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Research Article

Anti-Inflammatory Properties of Extract of the Hairy Root of Native *Portulaca Oleracea* L. and Its Effect on Some Inflammatory Genes Expression in Rat Microglial Cells

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Background: Most herbs play significant roles in the treatment of various diseases. Because dopamine functions in the anti-inflammatory process and the presence of this substance in *Portulaca Oleracea L.* native plant, investigating this plant's anti-inflammatory properties in treating neurological diseases is interesting.

Objectives: The objective of this study was to estimate the NO production and the expression level of inflammatory genes in lipopolysaccharide (LPS)-treated microglial cells affected by *P. oleracea L.* extraction.

Materials and Methods: *P. oleracea L.* hairy root extract was isolated, and the primary microglial cell of the rat was isolated from glial cells and confirmed by immunocytochemistry analysis. Microglial cells were pretreated with different concentrations of *P. oleracea L.* extract and then treated with 1 $\mu g.mL^{-1}$ LPS. The control group did not receive any treatment. The NO level in culture supernatants was measured by the Griess method. The mRNA expression levels of *iNOS* (inducible Nitric oxide synthase) and *TNF-α* (tumor necrosis factor-alpha) in LPS-treated microglial cells were evaluated using Real-Time PCR.

Results: The present study determined that 0.1 mg. mL^{-1} of the *P. oleracea L.* extract decreased the NO production in rat microglial cells. Different concentrations of the *P. oleracea L.* extract had no prominent effects on LPS-treated cell viability. The results of real-time PCR indicated that *P. oleracea L* extracts suppressed the mRNA expression levels of *iNOS* and *TNF-α* in LPS-treated cells. MTT assay determined that *P. oleracea L.* extract was not cytotoxic, and the anti-inflammatory *P. oleracea L.* extract effects observed were not because of cell death.

Conclusion: *P. oleracea L.* extract might be helpful as an anti-inflammatory agent in treating inflammatory diseases.

Keywords: Inflammation, *iNOS*, Microglia, *P. oleracea* L. extract, *TNF- α*

1. Background

Herbals and their extracts have been used for therapeutic applications for many decades. Some modern medicines are derived from herbal sources. Raw materials for medicine include plant extracts and active ingredients derived from or based on plant substances (1,2).

Hairy root culture techniques have been considered for years because of the production of beneficial metabolites in wild-type roots (3). The advantages of using a culture of the hairy root by *A.Rhizogenes*-mediated transformation are particularly its fast growth, biochemical stability, and relatively high production of secondary metabolites (4,5).

A. Rhizogenes, a Gram-negative bacterium belonging to the *Rhizobaceae* family, causes hairy root disease by infecting injured higher plants. DNA fragment (T-DNA region bounded by 25 bp direct nucleotide repeats) is transferred from large bacteria. The T-DNA region of the Root-inducing (Ri) plasmid enters into the genome of infected plants. This T-DNA contains coding sequences that encode some enzymes for the phytohormone auxin control and cytokinin biosynthesis (iaaM, iaaH, ipt) and encoding genes for opines (unusual amino acids). This new hormonal balance causes hairy root disease, which emerges at the wound site. Latitudinally branching, rapid, hormone-free growth, lack of geotropism, and genetic stability are all characteristics of the hairy root phenotype (3). These roots can also be used for therapeutic applications (6).

P. oleracea L. is widely distributed throughout Europe, India, and Asia. Iran is one of the origins of this plant (7). This plant has various pharmacological properties, including antioxidant, anticancer, antidiabetic, hypocholesterolemic, neuroprotective, hepatoprotective, anti-inflammatory, antimicrobial, wound-healing, and insecticidal properties (8).

P. oleracea L. has many bio-constituents, including catecholamines, noradrenaline, dopamine, l-dopa,α-amyrin, β-Amyrin, and Portuloside A (4,9). Studies have revealed that compounds such as noradrenaline and dopamine have anti-inflammatory effects (10,11). Inflammation is defined as a nonspecific immune response that occurs due to bodily injury (12). Nervous system inflammation may lead to the emergence and development of various nervous system diseases, such as Alzheimer, Parkinson, amyotrophic lateral sclerosis, neurotrophic viral infections, neoplasia, aging, and head trauma, which can damage the nervous system (13). The microglia are defined as the resident macrophage population of the central nervous system (CNS) and are active participants in adaptive immune responses in the neural tissue (14,15). Microglial cells in the healthy brain are characterized by a ramified morphology. Under neuropathological conditions, microglia are readily activated (16). Surface receptors expressed by microglial cells trigger various responses, such as migration to the injury site, proliferation, and phagocytosis. (17,18).

