Original Article

Moringa Oleifera Enhances the Cell Proliferation and Neural Differentiation of B65 and PC12 Cells

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Abstract

Background: Neurodegenerative diseases, marked by progressive symptoms, currently lack definitive, side-effect-free treatments. Research continues for effective therapies. Moringa oleifera extract (MOE), rich in phytochemicals like vitamins, carotenoids, and flavonoids, has demonstrated neuroprotective effects through antioxidant activity and inflammation reduction. The aim of this study was to investigate the effect of Moringa extract on the proliferation and cell differentiation.

Materials and Methods: This study investigated the effects of MOE on the proliferation and cell differentiation of B65 and PC12 cells *in vitro.* MOE was extracted from plant leaves using the Soxhlet method, and its antioxidant properties were confirmed through the Ferric Reducing Antioxidant Power assay. Cell proliferation was assessed using an MTT assay, while cell differentiation was evaluated through Real-time PCR and immunocytochemistry (ICC).

Results: MOE significantly increased cell proliferation in both cell lines in a dose-dependent manner. Real-time PCR results indicated that MOE upregulated the expression of the gap-43 gene, which encodes a protein, involved in regulating actin dynamics in axons and promoting axonal regeneration, as well as the nf200 gene, which encodes an intermediate filament protein in neurons. Furthermore, ICC results demonstrated that higher doses of MOE were associated with increased levels of the nf200 protein in both cell lines.

Conclusion: The findings demonstrate that MOE improves cell proliferation and facilitates neuronal differentiation. These effects, attributed to its high antioxidant content, suggest that MOE may serve as a promising therapeutic candidate for neurodegenerative disorders.

Keywords: Cell differentiation, cell proliferation, *in vitro*, moringa, PC12 cells

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Introduction

Neurodegenerative diseases (NDs) result in progressive neuron dysfunction or loss, leading to disturbances in consciousness, memory, aphasia, and vision impairment. Despite the high prevalence, significant disease burden, and high healthcare costs, there is still no definitive treatment without side effects.[1] Therefore, researchers are still looking for new therapeutic targets. Herbal medicine as a multicomponent agent is of interest.[2]

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Moringa oleifera Lam. (*M. oleifera*) belongs to Moringaceae family. This plant is a native of the tropics and subtropics of Asia and Africa[2,3] but India is the country with the most cultivation and production of *M. oleifera*. Due to its numerous economic, nutritional and medicinal properties, is called a "miracle tree" or "wonderful tree."[4]

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Almost all parts of the plant have been used in traditional medicine to treat many diseases such as infections, paralytic attacks, anemia, and sores.[3,5,6] However, it is a novel material in the Western world.[6] *M. oleifera* is highly nutritious and rich in several phytochemicals such as minerals like zinc, magnesium, calcium, and potassium, vitamins like vitamins C, D, E, vitamin B, flavonoids, amino acids, glycosides,[6] and phenolics.[3,7]

In recent decades, several studies have been conducted to investigate the effect of *M. oleifera* extract (MOE) on diseases such as cardiovascular and liver diseases. These researches have shown that it possesses immune boosting agents, anti-inflammatory, and antimicrobial activities.[4,8] In previous studies have shown the positive effects of MOE on the nervous system. The leaf extract displays antioxidant activity and demonstrates neurotrophic effects.[7] In a rat model of Alzheimer's disease, MOE has been shown to mitigate oxidative stress.[1] The researches have indicated a significant enhancement in spatial memory[7] and a reduction in neurodegeneration in the brain due to the MOE leaf extract.[2] MOE ameliorates function recovery following sciatic nerve injury and ischemic stroke.[9] This plant can prove an interesting and valuable target for the discovery of effective and affordable intervention against neurological disorders.[10]

While there is a considerable body of experiments detailing the influence of MOE on the nervous system *in vivo*, there have been fewer studies on the effects of MOE on both proliferation and differentiation of peripheral nervous system (PNS) neuronal cells. Therefore, in this study, we investigated the effect of leaf extract of MOE on proliferation and neural differentiation of PC12[11] and B65[12] cells that are widely accepted as an *in vitro* model for neuronal differentiation, neurosecretion, and neuronal injury studies.[13]

Materials and Methods

Preparation of MOE sample

The *M. oleifera* leaves were purchased from India Mart in India. The extraction was performed using Soxhlet method. For this purpose, 25 grams of *M. oleifera* leaves were dried in an oven at 40°C for 24 hours. Then, the samples were powdered using an electric grinder and passed through an 80-mesh sieve. The extraction was performed using 250 mL of 99% methanol (in a 1:10 ratio) (Merck, Germany), and the sample was placed inside filter paper and inserted into a Soxhlet apparatus (Buch and Holm, Denmark). Methanol was added to a flask, and the heater was set to 80°C.[14] To obtain a high-purity extract, excess solvent (methanol) was separated using a vacuum distillation apparatus (rotary evaporator) at 50°C. The obtained extract was stored in a sealed bottle at a –24°C temperature.

