THE EFFECT OF CANNABIDIVARIN ON T-CELL LINES PROLIFERATION AND ACTIVATION

Wilai LAOLEE[1], Koramit SUPPIPAT[2] and Nipan ISRASENA[3]
1 Faculty of Medicine, Chulalongkorn University, Thailand; Wilailaolee8@gmail.com
2 Cellular Immunotherapy Research Unit, Chulalongkorn University, Thailand; Koramiz@yahoo.com
3 Stem Cell and Cell Therapy Research Unit, Chulalongkorn University, Thailand; nipan.i@chula.ac.th

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ABSTRACT
Cannabidivarin (CBDV) is a non-psychotropic cannabinoid and a propyl analog of cannabidiol (CBD). Studies have suggested CBD has immunosuppression, but little is known about the effects of CBDV. Therefore, we explored the effects of CBDV on cell proliferation and activation of T-cell lines. The study was performed by investigating cell cytotoxicity, T-cell activation (expression of CD25 and CD69), and cell proliferation. Varying doses of CBDV and CBD were administered to Jurkat and MOLT-4 cell lines. CBDV demonstrated less toxicity than CBD at the higher concentrations in both Jurkat and MOLT-4 cell lines, with IC50 of 31.03 μM (CBDV) and 22.42 μM (CBD) in Jurkat cell lines, and 25.23 μM (CBDV) and 14.18 μM (CBD) in MOLT-4 cell lines. Furthermore, there was a significant increase in cell apoptosis at 16 and 32 μM in CBD, and at 32 μM in CBDV. Interestingly, when comparing CBDV to CBD, there was a significant difference in the suppression of T-cell proliferation at similar concentrations of 16 μM and 32 μM. Lastly, CBDV showed no significant difference in the expression of CD25 and CD69 compared to the vehicle control, while CBD, showed a significant decrease in CD25 and CD69 expression at 32 μM. Although CBDV is a propyl analog of CBD, there were major differences in pharmacodynamics between the two cannabinoids. CBDV showed lower levels of toxicity and a significant decrease in T-cell proliferation compared to CBD in both Jurkat and MOLT-4 cell lines. However, CBDV did not affect T-cell activation while CBD significantly reduced T-cell activation.
Keywords: Cannabidivarin, Cannabidiol, T-cell Proliferation, T-cell Activation, Cell Apoptosis

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INTRODUCTION

Cannabis is a plant genus of the Cannabaceae family which has approximately 700 different strains including *cannabis sativa*, *cannabis indica*, and *cannabis ruderalis* as three initial species. (Pattnaik et al., 2022). They contain a variety of physiologically active secondary metabolites, and due to their nontoxic and complex biological compounds, these plants have many potential uses in medicine (Helcman & Šmejkal, 2021). Cannabidiol (CBD), a cannabis derivative, shares structural similarities with tetrahydrocannabinol (THC). CBD is non-psychoactive, while THC is psychoactive and can induce euphoric moments. For this reason, researchers have begun to explore CBD as an alternative to THC (Gaoni & Mechoulam, 2002). CBD has been documented to have immunosuppressive action on the innate or adaptive immune systems. For instance, Watzl et al. (1991) found that CBD suppresses the production of TNF-α, IFN-γ, and IL-1β cytokines in human mononuclear cells. Additionally, treatment of many T-cell types, including Jurkat cells, MOLT-4 human T-cells, and HL-60 or basic human monocytic cells with CBD revealed that CBD promoted apoptotic activity. (Wu et al., 2010).
Recently, cannabidivarin (CBDV) garnered scientific interest for being a major homolog of CBD with a very similar structure as a propyl analog of cannabidiol (CBD). It is also non-psychotropic. CBDV was discovered by Vollner and his colleagues in 1969; however, there is limited documentation of the pharmacodynamics of CBDV. CBDV is postulated to have similar phytochemical properties to CBD (Pagano et al., 2019). Some studies on CBDV demonstrated the effects of CBDV in reducing inflammation through suppression of neutrophil infiltration, activation of intestinal permeability, and decreased release of cytokines such as IL-1β, IL-6, and MCP-1 chemokines (Alves et al., 2020; Pagano et al., 2019). Therefore, this study aims to evaluate the effect of CBDV on T-cell proliferation and activation to expand our knowledge and understanding of the pharmacodynamics of CBDV in therapy and as a potential alternative to THC and CBD.

