The protective effect of quercetin against hepatotoxicity induced by doxorubicin in male rats

Farzad Rahmani1, Parvaneh Najafizadeh2, Zahra Mousavi1*, Tayebeh Rastegar3, Elmira Barzegar4

1 Department of Pharmacology & Toxicology, Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
2 Department of Pharmacology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
3 Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
4 Molecular Research Lab, Department of Pharmacology and Toxicology, Tehran University of Medical Sciences, Tehran, Iran

Please cite this article as:

ABSTRACT
Quercetin is one of the major flavonoids finding in many fruits and vegetables. Quercetin has many health promoting advantages such as improvement of allergic, arthritis disorder, and reduction of cancer and cardiovascular risk. The aim of this study was to examine hepatoprotective effects of quercetin on doxorubicin induced toxicity in animal model. Thirty male Wistar rats (200-230 g) were randomly divided in to 5 groups including: Quercetin group was orally treated with 20 mg/kg of quercetin for 14 days. Doxorubicin group was treated with 25 mg/kg of doxorubicin i.p. for 3 days. Doxorubicin- Quercetin group (DQ) was orally pretreated of 20 mg/kg quercetin for 14 days with hepatotoxicity induced by i.p. injection of 25 mg/kg doxorubicin (12th, 13th and 14th days). The control and vehicle group were orally received saline (1 mL/kg), and DMSO (1 mL/kg) respectively. Sample of their livers were used to determine the myeloperoxidase (MPO) activity, superoxide dismutase (SOD), malondialdehyde (MDA), GPX, catalase, and histological analysis. The numerical data were evaluated by ANOVA, followed by the Tukey tests. The results show that doxorubicin could produce hepatotoxicity in rat. Also, increased liver enzymatic (ALT, AST, ALP), MPO, MDA (p<0.001), followed infiltration of inflammatory cell, and necrosis of hepatocytes also Pretreatment with quercetin reduces MPO activity (p=0.0034), MDA (p=0.0335) and the elevated liver ALT (0.009), ALP (p=0.0023) and AST activity (0.0074) in rats in DQ group. This study suggests that quercetin have a protective effect on the liver tissue against toxicity induced by doxorubicin.

INTRODUCTION
Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruits, vegetables, grains, bark roots, stem, flowers, tea and wine. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic, and anti-ulcer [1]. Quercetin (3,5,7,3',4'-pentahydroxyflavone) is a unique bioflavonoid that has been widely studied over the past decades. Quercetin is found in abundance in onion, broccoli, apple, and berries. Previous studies indicated that quercetin has antibacterial, antinociceptive (pain-relieving),...
Experimental treatment

Thirty animals were randomly divided into five groups (6 rats in each group) and weighed before and 14 days after treatment.

- Control group: Saline was administrated orally (1mL/kg) for 14 days
- Vehicle group: DMSO was administrated orally (1mL/kg) for 14 days
- Doxorubicin group: Doxorubicin was given doxorubicin interpretational (25 mg/kg) for 3 days.
- Quercetin group: Quercetin was given orally (20 mg/kg) for 14 days.
- Doxorubicin-quercetin group (DQ): Quercetin was given orally (20 mg/kg) for 14 days and doxorubicin (for inducing hepatotoxicity) was given interpretational (25 mg/kg on 12th, 13th and 14th days).

Evaluation of hepatic biochemical markers

One day after the administration of the last dose, the animals were sacrificed under ether anesthesia. Their livers were immediately removed and washed with saline. Then 10% of the liver was homogenized in buffered phosphate (pH=7.4) and centrifuged at 10000 rpm for 1min at 4°C. The supernatant was used for estimation of Biochemical parameters such as concentration of glutathione peroxidase (GPx), intensity of lipid peroxidation (LPx), CAT, and SOD (ZellBio GmbH: Germany).

The enzymatic activity was measured by Randex kinetic method, using bio-commercial kits and Hitachi 917 UV/VIS spectrophotometer (Hitachi Technologies, China). Liver tissue was homogenized in a Potter homogenizer with a buffered solution of TRIS-HCl sucrose with a ratio 1:3 at 4 °C. Liver homogenate was then prepared from 1 g of the homogenized liver tissue and filtered.

