

Potential of Cannabidiol for the Treatment of Viral Hepatitis

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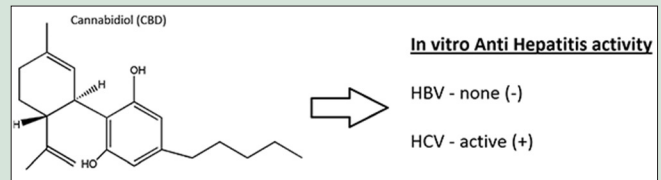
ABSTRACT

Viral hepatitis B (HBV) and hepatitis C (HCV) pose a major health problem globally and if untreated, both viruses lead to severe liver damage resulting in liver cirrhosis and cancer. While HBV has a vaccine, HCV has none at the moment. The risk of drug resistance, combined with the high cost of current therapies, makes it a necessity for cost-effective therapeutics to be discovered and developed. The recent surge in interest in Medical *Cannabis* has led to interest in evaluating and validating the therapeutic potentials of *Cannabis* and its metabolites against various diseases including viruses. Preliminary screening of cannabidiol (CBD) revealed that CBD is active against HCV but not against HBV *in vitro*. CBD inhibited HCV replication by 86.4% at a single concentration of 10 μ M with EC₅₀ of 3.163 μ M in a dose-response assay. These findings suggest that CBD could be further developed and used therapeutically against HCV.

Key words: *Cannabis*, hemp, hepatitis B, hepatitis C, cannabidiol

SUMMARY

- Cannabidiol exhibited *in vitro* activity against viral hepatitis C.



Abbreviations Used: CB2: Cannabis receptor 2, CBD: Cannabidiol, DNA: Deoxyribonucleic acid, HBV: Hepatitis B virus, HCV: Hepatitis C virus, HIV/AIDS: Human immunodeficiency virus/acquired immune deficiency syndrome, HSC: Hepatic stellate cells, MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, PCR: Polymerase chain reaction

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INTRODUCTION

Viral hepatitis is caused by a group of viruses divided into five types (A, B, C, D, and E), and they are primarily known to attach to the liver.^[1] Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most dangerous and prevalent of the five virus types.^[2,3] Chronic cases of HBV as well as HCV are among the leading causes of liver cirrhosis and hepatocellular carcinoma (HCC) in the world.^[4] HBV and HCV infections are also implicated in the development of other diseases including lymphoma, diabetes, and atherosclerosis.^[5,6] HBV is the most prevalent type worldwide and the leading cause of HCC in some countries, especially in Asia.^[7] Despite the fact that great strides have been made in the treatment and prevention of HBV and HCV, the global burden remains a major health problem. There is as such a great need to continue to search for new molecules with activity against hepatitis viruses.

Cannabidiol (CBD) is a nonpsychoactive cannabinoid found in the *Cannabis* plants and is credited for several pharmacological properties. It is also known to have beneficial effects against inflammation/pain, neurological conditions, cancer, and other ailments.^[8-11] The current surge in the interest in medical *Cannabis* has rekindled research to validate the medicinal properties of this molecule, especially given that it is nonpsychoactive compared to its close derivative tetrahydrocannabinol. While most of the studies on CBD and *Cannabis*, in general, have focused on the neuroprotective as well as anti-inflammatory properties, little is known about the antiviral activity of *Cannabis* and its CBDs. A search of the current literature did not reveal any report on the antiviral activity of *Cannabis* molecules against the hepatitis virus. In general, with regard to antiviral activity, medical *Cannabis* was reported to be used as an accompanying remedy by

HIV/AIDS patients to alleviate neuropathic pain, wasting, nausea, and vomiting.^[12-14] Other studies have focused on the effects of *Cannabis* use on patients undergoing treatment for HCV with mixed results.^[15,16] Given the increasing use and application of medical *Cannabis* along with its nonpsychoactive metabolite (CBD), and in line with our continuous effort to evaluate and validate the potential therapeutic properties of CBD, the major aim of this study was as such to evaluate the anti-HBV and anti-HCV activities of CBD *in vitro*.