Ferric reducing antioxidant power (FRAP) assay

For the identification of MOE and investigation of its antioxidant and neuroprotective properties, the FRAP assay was employed. For this purpose, 1 mL of MOE was prepared at different concentrations (10-20-40-60-80-100 mg/mL) and added to a solution consisting of 2.5 mL of phosphate buffer (pH 6.6) (Bioidea, Iran) with 2.5 mL of 1% potassium ferricyanide (Fe3K (CN) 6) (Sigma-Aldrich, United States). The resulting mixture was incubated at 50°C for 20 min. After adding 2.5 mL of 10% trichloroacetic acid (Bioidea, Iran), the resulting solution was centrifuged at 3000 rpm for 10 min. Then, 2.5 mL of the supernatant was carefully withdrawn and added to a tube containing 2.5 mL of dH_2O (Bioidea, Iran) with 0.5 mL of ferric chloride 1%. Finally, the absorption was measured at 593 nanometers using a UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan).[15]

Preparing MOE solution for in vitro studies

To use MOE for *in vitro* studies, the extract powder was dissolved in dimethyl sulfoxide (DMSO, 16 mg/mL) (Sigma-Aldrich, United States) at a concentration of 16 mg/mL to prepare a stock solution. The stock solution was then stored in a foil-wrapped vial at –20°C. Subsequently, it was diluted with DMEM (Bioidea, Iran) to obtain the final concentrations for the test samples of the extract.[16]

Culture and differentiation of PC12 and B65 cells

Pheochromocytoma (PC12) and Neuroblastoma (B65) cell lines, were cultured at a density of 1000/cm^2 in RPMI-1640 medium containing 10% horse serum (Gibco, New Zealand), 5% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin-streptomycin for PC12 cells, and in Dulbecco's Modified Eagle's Medium (DMEM, high glucose) medium containing 10% FBS and 100 mg/mL penicillin/streptomycin (all from Bioidea, Iran) for B65 cells. The cells were cultured under conditions of 37°C and 5% CO_2 for 14 days, and their morphology was investigated using an inverted microscope (Nikon, PS-100). The culture medium was changed every 2 days.[13]

In this study, the cells were assessed in the following groups: The cells exposed to the only culture medium (as the control group) and cells were then induced by culture medium and DMSO, MOE 15 (M15), 30 (M30), and 60 (M60) µg/mL of MOE (as the treated groups).[16]

Cell viability assay

To investigate the effect of MOE on cell viability and proliferation, the MTT assay was performed on days 1, 7, and 14 after culture. For this purpose, after medium removal, the cells were washed three times with phosphate buffered saline (PBS, Bioidea, 1038), then 0.5 mg/mL MTT (Bioidea, Iran) was added to each well and incubated for 4 hours. Subsequently, the supernatant was removed, and 200 µL DMSO was added. After 20 min, the optical density was measured at a wavelength of 540 nm by a microplate reader (Hyperion MPR 4+, Germany).[17]

Realtime PCR

To examine cell differentiation by Real-time PCR, we measured the expression of gap-43 and nf200 genes on day 14. For this

purpose, the High Pure RNA Isolation Kit (Roche, United States), following the manufacturer's instructions, was used to extract RNA from the B65 and PC12 cells. Subsequently, cDNA was synthesized from the extracted RNA using the Revert Aid™ First Strand cDNA Synthesis Kit (Thermo Scientific, United States). This study utilized the GAPDH gene as the housekeeping gene for normalization. Using Maxima SYBR Green Rox qPCR master mix kit (Thermo Scientific, United States) and the Step One Plus™ quantitative Real-time PCR detection System (Applied Biosystems, United States), the expression levels of the genes were determined. To calculate the relative expression levels of the target genes, the expression level in the control group was set to 1, and the levels in other groups were normalized to the control group using the $2^{-}\Delta\Delta^{CT}$ formula. The relative expression levels in the different groups were then compared to the control group. The primers used in this study were designed using Allele ID 7.6 software. Primer sequences are shown in Table 1.

Immunocytochemistry technique

To investigate cell differentiation, immunocytochemistry was performed for neural marker NF200. After 14 days of culture, the cells were fixed using 4% paraformaldehyde (Sigma-Aldrich, United States) for 30 min and permeabilized with 0.1% Triton X-100 (Sigma-Aldrich, United States) for 1 hour. Then primary antibody for NF200 (Sigma Aldrich, United States) was added. The ration of diluted primary antibodies was 1:500. The excess antibody was removed by washing with PBS and samples were incubated with secondary antibody conjugated with fluorescein isothiocyanate (FITC) (Sigma-Aldrich, United States). Nuclei were stained by DAPI (Sigma-Aldrich, United States) and samples were evaluated with fluorescent microscope (Olympus, BX51, Japan). Images were processed by image J software (NIH, MD, USA).[17]

Statistical analysis

Statistical analyses were performed using SPSS software (version 22.0; SPSS, Chicago, IL). The obtained data, which were all the result of triplicate measurements were evaluated using one-way ANOVA and Tukey's *post hoc* test and presented as Mean ± SEM (Standard Error of Mean).

