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Original article

Anti-inflammatory effects of essential oil, aerial parts and hairy roots extracts of *Nepeta pogonosperma* on rat brain mixed cells

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Abstract

Background and objectives: Many *Nepeta* species have been commonly used in Iranian folk medicine as tranquilizer, relaxant, carminative and restorative tonic for nervous and respiratory disorders. Inflammation is a problem in many diseases and has an important role in brain function that can cause neurodegenerative disorders. Inflamed glial cells can exacerbate neurodegenerative diseases by producing neurotoxins. In the present study, the anti-inflammatory effects of essential oil, aerial parts and hairy roots extracts of *Nepeta pogonosperma* Jamzad & Assadi have been evaluated on rat brain mixed cells. **Methods:** Inflamed mixed glial cells from rats' brains were treated with different concentrations of essential oil and extracts from aerial parts and hairy roots of *Nepeta pogonosperma* to evaluate their anti-inflammatory effects. No level as the indicator for inflammation was measured. **Results:** The results revealed that 0.5 µL/mL of the essential oil reduced NO production significantly. In addition, some hairy root extract concentrations led to reduce it, although the extract of the aerial parts of the plant did not affect NO production. **Conclusion:** This research has confirmed the anti-inflammatory potential of essential oil and hairy root extract of *Nepeta pogonosperma* on rat brain mixed cells.

Keywords*:* essential oil, inflammation, mixed glial cells, *Nepeta pogonosperma*

Introduction

Medicinal plants are the most important source of drugs for people all around the world; about 50 percent of drugs produced in the world are of natural origin [1]. According to the World Health Organization, about 80% of people rely on traditional remedies such as herbal drugs which are found in many modern medicinal formulations [2]. *Nepeta* contains about 300 species, which are distributed in central and southern Europe, East, central and southern Asia, among them Iran is one of the centers of origin of this genus with 75 species and approximately 53% endemics which have been used as herbal remedies. *Nepeta pogonosperma* Jamzad & Assadi was identified as a new species in 1984 [3]. Sefidkon and Akbari-Nia [4] demonstrated that 4aα-7α-7aβ-nepetalactone and 1,8-cineole were the main compounds in *N. pogonosperma*

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essential oil. Anti-inflammatory effects of these compounds have also been investigated in other studies [5-8].

Inflammation is an important aspect of many human diseases and reduction of this process could therefore be of therapeutic interest [9]. In neurodegenerative diseases such as amyotrophic lateral (ALS), Multiple sclerosis (MS) and Alzheimer, neuroinflammation is a key defense reaction [10,11].

Glial cells like astrocyte and microglia are the brain important immune cells [12]. Activation of microglia as resident microphages initiates to release several potentially cytotoxic substances such as reactive oxygen intermediates, nitric oxide, proteases, arachidonic acid derivatives, excitatory amino acids, and cytokines [13-15]. It is believed that these components contribute to the progressive damage in neurodegenerative diseases, so decrease in their levels in activated microglia can alleviate the severity of neurodegenerative diseases [16,17].

In the present study, essential oil and extracts from stems, leaves and hairy root of *N. pogonosperma* were used to alleviate LPS-Inflamed mixed glial cells from rat. We evaluated NO level as an indicator for inflammation. To insure that the decline in NO production was not because of the cell death, the viability of the cells was also measured.

Experimental

Establishment of hairy root culture

Seeds of *N. pogonosperma* were collected from aerial flowering parts which were provided from Alamout medicinal plant research center, Qazvin province, Iran. Hairy roots were induced by *Agrobacterium rhizogenes* strain MSU440 using transformation procedure [18]. Briefly, explants were randomly wounded using sterile needle and were inoculated with *A. rhizogenes* culture suspended in liquid inoculation medium for 10 min. The explants were dried by blotting with sterile filter paper and then placed on co-cultivation medium in dark. After 2 days of co-cultivation, the explant tissues were transferred to selective media (MS medium supplemented by 400 mg/L cefotaxime).

Plant material

The seeds were surface-disinfected with 70% alcohol and then 10 seeds were sown in 10 cm pots containing a sterilized mixture of field perlite, soil and leaf-compost at a rate of 1:1:1. The seedlings were grown in a greenhouse under natural condition at 22/18 °C (day/night) and 16 h photo period. The aerial parts were collected and air dried

Essential oil preparation

50 gram of dried aerial parts were hydro-distilled in 500 mL distillate water for 3.5 h in a Clevenger-type apparatus (Medicinal Plant Research Institute, Shahid Beheshti university) [19]. The essential oil was dissolved in dimethylsulfoxide 10% (DMSO 10%) [20].

