# The Effects of Cannabidiol and its Main Metabolites on Human Neural Stem Cells

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## Abstract

Cannabidiol (CBD) is used for a variety of purposes by different groups of people, including to reduce nausea in pregnancy. In recent years, demand for CBD or CBD containing products has increased at an alarming rate. When consumed during pregnancy, CBD can pass through the placenta and enter the fetal blood stream. There are concerns about the effects of CBD and its major metabolites (7-OH-CBD and 7-COOH-CBD) on fetal development. In the present study, human neural stem cells (NSCs) were treated with CBD and its metabolites at different concentrations and durations to understand how the drug may affect fetal brain development. Drug effects on NSC viability and proliferation rate were assessed using MTT reduction, LDH release and EdU incorporation assays. Cell cycle analysis was performed using flow cytometry after cellular DNA was stained with propidium iodide. CBD, 7-OH-CBD and 7-COOH-CBD dose-dependently reduced NSC viability. At clinically relevant concentrations (i.e. human plasma concentrations), CBD, 7-OH-CBD and 7-COOH-CBD caused more obvious cell death when exposure duration increased. CBD and 7-OH-CBD reduced NSC numbers in the G1 phase after 24h exposure. However, 24h exposure did not cause significant change in NSC proliferation. This study demonstrates that clinically relevant concentrations of CBD, 7-OH-CBD and 7-COOH-CBD affect basic physiological features of human NSCs, suggesting the drug may have adverse effects on the developing human brain in vivo.

## Introduction

Cannabis use has increased in the United States as more states legalize its use for medical and recreational purposes [1, 2]. Users of cannabis containing products include pregnant women who choose such products over typical medications for pregnancy related symptoms [3]. Some pregnant women view cannabis containing products as a more effective or a safer alternative to prescription medications [4, 5]. However, retrospective studies have shown that cannabis use significantly increases the odds of preterm birth, small for gestational age newborns, and death of the infant [6-8]. Additionally, children exposed in utero to cannabis, with or without THC, were more likely to have memory and learning troubles, hyperactivity, aggression, depression, and anxiety and these conditions can persist into young adulthood [7, 9, 10].

Cannabinoids are compounds from the *Cannabis stavia* plant. There are over 500 identified cannabinoids that can bind to the cannabinoid receptors in the human body (CB1 and CB2) [11, 12]. One of the most well-known cannabinoids is cannabidiol (CBD). CBD is a non-psychoactive component of cannabis that is used by some to self-medicate for a variety of medical purposes [11]. To date, the only CBD containing medicine that is FDA approved is Epidiolex which is used to treat rare seizure disorders [13]. CBD can be metabolized into hundreds of byproducts [11]. Two of the most common metabolites are 7-COOH-CBD and 7-OH-CBD [14]. The specific functions of CBD and these metabolites on human neural stem cells (NSCs) are unknown. Here we treated human NSCs with CBD, 7-OH-CBD, or 7-COOH-CBD as indicated in each graph and monitored cellular health by LDH, MTT, and cell cycle assays.

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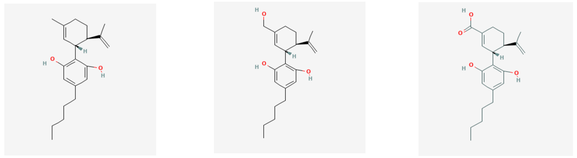
## Materials and Methods

CBD 7-OH-CBD 7-COOH-CBD



Chemical structures of cannabidiol (CBD) and two of its metabolites, 7-OH-CBD and 7-COOH-CBD. Images were taken from PubChem.

**Cell Culture:** Human hippocampal neural stem cells from a single donor were commercially obtained (PhoenixSongs Biologicals). Cells were maintained in a low oxygen (4%) incubator and fed neural stem cell growth media every other day. Cells from passages 8-12 were used in experiments.

**Treatment:** CBD, 7-OH-CBD, or 7-COOH-CBD was dissolved in growth media and fed to the cells at the indicated concentration and time points.

**LDH Assay:** Lactate dehydrogenase was detected in the cell culture medium using the cytotoxicity detection kit (Roche) according to manufacturer's directions. Assays were completed on days 1, 3, 5, and 7 following exposure.

**MTT Assay:** [3-(4,5-dimethylthiazolyl-2)-2,5 diphenyltetrazolium bromide] was dissolved in media. MTT solution was transferred to each well, and the plate was incubated at 37°C for 2 hours. Then, the media was removed and DMSO added to each well. After formazan crystals rehydrated the colorimetric changes were read at 595 nm. Assays were completed on days 1, 3, 5, and 7 following exposure.

**Cell Cycle Assay:** Cells were treated with CBD, 7-OH-CBD, or 7-COOH-CBD for 24 hours. Cells were then collected, fixed, RNA digested, and stained with propidium iodide (PI). Samples were ran on a BD LSR Fortessa flow cytometer and PI signal was analyzed in FCS Express version 10.07.

