JCHR (2025) 15(2), 405-413

Journal of Chemical Health Risks

sanad.iau.ir/journal/jchr

ORIGINAL ARTICLE

Investigation into Regeneration Effects of Ethyl acetate and N-butanol Fractions through the Evaluation of NGF Gene Expression

Fereshteh Naderiallaf[1], Maryam Tehranipour[*2], Farnaz Soheily[3]

[1]*MSc in Cell and Developmental Biology, Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran*

[2]*Department of Biology, Ma.C., Islamic Azad University, Mashhad, Iran*

[3]*MSc in Animal Physiology, Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran*

Received: 12 January 2024 Accepted: 10 March 2025)

KEYWORDS

NGF gene;
Sciatic nerve regeneration;
Compression;
Echinacea purpurea

ABSTRACT: Neural Growth Factor (NGF) is important for the maintenance and survival of *sympathetic* nerve cells; it also stimulates Schwann cell migration and reduces degeneration, enhances healing after environmental damage, and increases angiogenesis. *Echinacea purpurea* is reported to have antioxidant and anti-inflammatory effects. This study examined NGF gene expression changes after sciatic nerve compression in rats, in the presence of *Echinacea purpurea* extract. After *Echinacea purpurea* Soxhlet hydroalcoholic extraction, different fractions were prepared from it. 36 male Wistar rats were randomly divided into 6 groups including control, compression and treatment A (compression + hydroalcoholic extract in dose of 75 mg kg^{-1}), treatment B (compression + ethyl acetate fraction in dose of 75 mg kg^{-1}), Treatments C (compression + N butanol fraction in dose of 75 mg kg^{-1}) and D (compression + water phase with dose of 75 mg kg^{-1}), In these groups, the sciatic nerve right leg was compressed. The extract was injected intraperitoneally on two occasions. A biopsy was taken from the spinal cord segments L4-L6 on day 28, and finally, NGF gene expression changes were analyzed by ANOVA and t-test software ($p<0.05$). Results indicate that comparing gene expression between control and compression groups and the compression group showed a significant difference with the hydroalcoholic and N butanol groups and the aqueous phase ($p=0.000$). However, there was no significant difference between the compression group and the ethyl acetate group ($p=0.6$). Results show that *Echinacea purpurea* hydroalcoholic extract improves nerve regeneration via a significant increase in NGF gene expression.

INTRODUCTION

Research has shown that cutting the sciatic nerve reduces the number of neurons in the posterior root ganglion DRG (Dorsal Root Ganglion) of the spinal cord through optic death. After the lesion, the expression of the genes of neurotrophins, neurotransmitters, and their receptors changes, which is detrimental to neuronal repair. The family of neurotrophins includes BDNF (Brain-Derived-Neurotrophin), NGF (Nerve Growth Factor), and NT3 (Neurotrophic Factor 3) the most important growth factors in the nervous system are those that play an

*Corresponding author: maryam_tehranipour@iau.ac.ir (M. Tehranipour)
DOI: 10.60829/jchr.2025.1241943

