RESEARCH ARTICLE

Editorial Process: Submission:12/30/2024 Acceptance:01/05/2026 Published:01/21/2026

The Effect of *Prunus amygdalus* var. amara Essential Oil on Suppressing Morphine-Induced Cell Death via Inhibition of Inflammatory Cytokines and Nitric Oxide Production

Zhila Dinari[1], Hossein Zhaleh[2]*

Abstract

Objective: Elevated levels of morphine have been shown to promote cell death by enhancing cytotoxicity, as well as the production of nitric oxide (NO), inflammatory cytokines, and *Caspase-3* within the central nervous system (CNS). The objective of this study was to investigate the inhibitory effects of essential oil derived from *Prunus amygdalus* var. amara on morphine-induced cell death in neuron-like PC12 cells. **Material and Methods:** Gas Chromatography Mass Spectroscopy (GC-MS) was employed for the chemical characterization of the essential oil derived from *Prunus amygdalus* var. amara. The assessment of cell viability, proliferation, and cytotoxicity was conducted using Trypan blue and lactate dehydrogenase (LDH) assays, respectively. DNA fragmentation was evaluated using the Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Nitric oxide production was quantified via the Griess reaction. The levels of inflammatory cytokines, specifically *IL-1β, IL-6, INF-γ*, and TNF-α, were measured using the Rat V-Plex Kit. Additionally, mitochondrial membrane potential was assessed with rhodamine-123, and *Caspase-3* activity was determined using the *Caspase-3* Colorimetric Assay Kit. **Results:** Gas chromatography-mass spectrometry analysis revealed that benzaldehyde and benzoic acid are the predominant chemical constituents present in the essential oil of *Prunus amygdalus* var. amara. Treatments utilizing the essential oil from *Prunus amygdalus* var. amara demonstrated enhanced cell proliferation and viability, alongside reduced cytotoxicity and cell death indices when compared to cells treated with morphine. Furthermore, the presence of *Prunus amygdalus* var. amara essential oil resulted in a decrease in the production of nitric oxide, interleukin-1 beta, interleukin-6, interferon-gamma, tumor necrosis factor-alpha, and *Caspase-3*, as well as a reduction in mitochondrial membrane potential. **Conclusion:** Our findings indicate that the essential oil derived from *Prunus amygdalus* var. amara effectively mitigates cell death induced by morphine in PC12 cells. This essential oil demonstrates the ability to inhibit nitric oxide (NO) production. Furthermore, it appears to impede apoptosis by inhibiting *Caspase-3* activity, preventing DNA fragmentation, and disrupting the integrity of the mitochondrial membrane.

Keywords: Morphine- Cell Death- Inflammation- *Prunus amygdalus* var. amara essential oil

Asian Pac J Cancer Prev, **27 (1)**, 71-78

Introduction

Morphine and other opioids have direct damaging effects on nerve cells in humans and other mammals [1, 2]. High dose of morphine can disrupt cell function in the most important parts of the brain [3, 4]. It induces the cell death through many known and unknown mechanisms. Disruption of brain cell leads to dysfunction of the brain in morphine abusers and patients which are dependent on this opioid. Shuxian Hu et al. revealed that morphine induces apoptotic cell death in microglia as well as neurons, but astrocyte cell are resistance to morphine-induced cell death [5]. There are many questions about the mechanism of morphine in mammals' brains, and it is necessary to investigate the improvement processes against morphine-induced brain dysfunction. Morphine abuse leads to decreased neurogenesis in the hippocampus of adult rats [6]. Therefore, although morphine have many clinical properties such as pain depletion [7],its abuse in patients leads to disruptive events in the central nervous system (CNS) and immune system cells [8, 9]. These events might occur in apoptotic cell death of some brain and immune cells [10, 11].

Some plants have pharmaceutical properties because of their components. *Prunus amygdalus* var. amara, or bitter almond, is an Asian tree which cultivates in the Mediterranean region and western Asia [12]. Amygdalin is one of its components, which might be responsible for

*[1]Department of Medicinal Plants Institute of Higher Education, Kermanshah, Iran. [2]Department of Biology, Faculty of Sciences, Bu-Ali Sina Unversity, Hamedan, Iran. *For Correspondence: h.zhaleh@basu.ac.ir*

the antioxidant, antibacterial, and anticancer properties of bitter almond. The essential oil of bitter almond has previously been used for wound healing as well as the treatment of hemorrhoids, hair loss. It can also relieve joint pain [13]. Our propose was that Prunus amygdalus var. amara essential oil can decrease the side effects of morphine and reduces the morphine-induced cell death in neuron cells. In this study, we aimed to investigate the components of Prunus amygdalus var. amara essential oil. Then, we investigated the suppressor potential of this essential oil on cellular parameters of PC12 cells, as neuron-like cell line of rats.

