AMELIORATIVE EFFECT OF QUERCETIN AGAINST CADMIUM INDUCED TOXICITY IN LIVER OF WISTAR RATS

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Abstract: Cadmium (Cd) is an environmental and industrial cumulative pollutant that affects many organs especially the liver, kidney and testis. Quercetin is a naturally occurring flavonoid which has been reported to have a wide range of pharmacological properties. The present study was carried out to investigate the efficacy of quercetin on antioxidant and lipid peroxidation status in the liver of cadmium intoxicated rats. Oral administration of cadmium chloride (5 mg/kg b. wt./day) for 4 weeks resulted in a significant (p<0.05) elevation of serum hepatospecific markers such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT). It also elevated the levels of lipid peroxidation markers (thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides) and protein carbonyl content in liver. Contrary to these, cadmium intoxication revealed a significant reduction of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST) activities as well as reduced the levels of glutathione (GSH), vitamin C and vitamin E in liver. Oral administration of quercetin (50 mg/kg b. wt./day) along with cadmium significantly (p < 0.05) decreased the activities of serum enzymes lipid peroxidation. In addition quercetin significantly (p<0.05) recovered the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase (GST), as well as the level of GSH, vitamin C and vitamin E in the liver of cadmium intoxicated rats. These results suggested that quercetin exhibited antioxidant property and decreased the lipid peroxidation against cadmium induced oxidative stress in liver.

Key words: Cadmium, Quercetin, Lipid peroxidation, Antioxidant

INTRODUCTION

Cadmium is an inorganic toxicant of great environmental and occupational concern, which was classified as a group I human carcinogen [1]. Cadmium is discharged mainly from the industries such as electroplating, plastics, pigments, battery manufactures, fertilizers, pesticides etc. The wide environmental distribution of cadmium has led to an increased interest in its toxicity and biological effects [2]. Heavy metal stress can cause multiple direct and indirect effects on all physiological processes. The toxicity of many metals is due to their ability to cause oxidative damage such as lipid peroxidation, DNA damage and the oxidation of protein -SH groups etc.[3]. Unlike most hepatotoxicants, cadmium produces hepatic damage without biotransformation as it does not undergo enzymatic conjugation and there is no possibility of degradation. Endothelial cells are thought to be the initial target of Cd in the liver [4]. Cadmium intoxication, causes severe hepatic congestion, ischemia and hypoxia leading to neutrophil infiltration, Kupffer cell activation and inflammation which could potentially contribute to the widespread hepatocellular apoptosis and necrosis [5]. Although several chelating agents and antagonists are established to reduce the Cd toxicity, some of them are burned with undesirable side effects. Due to the intrinsic limitations and variability of efficacy
of heavy metal chelating agents, Cd intoxication therapy is looking for the development of new therapeutic agents especially from the flavonoids. Flavonoids have shown potential health benefits arising from their antioxidant properties [6]. In recent years, flavonoids as potent free radical scavengers have attracted tremendous interest as possible therapeutics, against free radical mediated diseases [7]. Quercetin (3,5,7,3′,4′-pentahydroxyflavon) is a plant bioflavonoid found in leafy vegetables, fruits (strawberry, apple etc.), beverages (tea, red wine, beer etc.). They have been reported to share a wide spectrum of pharmacological properties including anti-inflammatory [8] antiallergic [9] antitumour [10] and antioxidant abilities [11]. Quercetin directly scavenges the superoxide anion [12] and inhibits several superoxide generating enzymes such as xanthine oxidase [13] or the neutrophil membrane NADPH oxidase complex [14]. In addition quercetin has the ability to chelate metal [15]. Antithrombotic [16] hepatoprotective, antifibrogenic [17] free radical scavenger [18] anti-lipid peroxidative effects [19] have also been reported. In addition quercetin is a more potent antioxidant than the other antioxidant nutrients such as vitamin C, vitamin E and β-carotene etc. [20] and it can chelate transition metal ions including iron, thus preventing iron-catalyzed Fenton reaction [21]. Even though there are more evidences of the efficacy of quercetin against various ailments, its effects against cadmium induced hepatic dysfunction are scanty. In view of this, the present investigation has been made to evaluate the antioxidant and hepatoprotective activity of quercetin in cadmium induced hepatic dysfunction in rats.

