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Research Article

Anti-Inflammatory Effect of Artemia Protein Extract on The Lps-Induced Primary Rat Astrocyte Cells as A Model of Alzheimer's Disease

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Abstract:

Background: Artemia is a crustacean brine shrimp typically in inland saltwater lakes. Artemia can tolerate extremely high temperatures or low-oxygen waters. So, the function of some proteins of this organism had shown new functions been investigated, most notably Artemin and P26.

Objectives: In this study, the effect of Artemia Extracted Proteins was evaluated on the Function of the cellular Alzheimer's disease model using LPS-induced inflammation Astrocyte cells.

Materials and Methods: Artemia cysts were hatched and the protein content was extracted. Semi-purification of the protein content was performed using Ion exchange chromatography. On the other hand, astrocyte cells were isolated from Infant rats' brains according to protocols. The Alzheimer disease protective effect of isolated protein portion was evaluated in, 1, 2.5, 5, 7.5, 10 µg/ml concentrations on LPS-induced inflammation astrocytes using cell cytotoxicity and NO generation assay according to MTT assay and Griess test. The iNOS gene expression was evaluated using RT-PCR as the key signal of cellular NO generation.

Results: The results showed that among the different isolated protein fractions, 1, 2, 3, and 6 fractions had no toxic effect on astrocytes and played an important role in reducing the inflammation of these cells, so could evaluated as potent proteins for reducing the progression of Alzheimer's disease.

Conclusion: As known that Astrocytes inflammation contributes to the progression of Alzheimer's disease, mitigating the inflammation of the astrocytes by Artemia extracted proteins could be evaluated as potent therapeutic agents to improve and suspend the progress course of Alzheimer's disease.

Key words: artemia proteins; anti-inflammation; no; astrocytes; alzheimer's disease

1.Introduction

Artemia is a crustacean brine shrimp existing in the sea and is considered an excellent target for biochemical and biophysical study due to its good adaptation to environmental stressors [1]. This creature is capable of enduring high salinity, living in various climatic conditions from the desert to tropical and mountainous weather. Artemia cysts live in liquid nitrogen, and a percentage of them can survive at higher temperatures than boiling point. Artemia has 8 bisexual (2n=42) and parthenogenetic (diploid, triploid, tetraploid, pentaploid) species. In the latest research, 595 Artemia-living geographic regions have been reported worldwide. Nowadays, Artemia has valuable usage; in human life as nutrition; in research as a model in genetics laboratories or radiometric studies; in medicine as bio-capsule; in industrial manufacturing in bio-processing of chitin and chitosan, and its use in salt-producing pools has made it an integral part of the aquaculture industry and one of the requirements of this industry [2-6]. Artemia extract has an antiaging effect [7] and it has been applied in cosmetics for skin care [8]. However, the anti-inflammatory effect of artemia extract on the glial cells has not been studied. Alzheimer's disease (AD) is characterized by significant neuronal death throughout the brain, which immediately causes memory impairment and severe disruption in cognitive function. AD is also

characterized by significant reactive astrogliosis and activated microglia. The special role of astrocytes in AD pathogenesis was raised after discovering that astrocytes pose as Aβ and (in particular its shorter form) as Aβ42 natural scavengers. In the AD early stages, neurons commence producing large amounts of Aβ, which endangers dendrites, leading to their degeneration; this condition, however, activates astrocytes encompassing the endangered neuronal domain. Astrocytes eventually accumulate Aβ in excess, which would affect their function and prompt to reduce their support for other neuronal processes. This situation can also lead to subsequent neuronal degeneration and trigger Aβ accumulation in distant areas. Adjacent astrocytes detect and activate Aβ extracellular deposits, and send their projections toward the plagues, to remove additional Aβ. The repetition of this process ultimately involves more astrocytes, and through the astrocytes, the neurons are also engaged, indeed leading to the release of plagues [9-12]. Astrocytes are special glial cells, that occupy about 25 to 50% of the brain's volume, are ostensibly star-shaped, have many projections developing a non-synaptic connection with the neurons, and have perivascular feet covering about 85% of the existing capillaries' surface in the CNS [10, 13]. These cells are the only brain cells that store high-energy glycogen molecules and are connected with gap junctions [14], which could permeability them to ions and other molecules with molecular weights less than 1000 Daltons [15, 16]. At the molecular level, all types of cerebral damage such as neuroinflammation [17, 18] undergo hypertrophy of the astroglia projections and significant increases in astrocytes (reactive astrogliosis) [19-22]. Therefore, astrocyte cells have a major contribution to CNS homeostasis and CNS response to inflammation in Alzheimer's disease progression [18, 22]. Studies particularly targeting astrocytes to attenuate astrogliosis open up new ways to novel therapies for AD [11, 23]. On the other hand, in various pathological conditions, constitute neurodegenerative diseases and neuroinflammation inducible nitric oxide synthase (iNOS) gene overexpress and results in high-level production of nitric oxide (NO). NO is an important inflammatory mediator and therefore NO signaling pathway characterizes a crucial therapeutic target (24-27). So, finding substances that could eliminate inflammation or minimize it as low as possible in these cells would be an effective therapeutic goal with minimal side effects [18].

2.Objectives

In this study, the effect of Artemia partially purified protein fractions on Alzheimer's disease via inhibition of inflammatory signs in a cellular model on the primary rat astrocyte cells.

