

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-psychoactive 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 hepatotoxicity 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].

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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±1°C, 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 -20°C 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 -80°C and subsequently homogenized in cold potassium phosphate buffer (0.05M, pH 7.4). The homogenates were centrifuged at 5000rpm for 10min at 4°C. 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.

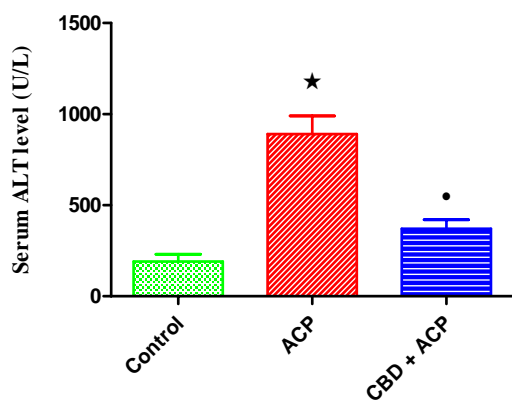


Fig. 1 Effect of cannabidiol (CBD) treatment on serum alanine aminotransferase (ALT) level in mice exposed to acetaminophen (ACP) hepatotoxicity. Data are mean ±S.E.M. of 8 mice, **P* <0.05 vs. control group, **P* <0.05 vs. ACP group

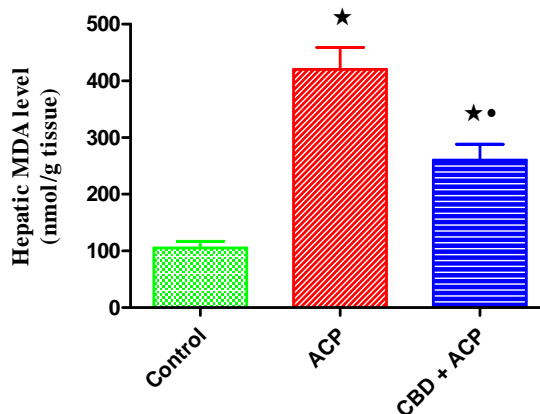


Fig. 2 Effect of cannabidiol (CBD) treatment on hepatic malondialdehyde (MDA) level in mice exposed to acetaminophen (ACP) hepatotoxicity. Data are mean ±S.E.M. of 8 mice, **P* <0.05 vs. control group, **P* <0.05 vs. ACP group

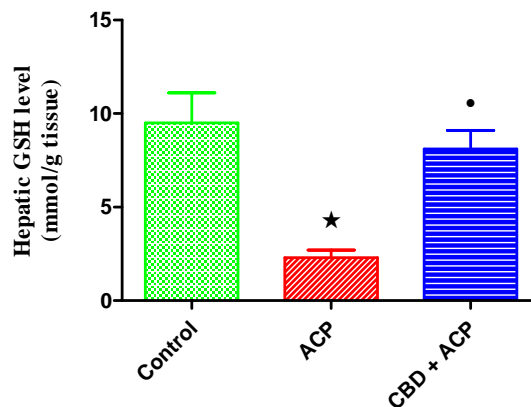


Fig. 3 Effect of cannabidiol (CBD) treatment on hepatic reduced glutathione (GSH) level in mice exposed to acetaminophen (ACP) hepatotoxicity. Data are mean ± S.E.M. of 8 mice, **P* <0.05 vs. control group, **P* <0.05 vs. ACP group

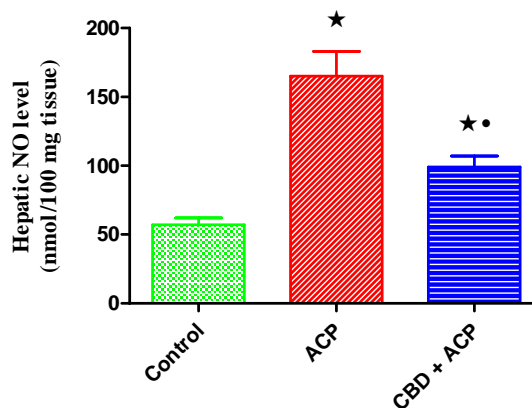


Fig. 4 Effect of cannabidiol (CBD) treatment on hepatic nitric oxide (NO) level in mice exposed to acetaminophen (ACP) hepatotoxicity. Data are mean ±S.E.M. of 8 mice, **P* <0.05 vs. control group, **P* <0.05 vs. ACP group

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).