**MATERIALS AND METHODS**

Male Wistar rats with body weight ranging 120-150 g were used for the present study. The rats were maintained under standard laboratory conditions (temperature 24 ± 2 °C, natural light-dark cycle). The rats had free access to drinking water and commercial standard pellet diet (Lipton India Ltd., Mumbai, India). The laboratory animal protocol used in this study was approved (Approval No. 160, 2007) by the committee for the purpose of control and supervision of experimental animals (CPCSEA) at Annamalai University.

**Drugs and chemicals:** Quercetin and cadmium chloride were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). All other chemicals used for the experiments were of analytical grade and obtained from local firms.

Experimental design: The animals were randomly divided into four groups of six animals in each group. The animals in group I received 0.5 ml of normal physiological saline (0.9%) for the period of 4 weeks and served as control. The animals in group II received cadmium chloride orally (5 mg/kg b. wt./day) for 4 weeks. The animals in group III received cadmium chloride (5 mg/kg b. wt./day) along with quercetin (50 mg/kg b. wt/day orally) prior to the administration of cadmium for 4 weeks. The animals in group IV received quercetin alone (50 mg/kg b. wt./day) orally for the period of 4 weeks.

At the end of the experimental period, all the animals were fasted overnight then they were killed by cervical decapitation with mild ether anesthesia. Blood was collected and centrifuged (1000 g for 15 min) for serum separation. The liver was dissected out, weighed and washed using chilled saline solution. Tissue was minced and homogenized (10% w/v) in appropriate buffer and centrifuged (3000 × g for 10 min). The resulting supernatant was used for various biochemical assays.

Levels of serum hepatic marker enzymes: The activities of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) were assayed spectrophotometrically according to the standard procedures using commercially available diagnostic kits (Sigma Diagnostics (I) Pvt. Ltd., Baroda, India).

Assay of hepatic lipid peroxidation lipid hydroperoxides and protein carbonyl contents: The lipid peroxidation indices namely TBARS and lipid hydroperoxides were estimated by the method of Fraga et al. [22] and Jiang et al. [23] respectively.
The protein carbonyl content was determined by the method of Levine et al. [24].

**Determination of antioxidants:** The levels of reduced glutathione (GSH) was determined by the method of Ellman [25]. Vitamin C (ascorbic acid) was estimated by the method of Omaye et al. [26] and vitamin E (α-tocopherol) was assayed by the method of Desai [27]. The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were assayed by the method of Kakkar et al. [28], Sinha [29] and Rotruck et al. [30] respectively. Glutathione S-transferase (GST) activity was determined by the method of Habig [31]. The total protein content in tissue homogenate was measured by the method of Lowry et al. [32] using bovine serum albumin as standard.

**Histopathological investigation:** The liver samples were fixed in 10% formal-saline and then dehydrated by passing successively in different mixtures of ethyl alcohol and water, cleaned in xylene and embedded in paraffin. Sections of liver (4-5 µm thick) were prepared and then stained with haematoxylin and eosin, and mounted in neutral DPX medium for microscopic observations.

Statistical analysis: All the data were expressed as mean ± SD of number of experiments (n=6). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 9.0 (SPSS, Cary, NC, USA) and the individual comparisons were obtained by Duncan’s Multiple Range Test (DMRT). Values were considered statistically significant when p<0.05.

**RESULTS**

Table 1 shows the levels of serum hepatic marker enzymes in control and experimental rats. Oral administration of cadmium for 4 weeks caused severe abnormalities in the levels of serum hepatic marker enzymes. The levels of serum hepatospecific enzymes such as aspartate transaminase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase and gamma glutamyl transferase were significantly increased (p < 0.05) in cadmium treated rats. Administration of quercetin along with cadmium significantly decreased (p < 0.05) the activities of serum hepatic markers when compared with cadmium alone treated rats.

The changes in the levels of lipid peroxidation products (TBARS), lipid hydroperoxides (LOOH) and protein carbonyl contents and the levels of non-enzymatic antioxidants (vitamin C and E) in control and experimental rats are shown in Table 2. The levels of thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides and protein carbonyl content were significantly higher (p < 0.05), whereas the levels of vitamin C and vitamin E were significantly lower (p < 0.05) in rats treated with cadmium. Administration of quercetin along with cadmium significantly increased (p < 0.05) the levels of vitamin C and vitamin E and also significantly decreased the level of lipid peroxidation products, lipid hydroperoxides and protein carbonyl content in liver.

Table 3 illustrates antioxidants and GSH levels were observed in cadmium treated rats as compared with control rats. Quercetin administration along with cadmium significantly (p < 0.05) increased the activities of these antioxidants when compared with cadmium treated rats.