Results

MOE possess antioxidant phytochemicals

The results of the FRAP assay indicated the presence of reducing agents (antioxidants) in the MOE. These reducing agents led to the reduction of ferricyanide complexes, transforming them into the ferrous form, resulting in a color change to Prussian blue in the solution [Figure 1a]. Additionally, by measuring the amount of Prussian blue color formed in the solution, which exhibits the highest absorption at 700 nm, the quantity of ferrous complexes and, consequently, the reductive capacity of the extract were determined. The results are illustrated in Figure 1b. In consideration of the preceding content and Figure 1c, the increase in absorption with the increase in extract concentration in the mixture indicates an enhancement in the antioxidant activity of the plant extract.

MOE enhances neural cell viability and proliferation

The MTT assay revealed no significant difference in cell viability between the DMSO and control groups for both B65 and PC12 cell lines [Figure 2]. In B65 cells, MOE treatment significantly enhanced cell viability at days 7 and 14, with a notable decrease in viability for M30 and M60 groups compared to M15 at day 14 (Day 1 ($F3, 8 = 3.82$, $P = 0.05$), Day 7 ($F3, 8 = 36.02$, $P = 0.00$), Day 14 ($F3, 8 = 9.16$, $P = 0.00$)). For PC12 cells, no significant differences were observed on day 1, but significant variations among treated groups emerged on days 7 and 14 (Day1 ($F3, 8 = 0.66$, $P = 0.59$), Day 7 ($F3, 8 = 17.47$, $P = 0.00$), Day 14 ($F3, 8 = 6.53$, $P = 0.00$)).

MOE induces neuronal differentiation

The images obtained from the inverted microscope of B65 and PC12 cells after 14 days of incubation indicate neurite outgrowth and neuronal morphology in MOE treated cells of both cell lines [Figure 2].

The expression levels of nf200 and gap-43 genes across different groups were evaluated using qRT-PCR. In the B65 cell line, qRT-PCR results showed a significant, dose-dependent increase in nf200 expression. Additionally, gap-43 expression was significantly higher in all treated groups compared to the control group, though a significant decrease was observed in the M60 group compared to the M30 group [nf200 ($F3, 21.56$, $P = 0.00$), gap-43 ($F3,8 = 28.00$, $P = 0.00$)]. In the PC12 cell line, qRT-PCR analysis revealed a significant increase in nf200 expression with higher extract dosages. Similarly, gap-43 expression was significantly elevated in all treated groups relative to the control group. However, a significant decrease was noted in the M30 group compared to the M60 group [nf200 ($F3,8 = 20.65$, $P = 0.00$), gap-43 ($F3,8 = 31.58$, $P = 0.00$)] [Figure 2].

Table 1: The sequences of primers used in this study

Gene	Primer sequences	Product (bp)	Size	Accession number
gapdh-F	CAAGTTCAACGGCACAGTCAAG		123	NM_017008.4
gapdh-R	ACATACTCAGCACCAGCATCAC			
nf200-F	AACACCACTAGATGGCGGG		176	NM_012607.2
nf200-R	ACGTGGAGCGTTCAGCAATA			
gap43-F	GATAACTCGCCGTCCTCCAA		187	NM_017195.3
gap43-R	CTACAGCTTCTTTCTCCTCCTCA			

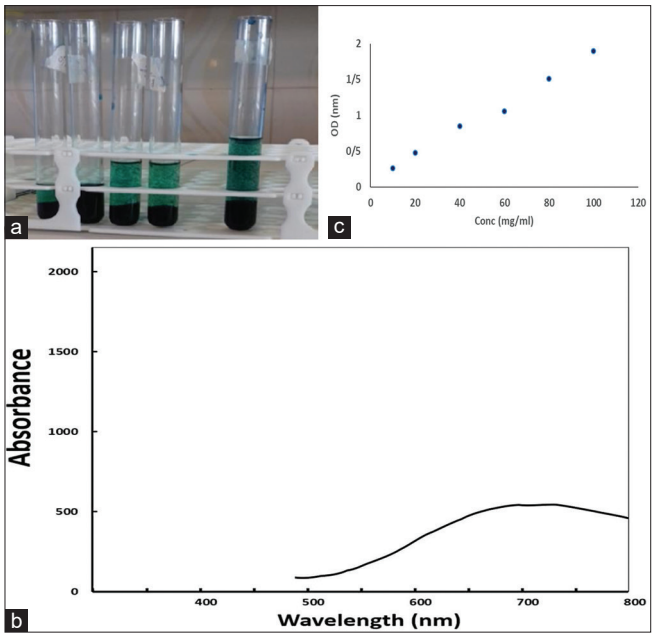


Figure 1: The ferric reducing antioxidant power assay results, (a) solution color change to Prussian blue, (b) quantity of ferrous complexes absorption at 700 nm, (c) absorption levels at different concentrations of the extract

