Preventive effects of quercetin on liver damages in high-fat diet-induced obesity

Semin Gedikli¹*, Seckin Ozkanlar², Cihan Gur², Emin Sengul³ and Volkan Gelen⁴

Abstract

**Background**: Obesity is a worldwide health problem and causes many important illnesses. The current study aimed to investigate the influence of quercetin over apoptosis by inflammatory activity on high-fat diet-induced liver damage.

**Material and methods**: Totally 18 adult female Sprague-Dawley rats were selected and randomly separated equally into three groups, followed as control, obesity and obesity-quercetin groups. Obesity and obesity-quercetin groups were fed a moderately high fat diet for 120 days. After obesity occurred, quercetin dissolved in the corn oil and given obesity-quercetin group at dose of 50 mg/kg by oral gastric way for 15 days. All animals were sacrificed with an anesthesia at the end of the experiment, and venous blood samples were collected from the right ventricle and hepatic markers were evaluated by using commercially available diagnostic kits. In addition, liver sections were analysed by histopathologic and immunohistochemical procedures. Also, we evaluated the expression of Bax and Bcl-2 by immunohistochemistry.

**Results**: Obesity induced important change (p<0.05) in the most of biochemical parameters, hepatic markers, and Bax immunoreactivity as well. But, the level of Bcl-2 was slightly increased, in contrast, the level of Bax decreased markedly (p<0.05) in the obesity-quercetin group.

**Conclusions**: Both immunohistochemical evidence and biochemical results showed that application of quercetin decreased the obesity mediated damage to the liver.

**Keywords**: Apoptosis, liver, obesity , oxidative stress, quercetin

Introduction

Obesity is described as extreme and abnormal fat accumulation that may adversely affect the health. Various factors such as body metabolism, diet, physical activity, familial tendency, hypothalamic dysfunction, environmental and genetic factors may lead to obesity [1,2]. In 2008, nearly 500 million human were obese and 1.5 billion persons were overweight in the world [3]. It is estimated that until 2030, approximately 1.12 billion of the world’s population will be obese and 2.16 billion will be overweight [4]. Therefore, obesity is accepted as one of the most important and complex health problems. And, it is considered to be a risk factor for development of various diseases including, hypertension, coronary heart disease, respiratory complications, cancer, osteoarthritis, [5], type 2 diabetes, nonalcoholic fatty liver disease and metabolic syndrome [6].

Obesity leads to nonalcoholic fatty liver disease (NAFLD) which causes liver injury that progress from steatosis to steatohepatitis [7]. The most aggressive form of NAFLD is nonalcoholic steatohepatitis (NASH) [8]. In case of obesity, the metabolic rate increases and this condition may be lead to an increase in reactive oxygen species (ROS) [9]. Additionally, overweight and obesity are associated with chronic inflammation, accumulation of macrophages, increased inflammatory markers and cytokines [10]. It is increasingly accepted that ROS is one of the causes of nonalcoholic steatohepatitis [11].

The excess generation of reactive oxygen may cause cellular dysfunction and may cause irreversible damage in tissues such as liver which is a vital organ that has an important role...
detoxification process [12,13]. Oxidative stress is a biochemical process that occurs when intracellular antioxidants are unable to neutralize ROS [14]. If liver damage has occurred, it can be determined by testing the liver enzymes’ serum levels such as lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [15]. Oxidative stress contributes to many pathological conditions, such as cancer, atherosclerosis, hypertension, neurological disorders, diabetes, ischemia/perfusion, asthma, acute respiratory distress syndrome and chronic obstructive pulmonary disease [16].

The body uses natural antioxidants such as vitamin E, vitamin C (ascorbate), glutathione and various enzymes to remove the reactive oxygen species [12]. Although endogenous antioxidant enzymes provide the primary defense against oxidative stress, antioxidants can be obtained through diet for protection against ROS [17]. These substances related to body defense, such as flavonoids and other phenolics have antioxidant capacity and may protect cells and tissues against the oxidative damage [18].

