ORIGINAL RESEARCH

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The Protective Effects of Cannabidiol on Chest Trauma-Induced Brain Injury by its Antioxidant and Anti-Inflammatory Effects

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Abstract

Objective
Chest trauma-induced brain injury (CTBI) is caused by the formation of inflammatory cytokines in the lungs and blood. Cannabidiol (CBD), a non-psychoactive agent, has antioxidant, anti-inflammatory, and antiapoptotic properties. In this study, we aimed to investigate the protective effects of CBD on CTBI.

Material and Method
Forty male Wistar Albino rats were divided into four groups: control, CTBI (200 g weight drop on the anterior chest wall from a height of 1 meter), CTBI+CBD (5 mg/kg, single dose intraperitoneally), and CBD. After 48 hours, rats were sacrificed under anesthesia, and brain tissues were placed in a 10% formaldehyde solution for histopathological and immunohistochemical examination.

Results
In the CTBI group, hemorrhagic areas, tumor necrosis factor-alpha, caspase-3, and malondialdehyde expressions increased in histological and immunohistochemical examinations compared to the control group. CBD treatment reduced hemorrhagic areas and reversed immune expressions.

Conclusion
Inflammation, apoptosis, and oxidative stress in brain tissue may develop in CTBI. These damages can be corrected with CBD treatment.

Keywords: Blunt chest trauma, brain, cannabidiol, apoptosis, malondialdehyde.

Introduction

Vehicle accidents and physical assaults are among the most common causes of chest trauma (CT) (1). Damage to the interstitial and vascular structures due to the pressure in the lung tissue as a result of trauma can cause local or systemic damage (2). Local damage mechanisms such as oxidative stress and inflammation can trigger more serious mechanisms such as apoptosis and necrosis. Oxidant and inflammatory substances produced as a result of such tissue damage and increased vascular permeability

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can enter the bloodstream and initiate distant organ damage, including brain tissue (3).

Peroxidation of lipids, especially those formed in cell membranes, plays an important role in the development of oxidative stress caused by free oxygen radicals in lung tissue. Increased levels of malondialdehyde (MDA), which is an indicator of lipid peroxidation at very low levels in healthy tissues, can be used as an indicator of oxidative stress. In addition to oxidative stress, tumor necrosis factor-alpha (TNF-α) can be used to show inflammation, and caspase 3 (Cas-3) can be used to show apoptosis (4).

CBD is a non-psychoactive phytocannabinoid derived from the Cannabis sativa plant. CBD has many beneficial effects, including anti-inflammatory and antioxidant properties. The lipophilicity of CBD and its ability to cross the blood-brain barrier make it a new candidate for central nervous system diseases (5). In an ischemia-reperfusion study, it was found that CBD supplementation decreased MDA levels in mouse hippocampal (HT22) neuron cells and thus reduced oxidative stress (6).

This study aimed to investigate the protective effect of CBD, which has known antioxidant and anti-inflammatory properties, on CT-induced brain injury (CTBI).

Material and Method

Animals and Ethical Approval

The design of all experimental procedures was carried out following the animal research guidelines of the National Institutes of Health and approved by the Local Ethics Committee for Animal Experiments of Suleyman Demirel University (Approval No. 2023-01/116). Animals were obtained from the Suleyman Demirel University Experimental Animal Laboratory and housed at 21-22 °C and 60% ± 5% humidity with a 12 h light:12 h dark cycle and fed ad libitum with standard commercial feed and water during the experiments.

Chemicals

Suleyman Demirel University, Natural Products Application and Research Center. The source of the CBD was the extract of Cannabis sativa L. (Cannabaceae). CBD content is >99.9 and the tetrahydrocannabinol content is <0.01. Limits of residual alcohol and heavy metals comply with USP and EU pharmacopeias. Ketamine HCl (Keta-Control 10%) and Xylazin HCl (Control 10%) were purchased from Doğa İlaç Turkey.