The results of immunostaining corroborated the qRT-PCR findings. Cells treated with MOE exhibited positive staining for nf200, in contrast to the control group. In both the B65 and PC12 cell lines [Figure 3], the expression levels of nf200 protein increased in a dose-dependent manner (B65 (F3,8 = 9.5, P = 0.00), PC12 (F3,8 = 6.51, P = 0.00)).

Discussion

Neurodegenerative diseases (NDs) are influenced by genetics, lifestyle, and environmental factors, making them difficult to treat.[2] With the limitations and side effects of chemical drugs, herbal medicine has emerged as a promising alternative for managing these conditions.[16]

MOE contains various phytochemicals that contribute to its pharmacological benefits, particularly flavonoids (like quercetin),[18] phenolics,[18,19] glucosinolates (GLSs),[19] and isothiocyanates (ITCs),[19] which are responsible for its therapeutic effects.[18,19] Recent studies indicate that glucosinolates protect against neurodegeneration by preventing DNA fragmentation and reducing oxidative stress.[20] Protease inhibitors like proline and alanine from MOE reduce axonal damage, while gallic acid prevents neurotoxicity and stabilizes IGF-1 expression. Additionally, chlorogenic acid exhibits neuroprotective effects by reducing oxidative stress and neuronal cell death.[2] This study evaluated the antioxidant activity of MOE and its effects on B65 and PC12 cell lines. Results showed that MOE enhanced cell proliferation and survival, while also promoting neuronal differentiation in both cell lines.

Inflammation can damage the nervous system. As a result, antioxidants play a crucial role in managing neurodegenerative diseases (NDs) by inhibiting signaling pathways related to neurodegeneration.[2,21] MOE has been shown to elicit immunomodulatory responses by modulating various cytokines, nitric oxide (NO), and the expression of NF-κB, TNF-α, IL-1, and IL-6. Research confirms its anti-neuroinflammatory properties.[22] Phenolic acids and flavonoids are potent neuroprotective agents that mediate cell signaling pathways related to inflammation, particularly involving mitogen-activated protein kinases (MAPK) and nuclear factor-kappa B (NF-κB) [Figure 4].[23] Many phenolic compounds have greater antioxidant effects than vitamin E, preventing lipid peroxidation by disrupting peroxyl radical chain reactions. Consequently, the high levels of antioxidants and polyphenolic components in MOE suggest its potential for neuroprotection.[24] The FRAP assay results confirm that MOE has a high reducing capacity and antioxidant potential, which increases in a dose-dependent manner. The strong antioxidant activity is likely due to the synergistic effects of the various polyphenols present in the extract.

Previous studies have shown that MOE contains carotenoids, β-sitosterol, phenols, and flavonoids, which contribute to its neuroprotective properties [Figure 4]. Baicalein, a flavonoid, has been found to reduce neuronal death by inhibiting MMP-9 activity in a brain ischemia mouse model.[19] Ghimire *et al*.[10] demonstrated that MOE's phytochemicals, including caffeic acid, ferulic acid, apigenin, luteolin, palmitic acid, and myristic acid, contribute to reducing apoptosis and cellular death [Figure 4]. Chang *et al*.[25] demonstrated that caffeic acid acts as a potent neuroprotective agent. Additionally, glucosinolates and their breakdown products from MOE have shown effectiveness against neurodegeneration.[20] Our findings support previous studies, showing a significant increase in cell proliferation and viability in MOE-treated groups compared to DMSO and control groups. Most treated groups exhibited a dose-dependent relationship, with higher MOE concentrations correlating with improved cell viability. These results suggest that MOE, due to its neuroprotective components, may enhance neural cell viability.[1,26,27] The decline in proliferation and cell viability observed between days 7 and 14 aligns with previous studies that attribute this decrease to reduced cellular proliferation during differentiation.[28]

It is suggested that MOE contains compounds with neurotrophic properties. Zeng *et al*.[9] demonstrated that MOE increased SYP and GAP-43 expression in a cerebral ischemia model, thereby promoting axonal. It also enhanced neurotrophic factors such as NGF, NT3, and BDNF [Figure 4]. A study reported that moringin from MOE promotes neural differentiation by increasing the expression of genes such as NESTIN, p75, GAP43, and BDNF. These findings highlight MOE's potential in neural development.[1] Numerous studies highlight β-carotene as the primary active component in MOE which enhances neurite.[29] These findings align with Lee *et al*.,[30] who reported that β-carotene promotes neurite outgrowth and neuronal differentiation by phosphorylating MAPK and