Flavonoids are polyphenolic compounds of plant origin and have some clinical features such as anti-allergic, anti-bacterial, anti-viral, anti-inflammatory and anti-tumor activities [19]. Quercetin compound (3,3',4',5,7-pentahydroxyflavone) is principal sample of the flavonoid subclass of flavonols in human diets found especially in fruits, vegetables, tea, red wine, coffee, beer and several medicinal plants [20-22]. Quercetin can protect many organs such as liver, kidney and pancreatic gland against oxidative stress [23]. Recent studies shown that quercetin has anti-inflammatory and antioxidant properties, free radical scavenging and metal chelating properties [24-26]. Also several epidemiological studies have supported that the antioxidant action of quercetin may reduce the risk of developing cancer, atherosclerosis, cardiovascular disease [27-29], chronic biliary obstruction, renal injury and liver fibrosis [30].

Our aim in this study is to investigate the effects of quercetin on apoptosis and inflammatory activity in obesity induced by high fat diet.

Materials and methods
Animals and groups
In this study, totally 18 adult female Sprague-Dawley rats (weighting 150-180 g and ten weeks old) were selected and separated equally into three groups of six animals, followed as Control group (Cont), obesity group (Ob), and obesity-quercetin group (Ob+Que). The experiments were performed in compliance with the ethical norms approved by the Local Ethics Committee of Atatürk University for Animal Experiments. During the study period, the animals were kept in metal cages under a 12 hr light/dark cycle and room temperature between 20-25°C.

Obesity procedure
Obesity and obesity-quercetin groups' animals were nourished a moderately high fat diet (HFD) (32.6% lipid, 43.8% carbohydrate, 23.5% protein) for 120 days according to previous studies [31,32]. During experiment, control group was given standard rat pellets and tap water ad libitum (10% kcal as fat) without applying experimental procedure. Body weight (BW) gains were measured weekly together with food consumptions. The BW gain ratio for obesity and control groups were performed as histogram for obesity evaluation. For the obesity, the ratio values of obese rats were defined as those with BW gains equal to or more than the heaviest control rats.

Obesity parameter
Obesity was determined by Lee index for each rat at months 4,5,6,7 of life each rat. This index was calculated by the cube root of body weight (g) x 10 / naso-anal length (mm), for which a value equal to or lower than 0.300 was classified as normal. Rats presenting values higher than 0.300 g/mm were classified as obese and included in this experiment [33].

Quercetin treatment
Quercetin was obtained from Sigma Chemical Company (St. Louis, MO, USA). After the occurring of obesity (according to Lee index), quercetin dissolved in 1 ml corn oil was orally given to the quercetin-obesity group in the dose of 50 mg/kg for 15 days. As for the obese group, corn oil was orally given 1 ml a day for 15 days. Following the study, all the animals were anesthetized with xylazine hydrochloride (10 mg/kg-1) (Rompun, Bayer, Turkey) and ketamine hydrochloride (40 mg/kg-1) (Ketalar, Pfizer, Turkey). After collection of the blood samples, the rats were sacrificed and their liver tissues were removed for biochemical and histological examination.

Biochemical assays
Animals' blood samples were centrifuged at 1500 rpm duration 10 min after 10 minutes from sacrfification. Before serum samples were analysed, it were stored in the −80°C freezer. Enzyme activity in serum glucose (GLU), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), amylase and albumin (ALB) were performed using diagnostic kits with auto-analyzer (Olympus Au-2700 auto-analyzer, Tokyo, Japan).

Histopathologic examination
All rats were sacrificed by under anesthesia at the end of the study, after liver tissues of rats were removed and fixed immediately in 10% neutral buffered formalin solution for 72 hours. Liver tissue samples were embedded in paraffin blocks after fixed liver tissues were dehydrated in ascendant ethanol series and cleared in xylene. 5-μm thickness sections were cut from paraffin blocks using a Leica RM2125RT microtome (Leica Microsystems, Wetzlar, Germany). The serial sections of all groups were stained with Mallory’s triple stain modified by Crossman for histopathologic examination. The stained specimens were visualized and examined under a light microscope (Nikon eclipse i50, Tokyo, Japan) and photo images were taken for
histopathological evaluation. For histopathological evaluation, each specimen was examined in regards of inflammatory cell density, sinusoidal dilatation and degenerated cell densities in ten randomly selected areas using an approximately x20 objective lens. The scores were derived semi-quantitatively using light microscopy on the preparations from each animal and were evaluated as follows: none: −, mild: +, moderate: ++, severe: ++++, and very strong: ++++. 