**DISCUSSION**

Liver dysfunction is accompanied by elevated levels of serum enzymes which are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [33]. High levels of aspartate transaminase and alanine transaminases are better parameters to detect liver damage [34]. Serum alkaline phosphatase and GGT levels are also related to the status and function of hepatic cells. Increase in the level of serum alkaline phosphatase is due to the increased synthesis in the presence of increasing biliary pressure [35]. Lactate dehydrogenase is an intracellular enzyme and its increased level in serum is also an indicator of cell damage [36]. In the present
Figs. 1 to 4 are histological sections of liver of male Wistar rats stained with Haematoxylin and eosin. X 100.

Fig. 1: Liver of control rat demonstrating normal structure.
Fig. 2: Cadmium (5 mg/kg) treated rat liver showing mild necrosis and fatty degenerative changes.
Fig. 3: Cadmium (5 mg/kg) + quercetin (50 mg/kg) treated rat liver showing necrotic with mild degenerative changes were reduced.
Fig. 4: Quercetin (50 mg/kg) treated rat liver demonstrating normal appearance of hepatocytes.
Values with different superscript letter differ significantly at p < 0.05 (DMRT)

Table 1: Effect of quercetin against cadmium induced changes in the activities of serum hepatic marker enzymes on control and experimental rats. Values are mean ± SD for 6 rats in each group. Values with different superscript letter differ significantly at p < 0.05 (DMRT)

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>LDH (IU/L)</th>
<th>GGT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>57.24 ± 4.01</td>
<td>28.71 ± 1.68</td>
<td>89.71 ± 6.19</td>
<td>101.34 ± 6.27</td>
<td>81.28 ± 9.31</td>
</tr>
<tr>
<td>Cadmium</td>
<td>90.71 ± 6.13</td>
<td>50.94 ± 3.41</td>
<td>128.06 ± 8.02</td>
<td>154.97 ± 10.18</td>
<td>132.42 ± 12.63</td>
</tr>
<tr>
<td>Cd + Quercetin</td>
<td>68.57 ± 3.62</td>
<td>32.09 ± 1.51</td>
<td>101.05 ± 7.25</td>
<td>127.43 ± 9.71</td>
<td>98.71 ± 10.12</td>
</tr>
<tr>
<td>Quercetin</td>
<td>60.67 ± 3.87</td>
<td>30.17 ± 2.17</td>
<td>92.87 ± 5.64</td>
<td>112.24 ± 7.89</td>
<td>83.49 ± 9.69</td>
</tr>
</tbody>
</table>

Table 2: Effect of quercetin against cadmium on the levels of lipid peroxidation, lipid hydroperoxides and non-enzymatic antioxidants status in liver of control and experimental rats. Values are mean ± SD for 6 rats in each group. Values with different superscript letter differ significantly at p < 0.05 (DMRT)

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (mM/100 g tissue)</th>
<th>GPx (µmole/min/g protein)</th>
<th>Vitamin C (µ mole/g tissue)</th>
<th>Vitamin E (µ mole/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.91 ± 0.05</td>
<td>1.69 ± 8.7</td>
<td>1.44 ± 0.08</td>
<td>1.00 ± 0.06</td>
</tr>
<tr>
<td>Cadmium</td>
<td>1.59 ± 0.12</td>
<td>2.34 ± 18.7</td>
<td>0.95 ± 0.07</td>
<td>0.58 ± 0.04</td>
</tr>
<tr>
<td>Cd + Quercetin</td>
<td>1.13 ± 0.09</td>
<td>1.87 ± 9.2</td>
<td>1.19 ± 0.05</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.87 ± 0.07</td>
<td>1.57 ± 11.4</td>
<td>1.55 ± 0.13</td>
<td>1.03 ± 0.08</td>
</tr>
</tbody>
</table>