In Alzheimer's and other neurodegenerative diseases, microglia can become overactive and release cytotoxic substances such as tumor necrosis factor-α (TNF-α), nitric oxide (NO), and cytokines that can cause vascular damage in addition to neurodegeneration (19).

Studies have indicated that NO alters the chemical biology of protein function by reacting with cysteine residues of target proteins to form S-nitrosothiols; a process known as S-nitrosylation leads to the aggregation of misfolded proteins such as α-synuclein and Synfilin-1 in the Parkinson's patients and amyloid-beta and tau in the Alzheimer's patients (20,21). Microglial cells are potential therapeutic targets for neurological disorders (22).

Nitric oxide synthases (NOS) catalyze the production of NO from L-arginine. The three NOS isoforms differ in their localization and signaling properties (23). Inducible NOS (*iNOS*) is activated in macrophages and microglia in response to pro-inflammatory stimuli.

Anti-inflammatory drugs can inhibit *iNOS* expression, and neuronal damage can be reduced by low NO levels (18).

2. Objectives

Inflammation is induced by the addition of bacterial endotoxin lipopolysaccharide (LPS), an inflammatory mediator that stimulates microglia through Toll-like receptor 4 (TLR4), which significantly increases the release of pro-inflammatory molecules and concurrently increases *iNOS* expression and NO production (24).

Therefore, in the present study, the anti-inflammatory effects of the hairy root extract of native *P. oleracea L.* were investigated by examining *iNOS* and *TNF-α* expression levels in activated microglial cells. Our results provide some satisfactory conclusions that could be useful for controlling neurodegenerative disorders.

3. Materials and Methods

3.1. Reagents

All cell culture-related materials, such as fetal bovine serum (FBS), Griess reagent and Dulbecco's modified Eagle's medium (DMEM) were purchased from Thermo Fisher Scientific, Inc. (MA, USA). MTT assay kit, Bacterial LPS, antibiotics including penicillin and

streptomycin, Trypsin enzyme, and Dimethyl sulfoxide (DMSO) were purchased from Merck Millipore (Darmstadt, Germany). *P. aleracea* L. seeds were collected from Hamedan City's Medicinal Plant Garden, Iran. *A. Rhizogenes* strain ATCC15834 was obtained from the Agricultural Biotechnology Research Institute of Iran (Karaj, Iran).

3.2. Bacterial Preparation

A single colony of *A. Rhizogenes* strain ATCC 15834 (carrying pRi15834) grown on Luria–Bertani medium (LB containing yeast extract 5 $g.L^{-1}$, tripton 10 $g.L^{-1}$, NaCl 10 $g.L^{-1}$, and agar 15 $g.L^{-1}$, adjusted to pH 7.0) was inoculated into 10 mL of liquid LB medium containing 50 $mg.L^{-1}$ of antibiotic rifampicin under sterile conditions. At 28 °C and in the dark for 24 hours, the culture was shaken at 110 rpm using a rotary shaker. The concentration of the bacterial suspension was measured using a Lambda 45UV/Visible spectrophotometer at OD_{600} (Optical Density). For transformation, the OD was set at a wavelength of 600 nm between 0.2 to 0.8 and then used for infection of plant explants.

3.3. Sterilization of P. Oleracea L. Seeds

Ethanol (70%) was used for the surface sterilization of *P. oleracea L.* seeds. After several washes with sterile water, Sodium hypochlorite (2%) was used to sterilize the seeds and rinsed with sterile distilled water. The sterilized seeds were sown in a sterilized Petri dish containing wet filter paper soaked in ½ MS liquid.

3.4. Induction and Maintenance of P. Oleracea L.

Seedlings were grown for 14 days in a medium containing wet filter paper soaked in 1/2 MS liquid. The cotyledon leaves were then isolated and cut (length, 3 cm) into explants and pre-cultured on solid, growth regulator-free ½ MS medium for 24 h. To inoculate the explants with bacterial strains, 2–3 vertical wounds were placed on the explants using sterile syringes to stimulate the bacterium to infect the explants. The explants were then transferred to a bacterial suspension and shaken for 10 minutes. The explants were incubated at 25 °C in the dark for two days of co-cultivation.