Materials and Methods

Plant sample collection and essential oil extraction

In this empirical study, 1500 g of Prunus amygdalus var. amara seeds were collected in Kermanshah, Iran (geographical coordinates: 34.3277° N and 47.0778° E). The plant was identified, and a voucher specimen (no. 2886RUH) was deposited at the herbarium of the Research Center of the Faculty of Agriculture, Razi University, Kermanshah, Iran. The leaves of the plant were dried in shadow, and after grinding, each time 200 g of the obtained powder was dissolved in 2000 cc ethanol and put in a Clevenger apparatus for eight. Subsequently, the hydrolate was collected and centrifuged at 10,000 rpm for 10 minutes. The organic phase was removed with the aid of a Pasteur pipette and subsequently transferred to an Eppendorf tube, encased in parafilm and aluminum foil, and stored in a freezer at -20 °C. The yields of oils were assessed on the basis of the dry mass. The experiment was performed in triplicate.

Gas chromatography-Mass spectrometry (GC-MS)

The essential oil of Prunus amygdalus var. amara was analyzed by GC-MS (Thermo Quest Finningan, UK). The GC-MS device was equipped with an HP-5MS 5 % phenyl methyl silicone capillary column (30.00 m length × 0.25 mm ID and 0.25 µm film thickness). Helium flow rate with the split ratio Mohammad Mahdi Zangeneh et al., / TEOP 21 (5) 2018 1349 - 1358 1350 of 1:3 was kept at 2 mL/min. The GC analysis was performed three times. The components were quantified by a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector (GC-FID). A Supelco SPB-5 (5% diphenyl/95 % dimethylpolysiloxane) capillary column (30 m × 0.25 mm with a 0.25 µm film) was utilized, while making use of nitrogen as the carrier gas. The percentage, retention index, and time of each component were computed from the relative peak regions. The analyses were carried out twice.

Cell culture

PC12 (ATCC® CRL-1721TM) cells were cultured in Gibco RPMI 1640 cell culture medium. 10% fetal bovine serum (FBS, Gibco), 100 IU/ml penicillin (Sigma), and 100 µg/ml streptomycin (Sigma) were added to the cell culture as supplement. The cell were incubated at 37 ºC and 5% CO2, and kept in T-25 cm2 tissue culture. The essential oil of Prunus amygdalus var. amara was dissolved by DMSO and plated by a final volume of 0.1% for 12 h at 37°C. Then, the cells were washed with PBS and treated under different treatments for 48 h. The nine groups are listed below: morphine cell culture containing 100 mM morphine, Control: cell culture medium without morphine and Prunus amygdalus var. amara essential oil; Treatment-1: cell culture containing 100 mM morphine and 2.5 µg of Prunus amygdalus var. amara essential oil; Treatment-2: cell culture containing 100 mM morphine and 5 µg of Prunus amygdalus var. amara essential oil; Treatment-3: cell culture containing 100 mM morphine and 10 µg of Prunus amygdalus var. amara essential oil; Treatment-4: cell culture containing 100 mM morphine and 20 µg of Prunus amygdalus var. amara essential oil; Treatment-5: cell culture containing 100 mM morphine and 40 µg of Prunus amygdalus var. amara essential oil; Treatment-6: cell culture containing 100 mM morphine and 80 µg of *Prunus amygdalus* var. amara essential oil; and Treatment-7: cell culture containing 100 mM morphine and 160 µg of *Prunus amygdalus* var amara essential oil.

Cell viability

Cell viability was measured by Trypan blue 0.4% solution. The cells were plated in 96-well cell culture plates for 12 h at a density of 5×104 cells/ml. Then, the cells were incubated at 37 ºC in 5% CO2 for 48 h. So the cells were trypsinized and 20 µl of Trypan blue 0.4% was added to 100 µl of cell suspension. Viability of the cells was measured 1-2 minutes after suspension under Neubauer chamber. The cell viability is equal to the division of the number of uncolored cells by the total number of cells [14].

Cell proliferation

Different treatments incubated by 5 mgr/ml of MTT powder (sigma) for 3 h. The formazan crystals were dissolved by 100 µL of DMSO for 30 minutes at room temperature. Then 200µl of DMSO was added to each well, and an ELIZA Reader reported the absorption of different treatments in 570 nm with a reference of 630 nm [15].

Cell cytotoxicity

In this study, LDH cytotoxicity kit was used for measurement of the cell cytotoxicity through the company's protocol. At first, the cells were plated in 24 well culture plates in density of 1×10^4 cells/mL for 12 h. Then, the cells exposed to different treatments for 48 h. ELISA Reader (EL800; USA) measured the absorbance of samples at 490 nm which indicates the LDH activity. The references wavelength should be more than 600 nm [16].

Cell death index

An in situ cell death detection kit (Roche) was used to TUNEL staining tests. This test measures the cell death index and apoptosis in different treatments. The TUNEL positive cells were counted by an Olympus AX-70 fluorescent microscope in eight random wells in fixed PC12 cells. The ratio of the number of apoptotic cells to the number of total cells indicates the cell death index [17].

Nitric oxide test

In this study, we used the Griess reaction for the measurement of NO production amount in different treatments, after 48 h [18].

