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Cannabidiol Treatment Ameliorates Acetaminophen-Induced Hepatotoxicity in Mice

Amr A. Fouad, Waleed H. Albuali, and Iyad Jresat

***Abstract*—**The possible therapeutic effect of cannabidiol, the major non-psychotropic Cannabis constituent, was investigated against acute hepatotoxicity induced by a single oral dose of acetaminophen (500mg/kg) in mice. Cannabidiol (two intraperitoneal injections, 5mg/kg, each) was given 1 hour and 12 hours following acetaminophen administration. Acetaminophen administration caused significant elevations of serum alanine aminotransferase, and hepatic malondialdehyde, and nitric oxide levels, and a significant decrease in hepatic reduced glutathione. Cannabidiol significantly attenuated the deterioration in the measured biochemical parameters resulted from acetaminophen administration. Also, histopathological examination showed that cannabidiol markedly attenuated ameliorated acetaminophen-induced liver tissue damage. These results emphasize that cannabidiol represents a potential therapeutic option to protect against acetaminophen hepartotoxicity which is a common clinical problem.

***Keywords*—**cannabidiol, acetaminophen, liver, mice.

I. Introduction

ACETAMINOPHEN (paracetamol) is a commonly used analgesic and antipyretic agent. At therapeutic doses, it is usually safe and well tolerated. However, acute acetaminophen overdose causes severe and fatal hepatotoxicity [1]. A significant amount of acetaminophen is metabolized by the cytochrome P450 system to form the highly reactive intermediate metabolite, N-acetyl-p-benzoquinoneimine which depletes hepatic glutathione and then binds covalently to the intracellular proteins including mitochondrial proteins [2]. The resulting mitochondrial oxidant stress and peroxynitrite formation leads to mitochondrial dysfunction, adenosine triphosphate depletion, increased mitochondrial permeability transition and nuclear DNA fragmentation, which contribute to hepatocellular necrosis. Acetaminophen also activates Kupffer cells which release numerous cytokines and signaling molecules, including nitric oxide and superoxide with increased peroxynitrite formation [3].

Amr A. Fouad is with the Department of Biomedical Sciences, Pharmacology Division, College of Medicine, King Faisal University, Al-Ahsa, Saudi Arabia (Primary affiliation: Department of Pharmacology, Faculty of Medicine, Minia University, El-Minia, Egypt). (Corresponding author to provide phone: +966 501776517; e-mail: amrfouad65@yahoo.com).

Waleed H. Albuali is with the Department of Pediatrics, College of Medicine, King Faisal University, Al-Ahsa, Saudi Arabia.

Iyad Jresat is with the Department of Biomedical Sciences, Pathology Division, College of Medicine, King Faisal University, Al-Ahsa, Saudi Arabia.

Cannabidiol is the major non-psychoactive cannabinoid component derived from the plant Cannabis sativa. It possesses powerful antioxidant and anti-inflammatory activities [4], [5]. However, the exact mechanisms of action of cannabidiol remain obscure. Previous reports proved that cannabidiol may have therapeutic utility in a number of conditions involving inflammation and oxidative stress, including diabetes mellitus, rheumatoid arthritis and neurodegenerative disorders [6]-[8]. However, to the best of our knowledge, the protective effect of cannabidiol against acetaminophen-induced hepatotoxicity was not studied before.

II. Materials and Methods

A. Animals

Male Swiss albino mice, weighing 25-30g were obtained from the Animal House, College of Medicine, King Faisal University. The animals were housed at 24±1ºC, 45±5% humidity and 12h light-12h dark cycle. They were supplied with standard laboratory chow and water *ad libitum*, and left to acclimatize for 1 week before the experiments. The experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

B. Drugs and Chemicals

Cannabidiol powder (Cayman Chemical Company, USA) was prepared in 1% aqueous solution of Tween 80. Acetaminophen powder (Sigma-Aldrich Co., USA) was prepared in normal saline stabilized by 0.2% gum. The doses of cannabidiol and acetaminophen used in the present work were selected bases on our preliminary experiments and in accordance with previous reports [9], [10].

C. Experimental Design

The mice were randomly allocated to three groups (n=8, each). The first group received a single oral dose of normal saline stabilized by 0.2% gum (vehicle of acetaminophen), and served as control group. Hepatotoxicity was induced in mice of the second and third groups by a single oral dose of acetaminophen (500mg/kg). The animals of the second and third groups respectively received two intraperitoneal injections of the vehicle of cannabidiol (1% aqueous solution of Tween 80) or cannabidiol (5mg/kg, each), given 1 and 12 hours following acetaminophen administration.

D. Sample Preparation and Biochemical Analysis

The mice were euthanized 24 hours following the acetaminophen administration. Blood samples were collected,

left to clot for 60min, and centrifuged for 10min at 5000rpm. The obtained clear sera were stored at −20ºC until alanine aminotransferase (ALT) level was measured using colorimetric assay kit following the instructions of the manufacturer (Biodiagnostic, Egypt).

