Curcumin prevents oxidative renal damage induced by acetaminophen in rats

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1. Introduction

Acetaminophen (APAP), also known as paracetamol, is most widely used in the world as an analgesic and antipyretic drug that is safe at therapeutic dosages (Kanno et al., 2006) and is the most commonly reported toxic ingestion in the United States (Mazer and Perrone, 2008). APAP is known to cause hepatic necrosis and renal failure in both humans (O’Grady, 1997; Prescott et al., 1971; Egwui and Materson, 1997) and animals (Potter et al., 1973; Moller-Hartmann and Seigers, 1992) when administered in overdoses. Renal damage and acute renal failure can occur even in the absence of liver injury (Prescott et al., 1971). Renal insufficiency occurs in approximately 1–2% of the patients with an overdose of APAP (Prescott, 1993).

The mechanism of APAP toxicity in the liver is well described, but in the kidney it is less clearly understood. APAP-induced renal insufficiency is consistent with acute tubular necrosis, an increase in the plasma creatinine level and a decrease in the glomerular filtration rate (Cobden et al., 1982; Blakely and McDonald, 1995). Oxidative stress is reported to play a role in the pathogenesis of APAP-induced renal damage, as evidenced by an increase in the lipid peroxidation (Balantz, 1996; Jaya et al., 1993) and the depletion of intracellular glutathione (GSH) (Li et al., 2003; Trumper et al., 1992).

Because APAP can cause life-threatening renal damages, the antidote or treatment of APAP-induced nephrotoxicity has a toxicological importance. Although N-acetyl-cysteine, a GSH precursor, protects against APAP hepatotoxicity in humans (Engelhardt and Hoppmann, 1996; Prescott, 1993), it is not protective against APAP-induced renal damage (Davenport and Finn, 1988; Blakely and McDonald, 1995). Antioxidants such as melatonin, vitamins E and C have also been used to prevent APAP-induced hepatotoxicity in mice (Sener et al., 2003; Abraham, 2005).

Curcumin (CMN) is a major yellow pigment in turmeric (the ground rhizome of Curcuma longa Linn), which is widely used as a spice and a colouring agent in several foods such as curry, mustard and potato chips as well as in cosmetics and drugs (Okada et al., 2001; Joe et al., 2004). It represents a class of anticancer agents (Gafner et al., 2004) and antioxidants (Kempaiah and Srinivasan, 2004) and has a strong potency in inhibiting the generation of reactive oxygen species (ROS). CMN administration has been reported to prevent renal lesions in streptococcal diabetic rats (Suresh and Srinivasan, 1998), and to protect against oxidative stress in renal cell lines (Okada et al., 2001; Balogun et al., 2003). CMN also

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A B S T R A C T

Acetaminophen (APAP) can cause life-threatening renal damages and there is no specific treatment for APAP-induced renal damage. The aim of this study was to investigate the protective effects of curcumin (CMN) on APAP-induced nephrotoxicity.

Nephrotoxicity was induced in male Wistar Albino rats by the administration of a single dose of 1000 mg/kg APAP intraperitoneally (i.p.). Some of these rats also received i.p. CMN (200 mg/kg) at 30 min after the administration of APAP. Twenty-four hours after the administration of APAP, all the rats were sacrificed with a high dose of ketamine. Urea and creatinine levels were measured in the blood, and the levels of malondialdehyde (MDA) and glutathione (GSH), and antioxidant enzyme activity were determined in the renal tissue. Histopathological changes were studied.

APAP administration caused elevated levels of renal MDA, and marked depletion of GSH levels and antioxidant enzyme activity, and deteriorated the renal functions as assessed by the increased plasma urea and creatinine levels as compared to control rats. CMN markedly reduced the elevated MDA levels, significantly increased the antioxidant enzyme activity and normalized the altered renal morphology in rats treated with APAP.

CMN might be a potential candidate agent against APAP-induced nephrotoxicity, but further studies are required to identify this issue before clinical application becomes possible.

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prevents or attenuates nephrotoxicity caused by cisplatin (Antunes et al., 2001) and Adriamycin (Venkatesan et al., 2000).