Materials and Methods

3.1 Artemia Cysts Hatching and Protein Extraction

Artemia urmiana cysts were provided from Urmia Lake (West-North of Iran). 10 gr of cysts were hydrated in tap and/or seawater at room temperature for one hour. Then the cysts were decolorized by Sodium Hypochlorite (NaOCl 5% W/V) until the cysts' color changed from brown to orange. Then the cysts were washed with 500 ml of cold distilled water. The decolorized cysts were hatched in 2 liters of artificial seawater (0.4 M NaCl, 0.009 M KCl, 0.05 M MgCl2, 0.009M CaCl2, and 0.028 M Na2SO4, pH= 8.0) at room temperature and/or 37 ºC for 24 hr. under light chamber in a shaking incubator according to the procedure reported by Liu and Mclennan (Liu and Mclennan, 1994). Freshly hatched nauplius is phototropism, the nauplius was collected by attraction to light. the tube connected to the air pump is putplaced in a loop containing artemiawith Artemia cysts to oxygenatesupply the cysts with oxygen. After passing about 24 hours, at have passed, first, the pump or the filter doingthat does the air conditioningaeration is slowly removed slowly, so that the aeration would be completely cutturned off. It is observed after After a while, observe that the shellsmussels come to the surface of the water and the hatched nauplius orients down to the bottom of the water. In the next step, the shells and unhatched cysts are slowly segregated with a pipette in orderthemselves to calculate the amount of naupliies obtained. Finally, after calculating the weight of the obtained naupliies, they are transferred to a falcon 15 and are frozen. The next steps of the research were as follows:the bottom of the water. The collected nauplius was washed with 400 ml of cold distilled water. Embryos were suspended in 50 ml of PBS, pH=7.4, and centrifuged at 3000 rpm for 20 minutes. 1.0 gr of freshly isolated nauplius was dissolved in 5 ml of extraction buffer (Tris- HCl 50 mM, pH 6.8). Total proteins were extracted by liquid homogenization, high-frequency sound wave sonication, and 3 times freeze/thaw cycles in liquid nitrogen separately. The homogenate was filtered through two layers of Mira cloth into a 50 mL Falcon tube at room temperature. The filtered homogenate was kept at -20°C until protein purification. In all of the experiments, total protein concentration was measured by Bradford methods. Extracting the extract using a homogenizer crushing with buffer A and B. Determining the amount of protein in the extract with Bradford method. Purification of protein

3.2 Protein Purification by salting out method.

Buffer replacement and salt removal of soluble specimens by dialysis method.

Partial separation of the protein components existing in the Artemia raw extract with ion Anion-Exchange Chromatography (type of resin used in chromatography DEAE is Cellulose Fast Flow ion exchange). The protein content of crude extracts was precipitated with the salting out method by using Ammonium Sulfate 40% (W/V) at 4°C on the orbital shaker for 2hr. The supernatant was removed and the precipitate was centrifuged at 5000 rpm for 20 min at 4°C. Then the crude was dissolved in Tris-HCl 50 mM, NaCl 20mM, pH 6.5 buffer dialysis and dialyzed against the same buffer overnight at 4 ºC. Partial separation of the protein was performed using ion exchange chromatography. An 18 × 3 cm column with DE-52 Sepharose beads (Whatman) was used for fast-flow ion exchange chromatography. First, the column was equilibrated d by Tris-HCl 50 mM, NaCl 20 mM pH 6.5 binding buffer. Then the extract after dialysis was eluted 1: 100 with the same buffer. Then gradients of 0.1, 0.2, 0.3, and 1.0 M of NaCl in Tris-HCl 50 mM buffer pH 6.5 were applied. The samples were collected as 2.0 ml fraction by 0.5 ml/min flow rate. SDS-PAGE electrophoresis was performed for each one of the fractions to analyze the protein profile on 13% resolving SDS-PAGE gel using electrophoresis LKB-Pharmacia electrophoresis system (LKB Pharmacia, Uppsala, Sweden). After running; the gels were stained by Coomassie blue. Finally, the fractions were concentrated by freeze-drying and sterilized by filtration through 0.2 µm filter papers to use for further analysis.

3.3 Primary Astrocyte Cell Culture and Treatment

Primary astrocyte cell cultures were obtained from mixed glial cell cultures. To achieve this, with pH = 8.5 and at a temperature of 27-28 °C. Since Artemia cysts need light to hatch, they are lit by a moonlight bulb. At the endapproval of the process, the hoseIran Ethics Committee of the National Institute of Genetic Engineering and Biotechnology (Permit

Number: IR.NIGEB.EC.1395.4.1.C), The Wistar rat infants were decapitated and the brains were dissected under sterile conditions. The meninges and blood vessels of the cortex tissues were carefully taken away, and then mechanically split up in Dulbecco's modified eagle medium (DMEM) (GibcoBRL, Grand Island, NY, USA). Then these cells convey into the flasks containing DMEM complemented with 100 UI/ml penicillin G, 100 µg/ml streptomycin (Merk, Darmstadt, Germany), and 10% of heat-inactivated fetal bovine serum (FBS) (GibcoBRL, Grand Island, NY, USA). Firstly, Mixed glial cultures were acquired and they were maintained at 37ºC in a 5% CO2 incubator (Binder, USA). The cell medium was renewed twice a week. To get pure astrocytes, the mixed glial cultures were trypsinized 3-4 times covering the bottom of the flask after 14 days. Typically, pure astrocytes can be obtained after the fourth passage. 20×103 and 5×104 astrocyte cells were seeded into each well of 96 and 24 well plates, respectively. The cells were incubated with 10% fetal bovine serum (FBS) for 24 hours. After that, Primary cells were pretreated with increasing concentrations (0, 1, 5, 10, 50, and 100 µg/ml) of sterile crude extract and/or partially purified fractions in a fresh medium containing 1% FBS for 1 h before LPS (10 mg.ml-1) was added and then were incubated for 48 h. All assays were examined in three replicates.