Secretion of anti-inflammatory and anti-inflammatory cytokines

Rat inflammatory cytokine assay kit, Rat Kit V-Plex was used for the measurement of pre-inflammatory cytokines *IL-1β, IL-6, INFγ*, and *TNFα* in different treatments, after 48 h.

Mitochondrial membrane potential (MMP)

Rhodamine 123 (sigma) detected the potential changes in the mitochondrial membrane of different treatments after 48 h. In this regard, 10 mg/ml rhodamine-123 was used for each treatment for half an hour. Then, the cells were washed by PBS and 900 µl triton-x100 (sigma) was added to each treatment for 2 h in 4ºC. Continuing, the cells were centrifuged at 130000 rpm for 15 min. Finally, the cells were moved to a fluorescence microplate reader for measurement of fluorescence at 488 nm excitation and 520 nm emission wavelength [19].

Caspase-3 activity

Activity of *Caspase-3* indicates apoptosis occurrence. In this study, the *Caspase-3* Colorimetric Assay Kit and Biotek (USA) Spectrophotometer were used to determine of *Caspase-3* activity, through the company's protocol [20].

Statistical analysis of results

In this study, statistical analyzes were performed using ANOVA and T-tests. In all cases, the P value of less than 0.05 was considered statistically significant.

Results

Chemical analysis

The essential oil yield of *Prunus amygdalus* var. amara was 6.99 (21.18 mL), calculated on the fresh seeds of plant. Overall, fourteen compounds, including 1,3-Xylene, Tetradecane, 1,4-Xylene, Dodecane, 1-Ethyl-3-methyl-benzene, Hexadecane, Mesitylene, Eicosane, Benzaldehyde, Dodecyl aldehyde, Benzyl alcohol, Benzyl cyanide, 3,5-Di-tert-butylphenol, and Benzoic acid were identified in the essential oil of *Prunus amygdalus* var. amara using GC-MS (Table 1). Benzaldehyde (41.9.0%) and Benzoic acid (22.9 %) were the most frequently detected compounds.

Viability of the cells

We measured the cell viability of different treatments through trypan blue, after 48h. The cell viability of the morphine group was 0% and for treatments 1-7 it was higher than the morphine group by intragroup significance ($p<0.05$). The cell viability of treatment-7 was similar to the control group but for treatments 1-6 it was lower than control treatment significantly (Figure 1).

Cell proliferation

In this study, the cell proliferation of different treatments was assessed through the MTT test after 48 h. Morphine totally suppressed the cell proliferation in the morphine group and the cell proliferation of treatments 1-7 was higher than the morphine group. Significant intragroup difference was observed between treatments 1-7 in terms of cell proliferation ($p<0.05$) (Figure 2).

Cytotoxicity of the cells

LDH kit results after 48h showed a cell cytotoxicity of 0% for the morphine group. Additionally, these results revealed that the cell cytotoxicity of treatments 1-7 were significantly lower than the morphine group and higher than the control group. A significant interg group

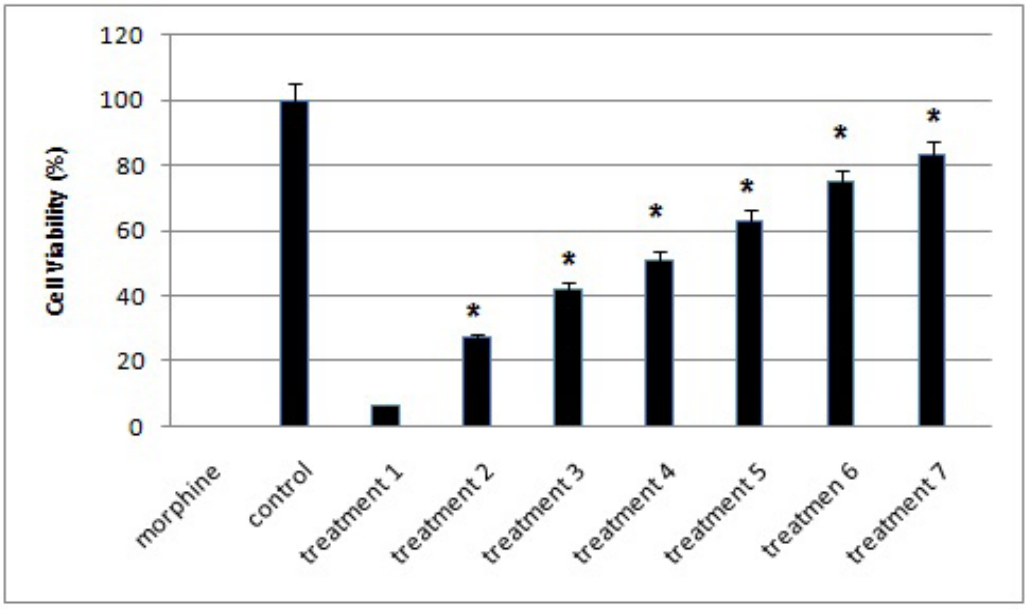

Figure 1. Viability of the Cell Treated by Morphine and *Prunus amygdalus* var. amara Essential Oil after 48 h.

