels of antidiuretic hormone and γ-glutamyl transpeptidase were reduced significantly in the quercetin-treated group compared with ethanol-treated rats, which could be partially attributed to the effect of accelerating ethanol metabolism and excretion by quercetin.

These findings are particularly appealing as they support the role of natural substances as a feasible preventive treatment for several ailments caused by inflammation, such as hepatic encephalopathy. Thus, the consumption of a diet containing quercetin might be an innovative approach in providing the patients with the opportunity of transplantation in a clinical priority. In summary, because of its potential beneficial effects on such diseases, cellular and molecular studies are needed to untie the role of dietary compounds, which are becoming more and more remarkable as alternative therapies. Further investigations should also be conducted to assess its adverse effects on clinical condition of the patients.

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Conflicts of interest
There are no conflicts of interest.

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