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*Short communication*

# *In vitro* effects of *Cannabis sativa* L. extract on lymphocyte viability and oxidative stress response

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## Introduction

Cannabis (*Cannabis sativa* L.), dioecious herbaceous plant from Cannabinaceae family, represents a rich source of bioactive compounds, including cannabinoids, terpenes, flavonoids, and other polyphenolic constituents (Bukowska, 2024). Among them, flavonoids such as quercetin, kaempferol, and cannflavins exhibit notable antioxidant activity by scavenging free radicals and reducing oxidative stress (Abdel-Kader et al., 2023). These polyphenolic components contribute significantly to the biological activity of cannabis and its effects on cellular and immune functions (Al-Khazaleh et al., 2024).

Oxidative stress results from an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, leading to cellular damage (Al-Khazaleh et al., 2024). Lymphocytes are particularly sensitive to oxidative stress due to their role in immune regulation, making them a suitable model for evaluating cytoprotective or cytotoxic effects of plant extracts. In this context, plant-derived antioxidants may modulate redox balance and influence lymphocyte viability.

The aim of this study was to investigate the effect of a *Cannabis sativa* L. plant extract on lymphocyte viability in order assess its potential biological activity and its impact on immune cell viability.

## Materials and methods

*Plant material extraction*

Plant material extraction was carried out using 0.5 g of dried, finely powdered plant material extracted with 50 mL of 50% ethanol (v/v). The extraction process was performed by ultrasonic-assisted extraction in an ultrasonic bath operating at 50/60 Hz and 720 W for 15 minutes. After sonication, the resulting extracts were filtered through filter paper and the volume was adjusted to 50 mL with 50% ethanol in a volumetric flask.

The solvent was subsequently removed using a rotary evaporator (BUCHI) until complete dryness was achieved. The dried residue was then reconstituted in water to obtain a stock solution with a concentration of 10 mg/mL. From this stock solution, three working dilutions were prepared at final concentrations of 50, 100, and 200 µg/mL, using RPMI medium as the diluent.

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*Isolation and Culture of Peripheral Blood Lymphocytes*

Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll density gradient centrifugation. After isolation, the lymphocyte-rich fraction was collected, resuspended in phosphate-buffered saline (PBS), and centrifuged to obtain a purified cell pellet. The cells were then reconstituted in RPMI-1640 culture medium supplemented with 10% fetal bovine serum (FBS) and antibiotics. Following preparation, equal volumes of the PBMC suspension were dispensed into 96-well culture plates. The cells were allocated into different experimental groups, including an untreated control, $H_2O_2$-treated wells, plant extract-treated wells, and wells receiving combined treatment with plant extract and $H_2O_2$. After treatment, the plates were incubated at 37 °C until further analysis was performed.

*Viability of lymphocites*

The viability of lymphocytes in each experimental group was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. At the designated time point, 20 µL of MTT solution (5 mg/mL) was added to each well, and the 96-well plates were incubated at 37 °C for 3 hours. After incubation, the supernatant was carefully removed, and the resulting formazan crystals were solubilized by adding 150 µL of dimethyl sulfoxide (DMSO). Absorbance was then recorded at 570 nm using a VICTOR microplate reader (PerkinElmer, USA).

## Results and discussion

Cannabis extracts at concentrations of 50, 100, and 200 µg/mL, resulted in reduced lymphocyte viability (45%, 46%, and 54%, respectively). Similar results were observed in the presence of $H_2O_2$, indicating no significant protective effect against oxidative stress. These findings indicate a concentration-dependent cytotoxic effect. Although cannabis contains cannabinoids and polyphenolic compounds with reported antioxidant properties, these constituents may also exert pro-oxidant effects depending on concentration and cellular context, leading to increased ROS production and reduced cell viability (Al-Khazaleh et al., 2024). Furthermore, cannabinoids have been shown to modulate immune cell function and reduce lymphocyte viability and proliferation in a dose-dependent manner (Bukowska, 2024). The absence of protection against $H_2O_2$ suggests insufficient antioxidant capacity under the applied conditions, while extract composition and extraction method may further influence the observed effects.Overall, the results are consistent with literature showing that the biological activity of polyphenol-rich extracts is highly concentration- and cell-type dependent (Bukowska, 2024).

## Conclusion

The effect of *C. sativa* extract on lymphocyte viability was evaluated at different concentrations. The results demonstrated that all tested concentrations did not provide antioxidant protection, but instead showed reduced cell viability, suggesting a potential cytotoxic effect.

These findings indicate that, under the applied experimental conditions, the extract does not exert a protective effect on lymphocytes and may induce dose-dependent adverse effects on cell viability. Further studies are needed to identify the specific bioactive compounds responsible for these effects and to evaluate the activity of *Cannabis sativa* extracts over a wider concentration range and in different cellular models.

## Reference

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