In order to assess the Myeloperoxidase (MPO) activity as a quantitative index of inflammation and marker of neutrophil infiltration in the tissue, hepatic samples were removed from freezer, 0.1g of each sample was weighted, homogenized in 50mM potassium phosphate buffer with pH 6 containing 0.5% hexadecyl trimethyl-ammonium bromide. The samples were then centrifuged with 11000 rpm for 10 min at 4 °C. Then, 0.1 ml of supernatant was combined with 2.9 ml of 50 mM phosphate buffer containing 0.167 mg/ml O-dianisidine hydrochloride and 0.005% H2O2. The Spectro-photometrical change of absorbance was measured (Shimadzu 160A UV-VIS spectrophotometer) at 460 nm. One unit of MPO activity in the final reaction was considered as the change in absorbance per minute at room temperature (25±1 °C); MPO activity (U g−1) =ΔX/weight of the separated piece of tissue, where X = 10×change in absorbance per minute/volume of supernatant taken in the final reaction.

Histological studies

Small pieces of liver tissue were fixed in 10% formalin.
The protective effect of quercetin against hepatotoxicity induced by doxorubicin

Table 1. Change in the body weight (Δ) and liver weight of the animals of control, Vehicle (DMSO) and experimental groups treated with Quercetin, doxorubicin and the combination of doxorubicin and Quercetin (DQ)

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Control</th>
<th>Vehicle</th>
<th>Doxorubicin</th>
<th>DQ</th>
<th>Quercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Body Weight (g)</td>
<td>1.82±2.4</td>
<td>-3.68±3.97</td>
<td>76.7±6.16**^ab#^&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-5.91±1.83</td>
<td>-0.37±2.2</td>
</tr>
<tr>
<td>Liver Weight (g)</td>
<td>11.23±0.58</td>
<td>10.63±0.45</td>
<td>7.60±0.58**^ab#^&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.55±0.62</td>
<td>11.77±0.58</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.E.M. (n= 6). Means were compared using one-way analysis of variance (ANOVA).

** show significant different between doxorubicin and control group (p<0.001)
*** show significant different between doxorubicin and Vehicle group (p<0.0001)
^ show significant different between doxorubicin and Quercetin group (p<0.0001)
# show significant different between doxorubicin and Vehicle group (p=0.015)
$$$ show significant different between doxorubicin and QD group (p<0.0001)
& & & show significant different between doxorubicin and Vehicle group (p<0.0001)
*** show significant different between doxorubicin and control group (p<0.0001)

used for histopathological studies. Then tissue processing was done as routine. Five-micron sections were obtained, stained with Harris Hematoxylin & Eosin and assessed for any change in histological structure under light microscope.

**Statistical analysis**

The data were expressed as mean ± SEM and statistical and correlation analyses were undertaken using the One-way ANOVA followed by a post-hoc Tukey test using GraphPad Prism 7. A p value < 0.05 was statistically significant.

**RESULTS AND DISCUSSION**

The present study confirmed that doxorubicin causes a series of side effects including decrease of the body and liver weight, elevation of hepatic ALT, AST and ALP levels, increase of MPO, infiltration of inflammatory cell, and necrosis of hepatocytes. These biochemical and pathological alterations in the rat model mostly resemble acute liver failure in human.

**Body weight changes**

The rate of gain of body weight during 2 weeks of treatment (Table 1) was different among the animal groups. Body weight gain of the animals treated with doxorubicin significantly (p<0.0001) decreased compared to control, vehicle, quercetin and DQ treated rats. A decrease in body weight was also observed in the experimental group treated with a combination of doxorubicin and quercetin (DQ) but was not significant in compared to vehicle group (p=0.5539).

**Liver weight**

Table 1 shows body and liver weight (Δ) changes in different groups. Data show that the liver and body weight of the animals treated by doxorubicin, significantly decreased compared to other groups. A decrease in liver weight was also observed in doxorubicin-quercetin group (DQ) but was not significant compared to vehicle group (p=0.97).

**Biochemical parameters of rat liver homogenate and liver enzymes activity**

The process of lipid peroxidation was significantly higher in the group treated with doxorubicin, compared to control, vehicle, quercetin and DQ groups (p<0.0001). Pretreatment with quercetin returned MDA content to normal levels and could be decrease MDA level compare to DQ group (Fig. 1).