important role in growth, repair, and regeneration, and ultimately the survival of neural tissue. These proteins play their role through two specific types of Trks receptors (Tropomyosin-related kinase receptors) (TrkA, TrkB, TrkC) and the general p75NTR (Neurotrophin P75 receptor). Neurotrophins are activated by intracellular processing through PC family enzymes (Proprotein Converteses enzymes) (furin, PC1/PC3, PC7/LPC, PACE4, PC5, PC4, PC2). Due to the extent of disability caused by spinal cord injuries and the increasing number of sufferers, many efforts have been made to repair this damage. In some cases, gene therapy and the introduction of effective genes into repairs, such as NGF, BDNF, and NT3, have been used in differentiated cells such as fibroblasts or stem cells. Expression of genes associated with axon growth, axon transport mechanisms, access to growth factors, extracellular matrix production, cell binding molecules, cytokine activity, and glial penetration all influence axon repair [1]. Renal tissue repair and regeneration directly affect the quality of life. Although much experience has been gained in the field of nerve repair, it does not seem to be sufficient or appropriate, and scientists are always looking for new ways to minimize the effects of the lesion and rebuild the damaged limb. In mammals of the neurotrophin family, it consists of four factors: BDNF, NGF, NT3, and NT-4/5 [2,3]. Other neurotrophins, such as NT7 and NT6, are known to be limited to fish and are not found in mammals. One of the members of this group is NGF, which is a specific factor for the survival of neurons. From the neurotrophin gene, a series of structural proteins are coded as precursors that are secreted into the extracellular space after processing in the form of a dimer [4,5]. This proteolytic failure occurs at specific sites and by the protease enzyme convertase enzymes (PCs) that are essential for neurotrophin function [2]. Unlike the central nervous system, peripheral nerve fibers can regenerate and innervate distal targets. Today, cell-dependent therapies, as well as molecules that affect nerve growth (neurotrophins), appear to have better results because they are based on strengthening the natural repair of nerve tissue. Cutting the sciatic nerve, which destroys nerve cells and destroys astrocytes, can lead to severe depletion of oxygen and nutrients needed by nerve cells at the site of injury. At this time astrocytes surrounding the healthy affected area should be the role neurotrophic intensified their work and, in addition, should majors cut distal (off-axis) with the help of macrophages to clean up space for the growth of the field proximal attached to the shaft be provided. Astrocytes exert their effect on the affected area by the molecules they produce. These molecules are called general neurotrophins, and we're looking at NGF, which belongs to the same family. How often this gene is expressed after injury and when it is reduced can greatly help in the treatment of neurodegenerative diseases and the timing of the use of neurotrophin-derived drugs. It is also possible to reconstruct the nerve by using gene therapy techniques and transferring genes that are effective in repairing nerves such as NT3, NGF, and BDNF within cells such as fibroblasts or undifferentiated stem cells [6]. Despite numerous reports confirming the neuroprotective capacity of many natural and synthetic chemical compounds, such as hormones and pharmacological drugs, however, due to their unwanted side effects, extensive efforts are being made to investigate the effects of neural protection on new substances such as herbal medicines. The history of treating diseases with medicinal plants is as old as the history of human life on the planet, and man treats himself with the help of medicinal plants. Humans are not only treated with chemical drugs, but all natural factors play a role in treatment, and medicine ultimately plays a preventive role against disease. The existence of plants in nature is one of the great blessings of God. Unlike chemical drugs, herbs do not have side effects, and their effects on the human body are far greater than those of chemical drugs. The inflorescence is more of a flowering plant with a medicinal use of the genus Echinacea in the chicory or Asteraceae family and is native to eastern North America and is now found wild in many parts of the eastern, southeastern, and central United States. The origin of all species of rosemary is North America, and this plant is of great importance to the natives of these regions [7]. This plant was first brought to Iran in 1993 by Dr. Reza Omidbeigi and was named after *Echinacea purpurea* by Dr. Seyed Mohammad Fakhr Tabatabai [7]. Because the extract of some plants can speed up the healing process or reduce the severity of the destruction of the nervous system herbal remedies usually have fewer

side effects, and due to the many properties of the herb, this study tries to examine its possible effects on the nervous system. In this study, changes in the expression of NGF gene in the spinal cord related to the sciatic nerve, in mice treated with aqueous, alcoholic, and hydroalcoholic extracts of redheads and untreated control mice were investigated.

