International Journal of Clinical and Experimental Medical Sciences
2022; 8(6): 78-86
http://www.sciencepublishinggroup.com/j/ijcems
doi: 10.11648/j.ijcems.20220806.11
ISSN: 2469-8024 (Print); ISSN: 2469-8032 (Online)

Evaluation of Alcoholic Extract of Elaeagnus Angustifolia L. in Diminishing Proinflammatory Genes in a Model of CA-II-Induced OA Mice

Amir Akbarnejad Eshkalak, Ladan Keyvan

Department of Biology, Payame Noor University (PNU), Tehran, Iran

Email address:

amiir.akbarnejad@gmail.com (Amir Akbarnejad Eshkalak), ladan_ky2000@yahoo.com (Ladan Keyvan)

To cite this article:

Amir Akbarnejad Eshkalak, Ladan Keyvan. Evaluation of Alcoholic Extract of Elaeagnus Angustifolia L. in Diminishing Proinflammatory Genes in a Model of CA-II-Induced OA Mice. *International Journal of Clinical and Experimental Medical Sciences*. Vol. 8, No. 6, 2022, pp. 78-86. doi: 10.11648/j.ijcems.20220806.11

Received: October 28, 2022; **Accepted**: December 8, 2022; **Published**: December 27, 2022

Abstract: In the present study, the analgesic and anti-inflammatory effects of alcoholic extract of Elaeagnus angustifolia L.(AEEA) in a model of CIA II-induced osteoarthritis in mice were investigated in vivo and in vitro. Production of inflammatory cytokines such as iL-1β, TNF-α, iL-6 as well as COX-2, iNOS by chondrocytes and synovial macrophages stimulates chondrocytes and produces metalloproteinases, especially MMP-3, MMP-1 and causes destruction of articular cartilage. Mice were divided into three groups: healthy, patient before treatment, and patient after treatment and infected OA using CIA II. After easy death, tryptophan blue cells were cultured using pentobarbital sodium. To determine the injectable doses of the extract, LD50 and LC50 of mouse cells were measured first. It was extracted from the blood of mice and converted to cDNA using a kit. Samples were analyzed by Real-Time PCR to express the expression of iL-1β, TNF-α, iL-6, COX-2, iNOS genes. Chondrocytes were treated with LPS in vitro to produce PGE2, NO. The PGE2 production of chondrocytes was measured by ELISA, and the NO production of chondrocytes was measured by chlorometric method. PGE2 and NO production was performed in three groups: chondrocytes, chondrocytes with LPS, and cells treated with LPS and AEEA. The results of gene expression in PGE2 were reduced by 89% and NO by 70%. The expression level of COX-2 and iNOS cytokines was significantly decreased after consumption of AEEA extract, and this mice was reported in chondrocytes with 89% decrease for COX-2 gene and 79% decrease for iNOS gene. Expression levels of iL-1β, TNF-α and iL-6 genes decreased by 76% (iL-1β), 89% (TNF-α) and 91% (iL-6), respectively, after consuming AEEA extract. Significant reduction in the expression of pro-inflammatory cytokines by AEEA demonstrates its analgesic and anti-inflammatory effects in mice with osteoarthritis. AEEA was able to effectively and dose-dependently reduce the production of PGE2, NO in LPS-stimulated chondrocytes (in vitro). It also reduces the expression of pro-inflammatory genes such as iL-1β, TNF-α, iL-6, COX-2, iNOS in the blood and plasma of mice with osteoarthritis in vivo.

Keywords: Osteoarthritis, CIA II, Elaeagnus Angustifolia L., Anti-Inflammatory, iL-1β, TNF-α, COX-2, iNOS

1. Introduction

Pain is a defense mechanism that occurs when a tissue is damaged and a person reacts and eliminates the pain-causing stimulus [1]. Pain occurs for a variety of reasons, for example: under the influence of harmful heat, trauma, rupture, strain, electric current, necrosis, inflammation and spasm [1]. Osteoarthritis is a joint failure or insufficiency [2]. It is a disease in which all joint structures (often coordinated) have undergone pathological changes. The pathological requirement of the disease is the loss of focal and initially non-uniform hyaline articular cartilage [3]. This phenomenon is associated with the following: increased thickness and sclerosis of the subcutaneous bone plate, protrusion of osteophytes at the margin of the joint, joint strain, mild synovitis in many affected joints, and muscle weakness that surrounds the joint. In the knees, meniscus damage is part of the disease. There are several pathways that lead to joint failure, but the first step is often joint damage due to failure or inadequacy of protective mechanisms [4].