Catalase enzyme activity in the experimental group treated with Quercetin was significantly higher (p<0.001),

![Figure 1. Effects of quercetin on hepatic MDA activity in rats treated with doxorubicin. Data are presented as mean ±S.E.M. (n= 6). Means were compared using one-way analysis of variance (ANOVA). Control (saline) group, vehicle (DMSO) group, DQ (Doxorubicin + Quercetin) group. *** show significant different between doxorubicin and control group (p<0.0001), ### show significant different between doxorubicin and control group (p<0.0001), & & & show significant different between doxorubicin and Vehicle group (p<0.0001), ^^^ show significant different between doxorubicin and Quercetin group (p<0.0001), $$ show significant different between doxorubicin and Vehicle group (p=0.015).](http://ijpt.iums.ac.ir)
Figure 2. Effects of quercetin on hepatic Catalase activity in rats treated with doxorubicin. Data are presented as mean ±S.E.M. (n= 6). Means were compared using one-way analysis of variance (ANOVA). Control (saline) group, vehicle (DMSO) group, DQ (Doxorubicin + Quercetin) group. *** show significant different between doxorubicin or quercetin and control group (p<0.0001), ### show significant different between quercetin and Vehicle group (p<0.0001), # show significant different between doxorubicin and Vehicle group (p=0.003), &&& show significant different between doxorubicin and Quercetin group (p<0.0001), and ^ show significant different between doxorubicin and QD group (p=0.0335).

Figure 3. Effects of quercetin on hepatic GSH-Px activity in rats treated with doxorubicin. Data are presented as mean ±S.E.M. (n= 6). Means were compared using one-way analysis of variance (ANOVA). Control (saline) group, vehicle (DMSO) group, DQ (Doxorubicin + Quercetin) group. *** show significant different between quercetin and control group (p<0.0001), ** show significant different between doxorubicin or vehicle and control group (p=0.0012), ### show significant different between quercetin and Vehicle group (p<0.0001), # show significant different between quercetin and DQ group (p<0.0001), and ^ show significant different between quercetin and QD group (p=0.0426).

Figure 4. Effects of quercetin on hepatic SOD activity in rats treated with doxorubicin. Data are presented as mean ±S.E.M. (n= 6). Means were compared using one-way analysis of variance (ANOVA). Control (saline) group, vehicle (DMSO) group, DQ (Doxorubicin + Quercetin) group. ** show significant different between quercetin and control group (p<0.0001), * show significant different between doxorubicin and control group (p=0.00041), & show significant different between DQ and Vehicle group (p=0.0026), &&& show significant different between doxorubicin and QD group (p=0.00001) and ^^^ show significant different between doxorubicin and QD group (p=0.0004).

quercetin (Fig. 2). DQ group increased significantly catalase enzyme activity compare doxorubicin group (p=0.0335).

There is statistically significant difference in the concentration of reduced glutathione between the experimental group, treated with doxorubicin, and the control group (p<0.001). Administrated separately, quercetin led to a statistically significant increase in glutathione peroxidase activity, compared to the enzyme activity of the animals in the control and experimental groups treated with doxorubicin and combination of doxorubicin and quercetin (p<0.0001). DQ group increased significantly catalase enzyme activity compare doxorubicin group (p=0.0335) but there wasn’t different (p=0.407) compare to control group (Fig. 3).

On the other hand, there was a marked reduction in the hepatic content of SOD in the animals treated with doxorubicin alone (p<0.001). Pretreatment with quercetin and combination of doxorubicin and quercetin improved the alterations in the hepatic SOD (Fig. 4).

MPO activity changes in hepatic homogenate of treated animals are shown in Figure 5. Doxorubicin induced a statistically significant MPO activity in comparison with the activity of control (p=0.0063) and vehicle groups (p=0.0226). Quercetin showed any significant change compared with control (p=0.71) and vehicle (=0.98) group. The MPO activity level significantly decreased in those groups treated with a combination of doxorubicin and
The protective effect of quercetin against hepatotoxicity induced by doxorubicin

Figure 5. Effects of quercetin on hepatic MPO activity in rats treated with doxorubicin. Data are presented as mean ±S.E.M. (n=6). Means were compared using one-way analysis of variance (ANOVA). Control (saline) group, Vehicle (DMSO) group, DQ (Doxorubicin + Quercetin) group.

Table 2. Liver enzymatic findings (U/L), in rats treated with saline, DMSO, Quercetin, doxorubicin and combination of Quercetin (DQ) and doxorubicin.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Control</th>
<th>Vehicle</th>
<th>Doxorubicin</th>
<th>DQ</th>
<th>Quercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>45.67±0.64</td>
<td>66.00±5.5</td>
<td>439.70±32.8³***</td>
<td>165.0±34.65³</td>
<td>148.30±33.14**</td>
</tr>
<tr>
<td>AST</td>
<td>6100±357.4</td>
<td>5087±138.9</td>
<td>8114±369.7⁶</td>
<td>4729±571.6⁶</td>
<td>4175±241.45²</td>
</tr>
<tr>
<td>ALT</td>
<td>2071±202.8</td>
<td>2710±193.8</td>
<td>4913±207.5⁵</td>
<td>3950±190.4⁵</td>
<td>1601±163.8⁶**</td>
</tr>
</tbody>
</table>

Data are presented as mean ±S.E.M. (n=6). Means were compared using one-way analysis of variance (ANOVA).