Chest Trauma Procedure

A bilateral CTBI model was developed as a trauma model by modifying the isolated bilateral pulmonary contusion model described by Raghavendran et al. (7). Lung contusion was induced by dropping a 200 g ball from a height of 1 meter onto the anterior thoracic wall of rats. The energy transferred to the chest wall was calculated as 1.96 joules using the formula E = mgh (E: energy, g: gravitational acceleration; 9.8 m/s², h: height; 100 cm, and m: dropped weight; 0.2 kg).

Anesthesia Procedure

Rats were anesthetized with 10 mg/kg Xylazine HCl + 50 mg/kg Ketamine HCl anesthesia before the thoracic trauma model. Before sacrification, 8-10 mg/kg Xylazine HCl + 90 mg/kg Ketamine HCl intraperitoneal (i.p.) anesthesia was administered.

Experimental Design

In the experimental project, 40 male Wistar Albino rats weighing 300-350 g were used. The animals were divided into four main groups of 10 animals each (Fig. 1). Groups;

Control group: Anesthesia was applied but no trauma was induced. Afterward, 0.5-1 mL saline injection was given i.p.

CTBI group: Thoracic trauma was applied under anesthesia. Afterward, rats were injected with 0.5-1 mL i.p. saline injection (7).

CTBI+CBD group: Thoracic trauma was applied under anesthesia. Afterwards, rats were given 5 mg/kg CBD i.p.

CBD group: Anesthesia was applied but no trauma was induced. Afterward, 5 mg/kg CBD was given to the rats i.p. (8).

48 hours after the beginning of the experiment, the rats were sacrificed under the anesthesia protocol. Brain tissues were removed and placed in a 10% formaldehyde solution for histopathologic and immunohistochemical examination.

Histopathological Method

The rat brains were removed from the skull by carefully opened with great care to prevent damage to the brain tissue during necropsy. All groups' brain samples underwent carefully gross examination. They were fixed in a 10% buffered formalin solution after this examination. Brain tissues were embedded in paraffin after undergoing standard tissue processing. Using

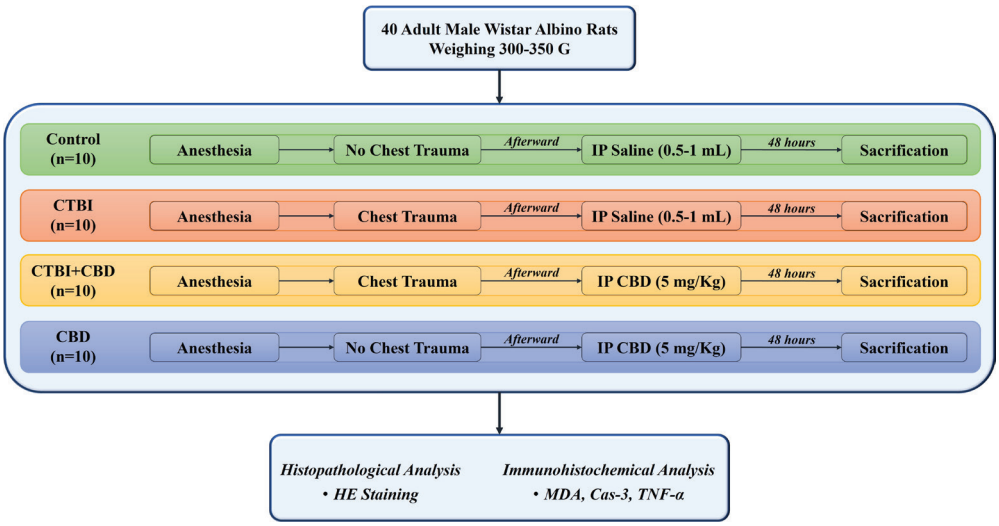

Figure 1
Experimental design

an automated rotary microtome, serial sections with a thickness of 5 microns were extracted from each tissue. Routine hematoxylin and eosin (HE) staining was used to stain sections and examined under a light microscope.

As in the previous studies (9), histopathological results were assessed using the standards given in Table 1. The scores ranged from 0 to 4.