The explants were transferred to ½ MS solid medium with 300 mg. L^{-1} cefotaxime to remove any remaining bacteria. The medium was maintained in a growth chamber at 25 °C under 16 $h.day^{-1}$ light to induce hairy roots. Simultaneously, control explants were placed in ½ MS medium without co-cultivation with *A. Rhizogenes*. From the incision site, roots that were 4-5 cm long were removed and placed on a medium of ½ MS for future growth.

Root lines were maintained by subculturing 3-4 cm long pieces of roots on ½ MS solid medium every four weeks. Additionally, the hairy root cultures were held in ½ MS liquid medium on a rotary shaker (110 rpm) at 25 °C, under 16 $h.day^{-1}$ light.

3.5. Extract from Hairy Roots

After air drying, the samples were crushed to create a hairy root extract, and 100 mg of the crushed sample was wrapped in filter paper and extracted for 1.5 hours at 4 °C using a Soxhlet with 250 mL of sterile distilled water. A rotary evaporator (Buchi Rotavapor R-200, Switzerland) was used to concentrate the extract at 20 °C.

3.6. PCR Analysis of Hairy Roots

Using the CTAB technique, DNA was extracted from every hairy root line and non-transformed roots from *in vitro*-germinated seedlings. For Polymerase chain reaction (PCR), specific primers of the *rolB* gene of T-DNA were applied to the plant genome. Briefly, a total volume of 50 µL was used for PCR and consisted of 200 ng of DNA, 10 µM primer, 200 mM dNTP, 1 U of Taq DNA polymerase, 1X PCR buffer, and 2 mM $MgCl_2$. The PCR conditions were as follows: 5 min denaturation at 94 °C, 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 60 °C for 1 min, and extension at 72 °C, followed by a final extension at 72 °C for 10 min.

3.7. Gel Electrophoresis of Amplified DNA

Amplified products were electrophoretically separated on a 0. 9 percent agarose gel in a 1X TAE (Tris-acetic acid) buffer; bands were detected by staining gel with ethidium bromide and then observed under UV illumination using a gel documentation system.

3.8. Cell Culture

Wister newborn rats, aged 1-4 days, were used to prepare primary mixed glial cultures from their cerebral cortices. Hank's buffer was used to remove meninges and blood vessels according to Giulian and Baker's method (31). Brain tissue was isolated and dissociated by pipetting into small pieces in DMEM. The cells were maintained in a 5 percent CO_2 incubator at 37 °C after

being transferred to T-25 tissue culture flasks containing DMEM supplemented with 10% heat-inactivated FBS (Fetal bovine serum). After four days, the culture medium was replaced with a fresh medium containing 10% FBS. After 70% confluence, the culture flask was shaken for 5-7 minutes to remove microglial cells from the bottom of the flask. The cell suspension was removed in a flask and transferred to a 15 mL sterile Falcon tube. Trypan blue (50 L) was used to stain the microglial cells (50 L), and the hemocytometer was used to count them. The cells were seeded onto 96-well plates at a density of 1×10^4 cells/well and allowed to adhere to the plate in 10% FBS by incubation at 37 °C in a humidified 5 % CO_2 atmosphere for 24 hours. After 24 h, the culture medium was replaced with fresh medium containing 1% FBS, and the cells were ready for treatment. The cells were pretreated with different concentrations of purslane extract (0.05, 0.1, 0.4 mg. mL^{-1}). After one hour, cells were stimulated with LPS (1 µg. mL^{-1}). The plates were then incubated for 48 hours in a 5 % CO_2 incubator.

3.9. Nitrite Quantification

The amount of nitrite secreted into the culture medium by microglial cells was measured using a colorimetric reaction with the Griess reagent. 48 h after treatment, 50 µL of the culture supernatant from each cell line was transferred to another 96-well plate. Fifty microliters of grease solution-1 were added to each cell. The plates were then incubated in the dark for 15 min. Fifty microliters of grease solution-2 were added to each cell. The plates were incubated in the dark for 15 min. Using a microplate reader, the mixture's absorbance at 540 nm wavelength was determined.

3.10. Cell Viability Assay

Using the MTT (3-4,5-dimethylthiazole-2-yl-2,5-di- phenyl-tetrazolium bromide) assay, cell viability after 48 hours was assessed. The MTT solution (1 mg. mL^{-1}) was added to a volume equal to 10% DMEM and incubated for 4 h at 37 °C in the dark. The MTT solution was removed, and forming formazan crystals dissolved in DMSO indicated the metabolic activity of the cells. Using a microplate reader, absorption at 580 nm was measured.