LITERATURE REVIEWS

The effect of cannabidiol (CBD) on T-cells

To test whether CBD had an antiproliferative effect, a study was performed using 1-100 g/ml of CBD to treat human primary T-cells. The results suggested that CBD significantly inhibits activated T-cell proliferation (IC50= 4.7 g/ml). The results were confirmed by cell cycle analysis whereby cells were treated with CBD for 72 h and the percentage of cell distribution in four phases of the cell cycle was determined. At the G0/G1 phase, CBD was found to suppress the cells in a dose-dependent manner, and the cell cycle remained suppressed in the S and G2/M phases (Devi et al., 2022). In addition, CBD was found to significantly suppress immune cells in both mesenteric lymph nodes (MLNs) and the spleen. The same report also showed that CBD significantly suppresses peripheral blood mononuclear cells (PBMCs) in both healthy donors and patients who have multiple sclerosis and non-seminomatous germ cell tumors (NSGCTs) (Zgair et al., 2017). In another study performed by treating splenocytes with CBD, researchers found that CBD enhanced apoptotic activities via T-cell populations (CD4+ and CD8+) in both time and dose-dependent manner. Furthermore, studies suggested that apoptosis was induced in cells treated with CBD, through the increase of DNA strand breakage and an increase in the percentage of hypodiploid cells (Rieder et al., 2010; Wu et al., 2008). Consequently, numerous further investigations have revealed that CBD triggers apoptotic activity by activating caspase-8 in a manner reliant on oxidative stress. With regards to the effect of CBD on T-cell activation through CD25 and CD69 expression, a study demonstrated CBD to significantly reduce CD25 and CD69 expression in a dose-dependently manner for CD4+ and CD8+ T-cells (Devi et al., 2022; Myers et al., 2019).

The effect of cannabidivarin (CBDV) on immune cells

At present, there is limited knowledge on the action of CBDV on immune function. However, since CBDV is a derivative of CBD with similar properties, the results of CBD were often extended to CBDV. For instance, studies have shown that CBD has anti-inflammatory properties (Burstein, 2015), which is also evidenced in CBDV through a study using CBDV on acute colitis which showed that CBDV significantly decreased neutrophil infiltration and intestinal permeability (Stone et al., 2021). Furthermore, CBDV selectively decreased pro-inflammatory cytokines (IL-1β and IL-6) without affecting the levels of the primary anti-inflammatory cytokines, IL-10. The same study further demonstrated CBDV to reduce colonic mRNA expression linked to the chemokine monocyte chemoattractant protein-1 (MCP-1), whose primary function is attracting macrophages to inflamed tissues (Stone et al., 2021). Furthermore, another study showed the effect of CBDV on monocytes. CD80 and CD86 are costimulatory molecules that aid in T-cell activation through the upregulation of monocytes. The study showed that CBDV significantly inhibits CD14+ monocyte-derived IL-1β, which caused a constant reduction of HLA-DR and CD80 expression. (Blevins et al., 2022).

Hypothesis

Cannabidivarin (CBDV) has an immunosuppressive effect on T-cell lines.