Table 3: Effect of quercetin against cadmium induced changes in the level of hepatic antioxidants in control and experimental rats. Values are mean ± SD for 6 rats in each group. Values with different superscript letter differ significantly at p < 0.05 (DMRT) SOD – One unit of activity was taken as the enzyme reaction which gave 50% inhibition of NBT reduction in one minute. CAT – µmoles of hydrogen peroxide consumed/min/mg protein. GPx – µg of glutathione consumed/min/mg protein, GST – µmole of CDNB-GSH conjugate formed/min/mg protein.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (units/mg protein)</th>
<th>CAT (units/mg protein)</th>
<th>GPx (µmole/mg protein)</th>
<th>GST (µmole/min/g)</th>
<th>GSH (mg/100 g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.17 ± 0.47</td>
<td>90.16 ± 5.49</td>
<td>6.13 ± 0.37</td>
<td>21.9 ± 1.5</td>
<td>42.08 ± 2.62</td>
</tr>
<tr>
<td>Cadmium</td>
<td>5.23 ± 0.39</td>
<td>57.15 ± 4.67</td>
<td>4.21 ± 0.29</td>
<td>9.6 ± 1.3</td>
<td>27.41 ± 2.25</td>
</tr>
<tr>
<td>Cd + Quercetin</td>
<td>7.13 ± 0.54</td>
<td>62.51 ± 5.12</td>
<td>5.52 ± 0.42</td>
<td>18.3 ± 1.9</td>
<td>37.71 ± 3.65</td>
</tr>
<tr>
<td>Quercetin</td>
<td>8.62 ± 0.71</td>
<td>97.91 ± 7.41</td>
<td>6.28 ± 0.53</td>
<td>24.2 ± 1.8</td>
<td>45.12 ± 3.01</td>
</tr>
</tbody>
</table>

study increased the level of hepatic serum markers suggested that an extensive liver injury was caused by cadmium due to increased lipid peroxidation indicating membrane damage. Cadmium causes structural and functional damage to the cell membrane and increased membrane permeability leading to the leakage of hepatic enzymes into blood. It is well established that Cd intoxication significantly elevated the serum hepatic marker enzymes [37,38]. Administration of quercetin effectively decreased the activities of these enzymes. This can be attributed to the antioxidant property of quercetin [16] and membrane stabilizing property [40]. It is reported that phenolic compounds scavenge free radicals [39].

Oxidative tissue injury induced by cadmium can be monitored in experimental animals by detecting lipid peroxidative products such as TBARS, HP and protein carbonyl content. In the present study cadmium administration resulted in an excessive production of free radicals such as hydroxyl radical, superoxide radical, peroxyl radical and hydrogen peroxide. All these radicals have a great potential to react rapidly with lipids which in turn leads to lipid peroxidation [41]. Decomposition products of lipid hydroperoxide such as malondialdehyde and 4-hydroxynonenal can cause chaotic cross-linkage with proteins and nucleic acids which plays an important role in the process of carcinogenesis. We observed a marked elevation of hepatic LPO following chronic Cd administration which was also reported earlier [42,43]. Furthermore, extensive damage to tissue via free radicals mediated LPO results in membrane damage and subsequently decreases the membrane fluidity [44].

Treatment with quercetin resulted in a significant decrease in the levels of TBARS, HP and protein...
carbonyl content when compared with Cd treated rats. The free radical scavenging efficiency of quercetin may be associated with the presence of two hydroxyl groups in the \( \beta \)-ring of its molecule [45]. The presence of polyunsaturated substitution on the \( \beta \)-ring of quercetin together with 2,3 double bond, a free 3-hydroxyl substitution and a 4-ketogroup confer potent antioxidant properties of quercetin [46]. Various reports indicated that LPO was blocked or prevented by quercetin administration [19,47]. Thus quercetin effectively quenches free radicals, inhibits LPO and protects the hepatic tissue from the cadmium induced oxidative damage. In addition to cellular lipids, studies have shown that cellular proteins may also be affected by free radical accumulation. The formation of carbonyl derivatives of proteins is suggested to be an useful measure of oxidative damage to proteins. The carbonyl derivatives of proteins may result from oxidative modification of amino acid side chains and reactive oxygen-mediated peptide cleavage [48]. Stohs and Bagchi [3] have reported that the primary target of the oxygen radical attack, promoted by cadmium, is represented on cellular proteins. Our results also support the above view, by showing the increased protein carbonyl content in the liver of cadmium treated rats. Treatment of quercetin along with cadmium significantly decreased the levels of protein carbonyl content, which may be due to the antioxidant property of quercetin. Quercetin by its free radical scavenging action, would prevent the attack of free radicals on amino acids and thus diminish the production of the protein carbonyl groups in quercetin along with Cd treated rats.

