Toxicology

Protective effect of cannabidiol against cadmium hepatotoxicity in rats

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A R T I C L E   I N F O

Article history:
Received 11 April 2013
Accepted 1 July 2013

Keywords:
Cannabidiol
Cadmium toxicity
Liver
Rats

A B S T R A C T

The protective effect of cannabidiol, the non-psychoactive component of Cannabis sativa, against liver toxicity induced by a single dose of cadmium chloride (6.5 mg kg−1 i.p.) was investigated in rats. Cannabidiol treatment (5 mg kg−1/day, i.p.) was applied for five days starting three days before cadmium administration. Cannabidiol significantly reduced serum alanine aminotransferase, and suppressed hepatic lipid peroxidation, prevented the depletion of reduced glutathione and nitric oxide, and catalase activity, and attenuated the elevation of cadmium level in the liver tissue resulted from cadmium administration. Histopathological examination showed that cadmium-induced liver tissue injury was ameliorated by cannabidiol treatment. Immunohistochemical analysis revealed that cannabidiol significantly decreased the cadmium-induced expression of tumor necrosis factor-α, cyclooxygenase-2, nuclear factor-κB, caspase-3, and caspase-9, and increased the expression of endothelial nitric oxide synthase in liver tissue. It was concluded that cannabidiol may represent a potential option to protect the liver tissue from the detrimental effects of cadmium toxicity.

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Introduction

The heavy metal cadmium is considered as one of the most common environmental pollutants in the biosphere [1]. It was stated that the provisional tolerable monthly intake for cadmium is 25 μg/kg body weight, and the safe cadmium levels are 3 μg/l in drinking water, and an annual average of 5 mg/m² in air [2]. However, hazardous exposure to cadmium occurs due to either occupational or non-occupational reasons. Industrial uses of cadmium in metal plating, pigments, plastics, glass, fertilizers, and batteries are the risk factors for occupational exposure. On the other hand, tobacco smoking, air pollution, and consumption of cadmium-contaminated drinking water are the major sources for non-occupational cadmium exposure [3]. Serious injury and dysfunction of different body organs were reported due to acute and chronic cadmium toxicity in humans and animals [4]. Acute cadmium exposure results primarily in accumulation of the metal in the liver and acute hepatotoxicity [5]. Growing evidence suggests that acute cadmium hepatotoxicity is a biphasic process including an initial phase caused by direct metal actions and ischemia, and a latter one due to inflammation and oxidative stress [6,7]. It is well-known that oxidative stress leads to activation of nuclear factor-κB signaling pathway which is crucial for regulation of many genes involved in inflammatory responses, as tumor necrosis factor-α, cyclooxygenase-2, nuclear factor-κB, caspase-3, and caspase-9, and increased the expression of endothelial nitric oxide synthase in liver tissue.

Cannabidiol is the major non-psychoactive cannabinoid component derived from the plant Cannabis sativa. It possesses powerful antioxidant and anti-inflammatory activities. However, the exact mechanisms of action of cannabidiol remain obscure. In contrast to the other cannabinoids, cannabidiol is known to have a very low affinity for the cannabinoid CB1 and CB2 receptors. The antioxidant and anti-inflammatory effects of cannabidiol may be due to its direct action or mediated through a new cannabinoid, non-CB1 and non-CB2, receptor [14,15]. Cannabidiol may also exert its beneficial effects by inhibiting adenosine uptake and activating transient receptor potential vanilloid-1 [16,17]. Previous reports demonstrated that cannabidiol may have therapeutic utility in a number of conditions involving inflammation and oxidative stress, including diabetes mellitus, rheumatoid arthritis, and neurodegenerative disorders [18–20]. The protective effect

The effects of cannabidiol (CBD) treatment on serum alanine aminotransferase (ALT), and hepatic malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO), and cadmium levels, and catalase and superoxide dismutase (SOD) activities in rats exposed to cadmium (Cd) toxicity.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vehicle + Cd</th>
<th>CBD + Cd</th>
<th>CBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ALT (U/L)</td>
<td>55.12 ± 3.91</td>
<td>861.26 ± 73.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>305.48 ± 34.17&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>71.49 ± 8.26</td>
</tr>
<tr>
<td>MDA (nmol/g tissue)</td>
<td>149.68 ± 16.27</td>
<td>371.94 ± 29.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>219.47 ± 25.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>138.75 ± 11.67</td>
</tr>
<tr>
<td>GSH (mmol/g tissue)</td>
<td>14.80 ± 2.62</td>
<td>3.71 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.11 ± 1.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.36 ± 1.34</td>
</tr>
<tr>
<td>NO (nmol/100 mg tissue)</td>
<td>117.49 ± 7.57</td>
<td>69.20 ± 5.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>163.98 ± 8.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>109.36 ± 11.18</td>
</tr>
<tr>
<td>Catalase (U/mg protein)</td>
<td>70.83 ± 8.12</td>
<td>19.44 ± 1.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.58 ± 6.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.39 ± 5.28</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>105.42 ± 9.56</td>
<td>33.86 ± 5.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.16 ± 3.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.83 ± 7.36</td>
</tr>
<tr>
<td>Cadmium (μg/g tissue)</td>
<td>1.05 ± 0.07</td>
<td>5.28 ± 0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.73 ± 0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.91 ± 0.09</td>
</tr>
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</table>