Immunohistochemical staining was performed by the standard avidin-biotin-peroxidase complex, apoptotic cells were determined with immunohistochemical staining method. For immunohistochemical examination, Bax and Bcl-2 primary antibodies were used. Bax and Bcl-2 antibodies were applied at a 1/50 dilution (Abcam, Cambridge, UK), and afterwards biotinylated secondary antibody (Dako-Universal LSAB Kit-K0690) was used. The binding sites of antibodies were visualized with DAB (Sigma). Negative control slides in the absence of primary antibody were included for each staining [34].

Statistical analysis
Because the data represent a normal distribution and the coefficient variables were more than 20%, differences between the three groups were tested with the one-way analysis of variation (ANOVA) following Post hoc Duncan test using SPSS 17.0 (SPSS Inc., Chicago, USA). All data were expressed as mean averages ± Standard deviation (a value p<0.05 was considered significant).

Results
Biochemically liver parameters
The biochemical serum parameters and liver function test of animals in whole groups are cited in Table 1. There were no statistically significant differences among the groups according to ALB levels (p>0.05). Serum GLU, LDH, AST, ALT, ALP and amylase levels were statistically higher in the obesity group with respect to the control group (p<0.05). However, quercetin administration significantly decreased GLU, LDH and amylase levels compared to the obesity group (p<0.05). In the quercetin treatment group, AST, ALT and ALP levels were lower respect to the obesity group, but there were no statistically significant differences among these groups (p>0.05).

Obesity results
High fat diet caused a significant rise in Lee’s index in obesity group rats according to control group rats (Table 2). Quercetin treatment caused important reduction body weight in relation to obesity+quercetin group. This weight loss was significant as statistical (p<0.05).

Histopathologic results
Then the slides were evaluated and graded for the histopathological changes under light microscope by two histologists and one pathologist blinded to group allocations. In the histopathologic analysis, the liver sections of control groups revealed usual histological structure. Besides the section of obesity group revealed increased inflammatory cell infiltration, sinusoidal dilatation and degenerated cells compared with control group. On the other hand, these pathologic structures of obesity+quercetin group were decreased. The scores of histopathologic evaluation for all groups were presented in Table 3.

In the present study, pro-apoptotic (Bax) and anti-apoptotic (Bcl-2) immunopositive reactions in the liver sections of all groups were examined with Bax and Bcl-2 antibodies, respectively. In stereologic estimation, Bax positive cells density was higher in obesity groups than obesity treated with quercetin group (p<0.05). Also, Bax positive cell density of control group was the little than other groups (p<0.05). In Bcl-2 cell density estimation, there was lower density in obesity group than other groups (p<0.05). Also, Bcl-2 cell density of control group was higher than obesity treated with quercetin group, but there were no statistically significant differences among these groups (p>0.05). Comparisons of pro-apoptotic and anti-apoptotic activities for all groups are shown in Table 4. Also, immunohistochemical and histopathologic sections of livers belong to all groups are seen Figure 1.

Discussion
Obesity is no longer considered only as an appearance problem. Several studies have shown that obesity is a serious problem that increases the risk of for the development of numerous health problems [35] such as liver and gall bladder disease, hypertension, coronary artery disease, stroke, sleep apnea, certain cancers, cardiovascular disease, type 2 diabetes mellitus, osteoarthritis, and gynecological problems [36,37]. Feeding with high fat diet (HFD) to rats was established to be a beneficial model of accepted effects of HFD in humans. When rats fed with high fat diets, they easily gain weight. So, rat model is suitable for forming obesity [38]. Our aim in this study is to investigate the effects of quercetin on apoptosis

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Table 1. Biochemical serum parameters and liver function test of rats in all groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GLU</th>
<th>LDH</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
<th>Amylase</th>
<th>ALBUM</th>
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<tr>
<td>Control</td>
<td>122.00±43.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>914.83±87.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>141.00±14.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.83±8.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>171.67±53.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1839.00±255.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.45±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Obesity</td>
<td>279.71±56.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1070.83±65.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>169.33±21.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.50±7.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>401.00±88.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2250.00±354.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.04±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Obesity-Quercetin</td>
<td>184.00±37.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>929.25±76.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>165.33±18.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58.33±9.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>400.50±75.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2006.40±301.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.30±1.1&lt;sup&gt;a&lt;/sup&gt;</td>
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Different letters (a,b,c) in the same column indicate significant differences among groups. P<0.05 by analysis of variance and post hoc Duncan test.
Table 2. Lee obesity index values of groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Obesity</th>
<th>Obesity+Quercetin</th>
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<tr>
<td>Lee index</td>
<td>0.29±0.03a</td>
<td>0.34±0.02b</td>
<td>0.30±0.01a</td>
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The letters indicate the statistical differences among groups; p-value was considered as 0.05.