The aim of this study was to investigate the protective effects of CMN on the experimentally APAP-induced renal toxicity.

2. Materials and methods

2.1. Chemicals

APAP was purchased from Sandoz Chemical Corp, Istanbul, Turkey, and CMN was obtained from Sigma Chemicals Corp, St. Louis, MO, USA. APAP was administered via gavage. CMN was dissolved in corn oil and was given orally.

2.2. Animals

Male Wistar Albino rats weighing approximately 340 ± 80 g were used in this study. The animals were housed in clean plastic cages in a temperature- and humidity-controlled facility with a constant 12 h light/dark cycle. Food and tap water were offered during the study period. All treatments were started after almost 1 week of stabilization from arrival. The use of animals and the experimental protocol were approved by the Institutional Animal Care and Use Committee and animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals of Research Council.

2.3. Treatment and experimental design

The animals were randomly divided into five groups, each consisting of six animals. APAP was dissolved in normal saline and injected i.p. at single doses of 1000 mg/kg/day. CMN was dissolved in corn oil and was injected i.p. at the dose of 200 mg/kg body weight. The choice of the dose of APAP was based on the results of previous studies (Abraham, 2005; Sener et al., 2003), and the dose of CMN was also selected based on the results of recent studies where the antioxidant effect of this agent was apparent (Chuang et al., 2000). Group 1 rats served as control and received a single dose of i.p. injection of 1 mL isotonic saline. Group 2 rats were treated with a single dose of APAP. Group 3 rats received APAP + CMN. CMN was administered 30 min after the injection of APAP. Group 4 rats were treated with CMN alone. Group 5 rats received APAP + corn oil (the curcumin vehicles).

Rats were anesthetized by intraperitoneal injection of ketamine, 24 h after the administration of APAP. Blood samples were taken from the vena cava to measure some biochemical parameters. Then, all the rats were sacrificed with a high dose of ketamine.

Kidneys were reached with an abdominal middle-line incision and were then quickly removed and separated from the surrounding tissues, washed twice with cold saline solution and one of the kidneys was stored at -80 °C to determine the level of renal malondialdehyde (MDA) and glutathione (GSH) and the activities of glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD). The other kidney was stored in formol solution for the histopathological examination.

2.4. Biochemical analysis

Serum samples were analyzed for urea, creatinine, Na + and K + (Marsh et al., 1965; Spencer, 1986). All biochemical variables were determined using an Olympus Autoanalyser (Olympus Instruments, Tokyo, Japan). Kidney tissue (300 mg) was homogenized in ice-cold tamponade containing 150 mM KCl for the determination of MDA. MDA levels were assayed for products of lipid peroxidation, MDA, which is referred to as thiobarbituric acid-reactive substance, was measured with thiobarbituric acid at 532 nm in a spectrophotometer as if we were reading it naturally.

GSH was determined by the spectrophotometric method, which was based on the use of Ellman’s reagent (Beutler, 1975).

Glutathione peroxidase (GSH-Px) activity was measured according to Paglia and Valentine (1987), by monitoring the oxidation of reduced NADPH at 340 nm. Enzyme units were defined as the number of micromoles of NADPH oxidized per minute and were calculated using the extinction coefficient of NADPH at 340 nm (6.22 × 105/mole/cm). The results are reported as units per gram protein.

CAT activity was determined according to the method of Aebi, by monitoring the initial rate of the disappearance of hydrogen peroxide at 240 nm in a spectrophotometer (Aebi, 1982). The results are reported as the constant rate per second per gram of protein.

SOD activity was measured according to Sun et al. by determining the reduction of nitroblue tetrazolium (NBT) by superoxide anion produced with xanthine and xanthine oxidase (Sun et al., 1988). Half unit of SOD is defined as the amount of protein that inhibited the rate of NBT reduction.