Immunohistochemical Method

Sections generated on slides coated with poly-L-lysine were subjected to streptavidin-biotin peroxidase immunohistochemical staining. Brain sections were subjected to immunohistochemical investigation using primary antibodies against MDA (Anti-malondialdehyde antibody [11E3] (ab243066)), cas-3 (Anti-Caspase-3 antibody [EPR18297] (ab184787)) and TNF-α (Recombinant Anti-TNF alpha antibody [TNFA/1172] (ab220210)). All of the primary antibodies used in the immunohistochemistry analyses were obtained from Abcam and diluted using antibody dilution solutions at a ratio of 1/100. The immunohistochemical procedure was performed according to the manufacturer's instructions. The secondary kit used in this study was the Mouse and Rabbit Specific HRP/DAB Detection Kit - Micropolymer (ab236466) from Abcam, Cambridge, UK. Other procedures were followed as directed; however, for the negative controls, antibody dilution solutions were applied to the sections at the primary antibody stage rather than primary antibodies.

Immunohistochemical analysis was conducted by counting the percentage of positive cells, and the results were compared and evaluated among the groups. Special attention was paid to evaluating cells in the same areas of the brain where CT was induced and in other groups. For this purpose, 100 cells were counted per area, with 20 cells randomly selected from each of five areas of the same brain region for each rat, using a 40X objective. The Image J 1.46r software (National Institutes of Health, Bethesda, MD) was used to determine the number of cells

Table 1 Histopathological scores of subarachnoid hemorrhages

0	Normal meningeal and parenchymal structure
1	No blood in the subarachnoid space, ventricles, or brain parenchyma.
2	No localized or diffuse thin subarachnoid hemorrhage, intraventricular, or intraparenchymal hemorrhage.
3	No diffuse or localized thick subarachnoid blood layers, intraventricular, or intraparenchymal hemorrhage.
4	Intraventricular or intraparenchymal hemorrhage in association with subarachnoid hemorrhage, regardless of thickness or location.

showing a positive immunohistochemical reaction. An Olympus CX41 model microscope was used for photographing the results, and the Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan) was used for microphotography.

Statistical Analysis

For the statistical analysis of histopathological scores and the number of immunohistochemically positive cells, the GraphPad Prism 8.0.2 software package was utilized. Group differences were determined using the One-way ANOVA test. Values with p≤0.001 were considered statistically significant.

Results

The sham group showed signs of mild meningeal hemorrhage during the histological evaluation. Rats in the CTBI group showed extensive and noticeable hemorrhage. The administration of CBD was found to significantly reduce hemorrhagic regions in the CTBI+CBD group. In the CBD group, normal brain histology was noted (Fig. 2). Histological evaluation was scored and presented graphically (Fig. 3).

Immunohistochemical Findings

Upon immunohistochemical investigation, the control and CBD groups exhibited mild to negative expressions of TNF-α, Cas-3, and MDA in their

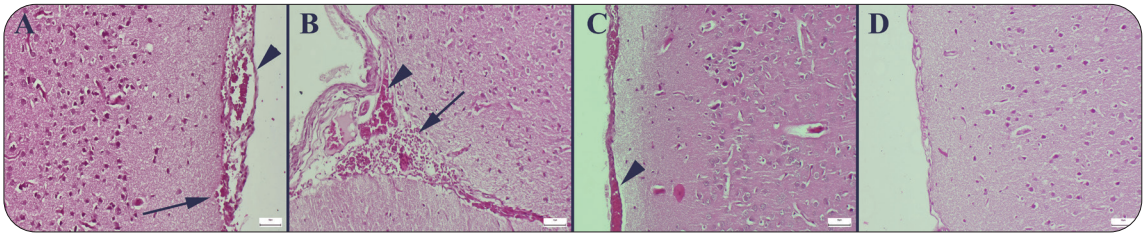

Figure 2

Histopathological appearance of brains between the groups

(A) Mild hyperemia (arrowhead) and slight hemorrhage (arrow) in meningeal vessels in the control group. (B) Marked hyperemia (arrowhead) and hemorrhage foci (arrow) in the brain of a rat in the CTBI group. (C) Markedly reduced hemorrhage area (arrowhead) in the CTBI+CBD group. (D) Normal brain and meningeal structure in a rat in the CBD group, HE, Scale bars=50μm.