Because the use of common analgesics has many side effects, for example: adverse effects of steroids on the

gastrointestinal tract or side effects of steroids in the form of delayed wound healing, increased risk of infection, edema, opioids as addiction, etc., researchers have been working to find safer painkillers around the world [4]. Due to the fact that arthritis in patients with osteoarthritis is one of the most prominent clinical symptoms of this disease, the use of corticosteroids and non-corticosteroids (NASID) to solve this problem leads to swelling and ulcers in the gastrointestinal tract [6]. Therefore, in order to study this group of plants, while studying the information of Iranian medicinal plants and the therapeutic properties of these plants, we realized the role of Elaeagnus angustifolia L. fruit in reducing pain symptoms, and treatment of disease and analgesic and inflammatory effects of this plant [7].

In this study, the effect of AEEA on the treatment of mice previously infected with arthritis by CIA-II (Collagen induce Arthritis - II) was investigated. In this method, first, clinical examination of mice was performed to determine their health and joint health [5]. The mice were then divided into 4 groups: 1- Healthy or control mice, 2- Untreated mice, 3- Mice treated with treatment, 4- Placebo-treated mice. The first group was tested as a control group and the next groups were tested, respectively. The plant extract was administered orally for treatment and daily control for 3 weeks. Sampling of mice was performed before death to measure cytokine levels. Mice were then killed by pentobarbital sodium anesthetic and after autopsy, all 4 groups were studied. In order to study the expression of cytokines gene after easy death, internal organs such as liver, kidney, spleen, heart and joints were isolated from mice and the expression level of cytokines gene was examined in vitro. In inflammatory diseases that destroy the cartilaginous structure, inflammatory cytokines including:: iL- 1β, TNF-α, iL-6, COX-2, iNOS are increased and exacerbate inflammation in the joints [6]. The above cytokines are described later in the chapter.

2. Methods

The materials used in this experiment are as follows:

Absolute ethyl alcohol, Collagenase Enzyme (CIA II), Ascorbic acid L, Hydrochloric acid, HBSS solution, Citric acid solution, Dextrose, Sodium bicarbonate, Sodium pyruvate, β-Mercaptoethanol, HEPES solution, Trypan Blue, Tris Base, Agarose, EDTA, PBS, Etidium bromide, NaOH, NaCl, KCl, Potassium dihydrogen phosphate, Sodium acetate, Potassium acetate, Monosodium dihydrogen phosphate, SDS, Xylene Cyanol, Bromo phenol blue, Lipopolysaccharide LPS (SIGMA), Dimethyl sulfoxide (DMSO).

Culture medium DMEM – F12, RPM I-1640, Antibiotics include: Penicillin, Streptomycin, Amphotericin B, Gentamicin (all from Recombinant Idea Biology Company, Iran). MTT Assay Kit, RNA Separator Kit ((Yekta Tajhiz Azma Company)), super PCR mastermix, Primers from Sinagen Company (Iran) RT-PCR kit, Sodium-Nitrate Cybergreen Kit (SIGMA), Primers from Sinagen Company (Iran) RT-PCR kit, Sodium-Nitrate Cybergreen Kit (SIGMA), Prostaglandin kit (invitrogen), Mgcl2 heat resistant DNA polymerase.

2.1. Preparation of Elaeagnus Angustifolia L. Extract

The sample extract of Elaeagnus angustifolia L. with a concentration of 1 g per 15 cc (1 g / 15cc) was prepared from the National Center for Genetic and Biological Resources of Iran. This plant consists of 3 genera and 50 species. Its main constituents include flavonoids and fatty acids and sterols and carbohydrates. The highest amount of fatty acid is related to the essential fatty acid linoleic acid, which together with oleic acid account for 92.8% of the total fatty acid.

2.2. Culture and Proliferation of Studied Cells

In this study, cartilage of the rat sternum was selected as a model for investigating the anti-inflammatory effects of Elm. After killing the rats with the method of easy death, they were quickly placed on a plate from behind and the animal's arms and legs were fixed. And to a plate containing 2 cc of culture medium 112 enriched with - LMPMD 10% FBS, 50µg / ml ascorbic acid) Sigma company (100 units of penicillin) Idea Zist company (50 µg / ml streptomycin) Idea Bist company (100 µg amphotericin B) Alchemy - drug was added and stored in a CO_2 incubator at 37°C and 5% CO_2 with 90% humidity. The cell division time of chondrocytes by mitosis is 24-72 hours. Chondrocyte cells were counted using a homocytometer slide and survival rate 2 was assessed by trypan blue method.