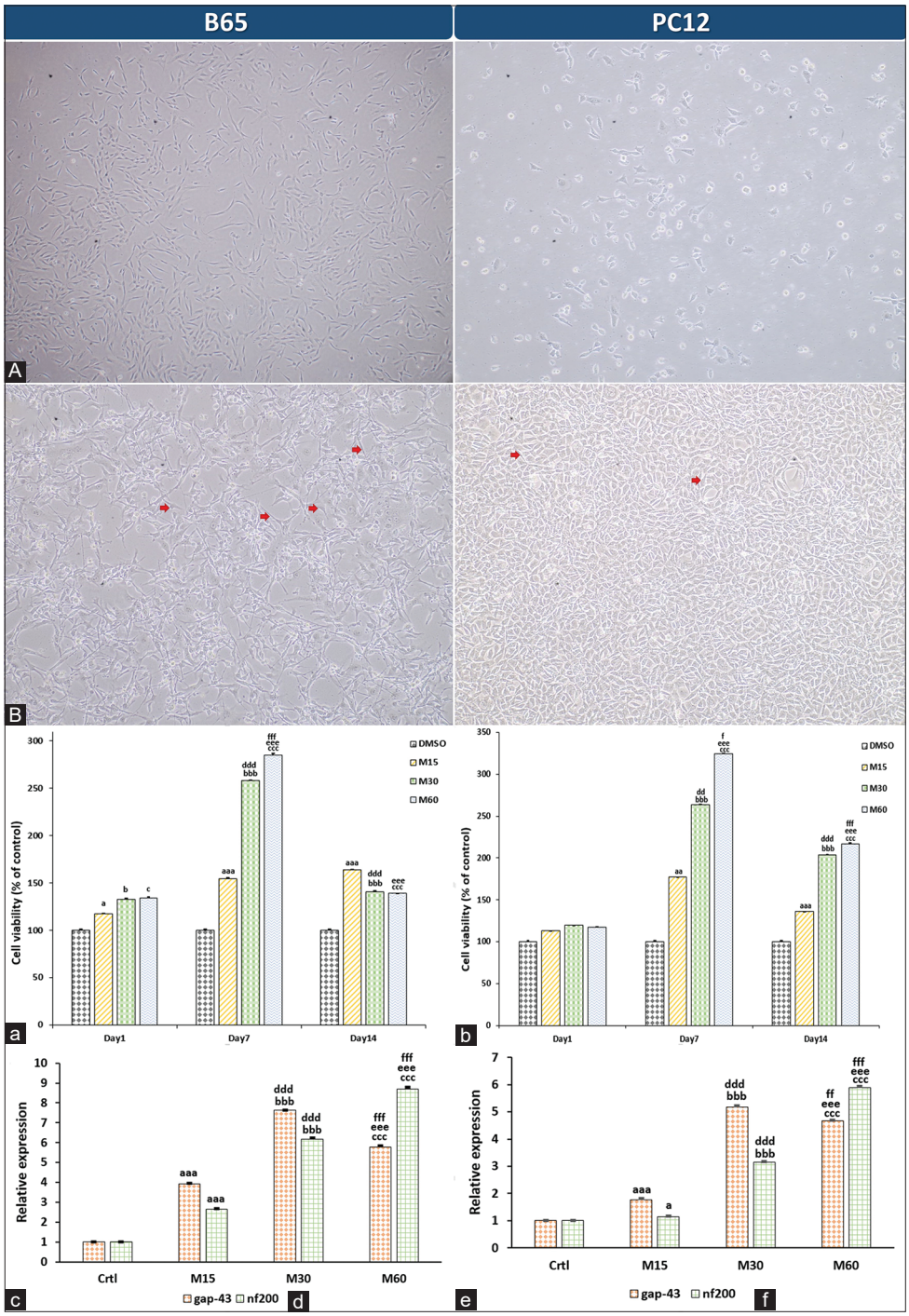


Figure 2: Phase contrast photomicrograph, MTT and qRT-PCR assay results, A cells before and B after 2 weeks (magnification 10X), (a) Comparison of M15 with Crtl, (b) Comparison of M30 with Crtl, (c) Comparison of M60 with Crtl, (d) Comparison of M30 with M15, (e) Comparison of M60 with M15, (f) Comparison of 60 with M30. One letter, two letters, and three letters present $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively

downregulating DLK1. Our findings support previous research, indicating that MOE enhances neural differentiation in B65 and PC12 cells. MOE treated cells showed neurite outgrowth and morphological changes from fibroblastic-like to nerve cells, signifying neural maturation. Two neural markers, GAP-43 and NF200, were used to confirm neuronal differentiation. GAP-43, a protein that regulates actin dynamics in axons, supports axonal regeneration and is a marker for developing neurons. NF200, a type of intermediate filament in neurons, appears later and serves as a marker for mature neurons.[31,32] In our study, MOE treated groups exhibited higher expression levels of GAP-43 and NF200 compared to the control group. This increase was dose-dependent, indicating that MOE promotes neuronal differentiation. The lower expression of GAP-43 and higher expression of NF200 in the M60 group, treated with a higher dose of MOE, suggest that MOE components