MATERIALS AND METHODS

This experimental research was conducted in 2017 in the Laboratory of Animal Physiology Research in the Department of Biology. The study used 36 Vistar male rats weighing about 250-200 kg, with an approximate age of three months. They were purchased from the animal department of Mashhad University of Medical Science. Until the animals were tested in standard daylight conditions and at a temperature of 22 to 24. C, they were kept in the animal room of the Faculty of Science of the Islamic Azad University, Mashhad Branch. The water required for the animals from the city's drinking water and their food also had a standard formula and was provided by the Javaneh Khorasan Company. Sampling and preparation of the plant were done in the greenhouse of Ferdowsi University of Mashhad and the herbarium code of the plant is 45950. *Echinacea purpurea* hydroalcoholic extract was prepared by the Souxleh method [8] and then different fractions were prepared from it. 36 Roster male rats were randomly assigned to 6 groups including control groups, compression, treatment A (compression + hydroalcoholic extract with a dose of 75 mg kg^{-1}), and treatment B (compression + ethyl acetate fraction with a dose of 75 mg kg^{-1}). Treatment C (compression + fractionation butanol with a dose of 75 mg kg^{-1}) and treatment D (compression + blue phase with a dose of 75 mg kg^{-1}) were divided. In the control group, the muscle in the sciatic nerve was split without injury. In the compression and treatment groups, the sciatic nerve of the right leg was compressed. The extract was injected intraperitoneally twice. The first injection was given immediately after compression and the second injection was given a week later [9]. On day 28, the rats underwent perforation and had their L4-L6 spinal cord parts sampled. RNA (Ribonucleic acid) was extracted from the samples. Extraction of RNA was performed using an extraction kit, prepared by Dena Bio. RNA was first administered to determine quantity and quality, nanodrop, and electrophoresis. After performing the RNA extraction steps, the purity of the extracted RNA was determined using a nanoparticle spectrophotometer. It was then made from cDNA (Complementary Deoxyribonucleic Acid) extracted from RNA. For synthesis, cDNA was synthesized using a Pars Toos synthesis kit and according to the RNA concentration of nano-therapy in the amount of 0. 5 to 1 microgram of RNA according to the instructions of the cDNA kit. Then, the Real-Time Polymerase Chain Reaction reaction was synthesized using the QIAGEN model real-time machine with a value of 3 µl of cDNA and using the Real-Time PCR kit. Samples were performed in triplicate for a dedicated primer as well as for housekeeping priming. The desire for gene coupling is to compare gene expression as a control. Then, Minitab software was used for the statistical analysis of data. GAPDH primers (Glyceraldehyde-3-phosphate dehydrogenase) related to GAPDHH rat gene cusping genes were also designed and purchased to evaluate, compare and measure cDNA quality and use it as a standard. Primers were designed using Prim3 software and then installed on the NCBI site to ensure proper BLAST connection. For each main sample, a sample containing GAPDH primer as well as NGF-specific gene primer was examined. The coincidence of the primers used and also the best annealing temperature for each pair of primers that are determined by the melting temperature of the primer based on the type of nucleotides are described in Table 1.

Table 1. The sequence of primers used to study the expression of the NGF gene

	Primer Sequence	TM
NGF	Forward**:** GTGTGTGGGTTGGAGATAAG	63 ºC
NGF	Reverse: CTTATCTCCAACCACACAC	63 ºC
GAPDH	Forward: TGCTGGTGCTGAGTATGTCG	60 ºC
GAPDH	Reverse: GCATGTCAGATCCACAACGG	60 ºC

NGF: Neural Growth Factor, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

RESULTS

Using a spectrophotometer and nanodrop device, the quality of RNA and its correct extraction were ensured. Then, by comparing the quality of the superior samples, cDNA was selected for synthesis. Table 2 shows the results of nano-therapy. The device automatically calculates the RNA concentration using light absorption at 260 nm. After preparing the sample and placing it in the sample location, the device calculates the concentration of the sample at the corresponding wavelength in nanograms per microliter [10].

Table 2. RNA concentration extracted using nanodrop device.