2.3. Chondrocyte Cell Washing

After counting the cells to pass through them, add 105 × 5 cells of chondrocytes to 5 ml of DMEM-F12 enriched medium and rotate slowly for 5 minutes and then inside the incubator at 37°C and CO_2-5% C and were stored for 4 to 5 days to increase the cell density in the environment above 80%. Differentiated chondrocytes look like spindle cells and chondroblasts (undifferentiated) look like round cells. Due to the inability of dead cells in the active transfer mechanism and inactivation of the dye cell membrane, dye enters the cell and causes discoloration. The dead cell nuclei appeared dark blue due to the adsorption of trypan blue (figure 1).

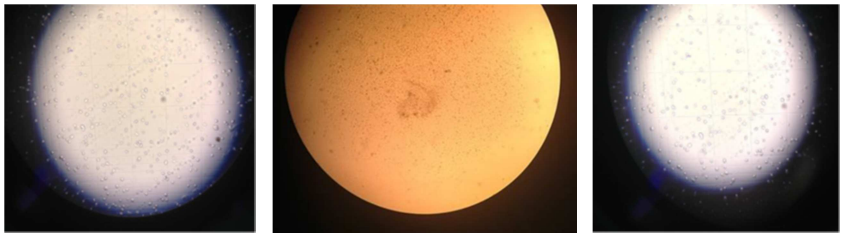

Figure 1. *Chondrocyte cells.*

2.4. Division of Study Groups and Cell Treatment

In this study, 25 mice weighing 30 to 38 g were used. The sexes of these mice were randomly divided into five groups. Mice were divided into the following 5 groups: Control and control groups, Patient group without treatment, Patient group treated with 6mg / kg AEEA, Patient group treated with 12mg / kg AEEA, Patient group treated with 18mg / kg AEEA.

2.5. Cytotoxicity Study by MTT Assay Method

MTT and Trypan Blue methods were used to evaluate the cytotoxic effect of AEEA as well as LC50. Dimethyl sulfoxide (DMSO) was added to dissolve the purple formans in the dye solution. The adsorption of this dye solution was measured by a spectrophotometer at a wavelength between 500 nm and 600 nm. Concentrations of 10, 15, 20, 25, 30, 40, 45, µg / ml were performed with DMEMF-12 medium injected into the medium. As the concentration of AEEA extract increases, the percentage of non-living cells increases and the number of living cells decreases. The LC50 is 30µg / ml and the median is 15µg / ml (figure 2).

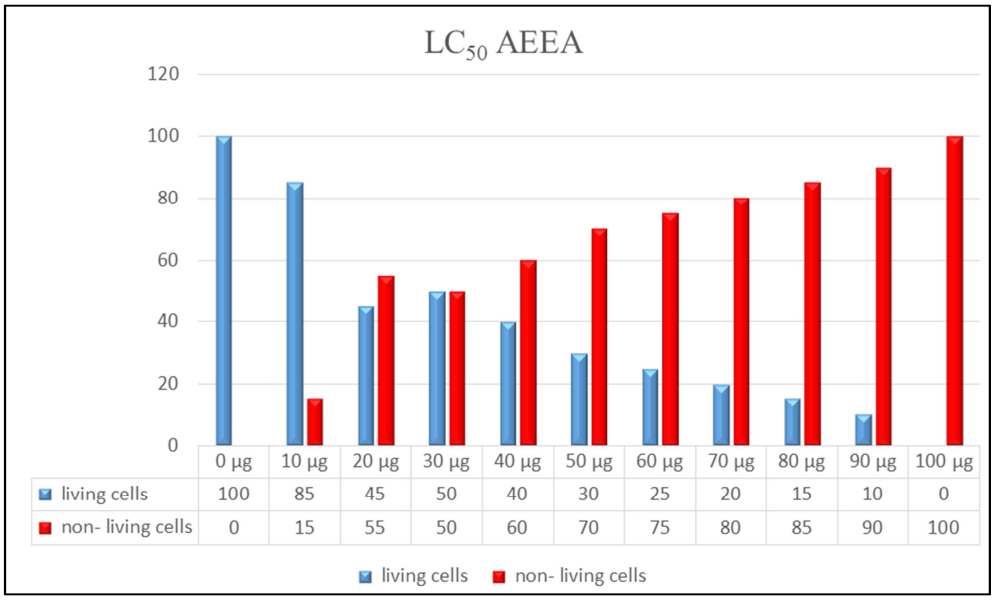

Figure 2. *Determination of the Cytotoxicity by Trypan blue, MTT assay for AEEA. in the concentration of 30 µg/ml dead cells and living cells are equal. Data are present the mean±S. E. M.n=6, *P<0.05.*

