Published Online: 2025 October 20 **Research Article**

Cytoprotective Effect of Ethyl Acetate Extract of *Artemisia turanica*

Leila Hosseinzadeh [1], Nasim Jamshidi [2], Shadi Mohammadi [2], Mahdi Mojarrab [3,4,*]

[1] Department of Pharmacology and Toxicology, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2] Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran
[3] Pharmaceutical Sciences Research Center, Research Institute for Health, Kermanshah University of Medical Sciences, Kermanshah, Iran
[4] Department of Pharmacognosy and Pharmaceutical Biotechnology, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran

[*] ***Corresponding Author:*** Pharmaceutical Sciences Research Center, Research Institute for Health, Kermanshah University of Medical Sciences, Kermanshah, Iran. Email: mmojarrab@kums.ac.ir

Received: 19 May, 2025; **Revised:** 8 October, 2025; **Accepted:** 12 October, 2025

Abstract

Background: Oxidative stress causes biological effects such as signaling changes, gene expression, mutagenesis, and apoptosis. Therefore, it is worthwhile to find new sources of natural antioxidants.

Objectives: The purpose of this research was to investigate the in vitro neuroprotective effects of different fractions of ethyl acetate (EA) extract of *Artemisia turanica* Krasch. against oxidative stress and apoptosis in PC12 cells. The extract was previously reported to be active in cytoprotective assays.

Methods: The EA extract was fractionated using the reversed-phase vacuum liquid chromatography (RP-VLC) method. Total phenolic (TPC) and flavonoid contents (TFC) of the fractions were evaluated. The protective effect of different fractions on the toxicity of hydrogen peroxide (H_2O_2) in PC12 cells was evaluated using the MTT assay. Additionally, the generation of reactive oxygen species (ROS), changes in mitochondrial membrane potential (MMP), and the activity of caspase 3 in PC12 cells were evaluated.

Results: The RP-VLC of the EA extract yielded seven fractions (F1 - F7). Pre-exposure of PC12 cells to F6 protected the cells against H_2O_2-induced toxicity and reduced ROS production. F6 also caused a significant decrease in caspase 3 activity. The TPC and TFC of F6 were relatively low.

Conclusions: The most active fraction of the EA extract of *A. turanica* is probably rich in non-phenolic antioxidant components.

Keywords: *Artemisia turanica*, Oxidative Stress, Hydrogen Peroxide, PC12 Cells

1. Background

Oxidative stress is known to cause damage to biological molecules such as proteins, lipids, amino acids, and nucleic acids (1). Studies have shown that free radicals slow down the function of neurons and cause a variety of neurological disorders (2, 3). Several natural substances have demonstrated efficacy against neurodegeneration (4). Herbal extracts may be preferred over purified phytoconstituents due to their potential ability to exert diverse biological effects and lower toxicity (5). *Artemisia turanica* Krasch. is one of the naturally growing plant species in Iran, belonging to the family Asteraceae (6). The ethyl acetate (EA) extract of *A. turanica* has been effective in in vitro antioxidant assays (7).

2. Objectives

This study was designed to compare the cytoprotective potential of different fractions of the EA extract of *A. turanica* Krasch. against oxidative stress and apoptosis in PC12 cells, which is an accepted model for in vitro neuroprotective studies. The extract was previously reported to be active in cytoprotective assays.

How to Cite: Hosseinzadeh L, Jamshidi N, Mohammadi S, Mojarrab M. Cytoprotective Effect of Ethyl Acetate Extract of *Artemisia turanica*. Jentashapir J Cell Mol Biol. 2025; 16 (4): e161752. https://doi.org/10.5812/jjcmb-161752.

3. Methods

3.1. Materials and Reagents

2′,7′-Dichlorodihydrofluorescein diacetate (DCF), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Triton-X-100, dimethyl sulfoxide (DMSO), and Rhodamine 123 were purchased from Sigma Aldrich (St. Louis, MO). Additionally, Trypsin-EDTA was supplied by Bon Yakhteh, Iran. Fetal bovine serum was purchased from Gabon, USA. RP18 (15 - 25 µm) and methanol were purchased from Merck, Germany. PC12 pheochromocytoma cells were provided by the Pasteur Institute of Iran (Tehran, Iran).

3.2. Preparation of Plant Extract and Fractions

The aerial parts of *A. turanica* were collected from Samie Abad, Torbat-e Jam, Razavi Khorasan province, Iran. A voucher specimen (with the identification number 12572) has been deposited in the Herbarium of Mashhad University of Medical Sciences. A total of 160 g of dried and ground plant material was extracted with petroleum ether (40 - 60), dichloromethane, and EA using the maceration method. The EA extract was concentrated under reduced pressure at a maximum temperature of 45°C. The dried extract was fractionated by the reversed-phase VLC method with different ratios of methanol (20 - 100%) in water as a mobile phase to afford six fractions (F1 - F6). The procedure was followed by using pure acetone as the eluting solvent to obtain F7.