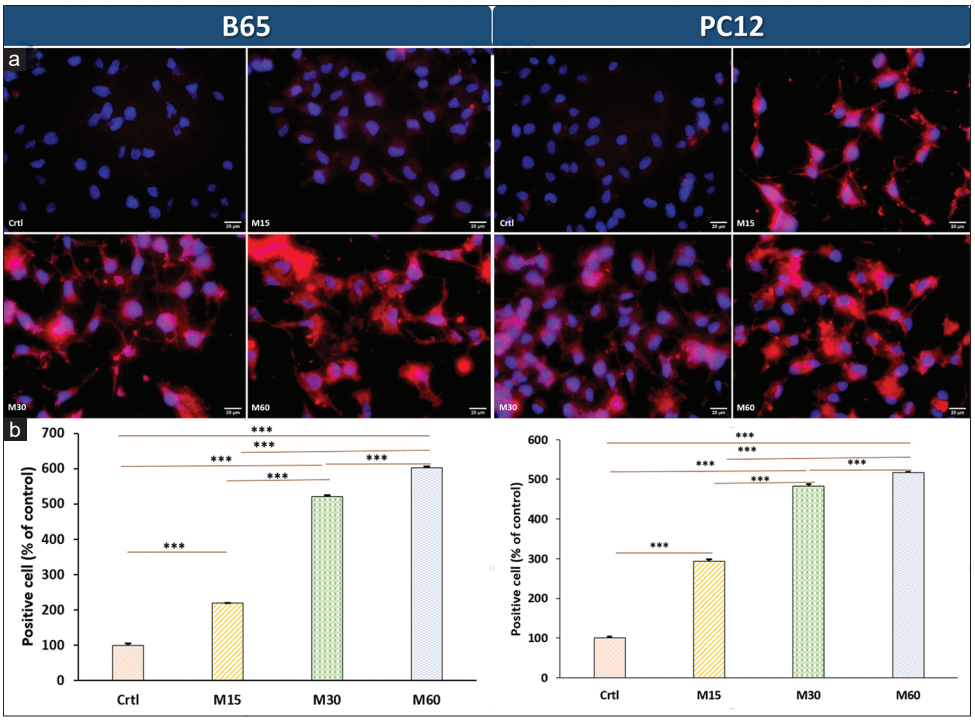


Figure 3: Neural differentiation of B65 and PC12 cells, (a) Immunocy to fluorescence photograph of B65 cells for nf200, (b) quantification of nf200 positive cells. *, **, and *** present $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively

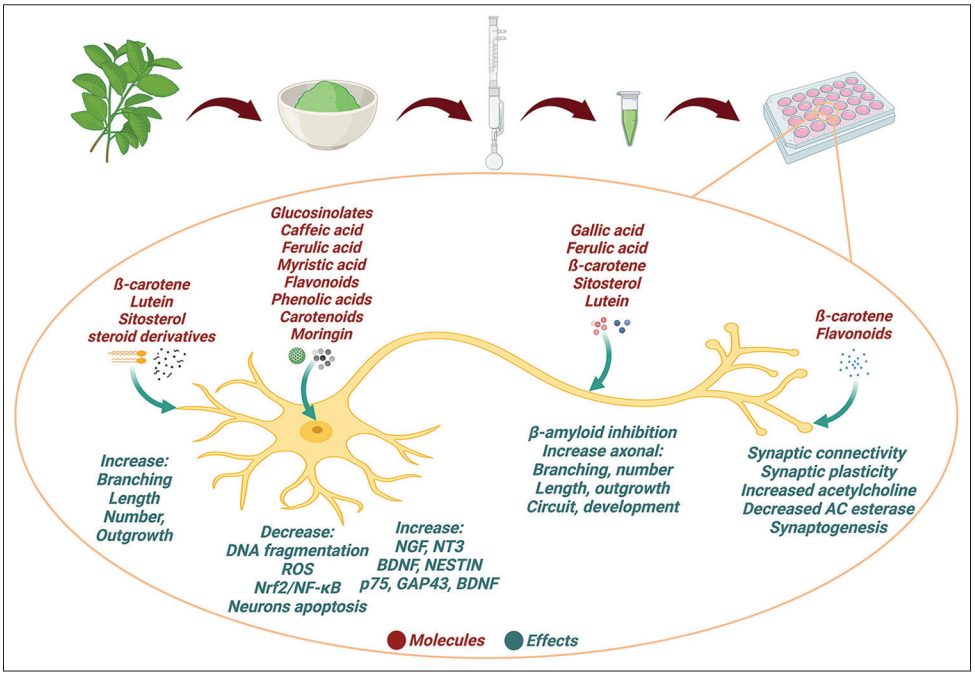


Figure 4: Preparation of Moringa extract, its constituents, and their effects on neuronal segments

accelerated the differentiation of B65 and PC12 cells into mature neurons.