All the values are expressed as mean ± S.E.M., n = 10 in each group.

<sup>a</sup> p < 0.05 vs. control group.
<sup>b</sup> p < 0.05 vs. vehicle + Cd group.

Cannabidiol was also demonstrated in animal models with cerebral and myocardial ischemia/reperfusion by attenuating the oxidative stress and inflammatory response [21–24]. Recent studies revealed that cannabidiol significantly protected against liver ischemia/reperfusion in mice and rats [25,26], and improved liver function in a fulminant hepatic failure-induced model of hepatic encephalopothy in mice [27]. However, to the best of our knowledge, the protective effect of cannabidiol against cadmium-induced hepatotoxicity was not yet investigated.

Therefore, the present study was conducted to evaluate the protective effect of cannabidiol against liver injury and dysfunction in rats exposed to acute cadmium toxicity. Also, the possible mechanisms underlying this effect were investigated.

Materials and methods

Animals

Male Sprague-Dawley rats, weighing 250 ± 10 g, were obtained from the Animal House, College of Medicine, King Faisal University. The animals were kept at standard housing facilities (24 ± 1°C, 45 ± 5% humidity and 12 h light/dark cycle). They were supplied with standard laboratory chow and water ad libitum, and left to

**Fig. 1.** Photomicrographs of rat liver (H&E) from: (A) (100×) control group showing normal liver histology; (B) (100×) and (C) (200×) cadmium group without cannabidiol treatment showing focal centrilobular necrosis (white arrow), microvesicular steatosis (black arrow) and ballooning degeneration with vacuolization of hepatocytes; (D) (100×) cadmium plus cannabidiol group showing a histological picture comparable to that of the control group with minimal liver tissue injury.

acclimatize for 1 week before the experiments. The experimental protocol was approved by the Ethical Committee, Deanship of Scientific Research, King Faisal University (approval number: 140095). The experimental procedures were carried out in accordance with the international guidelines for care and use of laboratory animals.

Drugs and chemicals

Cadmium chloride powder (Sigma–Aldrich Co., USA) was dissolved in normal saline. Cannabidiol powder (Cayman Chemical Company, USA) was prepared in 1% aqueous solution of Tween 80. The doses of cadmium chloride and cannabidiol used in the present work were selected based on our preliminary experiments and in accordance with previous reports [26,28].

Experimental protocol

The rats were randomly divided into four equal groups (n = 10, each). The first group received a single i.p. injection of normal saline (vehicle of cadmium chloride) and served as control. Hepatotoxicity was induced in animals of the second and third groups by a single dose of cadmium chloride (6.5 mg kg\(^{-1}\) i.p.). The second and third group animals respectively received the vehicle of cannabidiol (1% aqueous solution of Tween 80) or cannabidiol (5 mg kg\(^{-1}\) i.p.), for five consecutive days starting three days before cadmium administration. The rats of the fourth group received cannabidiol for five consecutive days without induction of cadmium hepatotoxicity.

Sample preparation and biochemical studies

The rats were euthanized 48 h following cadmium administration. Blood samples were collected, left for 60 min to clot, and then centrifuged for 10 min at 2430 × g to obtain clear sera which were stored at −20 °C. Subsequently, serum level of alanine aminotransferase (ALT) was measured using colorimetric assay kit according to the recommendations of the manufacturer (Biodiagnostic, Egypt).

The liver was removed, washed with ice-cold saline and kept at −80 °C and subsequently homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The homogenates were centrifuged at 2430 × g for 10 min at 4 °C. The resulting supernatant was used for determination of malondialdehyde (MDA), as an indicator for lipid peroxidation, and reduced glutathione (GSH) levels, and catalase and superoxide dismutase (SOD) activities using colorimetric assay kits according to the manufacturer's

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instructions (Biodiagnostic, Egypt). The level of nitric oxide (NO) was also assayed using colorimetric assay kit as indicated by the manufacturer (Cayman Chemical Company, USA).