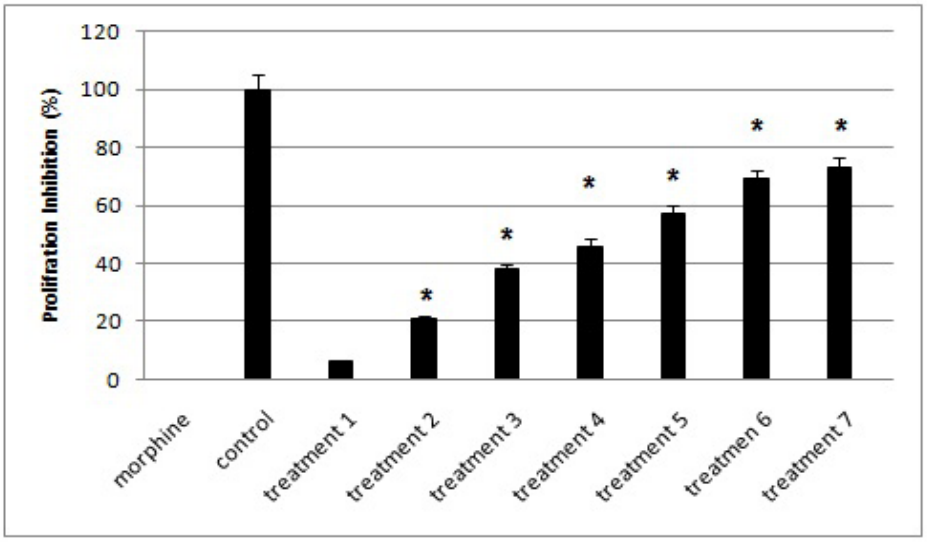

Figure 2. Proliferation of the Cell after 48 h Treatment by Morphine and *Prunus amygdalus* var. amara Essential Oil.

difference was observed in terms of cell cytotoxicity ($p<0.05$) (Figure 3).

DNA fragmentation and apoptosis: Cell death index

The DNA fragmentation indicates the apoptosis and determines the cell death index parameter. The images of the TUNEL test which were taken in different treatments after 48 h revealed that in the morphine group all the cell were exposed to DNA fragmentation and apoptosis, so the cell death index in this group was 100%. DNA fragmentation in treatments 1-7 was lower than the morphine group significantly. A significant interg groupdifference was observed in terms of cell death index ($p<0.05$) (Figure 4).

Nitric oxide production

In this study, we used Griess reaction assay to measure of nitric oxide (NO) production in different treatments after 48h. The results revealed that the concentration of NO in the morphine group was significantly higher than treatments 1-7. Concentration of NO in treatments 1-7 was lower than the control group with intragroup significant difference ($p<0.05$) (Figure 5).

Inflammatory cytokines

The concentration of *IL-1β, IL-6, INF-γ*, and *TNF-α* inflammatory cytokines in treatments 1-7 were lower than the morphine group and higher than the control group. An intra-group significant difference was observed in the concentration of these inflammatory cytokines during treatments 1-7 ($p<0.05$) (Supplementary Figure 1).

Mitochondrial membrane potential

In this study, we used rhodamine 123 for detection of mitochondrial membrane potential in different treatments after 48h. The results revealed that mitochondrial membrane potential of treatments 1-7 was higher than the morphine group and lower than the control group by significant intragroup difference ($p<0.05$) (Supplementary Figure 2).

Table 1. Identified Main Composition of the Essential Oil of Bitter Almond Seeds Using Gas Chromatography Mass Spectrometry Method

No.	Compound	RT	RI	Percent
1	1,3-Xylene	4.684	756	2.6
2	Tetradecane	4.987	794	1.1
3	1,4-Xylene	5.352	864	1.3
4	Dodecane	5.561	894	2.4
5	1-Ethyl-3-methyl-benzene	6.093	975	1.9
6	Hexadecane	6.304	1007	5.3
7	Mesitylene	7.197	1165	2.3
8	Eicosane	10.572	1354	3.1
9	Benzaldehyde	11.913	1500	41.9
10	Dodecyl aldehyde	14.687	1564	1.9
11	Benzyl alcohol	16.784	1721	2.2
12	Benzyl cyanide	17.487	1801	1.8
13	3,5-Di-tert-butylphenol	21.009	2017	1.9
14	Benzoic acid	22.796	2159	29.9
Total				99.6

RI, (Retention index); RT, (Retention time).

Caspas-3 activity: apoptosis

The activity of *Caspase-3* indicates apoptosis occurrence. This parameter in treatments 1-7 was lower than the morphine group and higher than the control group. A significant intra-group difference was observed in treatment 1-7 ($p<0.05$).