The liver was removed, washed with ice-cold saline and kept at −80ºC and subsequently homogenized in cold potassium phosphate buffer (0.05M, pH 7.4). The homogenates were centrifuged at 5000rpm for 10min at 4ºC. The resulting supernatant was used for determination of malondialdehyde (MDA), as an indicator for lipid peroxidation, and reduced glutathione (GSH), and nitric oxide (NO) levels using colorimetric assay kits according to the manufacturer's instructions (Biodiagnostic, Egypt).

E. Histopathological Examination

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

F. Statistical Analysis

The values are expressed as mean ±S.E.M. The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test for post hoc comparisons using SPSS for Windows (version 18). P <0.05 was selected as the criterion for statistical significance.

III. Results

Figs. 1-4 show that acetaminophen administration resulted in significant elevations of serum ALT, hepatic MDA and NO levels, and a significant decrease in hepatic GSH level as compared to the control values. However, cannabidiol-treated group showed significantly lower serum ALT, hepatic MDA and NO, and a significantly higher hepatic GSH level as compared to the acetaminophen group non-treated with cannabidiol.

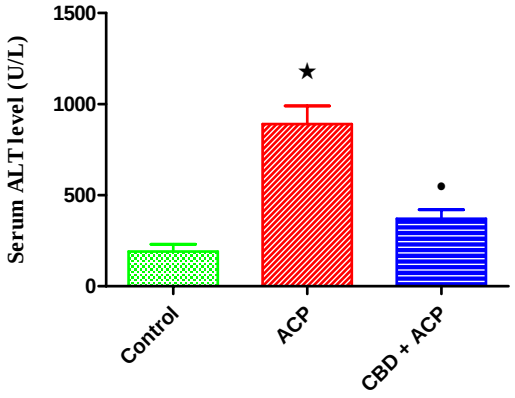

Fig. 1 Effect of cannabidiol (CBD) treatment on serum alanine aminotransferase (ALT) level in mice exposed to acetaminophen (ACP) hepatotoxicity. Data are mean ±S.E.M. of 8 mice, $^{*}P$ <0.05 vs. control group, $^{\bullet}P$ <0.05 vs. ACP group

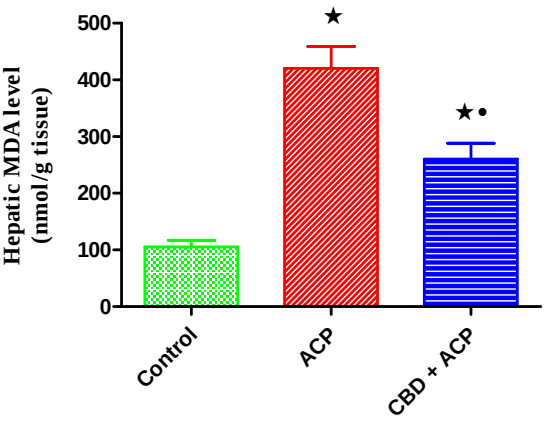

Fig. 2 Effect of cannabidiol (CBD) treatment on hepatic malondialdehyde (MDA) level in mice exposed to acetaminophen (ACP) hepatotoxicity. Data are mean ±S.E.M. of 8 mice, $^{*}P$ <0.05 vs. control group, $^{\bullet}P$ <0.05 vs. ACP group

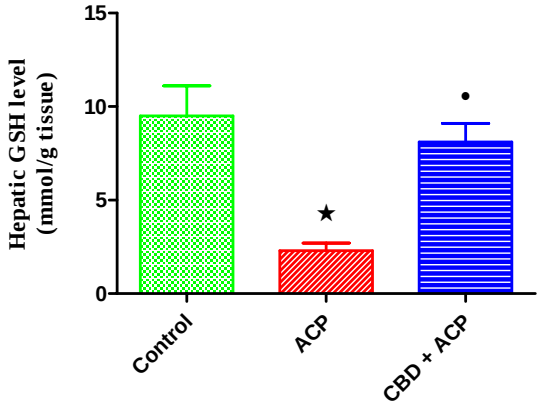

Fig. 3 Effect of cannabidiol (CBD) treatment on hepatic reduced glutathione (GSH) level in mice exposed to acetaminophen (ACP) hepatotoxicity. Data are mean ± S.E.M. of 8 mice, $^{*}P$ <0.05 vs. control group, $^{\bullet}P$ <0.05 vs. ACP group