Hairy root and aerial parts extract preparation

50 gram of dried aerial parts of plant and hairy roots were hydro-distilled in 500 mL distillate water for 5 h in Reflux (National Institute of Genetic Engineering and Biotechnology). Then extracts were filtered and the filtrate was concentrated using a freeze drier and kept at room temperature pending investigation. [21]

Primary cell culture

Primary cell cultures were prepared from 1 to 3 day-old newborn Wister rat brains and were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% heat-inactivated fetal bovine serum (FBS) at 37 ºC in a humidified incubator under 5% CO_2. After 24 h, the medium was replaced by 10% FBS and after 14 days the samples were used for treatment with different concentrations of the essential oil (0.25, 0.5, 0.75 and 0.9 µL/mL), aerial parts of plant and hairy root extracts (375, 675, 750, 900 and 1200 mg/mL) [22]. Animal experiments were performed in accordance with the approval of Bioethic Committee of Health Ministry.

Assessment of cell viability

Cell viability was determined by measuring the reduction of 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. Mix glial cells were seeded in 96-well plates at the density of 12×10^3/well and were treated with various concentrations of the essential oil, plant aerial parts and hairy root extracts for 48 h. After treatment, 0.5 mg/mL MTT was added and the cells were incubated at 37 ºC for 4 h. The formazan crystals were dissolved by dimethyl sulphoxide (DMSO). Absorbance was measured at 570 nm using a microplate reader. Each experiment was performed in triplicate [23].

Assessment of NO

Production of NO was assessed by measuring levels of nitrite in the culture medium using a colorimetric assay with Griess reagent. Mixed glial cells at the density of 12×10^3/well were seeded in 96-well plates and were stimulated with LPS (1 mg/mL) 1 h after treatment. After 48 h, 50 µL of culture supernatant was subjected to react with an equal volume of Griess reagent (Sigma) in 96-well plate for 20 min at room temperature in dark. Nitrite concentration was determined by using standard solutions of sodium nitrite prepared in the medium. The level of absorbance was determined at 540 nm using a microplate reader [24].

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's test and LSD. Statistical significance was set at $p<0.05$.

Results and Discussion

Human beings are exposed to many diseases and one of these most important diseases is neurodegenerative disorder. Inflammation is a defense reaction against diverse insults in neurodegenerative disorders [13] and has a primary role in almost every disease. Hence, using some methods to reduce inflammation can alleviate the effect of diseases. In CNS, microglia cells are the major effector cells involved in immune and inflammatory responses [25]. Natural compounds that have anti-inflammatory effects, may offer a promising strategy for therapeutic application. In this study, LPS was used as an agent for inflammation of microglia and NO production was investigated by Griess reagent.

The role of NO is very complicated because it has both positive and negative effects. Small amounts of NO produced by nitric oxide synthase may directly suppress pain by vasodilatation and increasing circulation and reduce nerve irritation and inflammation. Conversely, increased production of NO after activation of iNOS by inflammatory cytokines can increase pain and lead to cell death in different ways [26].

The essential oil of *N. pogonosperma* was investigated for its inhibitory effect on nitric oxide production in mixed glial cell culture inflamed by LPS. Different concentrations of the essential oil were tested. The level of NO production was decreased in 0.25, 0.5 and 0.75 µL/mL of essential oil but 0.5 µL/ml was determined as the best concentration of the essential oil for NO reduction (figure 1).

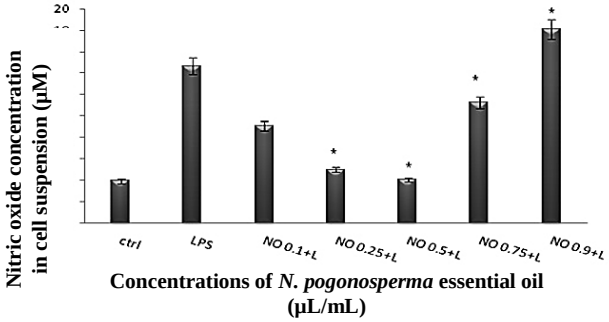

Figure 1. Effect of different concentrations of *N. pogonosperma* essential oil on NO Production. * means $p<0.05$ in comparison to LPS group. Data were obtained as the mean of three replications.