3.4 ICC (Immunocytochemical) Staining

Specific immunocytochemical staining was performed using a cell marker to identify astrocytes. Glial Fibrillary Acidic Protein (GFAP) Astrocyte cells were isolated from the brain of newborn rats (according to the method mentioned in cell culture) and cultured in a 96-well plate. Then, they were fixed with paraformaldehyde (Merck) and permeabilized with 2% Triton X100 permeabilizing solution. After adding 10% blocking solution for 45 minutes, a 1/200 dilution of the primary GFAP antibody (Santa Cruz Biotechnology) was added to the cells and they were kept at room temperature for one hour. Then, after treatment with anti-GFAP secondary antibody (Dako Cytomation) for one hour, streptovidine/HRP solution was replaced to an amount that covered the sample and kept at room temperature for one hour. After replacement with DAB-chrom solution (Dakof Cytomation) for 15 minutes, the cells were examined by fluorescence microscopy.

3.5 Cell Viability Assay and NO Assay

In the following step, after the effect of chromatography-obtained fractions on the astrocyte cells, the survival rate of these cells and the level of inflammation were measured by MTT and Griess, respectively. After 48 hrs., each well media was transferred to a new plate for NO assay and immediately was replaced with 100 µL of the medium, then 10 µl (0.5 mg.ml-1) of freshly prepared (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (Sigma, U.S.A.) solution) was added to each well plate. The plate was incubated at 37 ºC for 4 hrs. Finally, MTT solution was removed and 100 µl of MTT lysis solution, DMSO, (Merk, Darmstadt, Germany) was added to each well and incubated overnight. The absorbent was read at 580 nm on a Multiskan RC microplate reader (Labsystems, Finland) NO assay was evaluated in the cell-free media collected above, by a spectrophotometric method based on the Griess reaction (28, 29). The absorbent at 540 nm was measured using an ELISA reader. The nitrite concentration was calculated from a NaNO2 (Sigma) standard curve.

3.6 RNA Extraction and RT-PCR

Cellular mRNA was isolated using the Total RNA Extraction Kit (Gene all, Korea), and complementary DNA (cDNA) was prepared using viva 2–step RT-PCR kits, (vivantis technologies sdn bhd, USA), according to the manufacturers' protocols. Semi-Quantitative PCR was performed using the cDNA, master mix (Ampliqon, Denmark), and specific primers (Cinnagen, Tehran-Iran) in a Bio Rad PCR System with the following conditions: 40 cycles of 50 °C for 2 min, 95 °C for 2 min, 95 °C for 15 s, and 60 °C for 60 s with rat iNOS forward primer: 5′- GAC ATC GAC CAG AAG CTG TC-3′; rat iNOS reverse primer 5′- GGG CTC TGT TGA GGT CTA AAG-3′; rat actin. beta (β)-actin was amplified under the same conditions with forward primer 5′-TGAGAGGGAAATCGTGCGT-3′, and reverse primer 5′-TCATGGATGCCACAGGATTCC-3′ for rat beta-actin.

3.6 Statistical Analysis

SPSS software (IBM SPSS Statistics 16) was used for Statistical comparisons between treatments by One-way ANOVA One-Way Analysis of variance (ANOVA) Values were given as the mean ± Standard Deviation (SD) and analytical variables were compared by using the student's t-test. By convention, an α-level of $p<0.05$ was considered to be statistically significant.

4.Results

4.1 Artemia Cyst Culture

The purity of the cysts after hatching was measured by: The efficiency of the procedure is presented in Table 1.

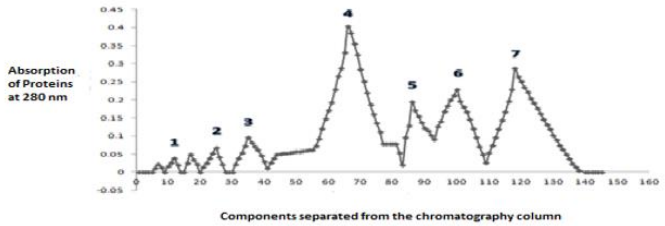
Repetition	Total Cysts	Total decapsulated	Embryo	Umbrella	Nauplii	Cyst Purity (%)
3	83gr	52gr	5.8gr	6.6gr	33.1gr	87.5

Table 1: Calculating the purity of cysts based on the numbers obtained during the research process.

4.2. Gel Electrophoresis of Proteins

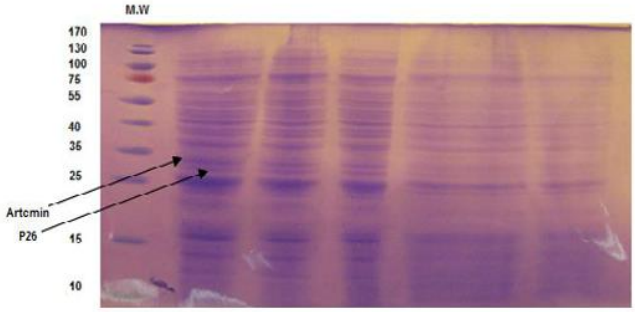
According to the applied method, total protein content was evaluated at about, 14±1 mg per gram of nauplii wet weight. Partial purification was done by DEAE ion exchange chromatography. The samples were plotted at 280 nm continuously indicating the chromatogram of purification (Figure. 1A).

A



The electrophoresis of the crude extract and the purified extract using ion exchange chromatography was performed on SDS-page acrylamide gel (Figure. 1B).

B



As shown the Protein separation pattern was unaffected with more sharpness and most effective separation. The Gel was examined by Alpha Ease FC and it was found that among the different proteins that had been separated along the gel, the two bands were around 26 kd could be assumed to be the main proteins Artemin and P26 that the content of both proteins is higher than other proteins present in the extract matrix related to more clearly visible sharp bands (Figure. 1C).