3.11. Reverse Transcription-Polymerase Chain Reaction (RT PCR) and Real-time PCR

Glial cells were pretreated with 0.1 $mg.mL^{-1}$ of purslane extract, after 1 h glial cells were stimulated with 1 $\mu g.ml^{-1}$ LPS. After 48 h, total RNA was extracted from the treated cell cultures. Using an RNA extraction kit (RNX-Plus, Cinna Gen Co.), total RNA was extracted from the treated cells. Using a Nano Drop spectrophotometer at a wavelength of 260 nm, the amount of total RNA was quantified. cDNA was produced using 1000 ng of RNA using intron's Power cDNA Synthesis Kit. Following the manufacturer's instructions, 95 °C for five minutes was followed by 45 °C for an hour during the cDNA synthesis process. PCR was used to amplify the cDNA. The PCR was carried out for 40 cycles with the following conditions: denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 60 s. The PCR products were separated by electrophoresis to verify the amplification of *iNOS* and *TNF-α* genes. The products were detected under UV light after being stained with ethidium bromide to make them visible.

Table 1. List of primers and the amplicon size applied in this study.

Gene name	Sequence	Amplicon Size (bp)
rolB	F: 5′-TTAGGCTTCTTTCATTCGGTTTACTGCAGC-3′	780
	R: 5′-ATGGATCCCAAATTGCTATTCCCCCACGA-3′	
iNOS	F: 5′- GAGATGTTGAACTACGTCCTATC-3′	166
	R: 5′- CCATGACCTTCCGCATTAG-3′	
TNF-α	F: 5′- GCTCCCTCTCATCAGTTCCA-3′	100
	R: 5′- TTGGTGGTTTGCTACGACG-3′	
β-actin	F: 5′- GCTCCCTCTCATCAGTTCCA-3′	155
	R: 5′- TTGGTGGTTTGCTACGACG-3′	

The newly synthesized cDNA, stored at -20 °C, was used for the *iNOS* and *TNF-α* gene expression assay by qRT-PCR. cDNA (1 µL) was amplified in 25 µL of the reaction mixture using 0.25X SYBR Green Supermix (Molecular Probes, Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed by monitoring the increase in the amount of SYBR-Green in real time using Rotor-Gene 6000 RT-PCR (Corbett Research, Sydney, Australia). The thermal cycling conditions were as follows: 95 °C for 15 min, 95 °C for 15 s, 1 min at 60 °C for 50 cycles, and 55 °C for 1 min. In this instance, we utilized the primers listed in **Table 1**. Primers were designed using the Gene Runner software.

The relative quantification of targeted *iNOS* and *TNF-α* mRNAs expression (*β-actin* was used as a housekeeping gene for normalization) was calculated using the $2^{-\Delta\Delta Ct}$ method.

$\Delta\Delta Ct$ = (Ct target gene – Ct Housekeeping gene) patients – (Ct target gene – Ct Housekeeping gene) control

Relative fold change of gene expression= $2^{-\Delta\Delta Ct}$

3.12. Statistical Analysis

A completely randomized design (CRD) was used in the statistical data analysis as factorial experiments.

The data were statistically analyzed using SPSS 16 software. In brief, one-way analysis of variance (ANOVA) and the least significant difference (LSD) test were applied to determine statistical differences among various cell groups. Three duplicates of every experiment were run. Statistical signifi cance was considered at P value < 0.05.

4. Results

4.1. Morphological and Genomic Evaluation of Transformed Hairy Roots

The morphological properties of the transformed hairy roots of all eight lines are shown in **Figure 1**. These transformed roots were also confirmed by PCR using *rolB* gene-specific primers, which results from PCR of the DNA of these roots, as shown in **Figure 2**.

A. Rhizogenes served as the positive control, and DNA from the non-transformed seedling roots served as the negative control. A 780 bp *rolB*-amplified product was present in all transform ants. In the control group, rolB was not detected **(Fig. 2).**

Figure 1. The image of transformed hairy root of *P. oleracea* by *A. rhizogenes*AR15834.