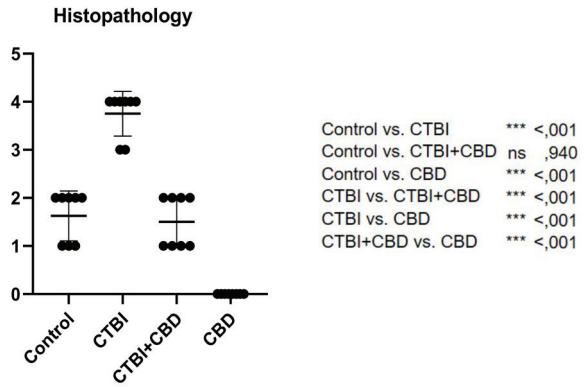

Figure 3

Statistical analyses of histopathological findings

CTBI: Chest trauma-induced brain injury, CBD: Cannabidiol. Values are presented as means ± standard deviation. A one-way ANOVA test was used. * p≤0.05, ** p≤0.01, *** p≤0.001.

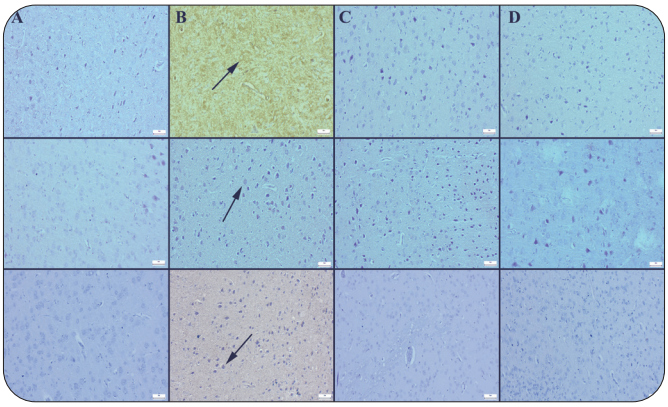

Figure 4

Expression of MDA (top row), Cas-3 (middle row), and TNF-α (bottom row) in the brain

(A) Negative MDA, Cas-3, and TNF-α expression in the control group. (B) Significant increase in MDA, Cas-3, and TNF-α expressions (arrows) in the CTBI group. (C) A marked decrease in MDA, Cas-3, and TNF-α expressions in the CTBI+CBD group. (D) No MDA, Cas-3, and TNF-α expressions in the CBD group, streptavidin-biotin peroxidase method, scale bars=50μm.

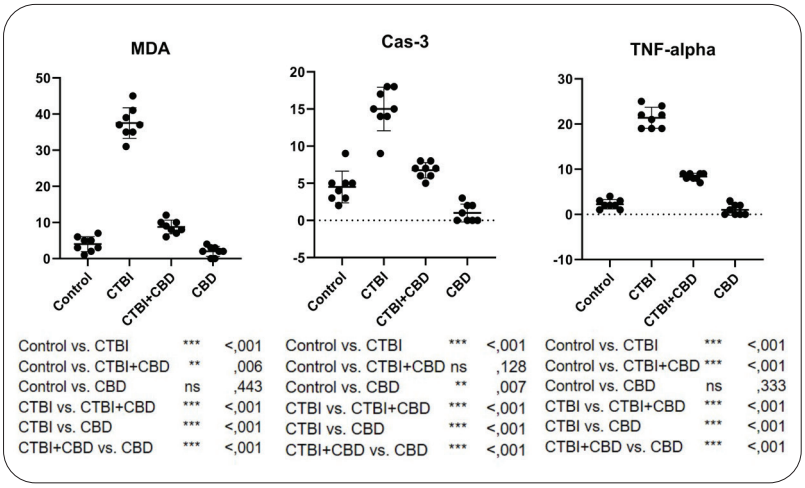

Figure 5

Statistical analyses of immunohistochemical findings

CTBI: Chest trauma-induced brain injury, CBD: Cannabidiol Values are presented as means ± standard deviation. A one-way ANOVA test was used. * p≤0.05, ** p≤0.01, *** p≤0.001

brains. Following the administration of CTBI, there was an increase in the expressions of TNF-α, Cas-3, and MDA. However, it was observed that CBD therapy caused all marker expressions to revert to normal (Fig. 4). Immunohistochemical evaluation was scored and presented graphically (Fig. 5).