C

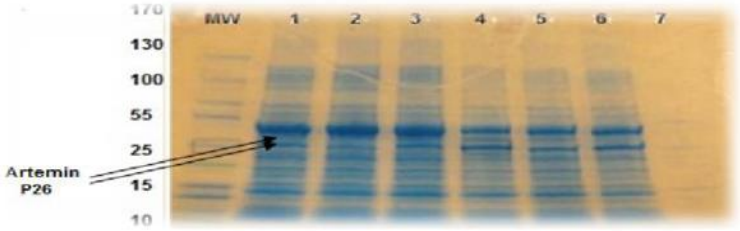


Figure 1: Chromatography and Acrylamide gel of Artemia protein extract. A) A Chromatogram from protein separation of chromatography column, DEAE-Sepharose resin, Flow rate 1ml/min. B) Acrylamide gel from Artemia-obtained protein extract. C) Components separated from the ion exchange chromatography column.

4.3. Astrocyte Cells Separation

After 7 days of the primary mixed glial cell culture, the types of glial cells were carpeting the flask floor. Up to the 14th day, the astrocyte cells were populated and propagated all around the flask floor. To achieve pure astrocyte, trypsin treatment of the glial mixed cells was performed 3-4 times. Typically, one can obtain pure astrocytes after the fourth subculture (Figure. 2).

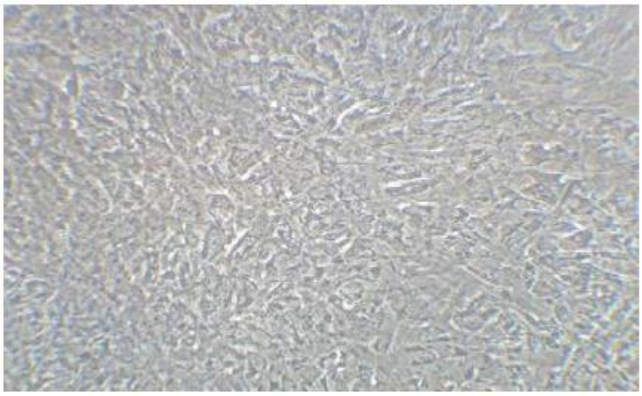
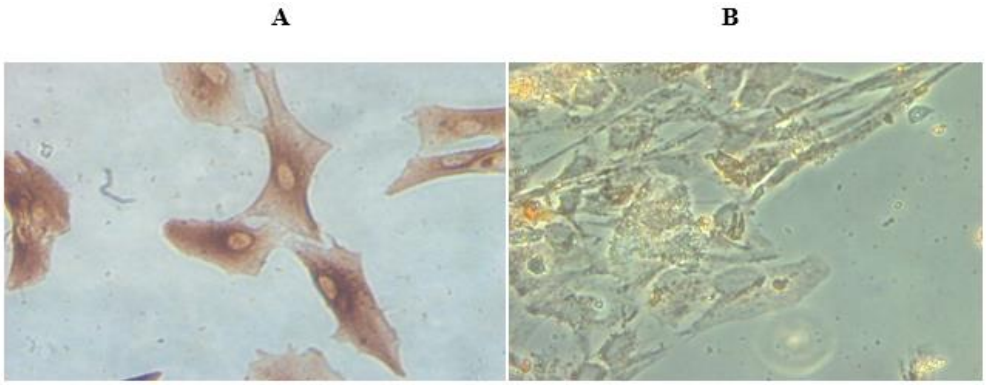


Figure 2: Primary astrocyte cell culture. Pure astrocyte after the fourth passage, observed with antimicrobial microscopy (magnification x75).

4.4. Specific Staining of Astrocytes by ICC

The results of specific immunocytochemical staining of astrocytes isolated from the neonatal rat brain indicate the high purity of the isolated astrocytes (Figure 3).



4.5. MTT test results

Evaluation of Toxicity of Different Concentrations of Artemia-extracted Protein extract was applied on Extract Fractions in the Inflamed Astrocyte Cells.

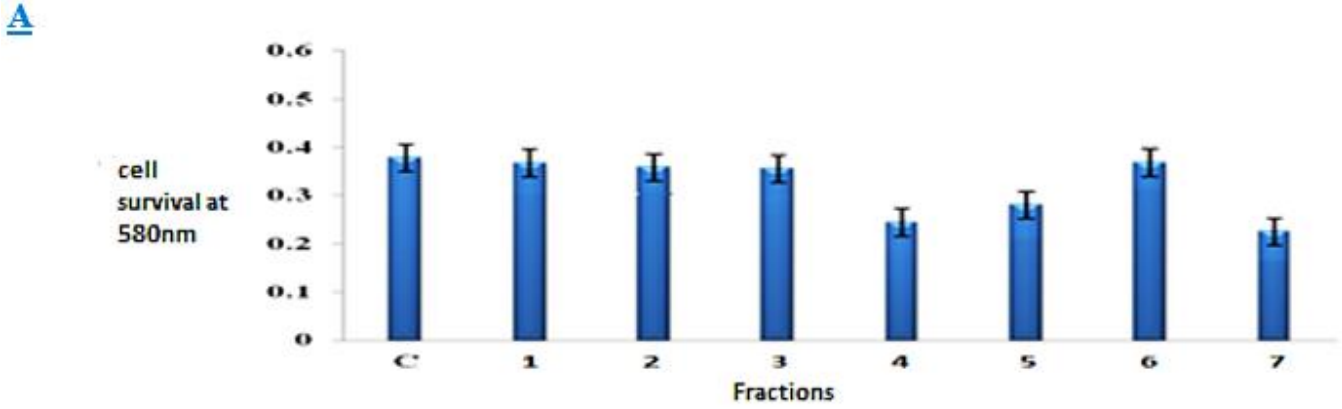
Artemia-extracted protein fractions were applied on the LPS-inflamed astrocyte cells at 1, 2.5, 5, 7.5, and 10 µg/ml concentrations separately. The survival rate of astrocytes was measured using MTT assay after 48 hours.