Discussion

Opiates provoke apoptosis in central nervous system cells [21]. Morphine is an opioid which is used in the treatment of acute and chronic pain. It acts directly on the central nervous system [22, 23]. Previous studies showed that morphine, as an opiate, reduces the cell proliferation and neurogenesis in the hippocampus of adult rats. A study confirmed that neuronal phenotypes altered in the dentate gyrus-CA3 region when exposed

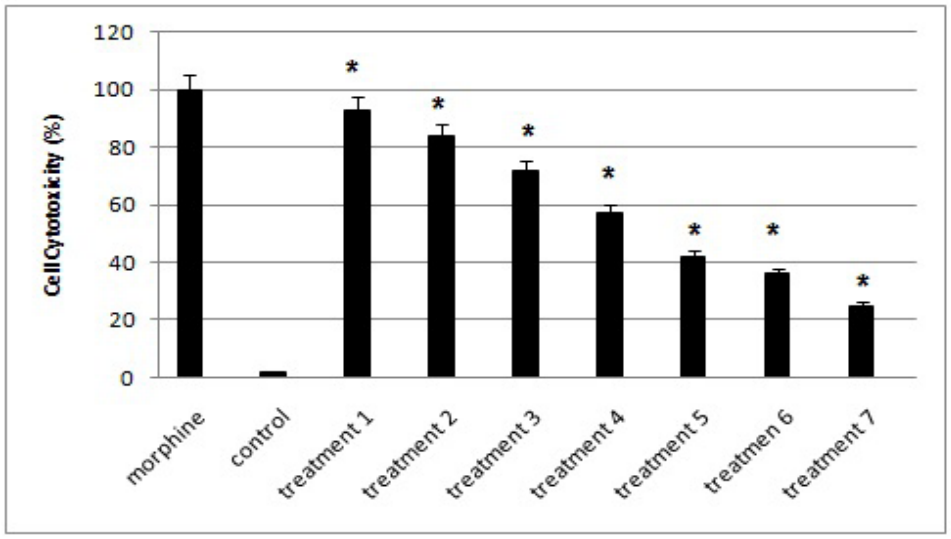

Figure 3. Cytotoxicity of the Cell Treated by Morphine and *Prunus amygdalus* var. amara Essential Oil after 48h.

to morphine chronically [24, 6]. Because of apoptosis blocked by naloxone, a powerful theory is that morphine induces apoptosis in microglia and neurons through a mechanism which applies to an opiate-receptor [25, 26]. Overexpression of p53 and Bax proteins was observed in morphine-treated cells [27]. In addition, evidence shows that morphine induces apoptosis through activation of *Caspase-3* [28]. In our study, morphine suppressed cell viability of PC12 cells through effects on different cellular parameters. It enhanced cell cytotoxicity and cell death index. DNA fragmentation or apoptosis is increased by morphine in neuron-like PC12 cells. An increase in NO production and mitochondrial membrane potential which are observed in the presence of morphine might lead to activation of *Caspase-3* and starting the apoptosis.

LuXiao et al. reported 58 volatiles in raw and in roasted almonds through an HS-SPME GC/MS method [29]. S.K. Sathe et al. analyzed fatty acids of eight cultivars of almond from different counties of California by CG-MS. They showed that oleic and linoleic together account for 91.16% to 94.29% of the total soluble lipids. Palmitic and α-linolenic were the other important lipids [30]. In a previous study, K. Pićurić-Jovanović et al. analyzed the composition of almond by headspace sampling, gas chromatography and mass spectrometry and revealed that benzene,n-alkanes, cycloalkanes, aromatics and furan compounds are the most important components [31]. Amygdalin as a cyanogenic diglucoside is a toxic compound for animals and its content determines the bitterness or sweetness of almond kernel [32, 33]. Huiling Geng et al. identified Twenty-one different components in the analysis of bitter almond through CG-MS. The Benzaldehyde had the highest amount and then Benzoic acid and Hexadecane were the most frequent components. They revealed that bitter almond oil has antifungal effects [34]. In this study, we analyzed the components of *Prunus amygdalus* var. amara or bitter almond essential oil by GC-MS. Benzaldehyde