In addition, parts of the liver tissue were dried overnight at 80 °C and the dry weight was recorded. The samples were then digested with equal volumes of 30% (w/v) H2O2 and 70% (w/v) nitric acid, and the clear digest was diluted with ultrapure water (1:3). Hepatic cadmium level was analyzed using inductively coupled plasma optical emission spectrometer (Optima 2100 DV, Perkin-Elmer, USA) at 228.8 nm, with sample-based standard.

Histopathological examination of liver tissue

Parts of liver tissue obtained from each animal were fixed in 10% formalin solution, dehydrated in ascending grades of alcohol, and embedded in paraffin. Sections at 4 μm-thickness were taken, stained with hematoxylin and eosin (H&E) and examined under light microscope by a pathologist unaware of the treatment protocol.

Immunohistochemical examinations of liver tissue

Four μm thick sections prepared from different animal groups were deparaffinized, rehydrated, and endogenous peroxidase activity was blocked with 3% H2O2 in methanol. Sections were pre-treated in citrate buffer (pH 6.0) in a microwave. Sections were incubated at room temperature with rabbit anti-endothelial nitric oxide synthase (eNOS), anti-cyclooxygenase-2 (COX-2), anti-nuclear factor-κB (NF-κB), anti-caspase-3, and anti-caspase-9 antibodies (Thermo Scientific, USA, dilution 1:1000), and anti-tumor necrosis factor-α (TNF-α) antibody (US Biological, USA, dilution 1:500). The sections were incubated with biotinylated goat anti-polyvalent, then with streptavidin peroxidase and finally with diaminobenzidine plus chromogen. Slides were counterstained with hematoxylin. The slides were visualized under light microscope and the extent of cell immunopositivity was assessed. The number of immunopositive cells was counted in five separate microscopic fields in each slide and the mean number for each slide was obtained, then the mean ± S.E.M. was calculated for each group (10 slides). The same procedures were repeated using normal rabbit
serum instead of the primary antibodies to obtain negative control and indicate the specificity of the used antibodies.

Statistical analysis

All values are expressed as mean ± S.E.M. The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test for multiple comparisons using SPSS for Windows (version 18). Differences were considered significant at $p < 0.05$.

Results

Biochemical analyses

Significant elevations of serum ALT, and hepatic MDA and cadmium levels, associated with significant decreases in hepatic GSH and NO levels, and catalase and SOD activities were observed in rats received a single dose of cadmium chloride (6.5 mg kg$^{-1}$ i.p.) as compared to the control animals. The rats treated with cannabidiol (5 mg kg$^{-1}$ i.p./day, for five days starting three days before cadmium administration) showed significant reductions of serum ALT, and hepatic MDA and cadmium levels, and significant increases in hepatic GSH and NO levels, and catalase activity as compared to the rats received cadmium without cannabidiol treatment. However, cannabidiol treatment did not significantly increase the SOD activity which was reduced as a result of cadmium chloride administration (Table 1).

Histopathological examination

Cadmium chloride administration caused marked liver damage in the form of microvesicular steatosis, focal hepatocyte centrizonal necrosis, ballooning degeneration and cytoplasmic vacuolation of hepatocytes with portal tract inflammation, and sinusoidal dilatation. Cannabidiol treatment markedly attenuated the cadmium-induced liver tissue injury and restored the same histological picture observed with the control group (Fig. 1).

Immunohistochemical examinations

Cadmium chloride administration resulted in significant increases in the immunoreactivity of COX-2, TNF-α, NF-κB, caspase-3, and caspase-9, and a significant decrease in eNOS immunostaining in the hepatocytes as compared to the control group. However, cannabidiol-treated animals showed significant reductions in the expression of COX-2, TNF-α, NF-κB, caspase-3,
and caspase-9, and a significant increase in eNOS expression in the liver tissue as compared to the cadmium group, non-treated with cannabidiol (Figs. 2–7). The slides from the cadmium group without cannabidiol treatment which were incubated with normal rabbit serum instead of the primary antibodies showed no staining at all indicating the specificity of the used antibodies (figures not shown).

Discussion

Acute cadmium hepatotoxicity is a biphasic process including two distinct steps. The first is the initial injury resulting from direct toxic effect of cadmium, and ischemia due to endothelial cell damage. The resultant ischemic hypoxia leads to Kupffer cell activation and neutrophil infiltration which trigger a complicated network of inflammatory mediators [6]. Activation of inflammatory cells increases generation of reactive oxygen species and lipid peroxidation which are key events in cadmium hepatotoxicity [29]. Inflammatory injury and oxidative stress eventually contribute to hepatocellular necrosis and apoptosis induced by cadmium [6,7].