MATERIALS AND METHODS

Cannabidiol

Research grade CBD (Item No.: C-045) [Figure 1] was purchased from Cerilliant Reference Standards (www.cerilliant.com).

Anti-hepatitis B assay

The anti-HBV assay was performed as previously described^[17,18] with modifications to use in real time quantitative polymerase chain reaction (PCR) (TaqMan) to measure extracellular HBV DNA copy number associated with virions released from HepG2 2.2.15 cells. The

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HepG2 2.2.15 cell line is a stable cell line producing high levels of the wild-type ayw1 strain of HBV. Antiviral compounds blocking any later step of viral replication such as transcription, translation, pregenome encapsidation, reverse transcription, particle assembly, and release can be identified and characterized using this cell line.

In brief, HepG2 2.2.15 cells are plated in 96-well microtiter plates. Only the interior wells were utilized to reduce "edge effects" observed during cell culture; the exterior wells were filled with complete medium to help minimize sample evaporation. After incubation with 5% CO₂ atmosphere at 37°C for 16–24 h, the confluent monolayer of HepG2 2.2.15 cells was washed and the medium was replaced with complete medium containing test compounds at a single concentration of 10 μM. Three days later, the culture medium was replaced with fresh medium and test compounds at a single concentration of 10 μM. Six days following the initial administration of the test compounds, the cell culture supernatant was collected, treated with pronase and DNase, and then used in a real-time quantitative TaqMan PCR assay. Antiviral activity was determined by calculating the reduction in HBV DNA levels compared to untreated virus control samples. Compound cytotoxicity was determined using MTS (CellTiter 96 Reagent, Promega) to measure cell viability as described above.

Anti-hepatitis C assay

The anti-HCV activity was carried out according to previously reported procedures.^[19,20] Huh7.5 cells (HD Biosciences) were grown in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin-streptomycin (pen-strep), and 1% nonessential amino acids. Cells were incubated at 37°C in a 5% CO₂ incubator. Huh7.5 cells were seeded at 1 × 10⁴ cells/well into 96-well plates. Test compounds were serially diluted with DMEM plus 5% FBS. The diluted compound in the amount of 50 μl was mixed with an equal volume of cell culture-derived HCV (HCVcc), and then applied to appropriate wells in the plate. Human recombinant interferon alpha-2b was included as a positive control compound. After incubation at 37°C for 72 h, the cells were lysed, and luciferase activity was measured using Renilla Luciferase Assay System (Promega) according to the manufacturer's instruction. The number of cells in each well was determined by CytoTox-1 reagent (Promega). CBD was first tested at a single concentration of 10 μM in triplicate to derive percentage inhibition of HCVcc. Based on the activity of the single dose, a dose-response assay was carried out to determine the IC₅₀. Sofosbuvir was used as a positive control.

Statistical analysis

Experiments were carried out in duplicate, and results were given as the mean of the two experiments. The data in all the experiments were analyzed using Microsoft Excel.