Table 3. Histopathological evaluation of liver sections of rats for all groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Inflammatory Cell</th>
<th>Sinusoidal Dilatation</th>
<th>Degenerated Cells</th>
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<tr>
<td>Control</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Obesity</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Obesity-Quercetin</td>
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<td>++</td>
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The scores were estimated as follows: none: −, mild: +, moderate: ++, severe: ++++, and very strong: +++++. These results brought out that quercetin could preserve rat liver against obesity-induced histopathologic changes. The possible therapeutic effects of quercetin on liver tissue may be associated with direct and indirect antioxidant effects.

Table 4. Stereologic estimation of Bax and Bcl-2 positive cell densities in the liver of rats for all groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Anti-Bax positive cell density per 1000 μm² area</th>
<th>Anti-Bcl-2 positive cell density per 1000 μm² area</th>
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<tr>
<td>Control</td>
<td>1.47±0.3a</td>
<td>2.44±0.4a</td>
</tr>
<tr>
<td>Obesity</td>
<td>2.89±0.5b</td>
<td>1.43±0.6b</td>
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<tr>
<td>Obesity-Quercetin</td>
<td>2.03±0.4c</td>
<td>2.21±0.5c</td>
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The letters indicate the statistical differences among groups; p-value was considered as 0.05.

Figure 1. Illustration of immunohistochemical and histopathologic sections of livers for all groups (Crossman modification triple stain, anti-Bax and anti-Bcl-2 immunohistochemical stain). cv: central vena, arrow: Bax immunopositive cells stain, black arrowheads: Bcl-2 immunopositive cells, white arrowheads: sinusoidal dilatation, asterisk: inflammatory cells.
viability may be protected in livers of obese person because some beneficial adaptive responses to obesity. These adaptive responses include the stimulation of Bcl-2 gene family proteins, which contain both proapoptotic (such as Bax) and antiapoptotic members (such as Bcl-2) [48]. These proteins play an important role in deciding whether a cell will live or die [49]. An important ratio of Bax/Bcl-2 proteins plays a crucial role in mitochondrial outer-membrane permeabilization, release of cytochrome C, and, initiation of apoptosis [50]. Quercetin, is an abundant flavonoids found in various vegetables, fruits, and teas, has been shown to induce cell death by apoptosis in many human cancer cell lines, such as leukemia, hepatoma, colon and lung cancer cell lines. It can selectively obstruct the proliferation of tumor cells and initiate cell apoptosis [51-53]. The antioxidant properties of quercetin have been studied fairly extensively and there is evidence that it may protect against DNA-damage caused by ROS [54]. Recent studies indicated that quercetin could improve metabolic parameters, oxidative stress and increasing PPAR (peroxisome proliferator-activated receptor) expression [55]. In our study revealed that quercetin administration showed potential protective effect on oxidative stress-mediated apoptosis, and DNA damage. In the current work, quercetin administration decreased apoptosis in the hepatocyte during obesity because of the decreased expression of Bax and the increased expression of Bcl-2, respectively.

Serum levels of ALT, AST, ALP [56], and blood glucose [57] are the indicator enzymes for assessing liver damage. Therefore, to determine hepatocellular damage, changes in these enzymes should be taken into consideration [56]. The increase in biochemical liver markers has been shown to cause hepatic injury, because these enzymes are located in the cytoplasm and are released into blood in the event of alteration in the hepatocyte cell membranes permeability [58]. In the present study, GLU, LDH, AST, ALT, ALP and amylase levels were significantly increased (p<0.05) in obesity group compared with control groups. These findings indicate that obesity has a hepatodegenerative effect. However, there were a decrease in levels of GLU, LDH, AST, ALT, ALP and amylase of obesity treated with quercetin group compared with obesity group (p<0.05). Although, there were an increase in level of ALB of obesity treated with quercetin group compared obesity group, there was no important distinction between these groups (p>0.05). These outcomes may support the hepatoprotective effects of quercetin towards obesity.

Conclusion
In conclusion, quercetin, which is a strong antioxidant, can be used as a therapeutic agent in preventing from obesity mediated damage to the liver. Further studies are needed to define the potential therapeutic effects of quercetin with obesity.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions

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