The results of statistical analysis indicated the effects of fractions in the range of concentrations and their reciprocal effects (fraction × different concentrations of extracted protein extracts) on cell cytotoxicity were significant at P value >0.05 (Table 2).

Variation Sources	Degree of Freedom	Sum of Squares	Mean of Squares	F
Fraction	6	0.683	0.113	10.09**
Different Concentrations of Extract	4	1.225	0.306	27.11**
Fraction× Different Concentrations of Extract	24	0.968	0.040	3.57**
Experimental Error	72	0.813	0.011	-
Total	107	3.691	-	-

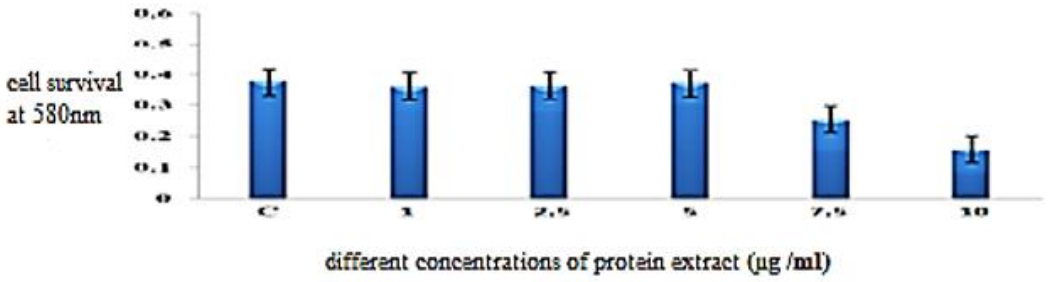
Table 2: MTT assay analysis of variance to evaluate the degree of toxicity produced by the desired protein extract on astrocyte cells (*p value <0.05).

These results exhibit different toxicity values for each fraction and cause differences in the viability rate of astrocytes (Figure. 4A).



The simultaneous application of varying fractions and concentrations of extracted protein extracts had different effects on the survival of astrocyte cells. The mean comparison results showed that Fraction no.6 had the least toxicity effect on astrocyte cells while owning the greatest survival effect on astrocyte cells. Fractions no.7 and 4 also had the highest toxicity effect on astrocyte cells, despite the lowest viable cells and the highest toxicity of both evaluated as 10 µg/ml concentration. The mean comparison results of the different concentrations showed that 5 µg/ml concentration had the lowest toxicity effect and the highest survival effect on cells for these two fractions. Along with this, 10 µg/ml concentration had the highest toxicity and the lowest survival effect on astrocyte cells for both (Figure. 4B).

B



The results of reciprocal effects (Fraction × different concentrations of extracted protein extract) had shown that fraction no.6 and 1, in 2.5 and 5 µg/ml concentrations had the least toxicity effects on astrocyte cells and the highest cell viability were monitored for these fractions (Figure. 4C).

C

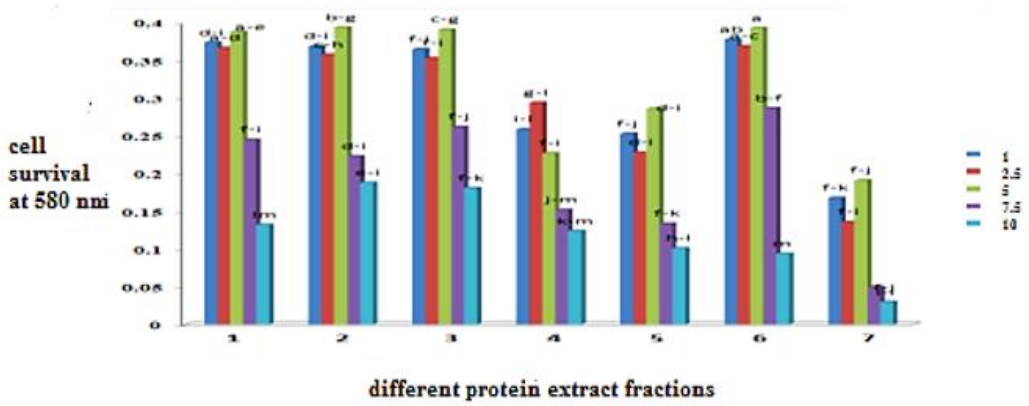


Figure 4: Cell viability measurement of different fractions and concentrations of protein extract. A) Effect of different fractions of the protein extract on the survival of astrocyte cells (* p value <0/05). B) Effect of different concentrations of the protein extract on the survival of astrocyte cells (* p value <0/05). C) Comparison of the mean reciprocal effect of different protein extract fractions at different concentrations of the protein extract on the survival of astrocyte cells.