3.3. Determination of Total Phenolic and Flavonoid Content of Fractions

The Folin-Ciocalteu method was used to determine the total phenolic content (TPC) of fractions (8). The TPC was expressed as mg of gallic acid equivalent (GAE) per gram of dried weight of the fractions. The calculation of the total flavonoid content (TFC) of fractions was performed using the colorimetric method with aluminum chloride (9). The TFC was expressed as mg of quercetin equivalent (QE) per gram of dried weight of the fractions.

3.4. Determination of Cytotoxicity Effects of Different Fractions of the Ethyl Acetate Extract and Hydrogen Peroxide by MTT Assay

The MTT method (10) was used to determine the cytotoxicity of hydrogen peroxide (H_2O_2) and plant fractions against PC12 cells. The IC_{50} value was described as the concentration at which 50% of the cells were killed.

3.5. Determination of the Protective Effect of Fractions Against Hydrogen Peroxide-Induced Cytotoxicity

PC12 cells were pretreated with non-toxic concentrations of different plant fractions (determined after the MTT study) for 24 hours and subsequently treated with H_2O_2 (5.0 mg/mL) for another 24 hours. The MTT method (10) was used to determine the protective effect of fractions against H_2O_2-induced cytotoxicity.

3.6. Evaluation of Intracellular Reactive Oxygen Species

The intracellular reactive oxygen species (ROS) was evaluated using the DCF-DA indicator (10). The intracellular ROS formation was studied in three groups of cells: (A) control, (B) cells treated with H_2O_2 (5.0 mg/mL) for 24 hours, and (C) cells pretreated with a non-toxic concentration of the selected fraction (2.5 µg/mL) for 24 hours and subsequently treated with H_2O_2 (5.0 mg/mL) for another 24 hours.

3.7. Measurement of Mitochondrial Membrane Potential

The cells were seeded in 6-well tissue culture plates and incubated for 24 hours, after which the selected fraction (2.5 µg/mL) was added to the wells. After 24 hours, the IC_{50} of H_2O_2 was added to the cells and incubated for another 4 hours. At the end of the treatment, the mitochondrial membrane potential (MMP) was assessed using Rhodamine 123 as a fluorescent dye (7).

3.8. Caspase-3 Activity Assay

The PC12 cells were seeded in 6-well tissue culture plates and incubated for 24 hours. Then, the selected fraction (2.5 µg/mL) was added to the wells and incubated for the next 24 hours. The H_2O_2 (5.0 mg/mL) was added to the treated cells and incubated for a further 4 hours. Caspase-3 activity was determined using the Sigma colorimetric caspase kit (7).

4. Results

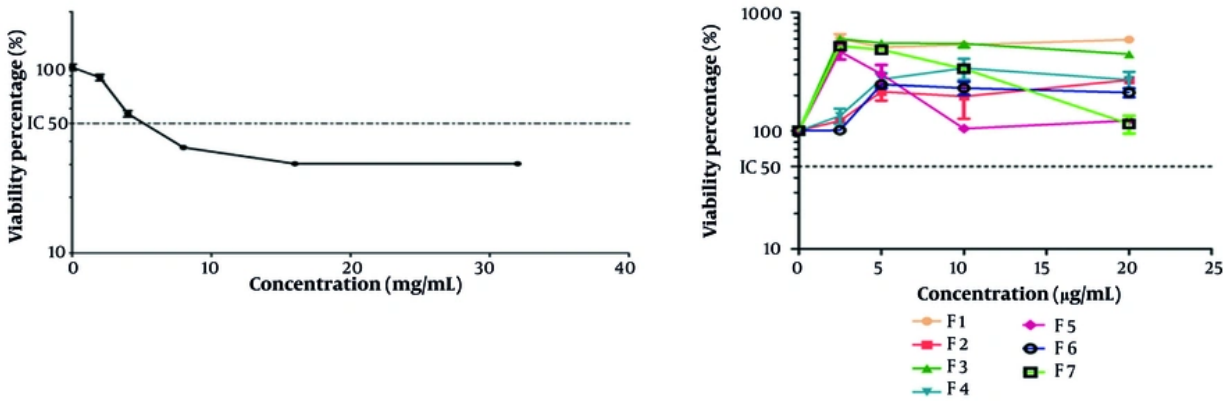

Figure 1. The cytotoxic effects of A, hydrogen peroxide (H_2O_2); and B, fractions on PC12 cells (data are presented as the mean ± SEM, n = 3).