No toxic effect was observed from different concentrations of essential oil that were tested (figure 2). The effect of the essential oil on mixed glial cells morphology has been previously investigated. Ali and Ricci demonstrated the anti-inflammatory effect of *Nepeta pogonosperma*

(and *N. cataria, N.sibthorpii* essential oils. They attributed the anti-inflammatory effect of essential oils to nepetalactone [6,27,28]

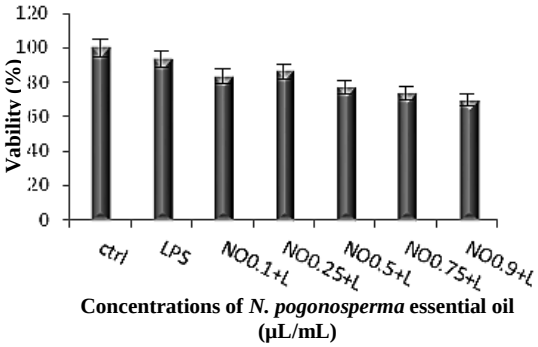

Figure 2. Effect of different concentrations of *N. pogonosperma* essential oil on cell viability.

Nepetalactone, 1,8-cineole, α-pinene, α-terpineol and caryophyllene oxide were the main metabolites that had been found in the studied species of the genus *Nepeta* [29]; furthermore, research by Sefidkon and Akbari-Nia [4] identified nepetalactone as a major component of *N. pogonosperma* essential oil. 1, 8-cineol, an oxygenated monoterpene, showed inhibitory effect on carrageenan induced paw edema and cotton-pellet induced granuloma in rats [30]. It has also been demonstrated that this terpenoid oxide has strong inhibitory effect on cytokine production in cultured human lymphocytes and monocytes [31]. In addition, different isomers of nepetalactones were reported to have considerable sedative and analgesic activity. 4aα, 7α, 7aβ-nepetalactone, the key constituent of *Nepeta ceasarea* Boiss, was suggested to have a specific opioid receptor agonistic activity [32].

We suggested that nepetalactone might be responsible for anti-inflammatory activity of the essential oil in LPS stimulated mixed glial cells.

Since no toxic effect was observed from the essential oil, its compound could be extracted and used as anti-inflammatory agents. However, the pharmacological actions on neuroinflammation mediated by microglial activation and the intrinsic mechanisms have not been investigated.

The aqueous extract of *N. pogonosperma* aerial parts was investigated for its inhibitory effect in nitric oxide production in mixed glial cell culture inflamed by LPS. Different concentrations of the extract were tested which showed no effect on NO production and had no toxic effect on cell viability.

NO production was decreased by using 675 and 750 mg/mL of hairy root aqueous extract and no toxic effect was observed in these concentrations (figure 3). Cell viability was decreased in 900 and 1200 mg/ml of hairy root aqueous extract (figure 4).

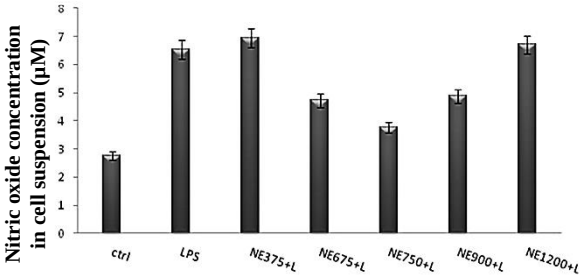

Figure 3. Effect of different concentrations of *N. pogonosperma* hairy root extract on NO production. * means p <0.05 compared to LPS group. Data were obtained as the mean of three replications

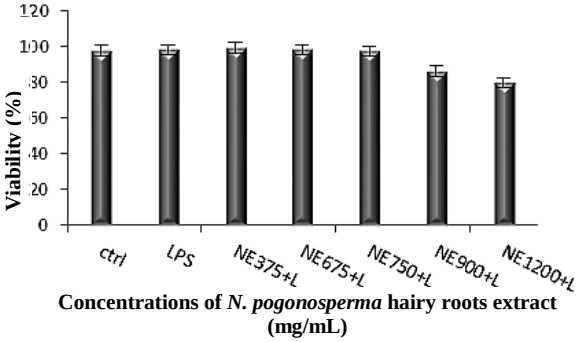

Figure 4. Effect of different concentrations of *N. pogonosperma* hairy root extract on cell viability

This research has confirmed the anti-inflammatory potential of some concentrations of essential oil and hairy root extract of *Nepeta*

pogonosperma Jamzad & Assadi on rat brain mixed cells. So the effective metabolites can be extracted and investigated in further studies.

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Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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