*** show significant different between doxorubicin and control group in ALP activity (p=0.0001)

## show significant different between doxorubicin and Vehicle group in ALP activity (p=0.0002)

# show significant different between doxorubicin and Vehicle group in AST (p=0.014) and ALT activity (p=0.014)

&& show significant different between doxorubicin and Quercetin group in ALP (p=0.014), AST (p=0.027) and ALT activity (p=0.0034)

^ show significant different between doxorubicin and Vehicle group in AST activity (p=0.014) and ALT activity (p=0.014)

** show significant different between doxorubicin and control group in ALP activity (p=0.0001)

### show significant different between doxorubicin and control group in AST activity (p=0.0001)

\[\text{http://ijpt.iiums.ac.ir} \]
activity, which can be prevented if quercetin is used together with doxorubicin.

Low levels of glutathione were observed during an increase in oxidative damage caused by doxorubicin administration. GSH is essential in maintaining the reduced status of the cell/tissue, and its severe depletion is reported to lead to liver injury. The impaired mitochondrial function, which may contribute to the pathogenesis of doxorubicin toxicity [6], might be a reason for the observed changes in the activities of the typical markers for oxidative status in damaged liver tissue. Hepatocytes are the likely targets of reactive oxygen species (ROS) which attack to the failing liver. It is possible that free radicals cause damage in their formation. Consequently, as a major source of ROS production, mitochondria could also be the major targets susceptible to ROS attacks [12]. The deficiency in the structural design of mitochondrial would lead to the adaptation of mitochondrial metabolism, which in return decreases the activity of mitochondrial enzymes in the doxorubicin intoxicated liver [17]. Therefore, it is considered as a key factor in dysfunction of intrinsic cell [18]. Mitochondrial degeneration and dysfunction has been reported to be associated with in doxorubicin administration [14]. The increased levels of oxidative stress enzymes (SOD, GSH-Px, CAT) were observed and confirmed in doxorubicin-induced rats [19].

In vitro experiments demonstrated rapid uptake of quercetin by cells, resulting in significant intracellular accumulation. A significant uptake by isolated mitochondria was also observed which shows that quercetin is possible to be stored there and released into the cytosol when needed [20]. In vivo experiments showed that quercetin affects the ratio of reduced glutathione (GSH) and oxidized glutathione (GSSG) [21]. It is suggested that quercetin and its metabolites tend to accumulate in the organs involved in its metabolism and excretion. Moreover, it is probable that mitochondria area place for concentration of quercetin within cells. Many in vitro and in vivo animal studies have considered the antioxidant potential of quercetin [22]. They suggest that antioxidant effects of quercetin can protect brain, heart, and other tissues against ischemia-reperfusion injury, toxicity and other factors capable of inducing oxidative stress. Under some conditions, quercetin seems to have pro-oxidant activity.

Long-term administration of quercetin (20mg/day) to Sprague-Dawley rats increased the concentrations of serum and liver alpha-tocopherol and significantly decreased malondialdehyde concentrations. It also decreased the GSH concentrations and activity of glutathione reductase significantly [23]. Daily intake of 1 mg/day of quercetin in an animal study increased the ratio of GSH:GSSG in hepatic tissue [21].

Quercetin is one of the major flavonoids belonging to the class of flavonols which is found in many fruits and vegetables with many pharmacological and therapeutic activities like antibacterial, antinociceptive (pain-relieving),...
The protective effect of quercetin against hepatotoxicity induced by doxorubicin…  7

... agent against nephrotoxicity and/or oxidative kidney damage: a detailed systematic review. Sci World J 2014;2014.

... intercellular adhesion molecules, which may help to form aggregates and increase the formation of atherosclerotic plaques [2]. Quercetin has been shown to inhibit the formation of atherosclerotic plaques in vivo [3]. This antioxidant property of quercetin might explain its protective effects against atherosclerosis.

In conclusion, quercetin has been shown to have a variety of beneficial effects on cardiovascular health. Further research is needed to better understand the mechanisms underlying its protective effects and to develop new therapeutic strategies to prevent and treat cardiovascular diseases.