4.1. Total Phenolic and Flavonoid Contents of Fractions

The TPC (mg of GAE/g of each fraction) and TFC (mg of QE/g of each fraction) values are presented in Table 1. The TFC for the EA fractions varied from 13.89 ± 0.42 (F7) to 236.11 ± 4.86 (F2), while TPC values varied from 14.25 ± 0.25 (F7) to 352.69 ± 27.08 (F2).

Table 1. Total Phenolic and Flavonoid Content of Fractions of the Ethyl Acetate Extract [a]

Sample	TFC (mg of QE/g)	TPC (mg of GAE/g)
F1	23.73 ± 0.81	146.89 ± 1.26
F2	236.11 ± 4.86	352.69 ± 27.08
F3	139.87 ± 3.25	144.67 ± 22.40
F4	112.99 ± 1.23	115.34 ± 8.47
F5	58.97 ± 1.11	62.45 ± 1.92
F6	31.8 ± 0.42	33.41 ± 0.36
F7	13.89 ± 0.42	14.25 ± 0.25

Abbreviations: TFC, total flavonoid content; QE, quercetin equivalent; TPC, total phenolic content; GAE, gallic acid equivalent.

[a] Values are expressed as mean ± SD.

4.2. Cytotoxicity of Hydrogen Peroxide and Fractions on PC12 Cells

MTT results showed that the IC_{50} value of H_2O_2 was 5.0 mg/mL. All the fractions of the EA extract (at concentrations up to 20 µg/mL) did not induce any toxicity in PC12 cells (Figure 1).

4.3. The Protective Effect of Fractions on Hydrogen Peroxide-Induced Cytotoxicity

F6, at a concentration of 2.5 µg/mL, had a significant protective effect against the oxidative stress induced by H_2O_2 (Figure 2).

4.4. The Effect of Selected Fraction on Hydrogen Peroxide-Induced Intracellular Reactive Oxygen Species Generation

According to Figure 3, ROS generation increased significantly after treatment of cells with the IC_{50} of H_2O_2. F6, at a concentration of 2.5 µg/mL, had a significant inhibitory effect on ROS production.

4.5. Effect of Selected Fraction on the Mitochondrial Membrane Potential

As seen in Figure 4, F6 (2.5 µg/mL) was not able to significantly inhibit the reduction of MMP.

4.6. Effect of Selected Fraction on Caspase-3 Activity

According to Figure 5, pretreatment of cells with F6 at a concentration of 2.5 µg/mL significantly decreased the activity of caspase-3.

5. Discussion

In the present study, the cytoprotective potential of different fractions of the EA extract of *A. turanica* on H_2O_2-induced oxidative stress and apoptosis in PC12

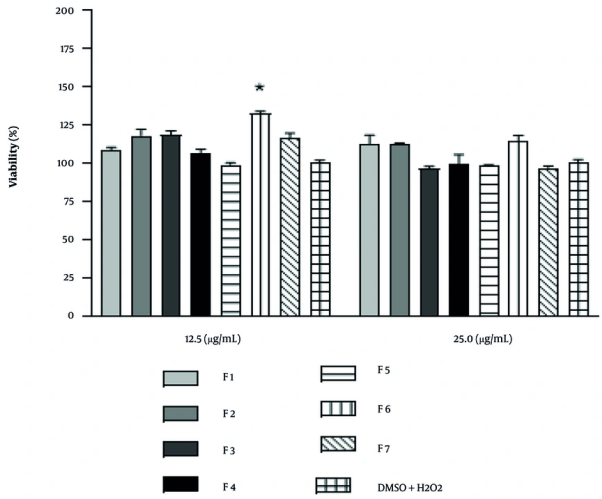

Figure 2. The protective effect of fractions on hydrogen peroxide (H_2O_2)-induced cytotoxicity in PC12 cells (data are presented as the mean ± SEM, n = 3; * P < 0.05 significant differences compared to the H_2O_2-treated group).