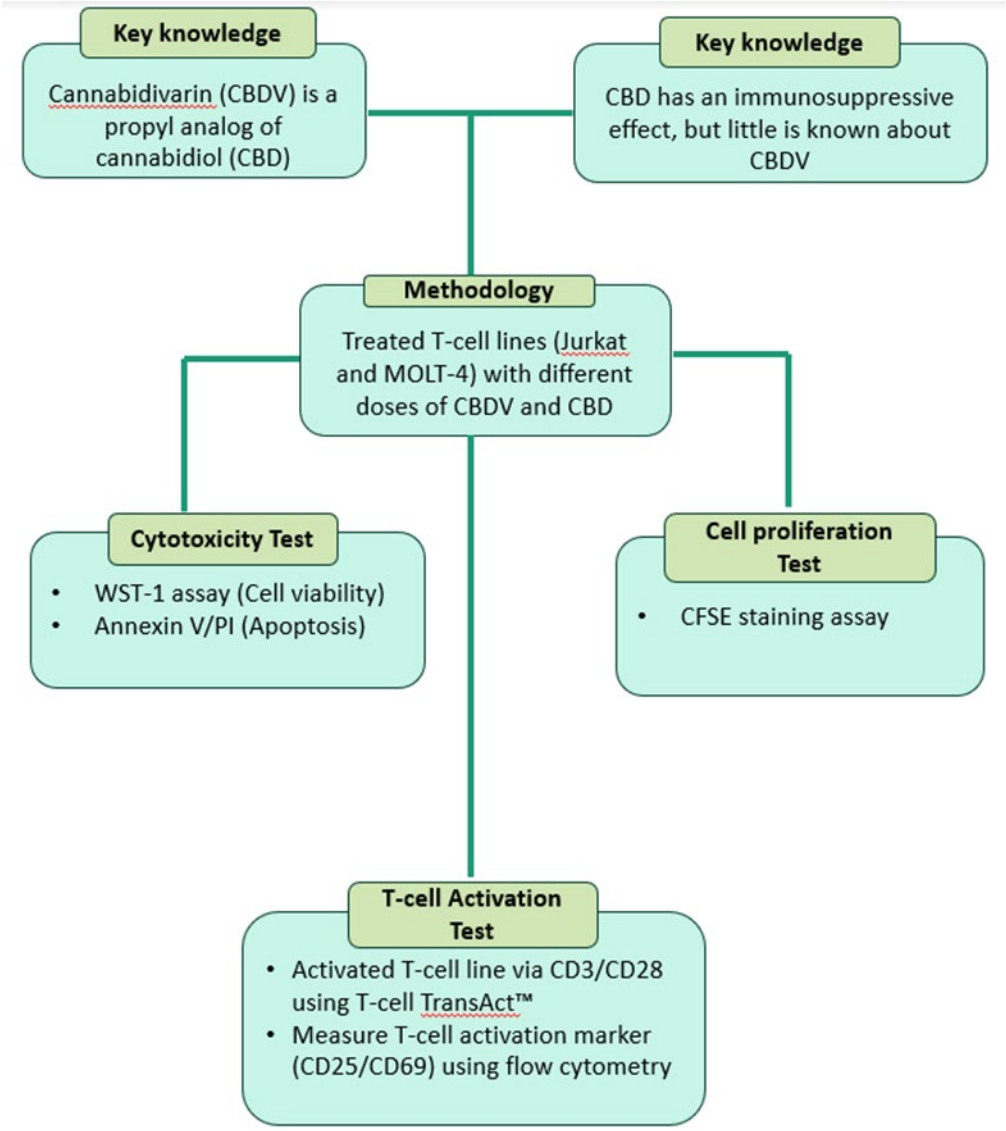

Figure 1 research framework

RESEARCH METHODOLOGY

Cannabinoid compound

1) Cannabidiol (CBD)

CBD (Batch: DWO180.707-1) was obtained from THC Pharm GmbH. For the biological assay, dimethyl sulfoxide (DMSO) was used to dissolve the CBD, which was then kept at -20°C for storage. This solution was further diluted to the desired concentration with RPMI 1640 medium.

2) Cannabidivarin (CBDV)

CBDV was graciously provided by Prof. Dr. Tirayut Vilaiwan., Ph.D., Faculty of Sciences, Chulalongkorn University.

Cell lines

The T lymphoblast cell lines Jurkat (clone E6-1; ATCC TIB-152) and MOLT-4 (ATCC CRL-1582) were obtained from ATCC®. Cells were cultured with Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% Glutamax, and 1% penicillin and streptomycin. All cells were cultivated and kept alive in a humidified incubator at 37°C with 5% CO_2 and 95% air.

Measurement of cell viability

CBDV and CBD were tested for their cytotoxic effect on Jurkat and MOLT-4 T-cell lines. Cells were cultured in 96 well plates at $2x10^4$ cells/well, treated with CBDV versus CBD at the concentration of 2 µM, 4 µM, 8 µM, 16 µM, 32 µM, and 64 µM, and then kept at 37 °C for 24 h in a humid incubator with 5% CO_2. DMSO was used as the vehicle control. Each well received 5 µM WST-1 assay reagent after 24 h, followed by 2 h of incubation. The absorbance was then measured using a CLARIOstar Plus Microplate Reader at 450 and 690 nm. The nontoxic dose of CBD and CBDV was determined by this method and used in subsequent experiments.