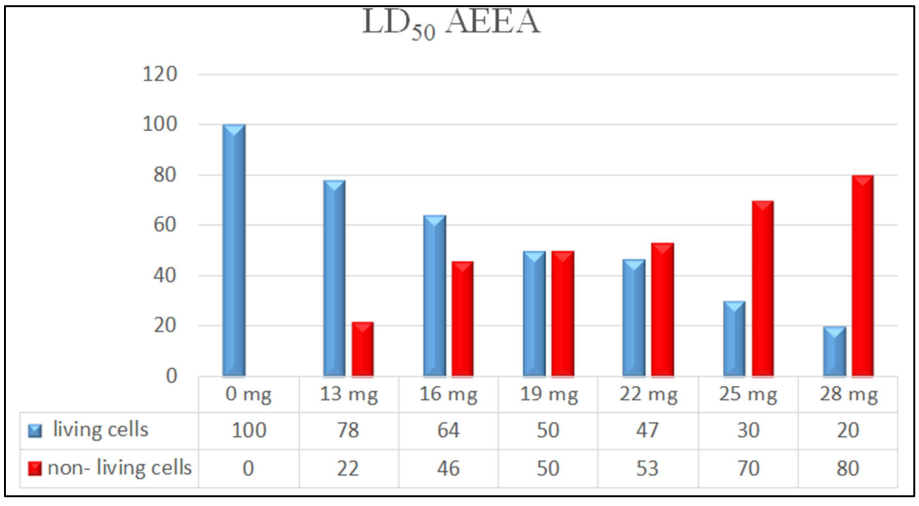

Figure 3. *Determination of the Lethal Dose of the AEEA by Moving Average Method. in the concentration of 19 mg/kg dead cells and living cells are equal. Data are present the mean±S.E.M.n=6, *P<0.05.*

2.6. Evaluation of LD50 of AEEA by Moving Average Method

To determine this dose, plant extracts were injected into mice in 6 doses of 13, 16, 19, 22, 25, 28 mg / kg with an exponential ratio of 1.2. After 24 hours, based on the mortality rate, we report the death toll as a percentage. The lethal dose was 19 mg / kg (figure 3).

2.7. Treatment of Chondrocytes with LPS to Produce NO and PGE2

After LC50 detection, 5×10^6 monocyte / macrophage cells with 4 $_{cc}$ of DMEMF-12 enriched medium were added to 22 $_{cm^2}$ plates and rotated for 2-3 minutes to spread evenly on the plate bottom. Then the plates were kept in an incubator at 90% humidity and 37 ° $_C$ and 5% CO_2 for 20-30 minutes and the alcoholic extract of the plant was added at the rate of 5 µg / ml and PBS and distilled water. The plates were incubated under standard conditions for 72 hours. At the end of this stage, the cells were treated with lipopolysaccharide at a concentration of 20 ng / ml and all plates were incubated for 24 hours to produce prostaglandin and nitrite oxide at 37 ° $_C$ and 5% CO_2 was stored at 90% humidity. The reason for adding distilled water and PBS in the medium enriched and stimulated by LPS as placebo is to indicate that these compounds do not have the power to inhibit or reduce the expression of cytokines and can not alter the expression of cytokines (Table 1).

Table 1. *Treatment of cells for the production of prostaglandins and nitrite oxide.*

Distilled water	PBS	LPS	Alcoholic extract	Chondrocytes	Plate
-	-	-	-	+	1
-	-	+	-	+	2
-	-	+	+	+	3
-	+	+	-	+	4
+	-	+	-	+	5

2.8. Extraction and Conversion of RNA to Cdna

RNA isolation and conversion to cDNA was performed according to the relevant instructions. Lysis of cells from trizol reagent and RNA extraction with chloroform were performed. RNA was converted to cDNA by two-step RT-PCR. Semi-quantitative PCR was used for each of the studied cytokines using specific primers and GAPDH gene as housekeeping gene. The PCR product was controlled in 1.5% agarose gel. Real-time PCR was performed using the same primers used for qualitative PCR and evergreen was used as the mastermix. The results obtained from CTs (threshold cycle) were evaluated by two standard curve methods and Pfaffi method. The importance of CTs was first assessed using ANOVA and secondly by Student-Newman Keuls (SNK), REST-2000. Finally, Pfaffi method was used to determine the amount of gene expression.