2.5. Histopathological examination

Histopathological evaluation was done in kidney tissues. Paraffin-embedded specimens were cut into 6-μm/thickness sections and were stained with hematoxylin–eosin for light microscope examination (Olympus, BH-2, Tokyo, Japan). All the sections of the kidney samples were examined for characteristic histological changes including tubular epithelial alterations (vacuolization, degeneration, and cell desquamation), and cortical interstitial congestion. The kidney sections were analyzed semi-quantitatively using the technique of Houghton et al. (1978). The lesions were graded as follows: 0, normal; 1, areas of focal granulo-vacular epithelial cell degeneration and granular debris in the tubular lumen, with or without evidence of tubular epithelial cell desquamation in small foci (<1% of the tubule population involved by desquamation); 2, tubular epithelial necrosis and desquamation easily seen but involving less than half of the cortical tubules; 3, more than half of the proximal tubules showing desquamation and necrosis but involved tubules easily found; 4, complete or almost complete proximal tubular necrosis.

2.6. Statistics

The results of all the groups are shown as mean values ± standard deviation (SD). Statistical analyses of the biochemical data were done by the Mann–Whitney U-test. P < 0.05 was accepted as a statistically significant value.

3. Results

No deaths or remarkable signs of external toxicity were observed in the groups of rats that were given APAP either alone or in combination with CMN. The biochemical and histopathological results were similar for APAP and APAP + vehicle groups and control and CMN groups, and we decided to consider them without distinction and report only those for the APAP and control groups, respectively. The effect of CMN on plasma urea and creatinine levels in rats treated with APAP is reported in Table 1. Significant increase in the plasma urea and creatinine levels was noted in the rats treated with APAP alone in comparison with the control, and administration of CMN to rats treated with APAP prevented the APAP-induced increase in plasma urea and creatinine levels (p < 0.01 Table 1).

Table 1 shows the effect of CMN on the activities of antioxidant enzymes such as GSH-Px, CAT and SOD, and lipid peroxidation, which were assayed for products of lipid peroxidation, MDA, which is referred to as thiobarbituric acid-reactive substance, was measured with thiobarbituric acid at 532 nm in a spectrophotometer as if we were reading it naturally.

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>APAP</th>
<th>APAP + CMN</th>
<th>CMN</th>
<th>APAP + vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.24 ± 0.04</td>
<td>1.24 ± 0.43 ***</td>
<td>0.34 ± 0.07 ***</td>
<td>0.22 ± 0.03</td>
<td>1.30 ± 0.4</td>
<td></td>
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<tr>
<td>BUN (mg/dL)</td>
<td>19.6 ± 2.1</td>
<td>80.1 ± 12.8 ***</td>
<td>28.3 ± 8.3 ***</td>
<td>21.3 ± 1.4</td>
<td>84.5 ± 8.6</td>
<td></td>
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<tr>
<td>Na+ (mmol/L)</td>
<td>137.2 ± 2.6</td>
<td>141.1 ± 2.9 ***</td>
<td>143.1 ± 3.8 ***</td>
<td>139.4 ± 2.4</td>
<td>143 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>K+ (mmol/L)</td>
<td>4.0 ± 0.3</td>
<td>4.1 ± 0.5 ***</td>
<td>4.1 ± 0.3 ***</td>
<td>3.9 ± 0.6</td>
<td>4.1 ± 0.7</td>
<td></td>
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</tbody>
</table>

Values are expressed as mean ± SD for six rats in each group.

* Compared with control group.

** Compared with APAP group.

*** P < 0.05.

**** P < 0.01.
levels were significantly increased in groups treated with APAP or ameliorated by treatment with CMN. Plasma urea and creatinine effects caused by acute administration of APAP could be prevented pared with the APAP group (Fig. 1 C).

In rats treated with APAP + CMN, despite the presence of mild tubular degeneration and epithelial vacuolization in the proximal tubules, cellular desquamation was minimal and glomeruli maintained a better morphology (grade of tubular necrosis: 0–2) compared with the APAP group (Fig. 1C).

4. Discussion

Nephrotoxicity and hepatotoxicity are the potential complications of APAP, which is widely used in general medicine, and an assessment of its relative toxicity is important. A number of drugs or chemicals such as melatonin, vitamin E and N-acetyl-cysteine have been used to prevent APAP-induced hepatic and renal injury (Sener et al., 2003).