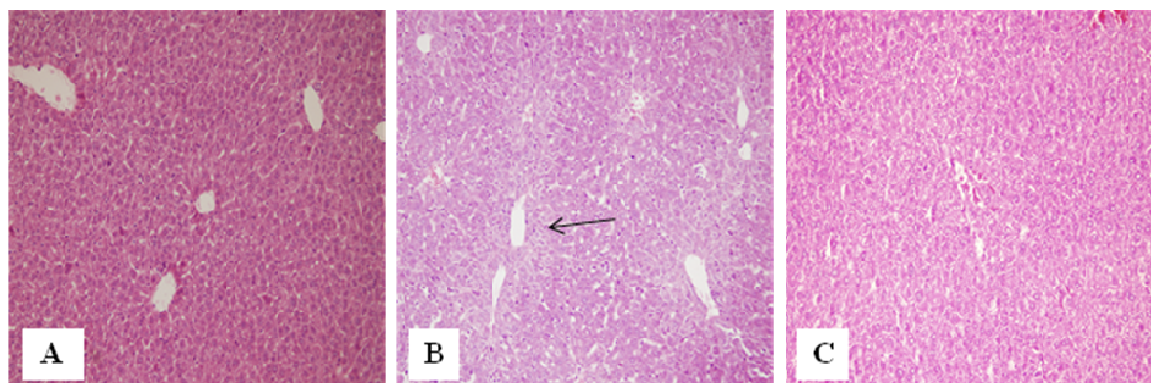


Fig. 5 Photomicrographs of mice liver (H&E, 200 \times) from: (A) control group showing normal liver histology; (B) acetaminophen group without cannabidiol treatment showing extensive centrilobular necrosis (black arrow), cytoplasmic vacuolization, and ballooning degeneration of hepatocytes; (C) acetaminophen 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-CB₁ and non-CB₂ 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.

REFERENCES

- [1] W.C. Maddrey, "Drug induced hepatotoxicity," *J. Clin. Gastroenterol.*, vol. 39, pp. 883-889, 2005.
- [2] L.P. James, P.R. Mayeux, J.A. Hinson, "Acetaminophen-induced hepatotoxicity," *Drug Metab. Dispos.*, vol. 31, pp. 1499-1506, 2003.
- [3] H. Jaeschke, M.R. McGill, C.D. Williams, A. Ramachandran, "Current tissues with acetaminophen hepatotoxicity—a clinically relevant model to test the efficacy of natural products," *LifeSci.*, vol. 88, pp. 737-745, 2011.
- [4] P. Mukhopadhyay, M. Rajesh, B. Horváth, S. Bátkai, O. Park, G. Tanashian, R.Y. Gao, V. Patel, D.A. Wink, L. Liaudet, G. Haskó, R. Mechoulam, P. Pacher, "Cannabidiol protects against hepatic ischemia/reperfusion injury by attenuating inflammatory signaling and response, oxidative/nitrative stress, and cell death," *Free Radic. Biol. Med.*, vol. 50, pp. 1368-1381, 2011.
- [5] M.R. Pazos, V. Cinquina, A. Gómez, R. Layunta, M. Santos, J. Fernández-Ruiz, J. Martínez-Orgado, "Cannabidiol administration after hypoxia-ischemia to newborn rats reduces long-term brain injury and restores neurobehavioral function," *Neuropharmacology*, vol. 63, pp. 776-783, 2012.
- [6] T. Iuvone, G. Esposito, D. De Filippis, C. Scuderi, L. Steardo, "Cannabidiol: a promising drug for neurodegenerative disorders?," *CNS Neurosci. Ther.*, vol. 15, pp. 65-75, 2009.
- [7] D.R. Blake, P. Robson, M. Ho, R.W. Jubbs, C.S. McCabe, "Preliminary assessment of the efficacy, tolerability and safety of a cannabis-based medicine (Sativex) in the treatment of pain caused by rheumatoid arthritis," *Rheumatology (Oxford)*, vol. 45, pp. 50-52, 2006.
- [8] M. Rajesh, P. Mukhopadhyay, S. Bátkai, V. Patel, K. Saito, S. Matsumoto, Y. Kashiwaya, B. Horvath, B. Mukhopadhyay, L. Becker, G. Haskó, L. Liaudet, D.A. Wink, A. Veves, R. Mechoulam, P. Pacher, "Cannabidiol attenuates cardiac dysfunction, oxidative stress, fibrosis, and inflammatory and cell death signaling pathways in diabetic cardiomyopathy," *J. Am. Coll. Cardiol.*, vol. 56, pp. 2115-2125, 2010.
- [9] R. Durst, H. Danenberg, R. Gallily, R. Mechoulam, K. Meir, E. Grad, R. Beeri, T. Pugatsch, E. Tarsish, C. Lotan, "Cannabidiol, a non-psychoactive Cannabis constituent, protects against myocardial ischemic