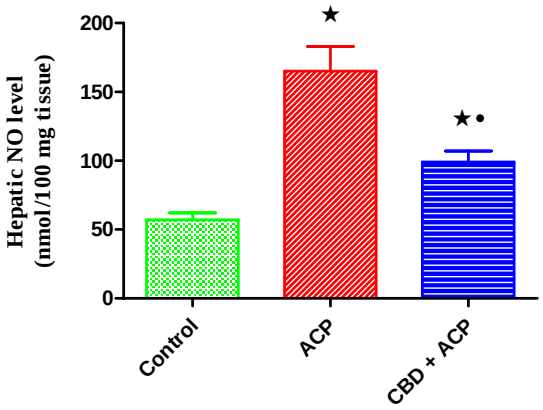

Fig. 4 Effect of cannabidiol (CBD) treatment on hepatic nitric oxide (NO) level in mice exposed to acetaminophen (ACP) hepatotoxicity. Data are mean ±S.E.M. of 8 mice, $^{*}P$ <0.05 vs. control group, $^{\bullet}P$ <0.05 vs. ACP group

Also, histopathological examination showed that acetaminophen overdose caused marked liver damage in the form of centrilobular necrosis, ballooning degeneration, and cytoplasmic vacuolation of hepatocytes with sinusoidal congestion. Cannabidiol treatment markedly attenuated acetaminophen-induced liver tissue damage with a histological picture similar to the control group and minimal damage of liver tissue (Fig. 5).

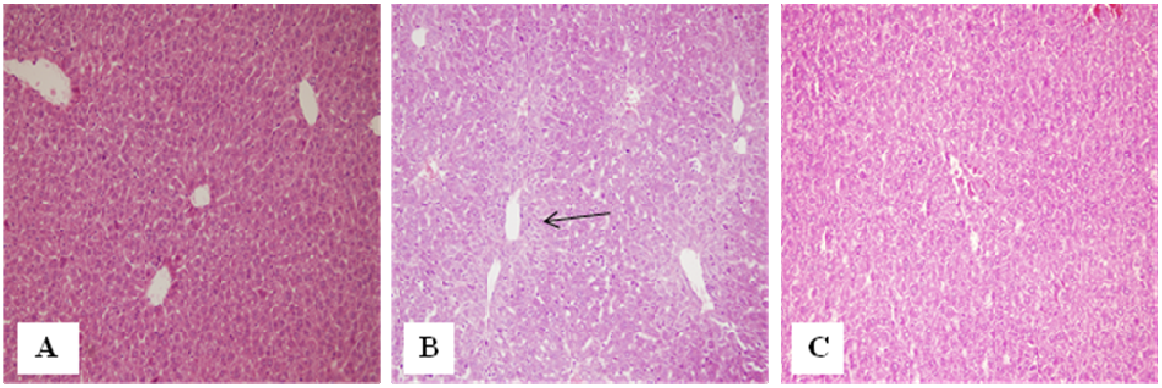

Fig. 5 Photomicrographs of mice liver (H&E, 200×) from: (A) control group showing normal liver histology; (B) acetaminophen group without cannabidiol treatment showing extensive centrilobular necrosis (black arrow), cytoplasmic vacuolization, and ballooning degeneration of hepatocytes; (C) acetaminophen plus cannabidiol group showing a histological picture comparable to that of the control group with minimal injury

IV. Conclusion

The present work, in agreement with previous studies, clearly demonstrated that oxidative stress with increased lipid peroxidation, depletion of antioxidant defenses and increased release of inflammatory mediators play a crucial role in the pathogenesis of acetaminophen hepatotoxicity [10], [11], [12], [13]. In addition, increased NO production in the liver tissue was reported to be involved in the pathogenesis of liver injury induced by acetaminophen overdose [14]. Excess NO reacts with superoxide anion to generate peroxynitrite radical which causes further cell damage by oxidizing and nitrating cellular macromolecules. Also, excess NO depletes intracellular GSH increasing the susceptibility to oxidative stress [15]. Several studies showed that antioxidants and anti-inflammatory agents effectively protected against acute hepatotoxicity induced by acetaminophen overdose [10], [11], [12], [13].

Cannabidiol has been shown to have prominent antioxidant and antinitrative properties in several disease models. It inhibits NADPH oxidases [16] implicated in the generation of reactive oxygen species during liver ischemia/reperfusion [17]. It also scavenges lipid peroxidation products during free radical reactions [18], and suppresses excess nitric oxide production preventing nitrosative stress [19]. In addition, cannabidiol exhibits anti-inflammatory activity by reducing the release of proinflammatory cytokines and inflammatory prostaglandins [20]. The antioxidant and anti-inflammatory effects of cannabidiol may be due to its direct action or mediated through a new cannabinoid, non-CB_1 and non-CB_2 receptor [21]. Cannabidiol may also exert its beneficial effects by inhibiting adenosine uptake and activating transient receptor potential vanilloid-1 [22], [23].

The results of the present study indicate that cannabidiol significantly protected against acute acetaminophen hepatotoxicity in mice. The hepatoprotective effect afforded by cannabidiol can be attributed to its antioxidant and anti-inflammatory activities. Therefore, cannabidiol may represent a feasible candidate to protect against acetaminophen hepatotoxicity.

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