In the present study, we assessed whether the nephrotoxic effects caused by acute administration of APAP could be prevented or ameliorated by treatment with CMN. Plasma urea and creatinine levels were significantly increased in groups treated with APAP alone, demonstrating the deterioration of the renal function, in comparison with those of the control and APAP + CMN groups. These findings are consistent with the results of a previous study in which APAP was administered to rats (Sener et al., 2003).

Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>APAP</th>
<th>APAP + CMN</th>
<th>CMN</th>
<th>APAP + vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/g wet tissue)</td>
<td>35.3 ± 5.9</td>
<td>60.4 ± 4.9&lt;sup&gt;∗∗&lt;/sup&gt;</td>
<td>34.8 ± 6.9&lt;sup&gt;∗&lt;/sup&gt;</td>
<td>36.1 ± 4.6</td>
<td>63.5 ± 5.3</td>
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<tr>
<td>GSH (µg wet tissue)</td>
<td>1.9 ± 0.2</td>
<td>0.96 ± 0.1&lt;sup&gt;∗∗&lt;/sup&gt;</td>
<td>1.7 ± 0.5&lt;sup&gt;∗&lt;/sup&gt;</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.3</td>
<td></td>
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<tr>
<td>NO (nmol/g wet tissue)</td>
<td>44.1 ± 11.2</td>
<td>92.6 ± 12.6&lt;sup&gt;∗∗&lt;/sup&gt;</td>
<td>52.2 ± 9.5&lt;sup&gt;∗&lt;/sup&gt;</td>
<td>46.5 ± 6.8</td>
<td>96.4 ± 8.4</td>
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<tr>
<td>GSH-Px (IU/mg protein)</td>
<td>8.57 ± 1.6</td>
<td>7.1 ± 0.8&lt;sup&gt;∗∗&lt;/sup&gt;</td>
<td>8.39 ± 0.56&lt;sup&gt;∗&lt;/sup&gt;</td>
<td>8.6 ± 0.7</td>
<td>6.9 ± 0.4</td>
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<tr>
<td>CAT (k/s/mg protein)</td>
<td>28 ± 0.6</td>
<td>20 ± 1.5&lt;sup&gt;∗∗&lt;/sup&gt;</td>
<td>28 ± 2.1&lt;sup&gt;∗&lt;/sup&gt;</td>
<td>29 ± 0.5</td>
<td>19 ± 2.3</td>
<td></td>
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<tr>
<td>SOD (U/mg protein)</td>
<td>159 ± 1.6</td>
<td>118.4 ± 2.1&lt;sup&gt;∗∗&lt;/sup&gt;</td>
<td>127 ± 4.3&lt;sup&gt;∗&lt;/sup&gt;</td>
<td>160 ± 1.8</td>
<td>114.5 ± 3.4</td>
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</tbody>
</table>

Values are expressed as mean ± SD for six rats in each group. CMN; curcumin, APAP; acetaminophen 1000 mg/kg, MDA; malondialdehyde, GSH; reduced glutathione, GSH-Px; glutathione peroxidase, CAT; catalase and, SOD; superoxide dismutase.

**P < 0.01.  
*P < 0.05.

Fig. 1. Kidney morphology in (A) a control rat, (B) a rat treated with APAP, (C) a rat treated with APAP plus CMN (hema-toxylin and eosin × 200).

In the present study, we assessed whether the nephrotoxic effects caused by acute administration of APAP could be prevented or ameliorated by treatment with CMN. Plasma urea and creatinine levels were significantly increased in groups treated with APAP alone, demonstrating the deterioration of the renal function, in comparison with those of the control and APAP + CMN groups. These findings are consistent with the results of a previous study in which APAP was administered to rats (Sener et al., 2003).

Administration of CMN modified the plasma urea and creatinine levels in all the rats treated with APAP. Earlier studies have also shown that CMN pre-treatment decreases ischemia-reperfusion-induced rise in serum creatinine levels in rats (Shoskes, 1998).