Discussion

The lung is a vital organ that meets the body's need for oxygen, is rich in blood vessels, and therefore ensures the continuity of the functions of other organs. When this tissue is damaged, hypoxia, which is a picture of oxygen deficiency, can trigger inflammatory reactions in many tissues (10). The inflammatory picture in lung tissue damage allows oxidant substances and proinflammatory cytokines, whose synthesis increases with increased capillary membrane permeability, to enter the blood. These substances passing into the bloodstream are known to bind to their receptors in distant organs and activate various intracellular pathways (3). Inflammatory cytokines circulating

freely in the blood may also increase the permeability of the blood-brain barrier. These substances passing into the brain may cause damage (11). In this study, the presence of intense hemorrhagic areas in the CTBI group detected histologically, and the increase in TNF-α expression detected in immunostaining indicates that the experimental model is established, inflammation is triggered, and brain damage develops. Intracellular mechanisms play an important role in the cellular response to various stimuli. It has been found that these pathways, either on their own or by activating other pathways, cause the response to grow. As the number of affected cells increases, clinical progression worsens and loss of tissue function occurs. Scientists are trying to reverse cell damage mechanisms such as oxidative stress, inflammation, and apoptosis by trying many different active substances (12). The decrease in hemorrhagic areas in the brain tissue, which is an indicator of the inflammatory reaction histologically of CBD used in this study shows that the drug can both pass into the brain tissue and provide anti-inflammatory activity. The decrease in TNF-α expressions detected in immunohistochemical analysis is another indicator of CBD's anti-inflammatory activity.

As known, oxidative stress and inflammation can trigger each other and cause a rapid and aggressive course. The increase in MDA levels in parallel with the increase in the expression of inflammatory cytokines indicates the development of oxidative stress caused by lipid peroxidation (4). The decrease in MDA levels with the use of CBD may be interpreted as the drug providing antioxidant activity by increasing antioxidant enzyme levels or may reduce secondary oxidative stress by regressing inflammation by another mechanism (6). To clarify this situation, studies in which CBD alone is used and antioxidant enzyme levels are examined in such models are needed.

As a result of the occurrence of apoptosis, which has more severe consequences than the mechanisms mentioned above and is also stimulated by these mechanisms, death occurs in cells (12). It is very important to prevent this irreversible process. The reduction of apoptotic responses observed in cells in the CTBI model with CBD treatment also shows that the active substance has an antiapoptotic effect.

In conclusion, oxidative stress, inflammation, and apoptosis develop in brain tissue as a result of chest trauma, resulting in neuronal damage. It is possible that this damage can be reversed with CBD treatment, but more detailed studies investigating molecular mechanisms are needed.

Conflict of Interest Statement
Milletsever A and the co-authors have no conflicts of interest to declare in association with this study.

Ethical Approval
The study was carried out at Süleyman Demirel University Experimental Animal Production and Experimental Research Laboratory. Ethical approval was obtained from the National Institutes of Health and approved by the Local Ethics Committee for Animal Experiments of Suleyman Demirel University (Approval No. 2023-01/116).

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Availability of Data and Materials
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors Contributions
A.M: Conceptualization; Data curation; Investigation; Formal Analysis; Methodology; Validation; Visualization; Writing review & editing; Writing original draft.

A.G: Conceptualization; Data curation; Investigation; Formal Analysis; Methodology; Validation; Visualization; Writing original draft

H.A: Conceptualization; Data curation; Investigation; Formal Analysis; Methodology; Validation; Visualization; Writing review & editing; Writing original draft

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