Group	RNA concentration (µg μL^{-1})
Control	2.533
Compression	2.755
Compression + hydroalcoholic extract with a dose of 75 mg	4.384
Compression + Ethyl acetate fraction with a dose of 75 mg	2.844
Compression + N-butanol fraction with a dose of 75 mg	2.63
Compression + blue phase with a dose of 75 mg	2.977

The extracted RNA samples were prepared to check their quality on the electrophoresis gel. To perform the electrophoresis operation, 1.5% agarose gel (along with ethidium bromide) was first prepared. Then 2 microliters of RNA extracted with buffer loading were poured into gel wells. The gel was rubbed into the electrophoresis device for 40 minutes at a voltage of 100-90. The gel was then photographed with a UV Tec [11]. The existing RNA columns are shown separately in 3 bands. Figure 1 shows the extracted RNA samples on Agars 1.5% gel. As can be seen in the Figure 1, some of the columns show better quality with three distinct bands. In repetitions used, suitable samples were used for cDNA synthesis. Following the RT-PCR (Reverse Transcription Polymerase chain reaction) reaction, which was performed in triplets by the real-time QIAGEN device, the gene expression chart was plotted.

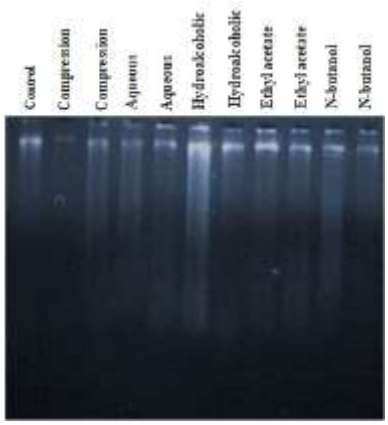

Figure 1. The figure shows the RNA samples extracted from Agarose gel 1. 5%. From left to right, column 1 of control, columns 2 and 3 of compression, columns 4 and 5 of aqueous, columns 6 and 7 of hydroalcoholic, columns 8 and 9 of ethyl acetate, and columns 10 and 11 of N-butanol.

According to Figure 2, the horizontal axis of the sample type and the vertical axis show the percentage of gene expression of control samples, compression, and treatment with aqueous, alcoholic, and hydroalcoholic extracts of *Echinacea purpurea* for 28 days. According to the chart, the expression of the NGF gene shows a significant difference after 28 days in the compression group compared to the hydroalcoholic and butanol groups and the blue phase (P = 0.000), while the expression of the NGF gene Comparing the compression group with the ethyl acetate group does not show a significant difference (P = 0.6). Data analysis showed that the intensity of NGF gene expression in the hydroalcoholic treatment group increased significantly compared to the compression group (P = 0.000). The highest and most significant gene expression is related to the hydroalcoholic treatment group on the 28th.

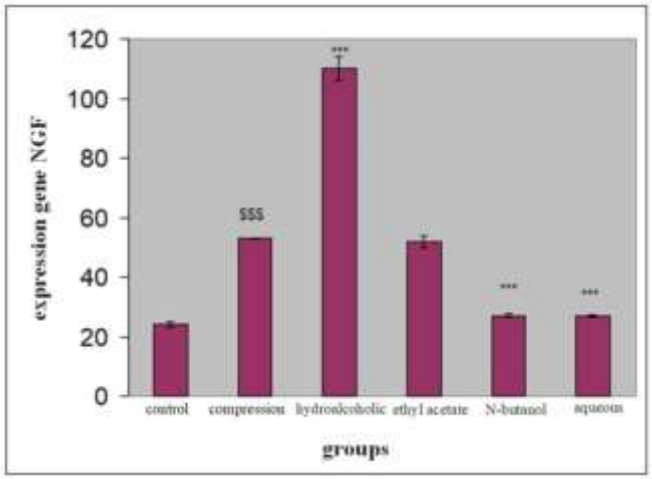

Figure 2. Comparison of gene expression intensity in sciatic nerve between control groups, compression and aqueous, alcoholic, and hydroalcoholic extracts of *Echinacea purpurea* (dose 75 mg kg^{-1}) on day 28
$$$: Comparison of gene expression between the control group and compression group.
***: Comparison of gene expression intensity between compression group and treatment groups shows.