4.6. Effect of Protein Extract Fractions on the NO Production in Inflamed Astrocyte Cells

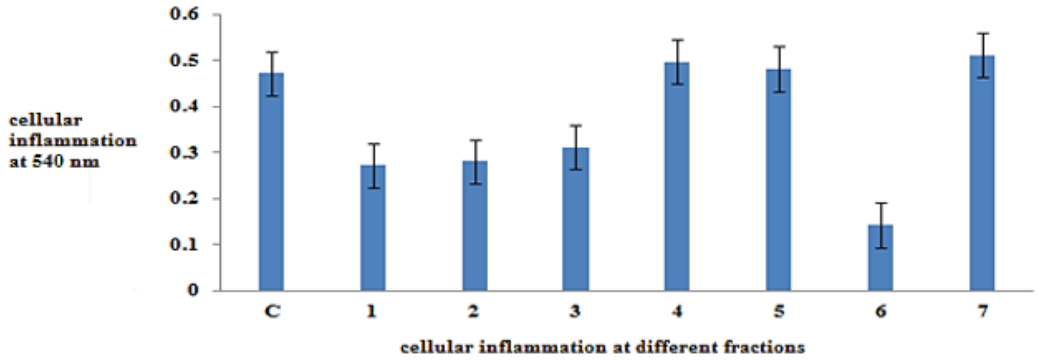
The results indicated that the effects of Fractions, different concentrations of Artemia protein extract, and reciprocal effects (Fraction × different concentrations of protein extract) on the amount of cellular inflammation in astrocyte cells were significantly different at the 1% level (Table 3).

Variation Sources	Degree of Freedom	Sum of Squares	Mean of Squares	F
Fraction	6	0.035	0.005	34.29**
Different Concentrations of Extract	4	0.486	0.121	695.81**
Fraction× Different Concentrations of Extract	24	0.012	0.0005	3.05**
Experimental Error	72	0.012	0.0001	-
Total	107	0.833	-	-

Table 3: Griess test analysis of variance to examine the reduction level of inflammation in astrocyte cells by the protein extract (p-value <0.05).

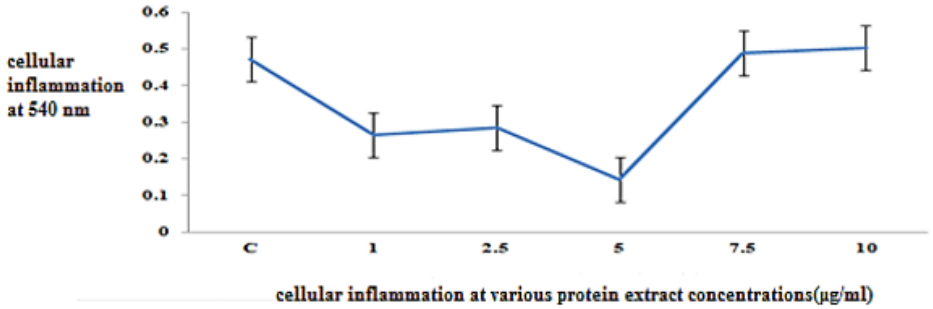
These results reveal that different fractions have different effects on the level of astrocyte cells' cellular inflammation. The results of mean comparison indicated that Fraction no.6 had the highest effect on the level of astrocyte cellular inflammation via generated NO reduction, which significantly reduced inflammation rates compared to other fractions; contrarily Fractions no.7 and 4 had the least effect on the level of astrocyte cellular inflammation (Figure. 5A).

A



The mean comparison results of different concentrations pertained to protein extract over the level of astrocyte cells' inflammation unveiled that the most effect on inflammation rate was observed at concentrations of 1, 2.5, and 5µg/ml leading to its remarkable decrease. Additionally, the lowest effect was seen at 10 µg/ml pharmaceutical concentration (Figure. 5B).

B



So, getting together the results demonstrated that Fraction no.6 in 5µg/ml concentration had the highest effect on cellular inflammation, significantly reducing inflammation via decreasing the NO generation compared to another fraction (Figure 5C).

C

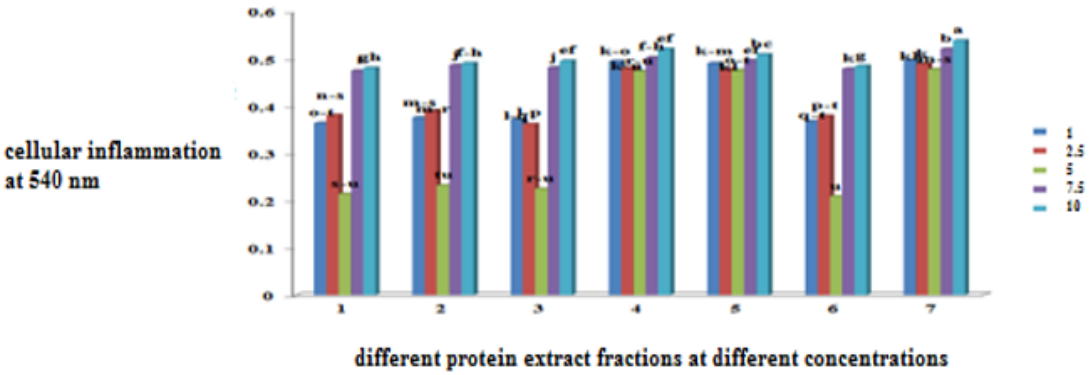
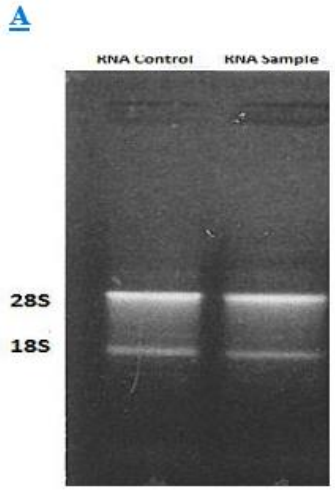


Figure 5: NO production assay of different fractions and concentrations of protein extract on the inflamed astrocyte cells. A) Level of cellular inflammation at different fractions of the protein extract (p-value <0/05). B) Level of cellular inflammation at different protein extract concentrations. C) Comparison of the mean reciprocal effect of different protein extract fractions at different concentrations of the protein extract on the cellular inflammation levels.