Determination of cell apoptosis

In 96 well plates, Jurkat and MOLT-4 cell lines were grown followed by treatment with CBDV versus CBD at concentrations of 8 µM, 16 µM, and 32 µM. The cells were grown for 24 h at 37°C with 5% CO_2 in a humid incubator. Then, in accordance with the manufacturer's recommendations, apoptosis was examined using an Annexin V-PI apoptosis kit. Briefly, Annexin V and PI staining was performed on the cells, followed by a 10-minute incubation period at room temperature, in the dark. Using flow cytometry (FACS Calibur, BD Bioscience) at 20,000 events, the cell apoptosis percentage was analyzed.

Determination of T-cell proliferation

A prepared solution of 1.25 µM carboxyfluorescein diacetate succinimidyl ester (CFSE, Bio legend) was employed to stain Jurkat and MOLT-4 cell lines according to the standard protocol. After staining, the cells were treated with CBDV versus CBD at the concentrations of 8 µM, 16 µM, and 32 µM. Afterwards, a 72-hour incubation period at 37 °C in a humidified incubator with 5% CO_2 was conducted. Flow cytometry (FACS Calibur, BD Bioscience), performed at 10,000 events, was used to measure cell proliferation.

Determination of CD25 and CD69 activation markers

Jurkat cells were activated via CD3 and CD28 using T-Cell TransAct™ and then treated with CBDV versus CBD at concentrations of 8 µM, 16 µM, and 32 µM. Following a 24-hour incubation period at 37 °C in a humidified incubator with 5% CO_2, the cells were harvested and stained with anti-human CD25 and CD69 antibody, followed by measuring T-cell activation marker (CD25 and CD69) using flow cytometry (FACS Calibur, BD Bioscience) at 10,000 events.

Statistical analysis

All data was analyzed using Graph Pad version 8.0. Data are presented as the mean ± SEM. Statistical significance of the data was considered at $P < 0.05$.

RESEARCH RESULTS

The effect of CBDV and CBD on T-cell lines toxicity (cell viability and cell apoptosis)

A WST-1 assay was used to determine the toxicity of CBDV and CBD on Jurkat and MOLT-4 cell lines. While CBDV significantly lowered cell viability, this difference was not as large as CBD at 32 µM and 64 µM in the Jurkat cell line, and at 16 µM, 32 µM and 64 µM in MOLT-4 (Figure 1A-B), with IC50 values of 31.03 µM with CBDV, 22.42 µM with CBD in Jurkat cell lines, 25.23 µM with CBDV, and 14.18 µM with CBD in MOLT-4 cell lines (Figure 1C-D).