Histological results

The images prepared from spinal cord slices were used to investigate the results of the research on the spinal cord alpha in the right half of the anterior horn. The sciatic nerve compression causes central degeneration and degradation of the alpha-mutated cells of the anterior spinal cord. The cellular body is in the form of an ellipse or triangular. Also, the core changes its central position in the cell environment (Figures 3 and 4). Figure 5 shows the injection of extracts with (a dose of 75 mg kg^{-1}) after the sciatic nerve complex causes regeneration changes in the axon. Following the regeneration changes in the axon, in some groups, such as the hydroalcoholic group of the cellular body, cell inflation is low.

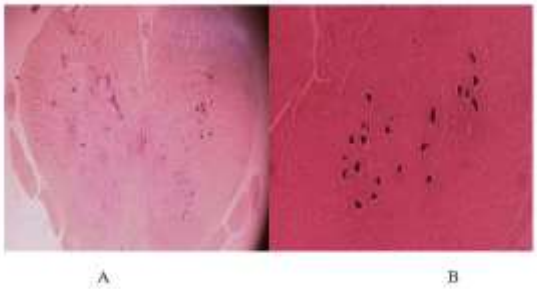

Figure 3. Cutting of spinal cord width in the control group, A: (200X magnitude), B: anterior spinal cord (400x magnitude), the blue coloring of toluidine

A B

Figure 4. Cutting of spinal cord width in the group of compression, A: (200X magnitude), B: anterior spinal cord (400x magnitude), the blue coloring of toluidine

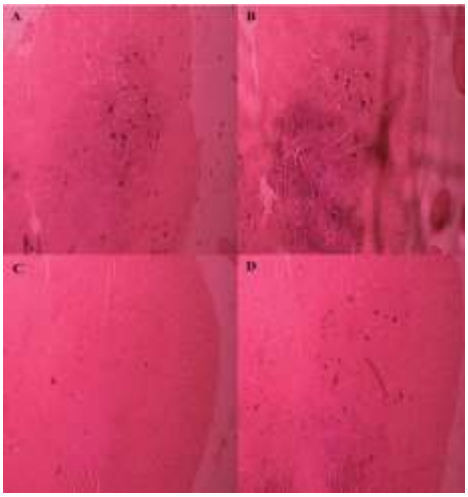

Figure 5. Cutting the spinal cord in treated groups (200X coarse). blue coloring of toluidine.

A: (Compression + Treatment with a dose of 75 mg kg^{-1} of hydroalcoholic) B: (Compression + Treatment with a dose of 75 mg kg^{-1} of Ethyl acetate) C :(Compression + treatment with a dose of 75 mg kg^{-1} of N_butanol) D: (Compression + treatment with a dose of 75 mg kg^{-1} of the aqueous phase)

DISCUSSION

The expression of the NGF gene after 28 days in the compression group showed a significant difference compared to the hydroalcoholic and butanol groups and the blue phase (P = 0.000), while the expression of the NGF gene compared to the group Compression does not show a significant difference with ethyl acetate group (P = 0.6). Based on the results of this study and data analysis, hydroalcoholic extract rats on the 28th day had a significant increase in expression of NGF gene compared to compression rats in their subgroup (P = 0.000). These data indicate the stimulant effects of echinacea extract on the expression of NGF gene in the neurotrophin family. Often after the sciatic nerve injury, the repair process begins, and the root ganglion, axons, and functional connections are rebuilt, with molecular mechanisms playing an important role in the neurotic repair process. Many types of cell-to-cell and cell-to-cell molecules are involved in the external matrix, including immunoglobulin, integrin, and cadherin, during nerve repair and development. These binding molecules play an important role in regulating the elongation of axons [12]. Cytokines support macrophages, lymphocytes, mast cells, and Schwann cells [13]. Cytokine interleukin 1 (IL-1) accelerates the repair of peripheral nerves by regulating the expression of growth factors. This increase in NGF is associated with an influx of macrophages [13]. Research shows that in normal adult mice, about half of the sensory lumbar neurons have high-affinity receptors for NGF. One month after the sciatic nerve is amputated, the average number of sites with high hypersensitivity to the neurons marked in the fifth lumbar vertebral ganglion reaches less than 20% normal, which is reduced due to receptor density and decreased cell volume [14], therefore, the use of substances that can increase the