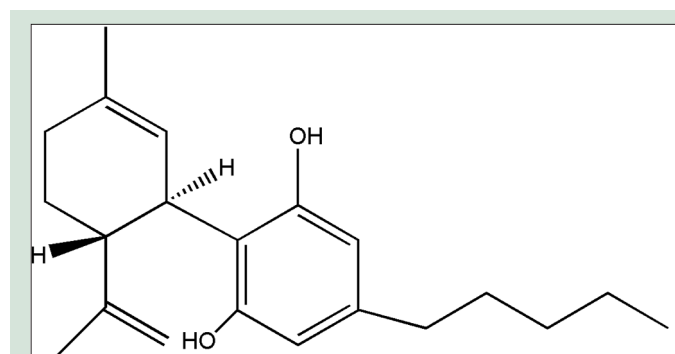


Figure 1: Chemical structure of cannabidiol

RESULTS AND DISCUSSIONS

The bioactivity of CBD against HBV and HCV is shown in Tables 1 and 2. CBD inhibited HCV replication by 86.4% at a single concentration of 10 μM. The compound was not active against the HBV virus *in vitro* but exhibited a significant cytotoxicity against HepG2 2.2.15 cells which were used to culture the virus. In the HCV assay, CBD inhibited the virus with minimal toxicity against the Huh7.5 cells that were used to culture the virus. Lamivudine and interferon alpha were used as positive controls against HBV and HCV, respectively, and they significantly inhibited viral replication at the single-dose assay. CBD was found to exhibit a dose-dependent inhibition of the HCV virus in the dose-response assay [Table 2].

The direct antiviral activity of CBD against the HCV indicates that the molecule has an effect against both the viral and nonviral hepatitis, otherwise known as autoimmune hepatitis. Autoimmune hepatitis is an inflammatory liver condition elicited by activated T-cells and macrophages. Studies have shown that CBD by interacting with the CB2 receptor induces apoptosis in thymocytes and splenocytes inhibiting the proliferation of T-cells and macrophages which are responsible for either attacking liver cells or inducing the release of pro-inflammatory cytokines that cause autoimmune hepatitis in the liver.^[21-23] CB2 receptors are expressed in immune and immune-derived cells and their activation is known to influence viral infections by altering host immune response, particularly inflammation.^[21] CB2 receptor activation is as such known to suppress inflammation and modulate immune responses to viral infection.^[24,25] Host inflammation is also said to be able to drive the progression of HBV and other viral infections where host inflammation is pathogenic and activation of the CB2 would as such be useful in the control of the HBV virus infection since it results in an anti-inflammatory effect.^[26] The benefit of CBD in alleviating liver fibrosis, which is one of the outcomes of untreated viral hepatitis, was also demonstrated in previous studies.^[27] The studies revealed that one of the most critical cellular events in the development and progression of liver fibrosis is the activation of hepatic stellate cells (HSCs), and CBD was shown to induce apoptosis in activated HSCs by interaction with the endoplasmic reticulum.^[27]

CONCLUSION

We report here for the first time *in vitro* studies to demonstrate the antiviral activity of CBD against HCV. CBD was shown to have activity against HCV *in vitro* but not against HBV. A review of the literature

Table 1: Inhibitory effect of cannabidiol against viral hepatitis B and C at a single dose of 10 (μM)

Hepatitis virus	Molecule	Percentage virus inhibition	Percentage cytotoxicity
HBV (μM)	CBD (10)	0	65.7
	Lamivudine (2)	97.7	0
HCVcc	CBD (10 μM)	84.6	2.7
	IFN-α (10 IU/mL)	85.4	22.6

HBV: Hepatitis B virus; HCV: Hepatitis C virus; HCVcc: Cell culture-derived HCV; IFN-α: Interferon-alpha; CBD: Cannabidiol

Table 2: Determination of EC₅₀ and CC₅₀ of cannabidiol against hepatitis C virus

	EC ₅₀ (μM)	CC ₅₀ (μM)	SI
Sofosbuvir	0.055±0.0104	>10	>181
CBD	3.163±0.133	15.670±0.250	4.954

CBD: Cannabidiol

seems to suggest that CBD may also have activity *in vivo* based on its interaction with the CB2 receptor and as such using a host mechanism to indirectly slow the pathogenic process of the HBV virus. Based on these findings, CBD as such has potential to be further developed as a treatment for viral hepatitis, especially as a combination therapy with the currently existing therapies. Further studies are in progress to further validate and assess the activity *in vivo*.

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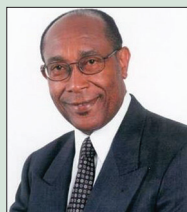
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Conflicts of interest

Authors Henry Lowe and Ngeh Toyang are on the management of Medicanja Jamaica Ltd., and Flavocure Biotech LLC.

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