4.7. Expression Analysis of iNOS Gene via RT-PCR

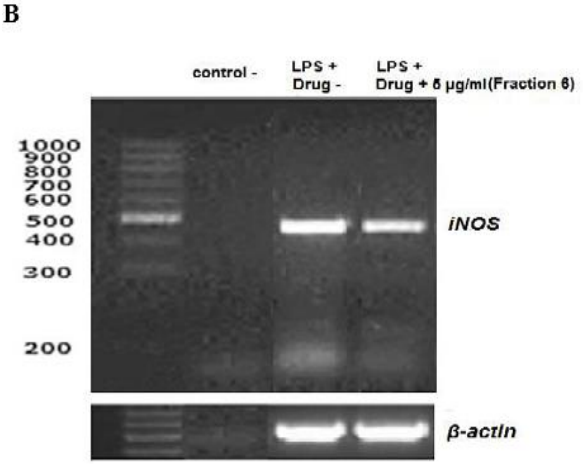
Transcriptional studies were performed using RT-PCR. RNA extraction from the sample was performed after 48 hours (Fig. 6A) and purity of the extracted RNA was measured using nanodrop (Table 4).



SAMPLE	Cdna Concentration Amount	260.280 NM
ASTROCYTE +/LPS +/DRUG –	2523 NG/ML	1.7
ASTROCYTE +/ LPS +/ DRUG +	3302 NG/ML	1.7

Table 4: Measurement of purity of the extracted RNA using nanodrop

After cDNA Synthesis, an RT-PCR reaction was performed using iNOS gene primers, and finally PCR products were taken over the 1.8% agarose gel. In Agarose gels, the molecular weight marker ladder indicates the bands formed by 350 bp correspond to the beta-actin gene (housekeeping gene), and the band in 253 bp corresponds to the iNOS gene, no band was observed in the negative control sample, confirming the absence of contamination in the work. Positive control samples were astrocytic cells exposed to LPS (LPS + cells). The results revealed that Fraction no.6 at a concentration of 5 µg/ml (which had the highest cellular viability and reduced inflammation as previously mentioned) was exerted on inflamed astrocyte cells. As shown in Figure 5B, Fraction no.6 at a concentration of 5 µg/ml significantly reduced the expression of iNOS (Figure. 6B).



Conversion of qualitative bands to semi-quantitative data by Total lab software revealed that expression of iNOS gene in the drug positive group considering beta-actin control group has reduced. (Figure.6C).

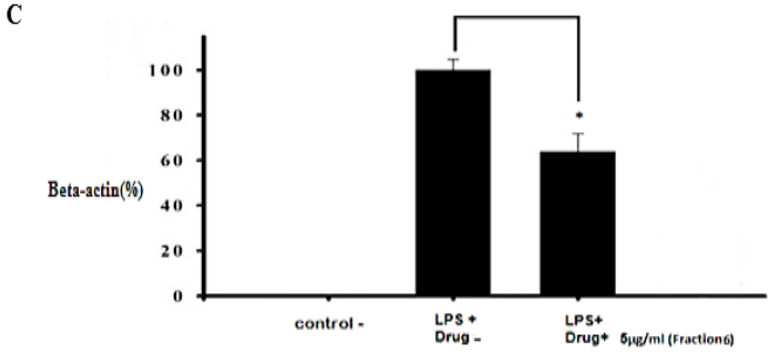


Figure 6: RNA Extraction and analysis of iNOS gene expression. A) RNA Extraction bands on the gel electrophoresis. B) RT-PCR electrophoresis pattern for the iNOS gene. C) Semiquantitative conversion of qualitative bands of iNOS gene using total lab software (* p value <0/05).