expression of NGF genes, such as echinacea extract, may be effective in accelerating the recovery process. NGF is a specific factor for the survival of neurons. NGF accelerates repair and promotes the development of lateral branches of damaged sensory axons and increases the number of myelinated axons. Following the cessation of the axon, Schwann cells begin to produce NGF. NGF stimulates the migration of Schwann cells and reduces degeneration and increases practical repair after peripheral nerve damage and increases angiogenesis. Prescribing NGF increases the number of myelinated axons, myelin thickness, and maturation of most endothelial layers [15]. The results of our study also showed that in the group receiving hydroalcoholic extracts, the expression of NGF gene increased and the acceleration of repair in their motor functions was evident. Other research suggests that NGF accelerates the repair of motor neurons. Under physiological conditions, the effects of NGF depend on the growth stage of the neurons. During the normal course of neuronal cell death, NGF regulates the survival rate of a specific population of neurons. NGF, for example, regulates the activity and synthesis of enzymes involved in neurotransmitter synthesis and neuropeptide synthesis. NGF prevents the breakdown of cholinergic neurons in the anterior cerebral cortex after axon removal and improves the behavioral function of damaged memory in older mice. NGF can also prevent neuronal degeneration after axon removal in the peripheral nervous system [16]. The presence of cellular messenger molecules and neurotrophic factors similar to those involved in anti-inflammatory responses play a role in the phenomenon of damage and repair. Neurotrophic factors such as NGF neuronal growth factor are other factors involved in this phenomenon [17]. According to the above reports, the results of the present study show that the administration of hydroalcoholic extract of *Echinacea purpurea* to mice with existing nerve crushing produces increased neurotrophic factors, including NGF nerve growth factor. Based on the results of this research and data analysis and comparison between control groups, compression, and treatment with aqueous, alcoholic, and hydroalcoholic extracts of *Echinacea purpurea* on the 28th day, the following results have been obtained. The expression of the NGF gene after 28 days in the compression group showed a significant difference compared to the hydroalcoholic and butanol groups and the blue phase (P = 0.000), however, the expression of NGF gene does not show a significant difference between the compression group and ethyl acetate group (P = 0.6). Data analysis showed that the intensity of NGF gene expression in the hydroalcoholic treatment group increased significantly compared to the compression group (P = 0.000). The highest and most significant gene expression is related to the hydroalcoholic treatment group on the 28th. In the process of nerve compression, inflammatory processes are activated, causing a harmful chemical environment and further damage [18]. The hydroalcoholic extract of *Echinacea purpurea* may prevent them from progressing with its anti-inflammatory effects. In the present study, the hydroalcoholic treatment group slowed the progression of the lesion compared with ethyl acetate, n-butanol, and aqueous extract. According to research by Nathan et al., Denaturation of proteins is one of the main causes of inflammation [19]. The chemical components of the plant's family species (echinacea) include lipophilic parts (such as alkaloids and polyacetylene), water-soluble polysaccharides, and caffeic acid derivatives such as echinacoside, chicory acid, and caffeic acid, and flavonoids. Caffeic acid is the main ingredient in many medicinal plants, including echinacea. The plant's first unique compound is echinacea, which contains caffeic acid, a derivative of caffeic acid, glucose, and rhamnose, all of which are attached to the central glucose molecule. Echinacea accumulates in the roots but is present in lower concentrations in flowers. Other caffeic acid derivatives with pharmacological effects include chicory acid, chlorogenic acid, and cinnarizine [20]. Studies have shown that echinacea stimulates the immune system with anti-inflammatory, anti-viral, and antibacterial effects. Echinacea polysaccharides have the property of stimulating the immune system and its polyacetylene has an anti-inflammatory effect. In animal studies, polysaccharide injectable polysaccharides induce tissue regeneration, and polyethylene has an anti-inflammatory effect [21]. Therefore, due to its anti-inflammatory and restorative effects and stimulation of the immune system, the extracts of *Echinacea purpurea* extract may have delayed the degeneration of neurons in the anterior spinal cord or have led to the development of

