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.

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 60 min, and centrifuged for 10 min at 5000 rpm. 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 5000 rpm 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), 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 4 µm 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×) from: (A) control group showing normal liver histology; (B) acetaminophen group without cannabidiol treatment showing extensive centrilobular necrosis (black arrow), cytoplasmic vacuolation, and ballooning degeneration of hepatocytes; (C) acetaminophen plus cannabidiol group showing a histological picture comparable to that of the control group with minimal injury](image)

**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 antinflammatory 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**