GSH is a tripeptide (L-\( \alpha \) glutamyl cysteiny1 glycine), an antioxidant and a powerful nucleophile, critical for cellular protection such as detoxification of ROS, conjugation with xenobiotics, excretion of toxic molecules and control of inflammatory cytokine cascade etc. [49]. Depletion of GSH in tissues leads to the impairment of cellular defense against ROS and may result in peroxidative tissue injury. In the present study the Cd treated rats showed a significant depletion of hepatic reduced glutathione (GSH) content along with the reduced levels of vitamin C and vitamin E in liver that implies Cd induced oxidative stress. Our findings are consistent with other published reports which quoted that GSH concentration decreased during chronic Cd intoxication [50]. Vitamin E, a major chain breaking antioxidant, found in the lipid phase of membrane, acts as a powerful terminator of LPO [51]. Vitamin C is the most potent water soluble antioxidant that scavenges a wide variety of reactive oxygen species and nitrogen species including superoxide radical [52]. Depleted levels of these hepatic non-enzymatic antioxidants in Cd intoxicated rats denotes the increased levels of free radical generation by Cd and could be effectively managed by vitamin C and E, which are effective free radical scavengers. Similar depletion in the level of hepatic antioxidants viz., GSH, vitamin C and vitamin E was reported in Cd intoxicated rats [53]. Under normal condition, animals maintain a balance between generation and neutralization of reactive oxygen species (ROS). However when organisms are subjected to xenobiotic compounds, rate of production of ROS, such as \( O_2^• \), \( H_2O_2 \), \( OH^- \), \( ROO^• \) exceeds their scavenging capacity. All organisms have their own cellular antioxidant defense system composed of both enzymatic and non-enzymatic components. Enzymatic pathway consists of SOD, CAT and GPx. Superoxide anion (\( O_2^• \)) dismutated by SOD to \( H_2O_2 \), which is reduced to water and molecular oxygen by CAT or is neutralized by GPx, that catalyzes the reduction of \( H_2O_2 \) to water and organic peroxide to alcohols using GSH as a source of reducing equivalent. GR regenerates GSH from oxidized glutathione (GSSG), which is a scavenger of (ROS) as well as a substrate for other enzymes. GST conjugates xenobiotics with GSH for excretion. The non-enzymatic components consists of small organic molecules such as \( \beta \)-carotene, GSH, vitamin E and vitamin C [54] some of these parameters could serve as stress indicators in animals exposed to environmental contaminants. Superoxide dismutase is considered to the first line of defense against the deleterious effects of oxygen radicals in the cells and it scavenges ROS by catalyzing the dismutation of superoxide to \( H_2O_2 \). Reports have shown that cadmium significantly depresses SOD activities [55]. The inhibition of SOD activity may result in an increased flux of superoxide in cellular compartments which may be the reason for the increased lipid peroxidative indices in our present study. Catalase acts as a preventive antioxidant and plays an important role in the protection against the deleterious effects of LPO, GPx and GST. Reports have shown that there is a significant decrease in the activities of hepatic
antioxidant enzymes in Cd intoxicated rats due to the over production of ROS (reactive oxygen species) by cadmium [56]. Glutathione peroxidase is also a first line of defense against oxidative damage by $H_2O_2$ or lipid hydroperoxides thus protecting the membrane from oxidative damage. GST is a second line of defense against xenobiotics by its direct conjugation with the expense of GSH. Both were glutathione dependent. In the present study their levels (GPx and GST) were significantly decreased in Cd intoxicated rats due to the overproduction of $H_2O_2$ by free Cd ions [38]. Thus the analysis of antioxidant status in our study indicates that the levels of both non-enzymatic and enzymatic antioxidants were decreased due to cadmium oxidative stress. Co-administration of quercetin along with cadmium significantly modulates the antioxidant status in liver, suggesting the enhancing effect of quercetin on cellular antioxidant defenses. The antioxidant role of quercetin may include the following interventions. Scavenging of $O_2^-$, $OH^•$, peroxyl radical and peroxynitrite [57]. Reports have shown that quercetin protects the DNA damage from cadmium intoxication [58]. Quercetin also enhances the GSH dependent protection and prevents the depletion of thiols during oxidative stress [59]. Thus quercetin might have quenched free radicals and inhibited LPO and ultimately decreased the burden of antioxidants in cadmium induced oxidative stress.

In conclusion, the present investigation shows that quercetin possesses an antioxidant property, which may be attributed to its protective action on lipid peroxidation and to the enhancing effect on cellular antioxidant defenses that might further contribute to the protection against oxidative damage in cadmium-induced hepatotoxicity.

REFERENCES