restorative processes. When nerve tissue is damaged, environmental conditions go in a direction that further destroys the nervous system. If the immune system is stimulated by any means, the repair process will be faster. *Echinacea purpurea* with its immune-stimulating properties has been able to improve the repair process. Studies show the analgesic and sedative effects of rosemary extract. These effects are very effective in repair processes, so these effects can also be effective in the role of neuronal protection of this plant. Gordon et al. Showed that in destroying the neural structure, the muscles being destroyed in the neural tissue negatively affect the functional outcomes. As a result, efforts are being made to restore the nerve as quickly as possible [22]. A comparison of data on the expression of NGF gene showed that the expression of this gene in the treatment group with hydroalcoholic extract has a significant increase compared to the compression group ($p<0.001$). The high restorative effect of this extract is due to the increased expression of NGF gene expression in neural tissue because this gene causes the growth of nerve cells, which naturally increases during nerve tissue damage, in the compression group compared to the group. Control also has a significant increase. Comparing gene expression in the N-butanol and ethyl acetate groups and the blue phase compared to the reduction group, it can be seen that this may be due to the presence of specific compounds in these extracts that can determine the effective components of each fraction. Therefore, another effective mechanism by which the hydroalcoholic extract of *Echinacea purpurea* can prevent cell death after sciatic nerve compression is the reduction of inflammation at the site of injury, and probably because of this, gene expression has increased significantly as time goes on. Most of the time, the amount of restoration and effectiveness of the extract have increased. Another solution is to use medications and substances that can increase the level of neurotrophic factors. Among these, we chose the *Echinacea purpurea* plant. The extract of this plant was used to evaluate the changes in gene expression so that the active ingredients in the red plant, both in water and in the alcoholic environment, could be easily dissolved and removed. Perhaps the effect of rosemary is applied by stimulating the increase in the production of Schwann cells during nerve damage. Schwann cells themselves increase the expression of NGF gene. It probably contains effective molecules that trigger cascading reactions that lead to more gene expression, preventing more neurons from dying, and speeding up the regeneration process.

CONCLUSIONS

The results of NGF gene expression confirm that *Echinacea purpurea* hydroalcoholic extract has nerve regeneration effects among the fractions, and butanol has very similar effects to hydroalcoholic extract, which indicates the presence of effective compounds in *Echinacea purpurea* including alkaloids, combined alkaloids. Caffeic and its derivatives, such as chicory acid, are in this fraction. Therefore, because of its anti-inflammatory and analgesic effects and stimulation of the immune system due to the presence of polyethylene and caffeic acid, the antioxidant effect of which has been identified, *Echinacea purpurea* can improve the repair processes of neural tissue.

ACKNOWLEDGEMENTS

We gratefully acknowledge the contributions of the data collection team and the individuals who participated in this study.

Conflict of interests

The authors have no conflict of interest to disclose

REFERENCES

1. Thanos P.K., Okajima S., Terzis J.K., 1998. Ultrastructure and cellular biology of nerve regeneration. J Reconstr Microsurg. United States. 14(4), 23–36.

2. Marandi M., Mowla S.J., Tavallaei M., Yaghoobi M.M., Jafarnejad S.M., 2007. Proprotein convertases 1 and 2 (PC1 and PC2) are expressed in neurally differentiated rat bone marrow stromal stem cells (BMSCs). Neurosci Lett. 42, 198–203.

3. Sieck G.C., Mantilla C.B., 2009. Role of neurotrophins in recovery of phrenic motor function following spinal cord injury. Respir Physiol Neurobiol. Elsevier. 16(9), 218–25.

4. Montazeri F., Esmaeili A., Miroliaei I., Moshtaghian S.J., 2011. Dual Role, Interactions and Signaling

Pathways of p75 NTR in the Nervous System. Genetics in the 3rd Millennium.