- reperfusion injury," *Am. J. Physiol. Heart Circ. Physiol.*, vol. 293, pp. H3602-H3607, 2007.
- [10] C. Girish, B.C. Koner, S. Jayanthi, K. Ramachandra Rao, B. Rajesh, S.C. Pradhan, "Hepatoprotective activity of picroliv, curcumin and ellagic acid compared to silymarin on paracetamol induced liver toxicity in mice," *Fundam. Clin. Pharmacol.*, vol. 23, pp. 735-745, 2009.
- [11] M.N. Nagi, H.A. Almakki, M.M. Sayed-Ahmed, A.M. Al-Bekairi, "Thymoquinone supplementation reverses acetaminophen-induced oxidative stress, nitric oxide production and energy decline in mice liver," *Food Chem. Toxicol.*, vol. 48, pp. 2361-2365, 2010.
- [12] H.S. Oz, T.S. Chen, "Green-tea polyphenols downregulate cyclooxygenase and Bcl-2 activity in acetaminophen-induced hepatotoxicity," *Dig. Dis. Sci.*, vol. 53, pp. 2980-2988, 2008.
- [13] S.L. Yan, S.T. Wu, M.C. Yin, H.T. Chen, H.C. Chen, "Protective effects from carnosine and histidine on acetaminophen-induced liver injury," *J. Food Sci.*, vol. 74, pp. H259-H265, 2009.
- [14] G. Kuvandik, M. Duru, A. Nacar, Z. Yonden, R. Helvacı, A. Koc, T. Kozlu, H. Kaya, S. Sogüt, "Effects of erdosteine on acetaminophen-induced hepatotoxicity in rats," *Toxicol. Pathol.*, vol. 36, pp. 714-719, 2008.
- [15] R.M. Clancy, S.B. Abramson, "Nitric oxide: a novel mediator of inflammation," *Proc. Soc. Exp. Biol. Med.*, vol. 210, pp. 93-101, 1995.
- [16] H. Pan, P. Mukhopadhyay, M. Rajesh, V. Patel, B. Mukhopadhyay, B. Gao, G. Hasko, P. Pacher, "Cannabidiol attenuates cisplatin-induced nephrotoxicity by decreasing oxidative/nitrosative stress, inflammation, and cell death," *J. Pharmacol. Exp. Ther.*, vol. 328, pp. 708-714, 2009.
- [17] I.H. Shaik, R. Mehvar, "Cytochrome P450 induction by Phenobarbital exacerbates warm hepatic ischemia-reperfusion injury in rat livers," *Free Radic. Res.*, vol. 44, pp. 441-453, 2010.
- [18] F. Borrelli, G. Aviello, B. Romano, P. Orlando, R. Capasso, F. Maiello, F. Guadagno, S. Petrosino, F. Capasso, V. Di Marzo, A.A. Izzo, "Cannabidiol, a safe and non-psychoactive ingredient of the marijuana plant *Cannabis sativa*, is protective in a murine model of colitis," *J. Mol. Med.*, vol. 87, pp. 1111-1121, 2009.
- [19] L. Ruiz-Valdepeñas, J.A. Martínez-Orgado, C. Benito, A. Millán, R.M. Tolón, J. Romero, "Cannabidiol reduces lipopolysaccharide-induced vascular changes and inflammation in the mouse brain: an intravital microscopy study," *J. Neuroinflammation*, vol. 8, p. 5, 2001.
- [20] B. Costa, M. Colleoni, S. Conti, D. Parolaro, C. Franke, A.E. Trovato, G. Giagnoni, "Oral anti-inflammatory activity of cannabidiol, a nonpsychoactive constituent of cannabis, in acute carrageenan-induced inflammation in the rat paw," *Naun Schm. Arch. Pharmacol.*, vol. 369, pp. 294-299, 2004.
- [21] M. Begg, P. Pacher, S. Batkai, D. Osei-Hyiaman, L. Offertaler, F.M. Mo, J. Liu, G. Kunos, "Evidence for novel cannabinoid receptors," *Pharmacol. Ther.*, vol. 106, pp. 133-145, 2005.
- [22] T. Bisogno, L. Hanus, L. De Petrocellis, S. Tchilibon, D.E. Ponde, I. Brandi, A.S. Moriello, J.B. Davis, R. Mechoulam, V. Di Marzo, «Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide," *Br. J. Pharmacol.*, vol. 134, pp. 845-852, 2001.
- [23] E.J. Carrier, J.A. Auchampach, C.J. Hillard, "Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression," *Proc. Natl. Acad. Sci. USA*, vol. 103, pp. 7895-7900, 2006.