Macedonian Pharmaceutical Bulletin, 72 (Suppl 1) 547 - 548 (2026)
Online ISSN: 1857-8969
DOI: 10.33320/maced.pharm.bull.2026.72.03.263
Short communication

Protective effects of *Verbena officinalis* extract against oxidative stress in human lymphocytes

Kamenka Nikolova[1*], Ana Trajkovska Matevska[1], Angela Joskovska[2], Dushko Shalabalija[3], Nadica Matevska Geshkovska[2], Marija Karapandzova[1], Iskra Davkova[1]

[1]*Institute of Pharmacognosy, Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia*
[2]*Center for Biomolecular and Pharmaceutical Analysis, Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa St. 47, 1000 Skopje, Republic of Macedonia*
[3]*Institute of Pharmaceutical Technology, Faculty of Pharmacy, Majka Tereza 47, Ss. Cyril and Methodius University in Skopje, 1000 Skopje, N. Macedonia*

Introduction

Verbena officinalis L. is a medicinal plant traditionally used in phytotherapy and widely recognized for its rich content of bioactive phytochemicals, particularly polyphenols, flavonoids, and phenolic acids. These compounds are considered major contributors to its biological activity, especially its antioxidant potential (Casanova et al. 2008).

Oxidative stress occurs when the generation of reactive oxygen species exceeds the capacity of cellular antioxidant defense systems. In lymphocytes, oxidative stress can lead to membrane lipid peroxidation, protein oxidation, DNA damage, and ultimately reduced cellular viability. Hydrogen peroxide is commonly used as an inducer of oxidative stress in *in vitro* models due to its ability to penetrate cellular membranes and generate reactive oxygen species intracellularly. Under such conditions, the balance between oxidative damage and antioxidant defense becomes a critical determinant of lymphocyte survival and functional integrity (García-Iñiguez de Cirano et al., 2011). The polyphenolic constituents of *V. officinalis* may confer cytoprotection against hydrogen peroxide-induced oxidative stress in lymphocytes by modulating redox homeostasis and limiting intracellular oxidative damage, thereby supporting cellular viability (Kaurinovic et al., 2022).

According to this, the aim of this study was to evaluate the effect of *Verbena officinalis* extract on lymphocyte viability and variability under hydrogen peroxide-induced oxidative stress conditions, using an *in vitro* experimental model.

Materials and methods

Plant material extraction

Plant material (0.5 g, dried and powdered) was extracted with 50 mL of 50% (v/v) ethanol. Ultrasonic-assisted extraction was performed in an ultrasonic bath (50/60 Hz, 720 W) for 15 min. The extract was filtered and the volume was adjusted to 50 mL with 50% ethanol in a volumetric flask. The solvent was removed using a rotary evaporator (BUCHI), and the dried extract was re-dissolved in water to obtain a stock solution of 10 mg/mL. Working solutions (50, 100, and 200 μg/mL) were prepared by dilution in RPMI medium.

*k.nikolova0713@gmail.com

Isolation and culture of peripheral blood lymphocytes

PBMCs were isolated by Ficoll density gradient centrifugation. The lymphocyte fraction was collected, washed with PBS, and resuspended in RPMI-1640 medium supplemented with 10% FBS and antibiotics. Cells were seeded into 96-well plates and divided into control, H_2O_2-treated, extract-treated, and combined treatment groups. Plates were incubated at 37 °C prior to analysis.

Viability of lymphocytes

Cell viability was determined using the MTT assay. After adding 20 µL MTT solution (5 mg/mL), plates were incubated for 3 h at 37 °C. Formazan crystals were dissolved in 150 µL DMSO, and absorbance was measured at 570 nm using a microplate reader.

Results and discussion

The present study demonstrated a concentration-dependent cytoprotective effect of *Verbena officinalis* extract on human peripheral blood lymphocytes. In the absence of oxidative stress, the extract significantly increased cell viability, showing 88% protection at 50 µg/mL, 97% at 100 µg/mL, and complete protection (100%) at 200 µg/mL, indicating strong supportive effects on lymphocyte metabolic activity. Under hydrogen peroxide-induced oxidative stress, the protective effect of the extract remained evident but was reduced at higher concentration. Combined treatment with H_2O_2 resulted in 87% protection at 50 and 100 µg/mL, while a decrease to 73% was observed at 200 µg/mL. This suggests a concentration-dependent modulation of redox balance, where higher doses may not further enhance protection under oxidative conditions.

The observed effects are likely related to the high polyphenolic content of *V. officinalis*, including verbascoside, flavonoids, and phenolic acids, which are known for strong antioxidant and radical-scavenging activity. These compounds can neutralize reactive oxygen species generated by H_2O_2 and support cellular antioxidant defenses, thereby preserving lymphocyte viability (Kaurinovic et al., 2022). However, at higher concentrations, polyphenols may exhibit pro-oxidant behavior under certain conditions, which could explain the reduced protective effect observed in the presence of H_2O_2 at 200 µg/mL. This dual behavior is consistent with the known concentration-dependent activity of plant-derived antioxidants (Kaurinovic et al., 2022).

Overall, the results indicate that *Verbena officinalis* extract exerts a significant cytoprotective and antioxidant effect on lymphocytes, contributing to the maintenance of cellular redox homeostasis under oxidative stress conditions.

Conclusion

This study demonstrates that *Verbena officinalis* extract exerts a cytoprotective effect on human peripheral blood lymphocytes under basal conditions and hydrogen peroxide-induced oxidative stress. The concentration-dependent increase in cell viability is likely due to polyphenolic compounds with strong antioxidant properties. By modulating redox balance and neutralizing reactive oxygen species, these constituents may support endogenous antioxidant defenses. The reduced efficacy at higher concentrations under oxidative stress indicates a concentration-dependent shift in activity.

V. officinalis extract exhibits promising cytoprotective and antioxidant potential in lymphocytes, warranting further investigation.

References

Casanova, E. et al., 2008. Antioxidant and antifungal activity of *Verbena officinalis* L. leaves. Plant Foods Hum. Nutr. *63*, 93–97. https://doi.org/10.1007/s11130-008-0073-0

García-Iñiguez de Cirano, M. et al., 2011. Chemical composition, mineral content and antioxidant activity of Verbena officinalis L. LWT – Food Science and Technology 44, 875–882. https://doi.org/10.1016/j.lwt.2010.11.035

Kaurinovic, B. et al., 2022. Phenylpropanoid glycoside and phenolic acid profiles and biological activities of biomass extracts from different types of *Verbena officinalis* microshoot cultures and soil-grown plant. I*ndustrial* Crops and Products 176, 114–123. https://doi.org/10.1016/j.indcrop.2021.114123