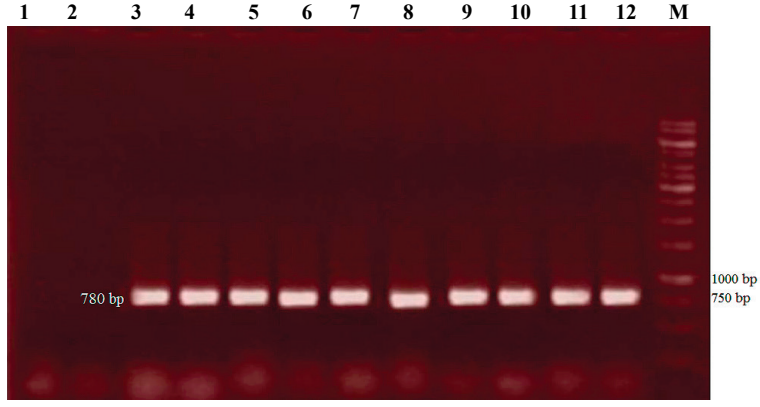

Figure 2. The electrophoresis gel of PCR-products of *rolB* gene in transformed root of *P. oleracea* by *A. rhizogenes* AR15834 in agarose gel, well M: molecular marker 1000 bp, wells 5-12: lines of transformed root, wells 1-2: lines of control root, wells 3-4 positive control (plasmid DNA).

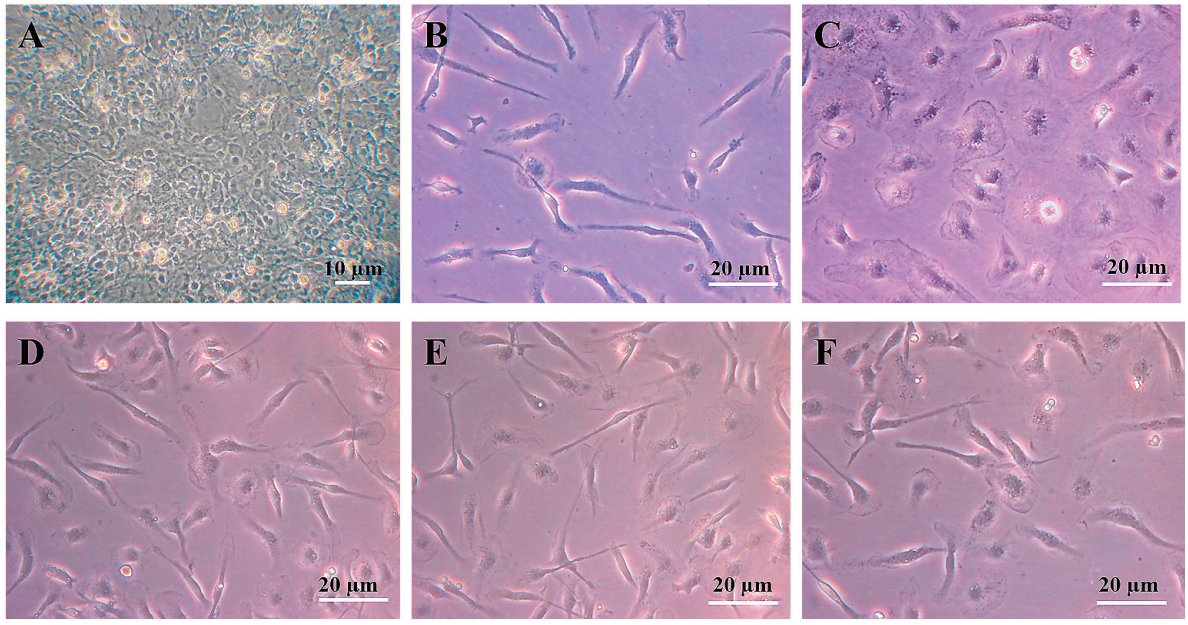

Figure 3. The bright field images of microglia cells. A) Mixed cell population containing neurons and other glial cell types. **B)** Purifi ed microglial cells. **C)** LPS-treated microglial cells. Pretreated microglial cells using different concentrations of purslane extract. **D)** 0.05 mg.mL^{-1}. **E)** 0.1 mg.mL^{-1}. **F)** 0.4 mg.mL^{-1}.

4.2. Microscopic Assay of Treated Cells by LPS

The treated and untreated cells were visualized using phase-contrast microscopy **(Fig. 3)**. Activation and morphological alterations in microglial cells by LPS treatment and pretreatment with purslane extract: In this study, we studied the effects of LPS treatment (1 µg. mL^{-1}) of microglia on cell morphology changes using phase-contrast microscopy. Phase-contrast microscopy showed mixed cell population containing neurons and other glial cell types **(Fig. 3A)**. Purified microglial cells showed the ramified shape of untreated cells as the control group **(Fig. 3B)**. Microglial cells activated with LPS showed an amoeboid morphology **(Fig. 3C)**. Different concentrations of purslane extract (0.05, 0.1, and 0.4 mg.mL^{-1}) was used to pretreat microglial cells **(Fig. 3D, 3E, 3F).**

4.3. NO Measurement

Effect of Purslane extract on lipopolysaccharide-induced nitric oxide production was investigated.