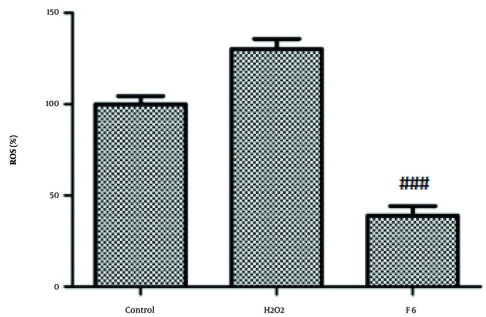

Figure 3. Effect of pre-exposure to the selected fraction of the ethyl acetate (EA) extract of *Artemisia turanica* on the induction of reactive oxygen species (ROS) in PC12 cells [data are presented as the mean ± SEM, n = 3; ### P < 0.001 significant differences compared to the hydrogen peroxide (H_2O_2)-treated group].

cells was investigated. This extract was selected based on the results of our previous study (7). Similar studies have reported the neuroprotective potential of other *Artemisia* species, such as *A. princeps* and *A. absinthium* L., via antiapoptotic activity and inhibition of intracellular ROS production (11, 12). The antioxidant capacity of plants is mostly attributed to their polyphenolic components (13).

Sterols, polyacetylenes, and terpenoids are some of the major non-phenolic constituents in the genus *Artemisia* (14). In the current study, the TPC and TFC of F6 — the selected fraction, based on in vitro neuroprotectivity — were relatively low. This finding indicates that non-phenolic components of this fraction may play a role in the bioactivity. Some non-phenolic secondary metabolites, such as terpenoids (15), polyacetylenes (16), and alkaloids (17) ,have shown notable antioxidant and neuroprotective effects. However, a phytochemical investigation can identify the key compounds of this fraction.

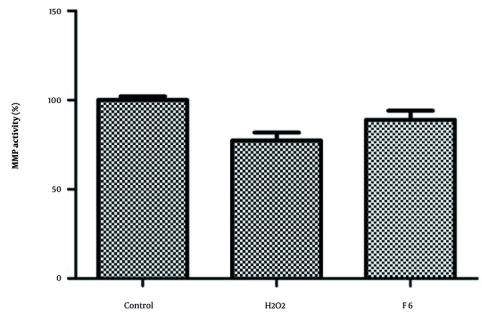

Figure 4. Effect of pre-exposure to the selected fraction of the ethyl acetate (EA) extract of *Artemisia turanica* on the inhibition of mitochondrial membrane potential (MMP) reduction (data are presented as the mean ± SEM, n = 3).

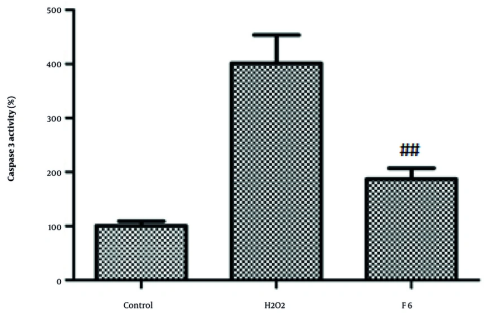

Figure 5. Effect of pre-exposure to the selected fraction of the ethyl acetate (EA) extract of *Artemisia turanica* on the reduction of caspase-3 activity [data are presented as the mean ± SEM, n = 3; ## P < 0.01 significant differences compared to the hydrogen peroxide (H_2O_2)-treated group].

5.1. Conclusions

The neuroprotective fraction of the EA extract of *A. turanica* is probably rich in non-phenolic antioxidant components.

Footnotes

Authors' Contribution: Study concept and design: M. M. and L. H.; Acquisition of data: S. M.; Analysis and interpretation of data: M. M. and L. H.; Drafting of the manuscript: N. J.; Critical revision of the manuscript for important intellectual content: M. M. and L. H.; Statistical analysis: L. H.; Study supervision: M. M. and L. H.

Conflict of Interests Statement: The authors have no conflict of interest.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: This study is approved under the ethical approval code of IR.KUMS.REC.1396.744.

Funding/Support: This study was supported by the Kermanshah University of Medical Sciences, Kermanshah, Iran (grant number: 97011).

References

1. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. *Clin Interv Aging*. 2018;**13**:757-72.

[PubMed ID: 29731617]. [PubMed Central ID: PMC5927356]. https://doi.org/10.2147/CIA.S158513.

2. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. *Nat Rev Drug Discov*. 2021;**20**(9):689-709. [PubMed ID: 34194012]. [PubMed Central ID: PMC8243062]. https://doi.org/10.1038/s41573-021-00233-1.
3. Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G, Serban AI. Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. *Eur J Med Chem*. 2021;**209**:112891. [PubMed ID: 33032084]. https://doi.org/10.1016/j.ejmech.2020.112891.
4. Goyal R, Mittal P, Gautam RK, Kamal MA, Perveen A, Garg V, et al. Natural products in the management of neurodegenerative diseases. *Nutr Metab*. 2024;**21**(1):26. [PubMed ID: 38755627]. [PubMed Central ID: PMC11100221]. https://doi.org/10.1186/s12986-024-00800-4.
5. Nobili S, Lippi D, Witort E, Donnini M, Bausi L, Mini E, et al. Natural compounds for cancer treatment and prevention. *Pharmacol Res*. 2009;**59**(6):365-78. [PubMed ID: 19429468]. https://doi.org/10.1016/j.phrs.2009.01.017.
6. Mozaffarian V. Assadi M, Maassoum AA, Mozaffarian V, editors. *Flora of Iran no. 59. Compositae: Anthemideae & Echinopeae*. Tehran, Iran: Research Institute of Forests and Rangelands; 2008.
7. Hosseinzadeh L, Malekshahi A, Ahmadi F, Emami SA, Hajialyani M, Mojarrab M. The Protective Effect of Different Extracts of Three Artemisia Species against H(2)O(2)-Induced Oxidative Stress and Apoptosis in PC12 Neuronal Cells. *Pharmacognosy Res*. 2018;**10**(1):64-71. [PubMed ID: 29568190]. [PubMed Central ID: PMC5855376]. https://doi.org/10.4103/pr.pr_98_17.
8. Mojarrab M, Nasseri S, Hosseinzadeh L, Farahani F. Evaluation of antioxidant and cytoprotective activities of Artemisia ciniformis extracts on PC12 cells. *Iran J Basic Med Sci*. 2016;**19**(4):430-8. [PubMed ID: 27279988]. [PubMed Central ID: PMC4887717].
9. Mohebi Z, Mojarrab M. The First Comprehensive Phytochemical Study of Nectaroscordum Tripedale (Trautv.) Grossh: Composition of Essential Oil, Antioxidant Activity, Total Phenolic and Flavonoid Content and Mineral Elements. *Pharm Chem J*. 2022;**55**(10):1071-9. https://doi.org/10.1007/s11094-021-02539-1.
10. Hosseinzadeh L, Mirzaei S, Hajialyani M, Ahmadi F, Emami SA, Mojarrab M. The protective effect of different extracts of aerial parts of Artemisia ciniformis against H2O2-induced oxidative stress and apoptosis in PC12 pheochromocytoma cells. *J Appl Pharm Sci*. 2019;**9**(4):16-23. https://doi.org/10.7324/japs.2019.90403.
11. Lee SG, Lee H, Nam TG, Eom SH, Heo HJ, Lee CY, et al. Neuroprotective Effect of Caffeoylquinic Acids from Artemisia princeps Pampanini against Oxidative Stress-Induced Toxicity in PC-12 Cells. *J Food Sci*. 2011;**76**(2):C250-6. https://doi.org/10.1111/j.1750-3841.2010.02010.x.
12. Hallal N, Kharoubi O. Evaluation of oxidative stress and neuroinflammation after mercuric-chloride and Artemisia absinthium L. administration. *Toxicol Lett*. 2016;**258**. https://doi.org/10.1016/j.toxlet.2016.06.1873.
13. Delazar A, Lasheni S, Fathi-Azad F, Nahar L, Rahman MM, Asnaashari S, et al. Free-radical Scavenging Flavonol 3-O-glycosides from the Leaves of Ribes biebersteinii Berl. *Rec Nat Prod*. 2010;**4**(2):96-100.
14. Bora KS, Sharma A. The genus Artemisia: a comprehensive review. *Pharm Biol*. 2011;**49**(1):101-9. [PubMed ID: 20681755]. https://doi.org/10.3109/13880209.2010.497815.
15. Porres-Martinez M, Gonzalez-Burgos E, Carretero ME, Gomez-Serranillos MP. Major selected monoterpenes alpha-pinene and 1,8-cineole found in Salvia lavandulifolia (Spanish sage) essential oil as regulators of cellular redox balance. *Pharm Biol*. 2015;**53**(6):921-9. [PubMed ID: 25474583]. https://doi.org/10.3109/13880209.2014.950672.
16. Zhu H, Wang WJ, Ding WL, Li F, He J. Effect of panaxydol on hypoxia-induced cell death and expression and secretion of neurotrophic factors (NTFs) in hypoxic primary cultured Schwann cells. *Chem Biol Interact*. 2008;**174**(1):44-50. [PubMed ID: 18541227]. https://doi.org/10.1016/j.cbi.2008.04.041.
17. Yu J, Piao BK, Pei YX, Qi X, Hua BJ. Protective effects of tetrahydropalmatine against gamma-radiation induced damage to human endothelial cells. *Life Sci*. 2010;**87**(1-2):55-63. [PubMed ID: 20562023]. https://doi.org/10.1016/j.lfs.2010.05.011.