The limitations of this study include the lack of detailed mechanistic insights into how MOE promotes neural differentiation. Further *in vivo* studies are necessary to validate these findings.

Conclusion

Overall, our research demonstrates that treatment with MOE is a promising strategy to enhance the viability and proliferation of neural cells, as well as to promote their neuronal differentiation into mature neurons, in a dose-dependent manner. Additionally, the MOE exhibits high antioxidant potential. Altogether, the

results of our research may pave the way for therapeutic breakthroughs in neurodegenerative diseases.

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Author contributions

All authors contributed to the study conception and design. Material preparation was performed by SSN, HAD and HS. SSN, MK, and HS performed data collection and analysis. The first draft of the manuscript was written by SSN and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was performed in line with the principles of the Declaration of Iran National Committee for Ethics in Biomedical Research. Approval was granted by the Ethics Committee of Isfahan University of Medical Sciences (29- 11-2021/Isfahan, Iran; IR.MUI.MED.REC.1400.665).

Availability of data and materials

Data and code are available from the corresponding author upon reasonable request.

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Conflicts of interest

There are no conflicts of interest.

Competing interests

The authors declare that they have no personal relationships, or relevant financial or non-financial interests that could have appeared to influence the work reported in this paper.

References

1. Romeo L, Diomede F, Gugliandolo A, Scionti D, Lo Giudice F, Lanza Cariccio V, *et al*. Moringin induces neural differentiation in the stem cell of the human periodontal ligament. Sci Rep 2018;8:9153.
2. Azlan UK, Khairul Annuar NA, Mediani A, Aizat WM, Damanhuri HA, Tong X, *et al*. An insight into the neuroprotective and anti-neuroinflammatory effects and mechanisms of *Moringa oleifera*. Front Pharmacol 2023;13:1035220.
3. Sreelatha S, Jeyachitra A, Padma P. Antiproliferation and induction of apoptosis by Moringa oleifera leaf extract on human cancer cells. Food Chem Toxicol 2011;49:1270-5.
4. Adebayo IA, Balogun WG, Arsad H. Moringa oleifera: An apoptosis inducer in cancer cells. Trop J Pharm Res 2017;16:2289-96.
5. Al-Asmari AK, Albalawi SM, Athar MT, Khan AQ, Al-Shahrani H, Islam M. Moringa oleifera as an anti-cancer agent against breast and colorectal cancer cell lines. PLoS One 2015;10:e0135814.
6. Berkovich L, Earon G, Ron I, Rimmon A, Vexler A, Lev-Ari S. Moringa Oleifera aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. BMC Complement Altern Med 2013;13:212.
7. Gopalakrishnan L, Doriya K, Kumar DS. Moringa oleifera: A review on nutritive importance and its medicinal application. Food Sci Human Wellness 2016;5:49-56.
8. Razzaq A, Ahmad Malik S, Saeed F, Imran A, Rasul A, Qasim M, *et al*. *Moringa oleifera* Lam. ameliorates the muscles function recovery following an induced insult to the Sciatic nerve in a mouse model. Food Sci Nutr 2020;8:4009-16.
9. Zeng K, Li Y, Yang W, Ge Y, Xu L, Ren T, *et al*. Moringa oleifera seed extract protects against brain damage in both the acute and delayed stages of ischemic stroke. Exp Gerontol 2019;122:99-108.
10. Ghimire S, Subedi L, Acharya N, Gaire BP. Moringa oleifera: A tree of life as a promising medicinal plant for neurodegenerative diseases. J Agric Food Chem 2021;69:14358-71.
11. Yang YJ, Lee HJ, Lee BK, Lim SC, Lee CK, Lee MK. Effects of scoparone on dopamine release in PC12 cells. Fitoterapia 2010;81:497-502.
12. Nakatani Y, Kobe A, Kuriya M, Hiroki Y, Yahagi T, Sakakibara I, *et al*. Neuroprotective effect of liquiritin as an antioxidant via an increase in glucose-6-phosphate dehydrogenase expression on B65 neuroblastoma cells. Eur J Pharmacol 2017;815:381-90.