After an hour of stimulation with LPS, the cells were pretreated with purslane extract. Nitrite concentration (µM) in the culture medium was assessed using the Griess reaction. The results showed that purslane extract decreased NO production in a dose-dependent manner **(Fig. 4A)**. The investigations revealed that with an increase in purslane extract concentration from 0.05 mg. mL^{-1} to 0.1 mg. mL^{-1}, NO levels decreased. The effective concentration of the purslane extract that controls the release of NO is 0.1 mg. mL^{-1}. A sodium nitrite solution ($NaNO_2$) in the range of 0–50 µM was used to draw the standard curve and determine the nitrate concentration **(Fig. 4B).**

4.4. The Effect of Purslane Extract on Cell Viability

MTT assay was performed to investigate the possibility of the cytotoxic action of purslane extract on the decrease in NO production and cell viability. Purslane extract did not significantly affect cell viability in the presence of LPS compared to the control group, according to forming formazan crystals in treated microglial cells with various concentrations of purslane extract **(Fig. 5)**.

4.5. Gene Expression of Treated Cells

Effect of the purslane extract on *iNOS* and *TNF-α* expression in glial cells treated with LPS was investigated.

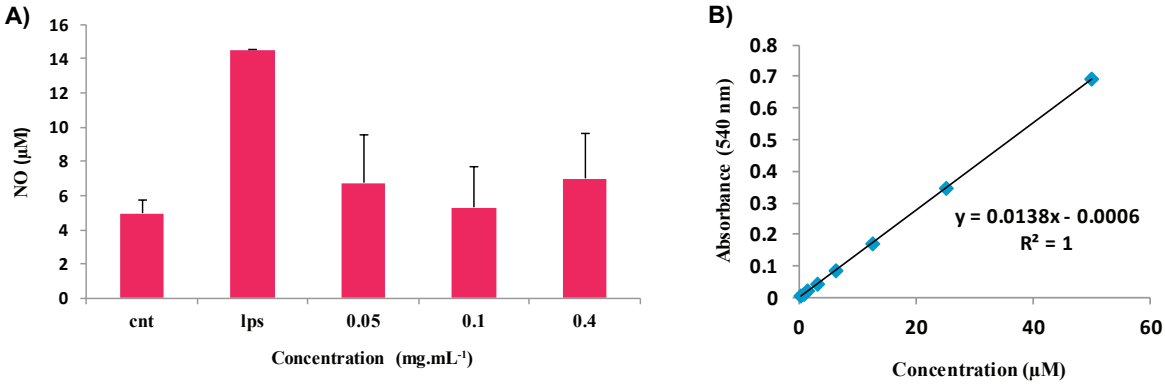

Figure 4. The effect of purslane extract on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated microglial cell. A) Main graph. **B)** Standard $NaNO_2$ curve for measurement of NO level.

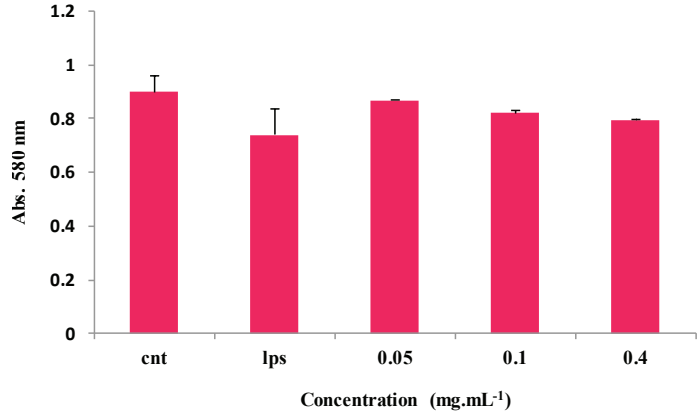

Figure 5. Viability of microglia cells after treatment with various concentrations of puslane extract. Values with $p<0.001$ are statistically significant.