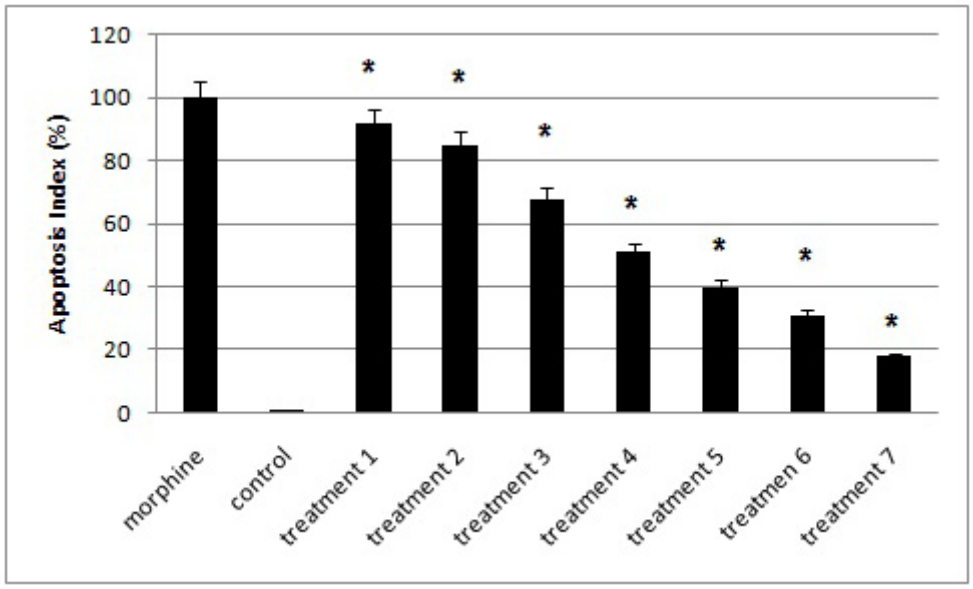

Figure 4. Death Index of the Cell Treated by Morphine and *Prunus amygdalus* var. amara Essential Oil after 48 h.

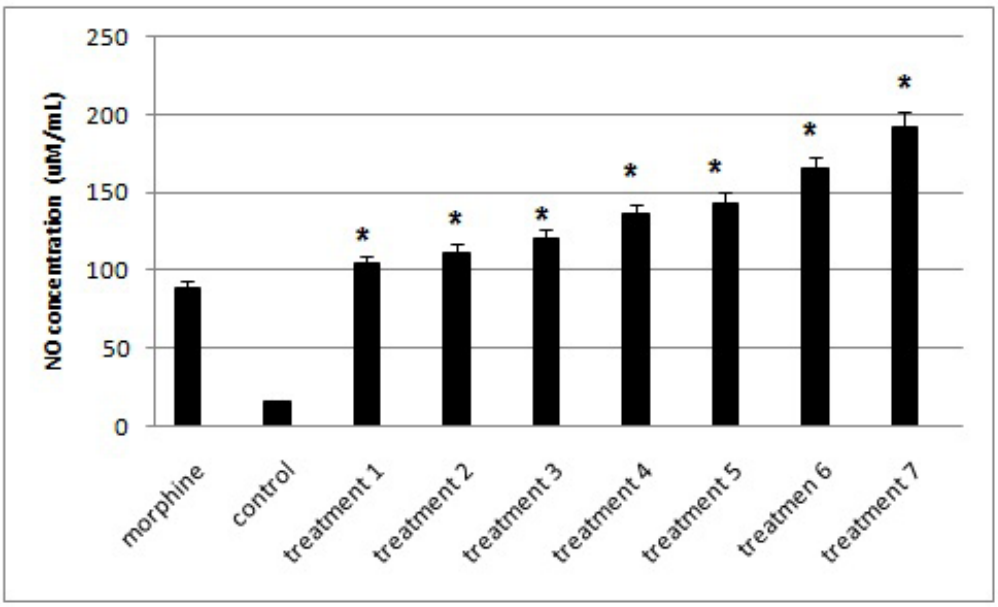

Figure 5. Production of NO in Cell Treated by Morphine and *Prunus amygdalus* var. amara Essential Oil after 48 h.

and Benzoic acid were the most frequent components in *Prunus amygdalus* var. amara essential oil which confirms previous studies [35]. In this study, we analyzed the effects of *Prunus amygdalus* var. amara essential oil, in different concentrations, on inhibition of cell death in PC12 cells which were exposed to high concentrations of morphine. Our observations revealed that this oil can reduce cell cytotoxicity and enhance cell viability. It suppressed NO and inflammatory cytokine production. It also inhibited the DNA fragmentation and activation of *Caspase-3* which indicates apoptosis. These events might be due to properties of the most frequent component of *Prunus amygdalus* var. amara essential oil.

The Benzaldehyde comes from amygdalin breakdown and can condense for production of 7-benzylidenenaltrexone (BNTX, 1a) the most important antagonist of δ-opioid [36-38].

In this study, we concluded that *Prunus amygdalus* var. amara essential oil can inhibit the cell death of neuron-like PC12 cells by high doses of morphine. These events confirmed by reduction of cell cytotoxicity and enhance the cell viability and proliferation of *Prunus amygdalus* var. amara essential oil. A high dose of morphine induces the production of NO, *IL-1β, IL-6, INF-γ*, and *TNF-α* inflammatory cytokines. It also, disrupts the mitochondrial membrane and activates the apoptosis through *Caspase-3* activation, but *Prunus amygdalus* var. amara essential oil suppresses these pathways in a dose-dependent manner.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

This work was fully supported by Dept. of Medicinal Plant of Kermanshah ACECR Institute of Higher Education, Iran.

Funding Statement:

This study is approved by the scientific committee of the Kermanshah ACECR Institute of Higher Education and is part of Ms. Zhila Dinari's thesis in collaboration with Dr. Hossein Zhaleh at the Dept. of Medicinal Plants of Kermanshah ACECR Institute of Higher Education, Iran.

Human and animal rights:

No Animals/Humans were used for studies that are base of this research.