13. Shariati Najafabadi S, Amirpour N, Amini S, Zare N, Kazemi M, Salehi H. Human adipose derived stem cell exosomes enhance the neural differentiation of PC12 cells. Mol Biol Rep 2021;48:5033-43.
14. Bhutada PR, Jadhav AJ, Pinjari DV, Nemade PR, Jain RD. Solvent assisted extraction of oil from Moringa oleifera Lam. seeds. Ind Crop Prod 2016;82:74-80.
15. Mukumbo FE, Descalzo AM, Collignan A, Hoffman LC, Servent A, Muchenje V, *et al*. Effect of Moringa oleifera leaf powder on drying kinetics, physico-chemical properties, ferric reducing antioxidant power, α-tocopherol, β-carotene, and lipid oxidation of dry pork sausages during processing and storage. J Food Process Preserv 2020;44:e14300.
16. Hannan MA, Kang JY, Mohibbullah M, Hong YK, Lee H, Choi JS, *et al*. Moringa oleifera with promising neuronal survival and neurite outgrowth promoting potentials. J Ethnopharmacol 2014;152:142-50.
17. Amini S, Saudi A, Amirpour N, Jahromi M, Najafabadi SS, Kazemi M, *et al*. Application of electrospun polycaprolactone fibers embedding lignin nanoparticle for peripheral nerve regeneration: *In vitro* and *in vivo* study. Int J Biol Macromol 2020;159:154-73.
18. Sutalangka C, Wattanathorn J, Muchimapura S, Thukham-mee W. Moringa oleifera mitigates memory impairment and neurodegeneration in animal model of age-related dementia. Oxid Med Cell Longev 2013;2013:695936.
19. Khan MF, Yadav S, Banerjee S. Review article on effects of moringa on central nervous system. J Young Pharm 2021;13:315-9.
20. Igado O, Olopade J. A review on the possible neuroprotective effects of Moringa oleifera leaf extract. Niger J Physiol Sci 2016;31:183-7.
21. Alqahtani WS, Albasher G. Moringa oleifera Lam. extract rescues lead-induced oxidative stress, inflammation, and apoptosis in the rat cerebral cortex. J Food Biochem 2021;45:e13579.
22. Jaja-Chimedza A, Graf BL, Simmler C, Kim Y, Kuhn P, Pauli GF, *et al*. Biochemical characterization and anti-inflammatory properties of an isothiocyanate-enriched moringa (Moringa oleifera) seed extract. PLoS One 2017;12:e0182658.
23. Jantan I, Haque MA, Arshad L, Harikrishnan H, Septama AW, Mohamed-Hussein ZA. Dietary polyphenols suppress chronic inflammation by modulation of multiple inflammation-associated cell signaling pathways. J Nutr Biochem 2021;93:108634.
24. Kim HS, Jeong CH, Lee JK. Neuroprotective effects of Moringa oleifera leaf extracts. Food Life 2022;2022:19-26.
25. Chang W, Huang D, Lo YM, Tee Q, Kuo P, Wu JS, *et al*. Protective effect of caffeic acid against Alzheimer's disease pathogenesis via modulating cerebral insulin signaling, β-amyloid accumulation, and synaptic plasticity in hyperinsulinemic rats. J Agric Food Chem 2019;67:7684-93.
26. Amara I, Ontario ML, Scuto M, Lo Dico GM, Sciuto S, Greco V, *et al*. *Moringa oleifera* protects SH-SY5YCells from DEHP-induced endoplasmic reticulum stress and apoptosis. Antioxidants 2021;10:532.
27. Lanza Cariccio V, Scionti D, Raffa A, Iori R, Pollastro F, Diomede F, *et al*. Treatment of periodontal ligament stem cells with MOR and CBD promotes cell survival and neuronal differentiation via the PI3K/Akt/mTOR pathway. Int J Mol Sci 2018;19:2341.
28. Gingold H, Tehler D, Christoffersen NR, Nielsen MM, Asmar F, Kooistra SM, *et al*. A dual program for translation regulation in cellular proliferation and differentiation. Cell 2014;158:1281-92.

29. Chen P, Li L, Gao Y, Xie Z, Zhang Y, Pan Z, *et al*. β-carotene provides neuroprotection after experimental traumatic brain injury via the Nrf2-ARE pathway. J Integr Neurosci 2019;18:153-61.
30. Lee HA, Park S, Kim Y. Effect of β-carotene on cancer cell stemness and differentiation in SK-N-BE (2) C neuroblastoma cells. Oncol Rep 2013;30:1869-77.
31. Sawant LA, Hasgekar NN, Vyasarayani LS. Developmental expression of neurofilament and glial filament proteins in rat cerebellum. Int J Dev Biol 2002;38:429-37.
32. Bako P, Bassiouni M, Eckhard A, Gerlinger I, Frick C, Löwenheim H, *et al*. Methyl methacrylate embedding to study the morphology and immunohistochemistry of adult guinea pig and mouse cochleae. J Neurosci Methods 2015;254:86-93.