We investigated the expression of inflammatory genes in activated microglial cells. *iNOS* and *TNF-α* play essential roles in inflammation progression. In this study, we used RT-PCR to detect the *iNOS* and *TNF-α* genes that produce relevant inflammatory factors. The results showed a 166 bp *iNOS* band and 100 bp *TNF-α* band. Pretreatment of microglia with a concentration of 0.1 mg. mL^{-1} of purslane extract reduced the band intensity.

Real-time PCR results for *iNOS* and *TNF-α* genes: The results showed significant differences in the quantitative expression of *iNOS* and *TNF-α* genes among the different treatment groups ($p<0.001$) **(Fig. 6)**.

5. Discussion

Herbals have been routinely used as one of the most critical therapeutic sources for thousands of years. In addition, natural products, such as herbal extracts, have been used as anti-inflammatory agents in some diseases (2). Using biotechnology techniques can increase the efficiency and productivity of medicinal plants as renewable sources for drug production using strategies

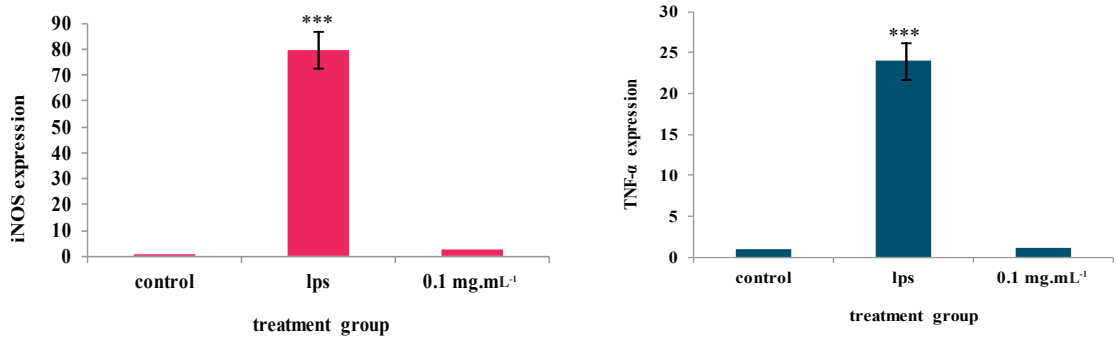

Figure 6. The results of *iNOS* and *TNF-α* genes expression levels in treated and non-treated microglia cells.

such as cell and tissue culture and genetic engineering. Hairy roots are helpful for the production of secondary metabolites, particularly medicinal metabolites, owing to their high stability and production in culture media. Our results showed that *A. Rhizogenes* strain AR15834 successfully established hairy roots in purslane.

These results concur with those reported by Kamada *et al*. (1986) and Mano *et al*. (1986), who first produced hairy roots to produce tropane alkaloids in *Atropa belladonna* and *Scopolia japonica* using *A. Rhizogenes* strain ATCC15834 (25,26).

According to a study by Dhakulkar *et al.* (2005), the successful transgenic purslane production by strain AR15834 is probably due to its high susceptibility to this strain (27). Hairy roots show rapid growth, high branching, and growth in a hormone-free medium. Wardell and Skoog (1967), in their studies, stated that the amount of hormone in the plant body plays a vital role in the formation and morphological characteristics of hairy roots (28). Batra *et al.* (2004) reported that the entry of both TL and TR segments or each of these segments alone could cause changes in the growth and morphology of the produced roots because of changes in their internal hormone balance (29). Tiwari *et al.* (2007) reported a direct relationship between the rate of *rolB* expression, root growth, and lateral branching of hairy roots (30). To ensure the transgenic origin of hairy roots, PCR has been used in most research for convenience, cheapness, and newer techniques, which require a specific primer for one of the rol gene families. In the present study, PCR with the *rolB* gene primer was used to confirm the transgenic form of the produced hairy roots, and the results confirmed the transgenic hairy roots. These results are in agreement with the results of the studies of Dhakkulkar *et al*. (2005) and Rahnama *et al*. (2008) (27). They confirmed the transgenicity of hairy roots produced in *Gmelina arborea* Roxb and *Silybum marianum* plants using PCR with a primer specific for *rolB* (780 bp).

Microglial cells are the smallest glial cells in the CNS. As brain macrophages play an important role in regulating innate immunity, they also play essential roles in pathological conditions such as brain tissue protection and phagocytosis of apoptotic neurons. In addition to morphological changes, inflammation of microglial cells is associated with the expression and release of proinflammatory factors such as interleukins and free radicals such as NO. Plant anti-inflammatory compounds, such as Dopamine, can affect cellular responses and inhibit the expression of inflammatory factors.