Availability of data and materials

All data generated during this study are included in this published article. Further data supporting the findings of the current article are available from the corresponding author (Dr. Hossein Zhaleh, Email: h.zhaleh@basu.ac.ir) on reasonable request.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

References

1. Vella-Brincat J, Macleod AD. Adverse effects of opioids on the central nervous systems of palliative care patients. J Pain Palliat Care Pharmacother. 2007;21(1):15-25.
2. Shirzad H, Shahrani M, Rafieian-Kopaei M. Comparison of morphine and tramadol effects on phagocytic activity of mice peritoneal phagocytes in vivo. Int Immunopharmacol. 2009;9(7-8):968-70. https://doi.org/10.1016/j.intimp.2009.04.002.
3. Sharma HS, Ali SF. Alterations in blood-brain barrier function by morphine and methamphetamine. Ann N Y Acad Sci. 2006;1074:198-224. https://doi.org/10.1196/annals.1369.020.
4. Williams JT, Christie MJ, Manzoni O. Cellular and synaptic adaptations mediating opioid dependence. Physiological Reviews. 2001;81(1):299-343. https://doi.org/10.1152/physrev.2001.81.1.299.

5. Hu S, Sheng WS, Lokensgard JR, Peterson PK. Morphine induces apoptosis of human microglia and neurons. Neuropharmacology. 2002;42(6):829-36. https://doi.org/https://doi.org/10.1016/S0028-3908(02)00030-8.
6. Eisch AJ, Barrot M, Schad CA, Self DW, Nestler EJ. Opiates inhibit neurogenesis in the adult rat hippocampus. Proc Natl Acad Sci U S A. 2000;97(13):7579-84. https://doi.org/doi:10.1073/pnas.120552597.
7. Behar M, Olshwang D, Magora F, Davidson JT. Epidural morphine in treatment of pain. Lancet. 1979;313(8115):527-9. https://doi.org/10.1016/S0140-6736(79)90947-4.
8. Preston KL, Jasinski DR, Testa M. Abuse potential and pharmacological comparison of tramadol and morphine. Drug Alcohol Depend. 1991;27(1):7-17. https://doi.org/https://doi.org/10.1016/0376-8716(91)90081-9.
9. Maurer HH, Sauer C, Theobald DS. Toxicokinetics of drugs of abuse: Current knowledge of the isoenzymes involved in the human metabolism of tetrahydrocannabinol, cocaine, heroin, morphine, and codeine. Ther Drug Monit. 2006;28(3):447-53. https://doi.org/10.1097/01.ftd.0000211812.27558.6e.
10. Honig LS, Rosenberg RN. Apoptosis and neurologic disease. Am J Med. 2000;108(4):317-30. https://doi.org/10.1016/S0002-9343(00)00291-6.
11. Martin LJ. Neuronal cell death in nervous system development, disease, and injury. Int J Mol Med. 2001 May 1;7(5):455-78.
12. Rosengarten Jr F. The book of edible nuts. Courier Corporation; 2004 Jun 1.
13. Moradi B, Heidari-Soureshjani S, Asadi-Samani M, Yang Q. A systematic review of phytochemical and phytotherapeutic characteristics of bitter almond. International Journal of Pharmaceutical and Phytopharmacological Research. 2017;7:1-9.
14. Strober W. Trypan blue exclusion test of cell viability. Curr Protoc Immunol. 2015;111(1):A3.B.1-A3.B. https://doi.org/10.1002/0471142735.ima03bs111.
15. Loveland BE, Johns TG, Mackay IR, Vaillant F, Wang ZX, Hertzog PJ. Validation of the mtt dye assay for enumeration of cells in proliferative and antiproliferative assays. Biochem Int. 1992;27(3):501-10.
16. Fotakis G, Timbrell JA. In vitro cytotoxicity assays: Comparison of ldh, neutral red, mtt and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicol Lett. 2006;160(2):171-7. https://doi.org/10.1016/j.toxlet.2005.07.001.
17. Kyrylkova K, Kyryachenko S, Leid M, Kioussi C. Detection of apoptosis by tunel assay. Methods Mol Biol. 2012;887:41-7. https://doi.org/10.1007/978-1-61779-860-3_5.
18. Sun J, Zhang X, Broderick M, Fein H. Measurement of nitric oxide production in biological systems by using griess reaction assay. Sensors. 2003;3:276-84. https://doi.org/10.3390/s30800276.
19. Baracca A, Sgarbi G, Solaini G, Lenaz G. Rhodamine 123 as a probe of mitochondrial membrane potential: Evaluation of proton flux through f(0) during atp synthesis. Biochim Biophys Acta. 2003;1606(1-3):137-46. https://doi.org/10.1016/s0005-2728(03)00110-5.
20. Gurtu V, Kain SR, Zhang G. Fluorometric and colorimetric detection of caspase activity associated with apoptosis. Analytical Biochemistry. 1997;251(1):98-102. https://doi.org/10.1006/abio.1997.2220.