5. Unsain N., Nuñez N., Anastasía A., Mascó D.H., 2008. Status epilepticus induces a TrkB to p75 neurotrophin receptor switch and increases brain-derived neurotrophic factor interaction with p75 neurotrophin receptor: an initial event in neuronal injury induction. Neuroscience. Elsevier. 15(4), 978–93.

6. Nazemieh H., Razavi SM., Asnaashari S., Talebpour AH., Ghahramani MA., Imani Y., 2009. Chemical composition of the essential oil of Nepeta menthoides Boiss & Buhse. Pharmaceutical Sciences. 14(4), 283-289.

7. Taghizadeh M., Jarvandi S., Yasa N., 2002. A review of Echinacea. J Med Plants. Journal of Medicinal Plants. 1,13–26.

8. Sc FMMM., D KJP., D TMP., Rasouli B., 2013. The Neuroprotective Effects of Hydroalcoholic Extract of Nigella Sativa on Alpha Motoneurons Degeneration After Sciatic Nerve Injury in Rats. Arak Med Univ J. 16, 79–86.

9. Tehranipour M., Ghadamyari T., 2010. The effects of root aquatic extract of Salvia staminea on neuronal density of alpha motoneurons in spinal cord anterior horn after sciatic nerve compression in rats. J Biol Sci. ANSInet, Asian Network for Scientific Information. 10, 48–52.

10. MrNasir F., Aghayi H.N.M., 2013. The role of lipocalin 2 molecule in processing damage and restoration sciatica nerveo. Sci Mag Med Univ Islam Repub Iran. 10, 198–206.

11. Naderi Allaf F., Tehranipour M., Nejad Shahrokh Abadi K., 2017. Investigation into Regeneration Mechanism of Hydroalcoholic Lavender (*Lavandula officianalis*) Extract through the Evaluation of NT3 Gene Expression after Sciatic Nerve Compression in Rats. J Arak Univ Med Sci. 20, 100–120.

12. Moro C., Palacios I., Lozano M., D'Arrigo M., Guillamón E., Villares A., 2012. Anti-inflammatory activity of methanolic extracts from edible mushrooms in LPS activated RAW 264.7 macrophages. Food Chem. Elsevier. 130, 350–5.

13. Ferrer I., Planas A.M., 2003. Signaling of cell death and cell survival following focal cerebral ischemia: life and death struggle in the penumbra. J Neuropathol Exp Neurol. 62, 329–39.

14. Barros L., Venturini BA., Baptista P., Estevinho L.M., Ferreira I.C.F.R., 2008. Chemical composition and biological properties of Portuguese wild mushrooms: a comprehensive study. J Agric Food Chem. 56–62.

15. Pan H., Hu X., Jacobowitz DM., Chen C., McDonough J., Van Shura K., 2012. Alpha-linolenic acid is a potent neuroprotective agent against soman-induced neuropathology. Neurotoxicology. Elsevie. 33(12), 19–29.

16. Jamalpoor Z., Asgari AR., Nourani MR., 2012. Skeletal muscle tissue engineering: Present and future. Journal Mil Med. 14, 77–84.

17. Fu L., Doreswamy V., Prakash R., 2014. The biochemical pathways of central nervous system neural degeneration in niacin deficiency. Neural Regen Res. 9, 15-19.

18. Sumathi T., Christinal J., 2016. Neuroprotective effect of Portulaca oleraceae ethanolic extract ameliorates methylmercury induced cognitive dysfunction and oxidative stress in cerebellum and cortex of rat brain. Biol Trace Elem Res. Springer. 17(2), 155–65.

19. Nathan C., 2002. Points of control in inflammation. Nature. Nature Publishing Group. 420, 846–52.

20. Bone K., Phyto D., 1997. Echinacea: When should it be used. Alt Med Rev. 2, 451–458.

21. Bauer R., Chemistry., 1997. analysis and immunological investigations of Echinacea phytopharmaceuticals. Immunomodulatory agents from plants. Springer. 41–88.

22. Gordon T., Tyreman N., Raji M.A., 2011. The basis for diminished functional recovery after delayed peripheral nerve repair. J Neurosci. 31(53), 25–34.