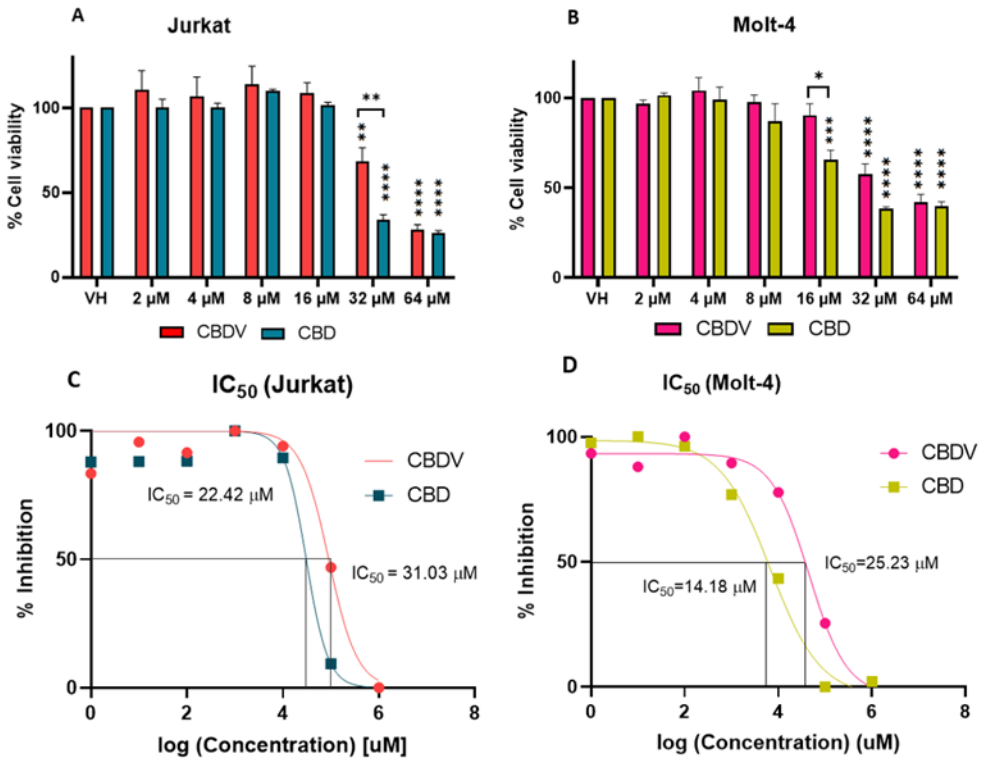

Figure 1 The toxicity of CBDV and CBD on Jurkat and MOLT-4 cell lines. (A-B) Jurkat and MOLT-4 cells ($2x10^4$ cells/100µl/well) were cultured to 96 well plates with varied concentrations (2 µM, 4 µM, 8 µM, 16 µM, 32 µM, and 64 µM) of CBDV versus CBD, cultured for 24 h. The data were then analyzed using a microplate reader at 450/690 nm. DMSO was used as the vehicle control (VH). (C-D) The half-maximum inhibitory concentration (IC50) was calculated based on the data of cell viability. The data was expressed as mean ±SD with comparison to the vehicle control. (Graph Pad version 8.0. *P<0.05, **P≤0.005, ***P≤0.001. ****P<0.0001)

Cannabinoid cytotoxicity to T-cell lines was confirmed by an apoptosis test. The test demonstrated a significant increase of cell apoptosis in both Jurkat and MOLT-4 cell lines at 16 µM and 32 µM in CBD and at 32 µM in CBDV, resulting in a lower number of apoptotic cells in CBDV compared to CBD (Figure 2A-D).