The present study showed that cannabidiol treatment significantly protected against cadmium-induced liver toxicity in rats. Also, the present work, in agreement with previous studies, clearly demonstrated that oxidative stress, increased lipid peroxidation, depletion of antioxidant defenses, and increased release of proinflammatory cytokines play a crucial role in the pathogenesis of cadmium hepatotoxicity [10–13].

The results of the present work also revealed that cannabidiol treatment significantly increased hepatic eNOS expression, and NO level in rats exposed to cadmium intoxication. This is in accordance with previous reports which showed that reduced eNOS expression and NO production were implicated in cadmium-induced tissue injury [12,30]. It is well known that endogenous NO protects against hepatocellular necrosis and apoptosis. This is probably due to conservation of cellular bioenergetics for maintenance of tissue homeostasis [31], attenuation of sinusoidal neutrophil adherence [32], and inhibition of platelet aggregation thus maintaining microvascular perfusion [33]. Also, it was found that the NO donors suppressed the cadmium-induced expression of caspase-3 in mice exposed to cadmium hepatotoxicity. This was attributed to the ability of NO to suppress the expression of NF-κB and therefore inhibiting the signal transduction pathways which lead to necrotic and apoptotic cell death [29]. Also, NO inhibits caspase-3 activity via S-nitrosylation of the enzyme [34], and by induction of heat shock protein 70 expression [35].
Cannabidiol has been shown to have prominent antioxidant and antiinflammatory properties in several disease models. It inhibits the generation of reactive oxygen species, and scavenges lipid peroxidation products during free radical reactions [36,37]. In addition, cannabidiol exhibits anti-inflammatory activity by reducing the release of proinflammatory cytokines and inflammatory prostaglandins [20,38]. Cadmium exposure is also known to induce NF-κB with subsequent cascade of events responsible for liver tissue injury [29]. Cannabidiol has ability to inhibit the activation of NF-κB signaling pathway which promotes the transcription of TNF-α and COX-2 genes [20,39]. This is in accordance with the present results which revealed that cannabidiol treatment significantly suppressed lipid peroxidation, attenuated the depletion of GSH, NO, and catalase activity, and reduced the expression of NF-κB, TNF-α, and COX-2 in the liver of rats exposed to cadmium toxicity. However, cannabidiol treatment in this study did not significantly increase the hepatic SOD activity which was reduced by cadmium administration. This may be attributed to the duration of cannabidiol treatment in the present work (five days) which may not be enough to affect SOD activity, as it was demonstrated in a previous study that daily cannabidiol administration for two weeks significantly increased SOD activity in the brain of a rat model of Parkinson’s disease [40]. However, this point needs to be investigated in a further study.

It was also reported that cadmium exposure can lead to liver cell apoptosis by activating the caspase family of proteases [41]. The present study also revealed that cannabidiol treatment significantly inhibited cadmium-induced expression of caspase-3, an executioner of cell apoptosis, and caspase-9 in liver tissue. This is in agreement with previous studies which demonstrated that cannabidiol treatment significantly reduced caspase-3 activity in the liver of rats exposed to ischemia-reperfusion liver injury [26], and inhibited caspase-9 activity in hypoxic-ischemic brain damage in mice [42]. The antiapoptotic activity observed with cannabidiol treatment can be attributed to its antioxidant action, reduced TNF-α production, and inhibition of NF-κB. However, this needs to be clarified by further investigations.

In the present study, cannabidiol significantly attenuated the increase in cadmium concentration in liver tissue resulting from cadmium chloride administration. The decreased hepatic cadmium overload in response to cannabidiol treatment may be due to the ability of cannabidiol to prevent cadmium-induced depletion of GSH which has antioxidant and metal-chelating activities. The thiol groups in GSH can bind heavy metals with high affinity and

play an important role in intracellular heavy metal detoxification [43,44].

The results of the present study indicate that cannabidiol provided a significant protective effect against cadmium-induced hepatotoxicity in rats. The antioxidant, anti-inflammatory and anti-apoptotic activities can be considered the main factors responsible for the hepatoprotective effect of cannabidiol. Therefore, cannabidiol may represent a potential candidate to prevent liver injury and dysfunction induced by cadmium toxicity.

Conflict of interest

All the authors declare that there are no conflicts of interest.

Acknowledgment

Special thanks to the Deanship of Scientific Research, King Faisal University for the continuous encouragement and support.

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