Discussion

Astrocytes, indeed, play an important role in controlling largely the creation of synapses, specifying the brain's microstructures and forming neuroglial-vascular units, integrating different cerebral structures and, receiving and releasing trans-mates. Additionally, Astroglia Syncytium connecting each other by gap junctions allows the direct transfer of ions, metabolic agents, and secondary messengers [13, 20] in neurodegenerative diseases such as AD Astrocyte suffering from neuroinflammation [17, 18] and more activated [11, 12]. In such pathological processes, the Expression of the iNOS gene increases in activated astrocytes, and NO as a critical inflammatory factor in neuroinflammation is overproduced [24-27]. Previous studies outlined substances that can suppress or regulate the activity of glial cells stand as ideal choices for a variety of neurodegenerative diseases such as Alzheimer's disease [18-21] especially targeting astrocytes for improving AD [11, 23]. Therefore, seeking the appropriate anti-inflammatory drugs mitigating the activity of glial cells could ultimately lead to the emergence of appropriate therapies against many nervous degeneration conditions. Artemia has a long-standing history of use in aquaculture as a highly nutritious live food source (with high protein content, especially amino acid [30], particularly for the larval stages of various fish and crustacean species. This widespread use in aquaculture implies a degree of biocompatibility and nutritional value that makes Artemia a subject of interest for broader biological applications [31-34]. Furthermore, traditional medicine practices, particularly in regions surrounding hypersaline lakes like Urmia Lake in Iran, have utilized Artemia extract and mud derived from these environments for the treatment of inflammatory conditions and skin diseases. This historical application provides an empirical basis for investigating the potential pharmacological properties of Artemia. Artemia contains proteins from the family of chaperones. They help artemia tolerate environmentally severe conditions, of which P26 shock protein poses the most important one accounting for about 10% of the proteins present in the extract [2, 3]. These organisms exhibit remarkable resilience, capable of surviving in conditions of extreme salinity, low oxygen tension, and significant temperature fluctuations [35]. This adaptability suggests the presence of sophisticated biological mechanisms and potentially unique biomolecules that facilitate survival under such challenging conditions [36]. Consequently, there has been a growing scientific interest in exploring the potential therapeutic applications of materials derived from marine organisms, including Artemia, due to their diverse bioactive properties. Many studies have focused on peptides and other biomaterials extracted from various marine animals, revealing promising anticancer and other pharmacological activities [35]. This increasing attention to marine resources underscores the potential for discovering novel therapeutic agents from organisms adapted to extreme environments. The cosmetic industry has also recognized the potential of Artemia extract, incorporating it into formulations for sunlight protection and anti-aging purposes. These applications likely stem from the antioxidant and potentially anti-inflammatory properties of Artemia-derived compounds, which can help protect the skin from environmental damage [37]. A patent (US20170042802A1) further highlights the cosmetic use of Artemia salina extract to protect the skin from thermal stress, including cold stress, which is also known to induce inflammatory responses. This protective effect against temperature extremes suggests that Artemia extract may contain components that help stabilize cellular functions and prevent the release of inflammatory mediators triggered by temperature fluctuations [8] Studies, such as the one by Vaseli-Hagh et al [7], have demonstrated the anti-aging effects of Artemia proteins on human fibroblasts. Treatment with fractions of Artemia extract resulted in a notable reduction in senescence-associated β-Galactosidase activity, a key marker of cellular aging. Additionally, these fractions induced the synthesis of collagen type I, and promoted fibroblast proliferation [7]. These findings suggest that Artemia proteins may play a role in mitigating age-related inflammation in the skin by reducing cellular senescence and supporting tissue regeneration. The traditional use of Artemia urmiana extract for treating inflammatory skin disorders in folk medicine provides further evidence for its potential in skin health. This historical application suggests the presence of bioactive compounds in Artemia that can alleviate skin inflammation [7]. Research into nano hyalurosomes containing Artemia franciscana extract has shown enhanced penetration into deeper skin layers, leading to anti-aging effects [38]. Furthermore, the active substance in Artemia franciscana has demonstrated anti-inflammatory properties and the ability to induce heat shock proteins, which are crucial in reducing UV stress, heat stress, and lowering the levels of inflammatory cytokines. In summary, the evidence indicates that Artemia extract and proteins possess anti-inflammatory effects on various skin cell types, contributing to anti-aging properties and overall skin protection. One study indicates that the hydrolytic extract of Artemia salina exhibits protective functions under oxidative stress, including safeguarding the central and ventral nervous systems. Oxidative stress is a significant factor in neuronal damage and often precedes or accompanies inflammation in neurodegenerative conditions. Therefore, the neuroprotective effect of Artemia extract against oxidative stress may suggest an indirect anti-inflammatory role by mitigating the initial triggers of inflammation in neuronal cells. Furthermore, another study reports that Artemia salina L. extract provided protection against carbon tetrachloride (CCl4)-induced toxicity in HepG2 cells, a liver cell line [39]. While this finding is not directly related to neuronal cells, it suggests a general cytoprotective capacity of Artemia extract that could potentially extend to other cell types, including neurons. However, further research is specifically needed to investigate the direct anti-inflammatory effects of Artemia extract and proteins on neuronal cells. However, the anti-inflammatory effects of Artemia extract and proteins on glial cells have not been studied. Beyond skin, neuronal, and glial cells, research has explored the anti-inflammatory effects of Artemia extract and proteins on other cell types. A study has been conducted on the antioxidant and anti-inflammatory activity of fractionated Artemia urmiana extract on human peripheral blood neutrophils, a type of immune cell. The study found that the Artemia extract increased the activity of superoxide dismutase (SOD), an important antioxidant enzyme, in these cells [40]. While nitric oxide production was not significantly affected, the observed increase in SOD activity suggests a potential mechanism for indirect anti-inflammatory effects. In another study Artemia urmiana extract and its protein fractions were tested on the human leukemic HL-60 cell line. Although this study primarily focused on cell growth, differentiation, and apoptosis, the observed bioactivity of the Artemia extract on these leukemic cells, which are involved in immune responses, suggests a potential link to inflammatory processes [37]. Another research showed that a methanolic extract of Artemia salina eggs exhibited anticancer activity by decreasing the cell viability of human colorectal cancer cells (HCT116) and melanoma cells (B16F10). Chronic inflammation is increasingly recognized as a contributing factor in cancer development, suggesting that the anticancer activity of Artemia extracts might, in part, be related to the modulation of inflammatory pathways [37]. As mentioned above the

documented medicinal benefits of Artemia extend beyond its potential anti-inflammatory effects. consequently, Artemia extract and proteins demonstrate promising anti-inflammatory effects, particularly on skin cells. While direct evidence for anti-inflammatory effects on neuronal and glial cells is limited in the provided material, neuroprotective potential under oxidative stress warrants further investigation. The medicinal benefits of Artemia are diverse, ranging from traditional remedies to applications in aquaculture drug delivery and its role in drug discovery. Further research into the specific bioactive compounds within Artemia and their mechanisms of action is crucial to fully unlock its therapeutic potential in medicine and related fields.

Conclusion

The perspective of these results revealed that Artemia-extracted protein affected the LPS-inflamed astrocyte cells after purifying and performing chromatography at concentrations of 1, 2.5, 5, 7.5, and 10 µg/ml. The result demonstrated that according to the results of the MTT assay, fractions 7 and 4 had a toxic effect on astrocyte cells and subsequently had no longer an influential contribution in diminishing the inflammation of astrocytic cells. Also, fractions 1, 2, 3, and 6 caused a valuable reduction of the inflammation in astrocytes and had no toxic effect on such cells, thus specifying that their anti-inflammatory effects are not motivated by cell death. It is conceivable to investigate upstream inflammatory pathways. Therefore, astrocyte cell inflammation decreased by the Artemia-extracted protein could be considered as a new therapeutic bio-potency to improve or stop the progress of the cited neural disease.

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