This study was designed to investigate the effects of *P. oleracea L.* hairy root extract on NO generation in LPS-stimulated microglial cells. The anti-inflammatory effect of the *P. oleracea L.* extract was investigated by the induction of inflammation with lipopolysaccharide, and its effect on the expression of inflammatory genes was studied. Our results showed that the method described by Giulian *et al*. is efficient for isolation and displays the appropriate morphology of inactive and resting microglial cells in the laboratory on a plate. It has been shown that when these cells are treated with LPS, an antigen of gram-negative bacteria, it simulates conditions equivalent to the attack of a pathogen in the

environment, and microglial cells are activated, leading to the expression of inflammatory genes (31).

Activation of brain microglial cells under severe conditions, such as infection by a pathogen or injury to the brain, occurs and changes morphology. Inflammatory cytokines help to kill pathogens, activate other immune cells, and are secreted to repair the damage. These factors include cytokines, chemokines, and prostaglandins, such as *TNF-α* and *IL-1β*. Our results showed that microglial cells underwent a significant morphological change from the spindle and branching to the amoebic form (quasi-macrophage). After treatment with *P. oleracea L.* root extract, real-time PCR showed decreased *TNF-α* and *iNOS* inflammatory gene expression.

The extract decreased inflammation in our cells activated with LPS, confirming the anti-inflammatory effect of *P. oleracea* L. extract.

Our results confirm the Miao *et al*. study (2019), in which they investigated the anti-inflammatory effects of purslane extract on LPS-stimulated RAW 264.7 cells and discovered that this extract decreased the level of *TNF-α* and NO. The production of *TNF-α* and *IL-6* was significantly decreased at higher dose of 400 $g.mL^{-1}$, according to previous report. The extract partially prevented the nuclear translocation of P65, which explains the inhibition of the NF-κB pathway. Three flavonoids, - luteolin, kaempferol, and quercitrin, -were also discovered in the extract by the authors, who hypothesized that they could be responsible for the anti-inflammatory effects of the extract. (32). In addition, Rahimi *et al*. (2019) have studied the anti-inflammatory effects of *P. oleracea* L. extract on LPS-induced rat lung injury that found a decrease in *IL-β*, *IL-6*, and *TNF-α* levels in response to *P. oleracea* L. extract treatment (33). These findings were consistent with our results.

Portulaca oleracea L. belongs to the Portulacaceae family and is commonly known as purslane. It is used in folk medicine and is commonly used in Mediterranean and tropical Asian nations. Flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, proteins, vitamins, and minerals are a few substances isolated from *Portulaca oleracea* (34). Dopamine is an essential secondary metabolite naturally produced by *P. oleracea* L. that has been introduced as a substance with anti-inflammatory properties (35, 36). The majority of immune cells have dopamine receptors. Dopamine is a peripheral chemical mediator and neurotransmitter. Dopamine or a dopamine receptor 1 (DRD1) agonist inhibits the activation of caspase-1 and the release of IL-1 from primed bone marrow-derived macrophages (BMDMs) (37). According to Martins *et al*., *P. oleracea* L. may be a potential therapeutic agent for Parkinson's disease (38). Numerous studies have supported the discovery of noradrenaline and dopamine in purslane (39, 40, 41). The presence of catecholamines in purslane supports the neuroprotective properties of the plant and raises the possibility of its use in treating neurodegenerative diseases (42). In this study, for the first time, we demonstrated the anti-inflammatory effect of a local *P. oleracea* L. hairy root extract on rat microglial cells, which is believed to be due to dopamine in *P. oleracea* L. hairy root.

6. Conclusion

In conclusion, the hairy root extract of *P. oleracea L.* decreased NO synthesis *in vitro* by exerting anti-inflammatory effects. In the activated microglial cells, the hairy root extract of *P. oleracea* L. prevented NO release. The present study determined that 0.1 $mg.mL^{-1}$ of *P. oleracea L.* extract significantly reduced the production of NO in rat microglial cells stimulated with 1 $\mu g.mL^{-1}$ LPS. *P. oleracea L.* extract may be a therapeutic component to control neurological disorders. The hairy root extract of *P. oleracea L.* can be considered a valuable agent for the neuroprotection and alleviation of symptoms in neurodegenerative diseases.

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Competing interests

The authors have no conflicts of interest.

Ethics approval and consent to participate

The study protocol was approved by the National Institute of Genetic Engineering and Biotechnology committee.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Consent for publication

Not applicable.

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