21. Goswami R, Dawson SA, Dawson G. Cyclic amp protects against staurosporine and wortmannin-induced apoptosis and opioid-enhanced apoptosis in both embryonic and immortalized (f-11kappa7) neurons. J Neurochem. 1998;70(4):1376-82. https://doi.org/10.1046/j.1471-4159.1998.70041376.x.
22. Borland M, Jacobs I, King B, O'Brien D. A randomized controlled trial comparing intranasal fentanyl to intravenous morphine for managing acute pain in children in the emergency department. Ann Emerg Med. 2007;49(3):335-40. https://doi.org/10.1016/j.annemergmed.2006.06.016.
23. Westin U, Piras E, Jansson B, Bergström U, Dahlin M, Brittebo E, et al. Transfer of morphine along the olfactory pathway to the central nervous system after nasal administration to rodents. Eur J Pharm Sci. 2005;24(5):565-73. https://doi.org/10.1016/j.ejps.2005.01.009.
24. Kahn L, Alonso G, Normand E, Manzoni OJ. Repeated morphine treatment alters polysialylated neural cell adhesion molecule, glutamate decarboxylase-67 expression and cell proliferation in the adult rat hippocampus. Eur J Neurosci. 2005;21(2):493-500. https://doi.org/10.1111/j.1460-9568.2005.03883.x.
25. Cahill CM, Morinville A, Lee M-C, Vincent J-P, Collier B, Beaudet A. Prolonged morphine treatment targets δ opioid receptors to neuronal plasma membranes and enhances δ-mediated antinociception. J Neurosci. 2001;21(19):7598. https://doi.org/10.1523/JNEUROSCI.21-19-07598.2001.
26. Tsai RY, Tai YH, Tzeng JI, Cherng CH, Yeh CC, Wong CS. Ultra-low dose naloxone restores the antinociceptive effect of morphine in pertussis toxin-treated rats by reversing the coupling of μ-opioid receptors from gs-protein to coupling to gi-protein. Neuroscience. 2009;164(2):435-43. https://doi.org/10.1016/j.neuroscience.2009.08.015.
27. Singhal PC, Sharma P, Kapasi AA, Reddy K, Franki N, Gibbons N. Morphine enhances macrophage apoptosis. J Immunol. 1998;160(4):1886-93.
28. Nasiraei-Moghadam S, Kazeminezhad B, Dargahi L, Ahmadiani A. Maternal oral consumption of morphine increases bax/bcl-2 ratio and caspase 3 activity during early neural system development in rat embryos. J Mol Neurosci. 2010;41(1):156-64. https://doi.org/10.1007/s12031-009-9312-6.
29. Xiao L, Lee J, Zhang G, Ebeler SE, Wickramasinghe N, Seiber J, et al. Hs-spme gc/ms characterization of volatiles in raw and dry-roasted almonds (prunus dulcis). Food Chem. 2014;151:31-9. https://doi.org/https://doi.org/10.1016/j.foodchem.2013.11.052.
30. Sathe SK, Seeram NP, Kshirsagar HH, Heber D, Lapsley KA. Fatty acid composition of california grown almonds. J Food Sci. 2008;73(9):C607-C14. https://doi.org/https://doi.org/10.1111/j.1750-3841.2008.00936.x.
31. Pićurić-Jovanović K, Milovanović M. Analysis of volatile compounds in almond and plum kernel oils. Journal of the American Oil Chemists' Society. 1993;70(11):1101-4..
32. Sánchez-Pérez R, Jørgensen K, Olsen CE, Dicenta F, Møller BL. Bitterness in almonds. Plant Physiol. 2008;146(3):1040-52. https://doi.org/10.1104/pp.107.112979.
33. Sánchez-Pérez R, Howad W, Garcia-Mas J, Arús P, Martínez-Gómez P, Dicenta F. Molecular markers for kernel bitterness in almond.Tree Genet. 2010 Feb;6(2):237-45.https://doi.org/10.1007/s11295-009-0244-7.
34. Geng H, Yu X, Lu A, Cao H, Zhou B, Zhou L, et al. Extraction, chemical composition, and antifungal activity of essential oil of bitter almond. Int J Mol Sci. 2016;17(9):1421. https://doi.org/10.3390/ijms17091421.
35. Zakarya D, Yahiaoui M, Fkih-Tetouani S. Structure-odor relations for bitter almond odorants. J Phys Org Chem. 2004;6:627-33. https://doi.org/10.1002/poc.610061106.
36. Palmer RB, Upthagrove AL, Nelson WL. (e)- and (z)-7-arylidenenaltrexones: Synthesis and opioid receptor radioligand displacement assays. J Med Chem. 1997;40(5):749-53. https://doi.org/10.1021/jm960573f.
37. Ohkawa S, Portoghese PS. 7-arylidenenaltrexones as

selective delta1 opioid receptor antagonists. J Med Chem. 1998;41(21):4177-80. https://doi.org/10.1021/jm980384s.
38. Ananthan S, Johnson CA, Carter RL, Clayton SD, Rice KC, Xu H, et al. Synthesis, opioid receptor binding, and bioassay of naltrindole analogues substituted in the indolic benzene moiety. J Med Chem. 1998;41(15):2872-81. https://doi.org/10.1021/jm980083i.