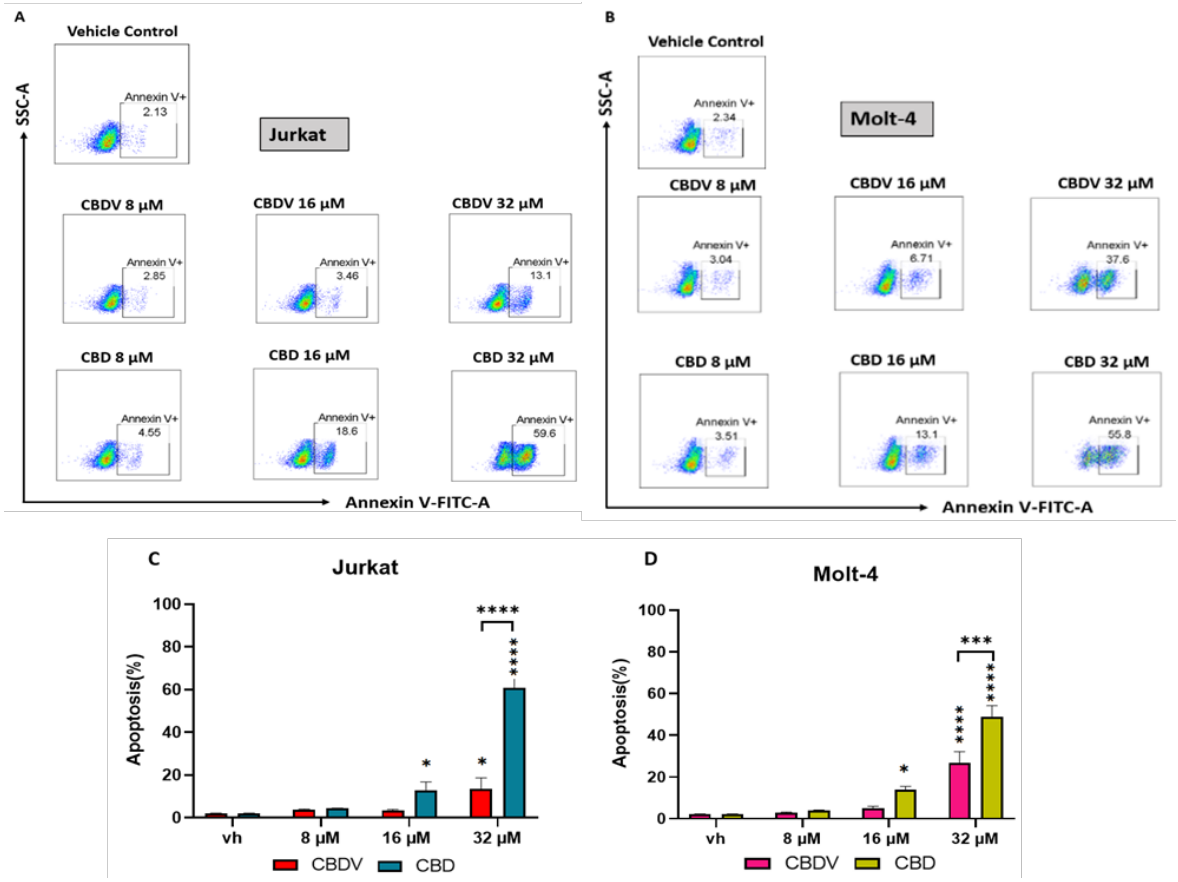

Figure 2 Effect of CBDV and CBD on T-cell line apoptosis. (A-D) Jurkat and MOLT-4 cells ($2x10^4$ cells/100 µl) administered at varied concentrations of CBDV versus CBD at 8 µM, 16 µM, and 32 µM in 96 well plates. DMSO was used for vehicle control (VH). After 24 h of culture, the cells were labeled through staining with Annexin V/PI, collected, and analyzed with a flow cytometer. The data (A-B) was presented as the total cell population percentage by using FlowJo 10.0 to determine the cell population. The data (C-D) was expressed as mean ±SD with comparison to the vehicle control. (Graph Pad version 8.0. *P<0.05, **P≤0.005, ***P≤0.001. ****P<0.0001)

The effect of CBDV and CBD on T-cell line proliferation

The effect of CBDV and CBD on T-cell line proliferation was studied using a CFSE staining assay. The study demonstrated that both CBDV and CBD significantly suppressed cell proliferation in Jurkat and MOLT-4 cell lines at similar concentrations of 16 µM and 32 µM (Figure 3A-D). However, CBDV had lower inhibition of T-cell proliferation compared to CBD.