## References

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## Results and Discussion

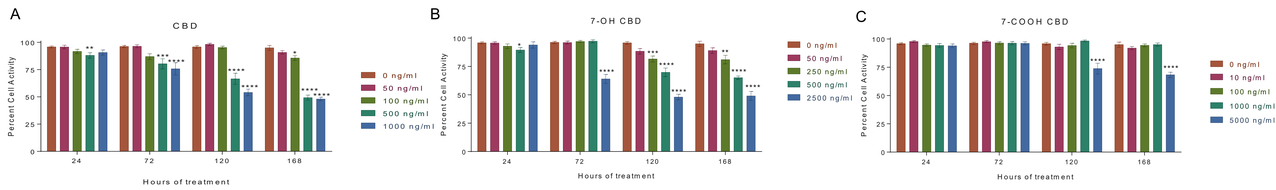


**Figure 1. CBD, 7-OH CBD and 7-COOH CBD reduce cell activity in a dose and time dependent manner.** Human Neural Stem Cells were grown to 80% confluency. NSCs were treated with the designated amount of (A) CBD, (B) 7-OH CBD, or (C) 7-COOH-CBD for 24, 72, 120, and 168 hours. At the indicated time points an MTT assay was completed to measure cellular activity. The displayed graphs show pooled data from three individual replicates. Significance was determined using a one-way ANOVA with Dunnett's correction to compare experimental values to the untreated values. * < 0.05; ** < 0.01; *** < 0.001; **** < 0.0001. Data is pooled from four replicates and error bars denote standard error of the mean.

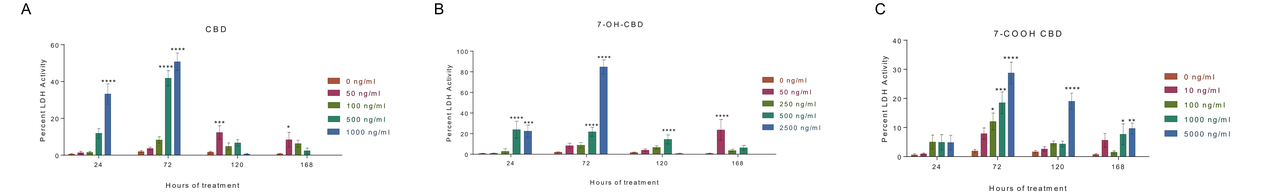


**Figure 2. CBD and its main metabolites, 7-OH-CBD and 7-COOH-CBD are toxic to human neural stem cells in a dose-dependent manner.** Human neural stem cells were grown to 80% confluency. NSCs were treated with the designated amount of (A) CBD, (B) 7-OH CBD, or (C) 7-COOH-CBD for 24, 72, 120, and 168 hours. At the indicated time points an LDH assay was completed to measure cell death. The displayed graphs show pooled data from three individual replicates. Significance was determined using a one-way ANOVA with Dunnett's correction to compare experimental values to the untreated values. * < 0.05; ** < 0.01; *** < 0.001; **** < 0.0001. Data is pooled from four replicates and error bars denote standard error of the mean.

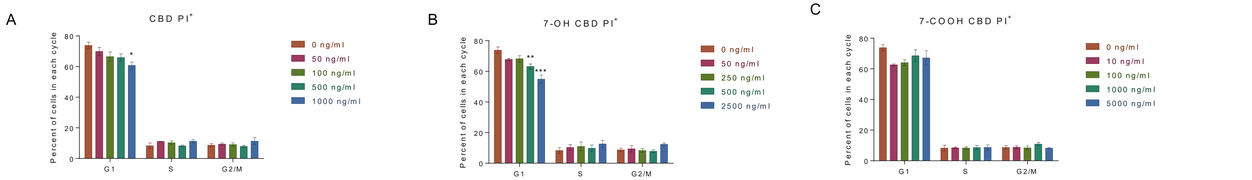


**Figure 3. High doses of CBD and 7-OH-CBD decreased the percentage of neural stem cells in the G1 phase of the cell cycle, but 7-COOH-CBD had no effect.** Human Neural Stem Cells were grown to 80% confluency. NSCs were treated with the designated amount of (A) CBD, (B) 7-OH CBD, or (C) 7-COOH-CBD for 24 hours. Cells were then stained with propidium iodide and read on a flow cytometer. Cell cycle phase and PI+ cells were analyzed via FCS Express. Significance was determined by a one-way ANOVA using Dunnett's correction to compare experimental values to the untreated values. * < 0.05; ** < 0.01; *** < 0.001. Data is pooled from three replicates and error bars denote standard error of the mean.

## Conclusion

- **CBD, 7-OH-CBD, and 7-COOH-CBD is toxic to human neural stem cells at high doses.**
- **Continual treatment of low dose CBD, 7-OH-CBD, and 7-COOH-CBD may also be toxic to human neural stem cells.**
- **The cell cycle is significantly altered at the G1 phase after exposure to CBD and 7-OH CBD.**
- **These results suggest that CBD and its metabolites may negatively impact neural stem cell development in utero.**