The primary toxicity of APAP is the result of drug metabolism in both the liver and extrahepatic tissues (Gu et al., 2005). At therapeu tic doses, APAP is metabolised via glucuronidation and sulfa tion reactions occurring primarily in the liver which result in the water-soluble metabolites that are excreted via the kidney. The result of the metabolic conversion of APAP by the microsomal P-450 enzyme system is that, a highly reactive intermediate, namely, N-acetyl-p-benzoquinone imine (NAPQI) is produced. This metabolite is then reduced by glutathione (GSH) (Bessens and Vermeulen, 2001). APAP-induced nephrotoxicity may be due to this metabolic activation of APAP to the reactive metabolite, NAPQI (Hart et al., 1994). When large doses of APAP are ingested, there is more severe GSH depletion as well as massive production of metabolites, which compounds the toxicity, leaving large amounts of reactive metabolite unbound. These intermediates then form covalent bindings with macromolecules on cellular protein (Bessens and Vermeulen, 2001). This process disrupts homeostasis and initiates apoptosis, or programmed cell death, leading to tissue necrosis and ultimately to organ dysfunction. The concentration of intracellular GSH, therefore, is the key determinant of the extent of APAP-induced renal injury, thus, interest has been focused on compounds that act as antioxidants and are capable of stimulating GSH synthesis.

Depletion of renal GSH is one of the primary factors which permits lipid peroxidation, suggested to be closely related to APAP tissue damage, and MDA is a good indicator of the degree of lipid
peroxidation (Ross, 1988; Sener et al., 2000). It has been reported that renal GSH levels, activities of renal GSH reductase and peroxidase, which are critical constituents of GSH-redox cycle, were significantly reduced due to Adriamycin treatment and the authors proposed that the impairment of the kidney antioxidant defence mechanisms could permit enhanced free radical-induced kidney damage in Adriamycin nephrotoxicity (Simic et al., 1996). Similarly, in the present study, administration of CMN to APAP-treated rats also increased GSH level and GSH-Px, CAT and SOD activities of renal tissue. This increase in both the non-enzymatic and enzymatic antioxidants may play a significant role in the mechanism of the nephroprotective effect of CMN (Sharma, 1976).

Furthermore, it has been suggested that lipid peroxidation might be a contributing factor to the development of renal toxicity. It is likely that the action of CMN in reducing the membrane damage is partially related to its ability to scavenge lipid peroxidation-initiating agents (Skrzydlewska et al., 2002). In this study, we also observed a significant increase in the MDA levels in the renal tissue of rats treated with APAP alone compared with the control.

APAP-induced renal damage is consistent with acute tubular necrosis. In the present study, the results of histopathological examination showed a clear evidence of nephrotoxicity following the administration of APAP in an overdose. Acute tubular necrosis was the most relevant histopathological change. These results are in agreement with those of the previous investigation describing the renal histological alterations following the administration of APAP in an overdose (Abraham, 2005). CMN co-treatment ameliorated the APAP-induced histopathological renal changes.

The preventive effect of CMN on the nephrotoxicity induced by APAP possibly depends on its ability to mainly eliminate the hydroxyl radical (Reddy and Lokes, 1994), superoxide radical (Sreejayan and Rao, 1996), singlet oxygen (Rao et al., 1995), nitrogen dioxide (Unnikrishnan and Rao, 1995), and NO (Sreejayan and Rao, 1997). It has also been demonstrated that CMN inhibits the generation of superoxide radical (Ruby et al., 1995). In the present study, APAP administration caused a significant deterioration of endogenous antioxidant profile as evidenced by the decrease in GSH-Px, CAT, and SOD activities, an effect which was effectively reversed by CMN treatment.

In summary, in the light of biochemical results and histopathological findings, the present data confirmed that CMN might be a potential candidate agent against experimentally induced APAP nephrotoxicity via its antioxidant and free radical-scavenging properties. However, further investigations are needed to demonstrate the exact mechanism of CMN on APAP-induced nephrotoxicity.

Conflict of interest

The authors declare that there are no conflicts of interest.

References