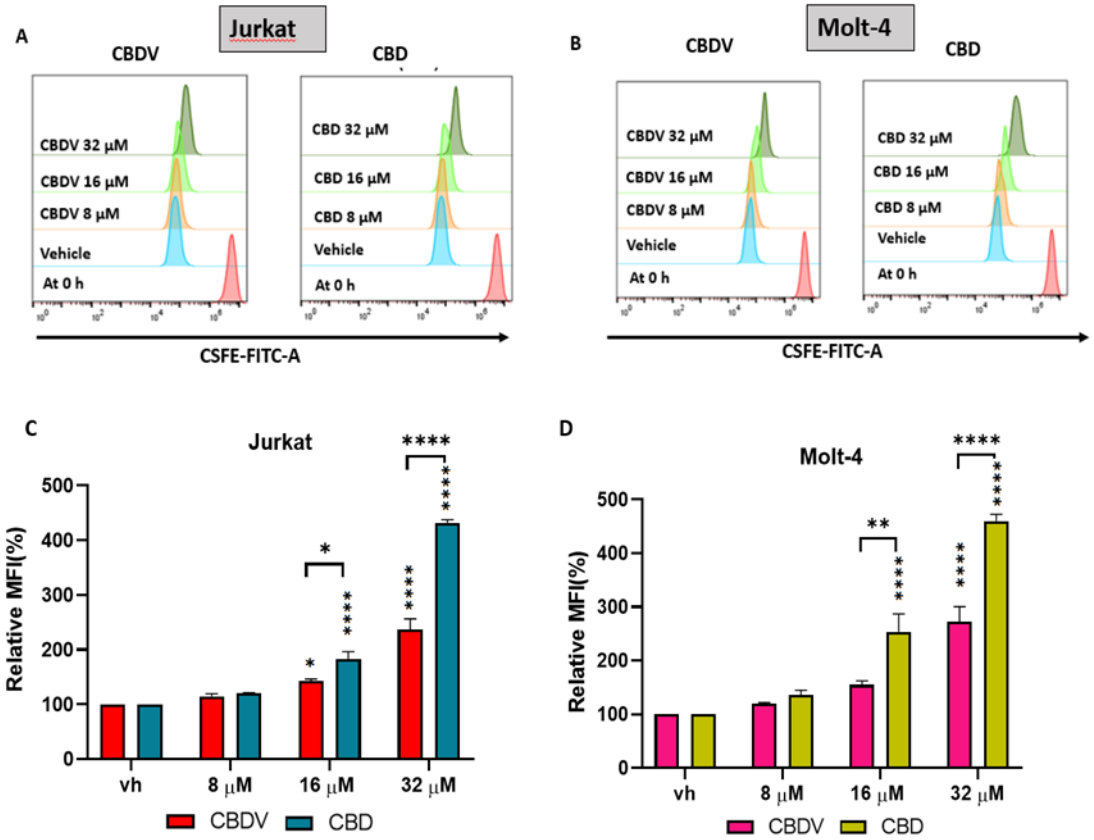

Figure 3 Effect of CBDV and CBD on T-cell line proliferation. (A-D) Jurkat and MOLT-4 cells were stained with CFSE (1.25 µM) and then administered with varied concentrations of CBDV and CBD, at 8 µM, 16 µM, and 32 µM in 96 well plates ($2x10^4$ cells/100µl/well). DMSO was used as vehicle control (VH). After 72 h of incubation, the cells were harvested and analyzed using a flow cytometer. The data (A-B) were analyzed using FlowJo 10.0. The data(C-D) are expressed as mean ±SD with comparison to the vehicle control. (Graph Pad version 8.0. *P<0.05, **P≤0.005, ***P≤0.001. ****P<0.0001)

The effect of CBDV and CBD on T-cell line activation

The effect of CBDV and CBD on Jurkat cell activation was determined by the cell's expression of CD25 and CD69 using a flow cytometer. MOLT-4 cell lines were not used since they were not activated with CD3/CD28 T-cell Transacts™ (Figure 4A-D). Our results demonstrated no significant effect of CBDV on T-cell activation compared to the control vehicle. However, CBD was found to significantly inhibit the expression of CD25 and CD69 at 32 µM (Figure 4 E-H).

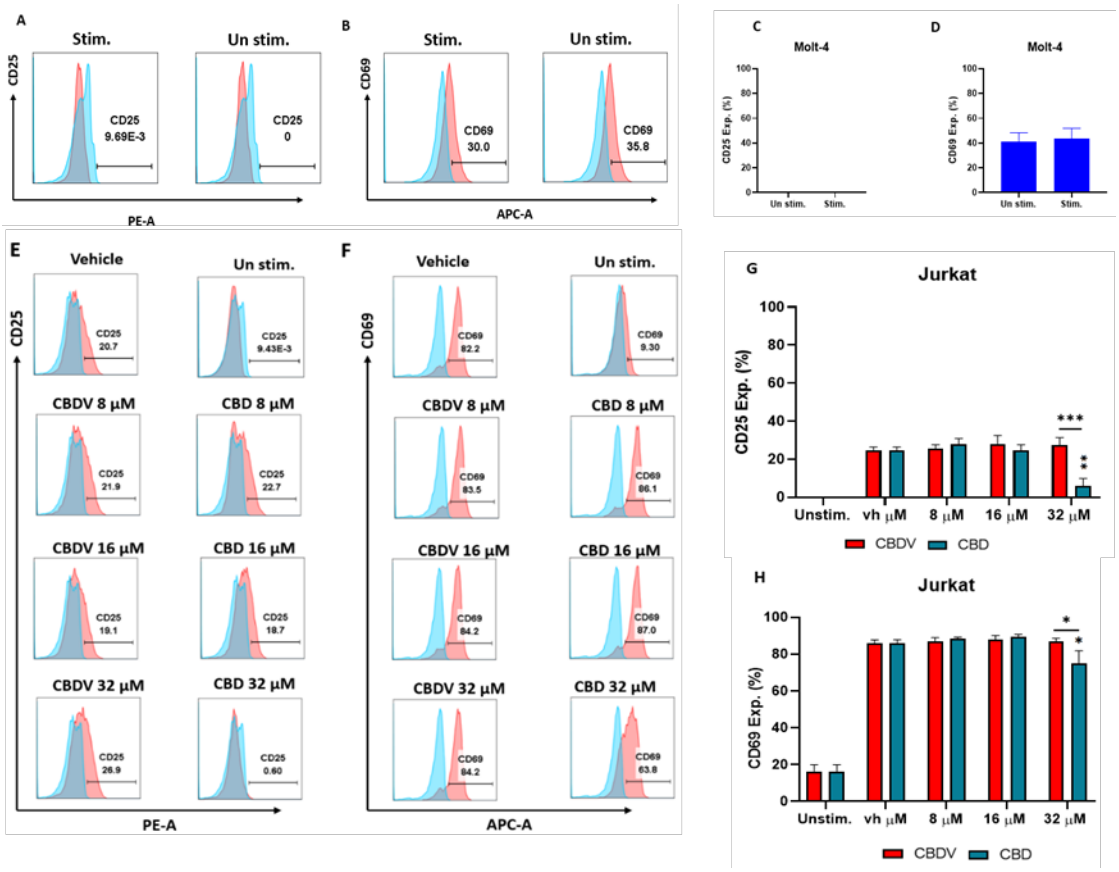

Figure 4 Effect of CBDV and CBD on T-cell line activation. (A-D) MOLT-4 cells ($1x10^6$ cells/ml) were activated using CD3/CD28 T-Cell TransAct™. An un-activated group was used as a control. (E-H) Jurkat cells ($1x10^6$ cells/ml) were activated by using CD3/CD28 T-Cell Transact™ and then administered various concentrations of CBDV and CBD at 8 µM, 16 µM, and 32 µM in 24 well plates. DMSO was used as vehicle control (VH). After 24 h of cell culture, the cells were harvested and stained with anti-human CD25 and CD69 antibody, followed by analyzing with a flow cytometer. The data (A-C, E-F) were analyzed using FlowJo 10.0. Data (D, G-H) are presented as the mean ± SD compared with the vehicle control. (Graph Pad version 8.0. *P<0.05, **P≤0.005, ***P≤0.001. ****P<0.0001)

DISCUSSION & CONCLUSION

Although cannabidivarin (CBDV) is a propyl analog of cannabidiol (CBD), it causes lower toxicity to cells than CBD (Figure 1). The cytotoxic effects of CBD were previously studied using the T-ALL model, reporting an IC50 of 12.1 µM (Olivas-Aguirre et al., 2020), which was comparable to the IC50 that we obtained in this study. Another study showed that CBD and CBDV were cytotoxic to HeGp2 cells at IC50 values of 15.8 µM and 19.74 µM respectively (Russo et al., 2021). Our results align with those of other researchers (Devi et al., 2022; Olivas-Aguirre et al., 2020; Russo et al., 2021). Our study also suggested that CBDV and CBD treatment significantly induced apoptosis in both Jurkat and MOLT-4 T-cell lines; however, CBDV was found to have lesser apoptotic induction than CBD (Figure 2). Similarly, other studies in murine splenocytes, primary T-cells, and human monocytes support the notion that CBD induces apoptosis in a dose-dependent manner in immune cells (Devi et al., 2022; Wu et al., 2018). With regards to cell proliferation, there was a significant decrease in T-cell proliferation on both CBDV and CBD (Figure 3). Nonetheless, CBD was found to have higher rates of T-cell proliferation suppression. A study which evaluated CBD effect on T-cell proliferation, cells were isolated from mesenteric lymph nodes (MLNs) and the spleen of rats

discovered strong suppression of cell proliferation in a dose-dependent manner (Zgair et al., 2017). Lastly, the study suggested that CBDV did not show a significant effect on T-cell activation compared to the vehicle control (Figure 4E-H). On the other hand, CBD significantly suppressed T-cell activation (Figure 4E-H). Interestingly, a study by Blevins et al. (2022) on the effect of CBDV on CD8+ T-cell activation and function, showed that CBDV caused a significant decrease in CD25 and CD69 expression at the concentration of 10 μM. This could be due to the differences in sensitivity of each cell type that allow them to have different results. Furthermore, Devi et al. (2022) have supported our results that CBD significantly suppressed the expression of both CD25 and CD69 in a dose-dependent manner. In conclusion, CBDV was suggested to have lower toxicity to cells compared to CBD and was found to have anti-proliferative effects. However, it showed a lower decrease in cell proliferation than CBD. Interestingly, CBDV did not significantly inhibit T-cell activation, whereas CBD significantly suppressed T-cell activation.

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Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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