2.9. Statistic Analysis

All data is expressed as mean $_{\pm}$ SD. Statistical analysis was performed using ANOVA, Student – Newman – Keuls, REST-2000. Cts was used for analysis. $P < 0.05$ is considered statistically significant.

3. Results

3.1. Estimation of NO Production by Chlorometric Method

To estimate NO, 5 × 5 x chondrocyte cells were cultured in plates of 12 cells. The order of the houses was as 1) normal cell 2-) standard solution 3-) cell composition + LPS + AEEA. They were treated for 72 hours. Then, for one hour, 20 ng LPS was treated and 100 Landa was used to measure NO. The standard curve was prepared based on the standard solution with concentrations of 0.5, 1, 1.5, 2, 2.5 nmol / L. 100 microliters of the surface of each culture medium with an equal volume of Griese reagent.

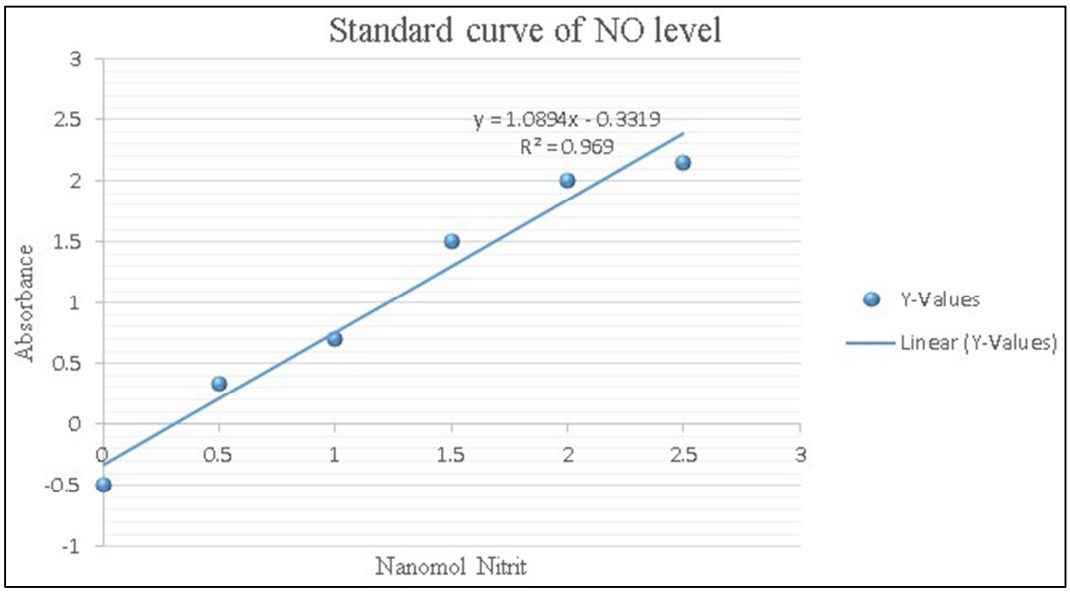

Figure 4. *The standard curve was prepared based on the standard solution with concentrations of 0.5, 1, 1.5, 2, 2.5 nmol / L. 100 microliters of the surface of each culture medium with an equal volume of Griese reagent. Nitrite levels were measured by adsorption at 540 nm by ELISA reader. All plates have 2 repetitions. optical density ST=0.084/ optical density Blank=0.01/ optical density Test=0.036.*

A portion of 0.1% naphthylene ethylene diamine and a portion of 1% sulfanilamide in 5% H3PO4 were reacted and

placed on a flat-bottomed 96-plate plate for 10 minutes at room temperature and in the dark. Nitrite levels were measured by adsorption at 540 nm by ELISA reader. All plates have 2 repetitions (figure 4).

3.2. Estimation of PGE2 Production by ELISA

Standard diluted prostaglandin solutions were used according to the following table: (Table 2).

Table 2. *Standard diluted PGE2 solutions.*

Buffer	Solution added	Standard (ng/ml)
900 µL 1x Tris Buffer	40 ng/mL standard solution from 100 µl prepared in step 4	4
500 µL 1x Tris Buffer	Standard solution 4 ng/ml from 500 µl	2
500 µL 1x Tris Buffer	Standard solution 2 ng/ml from 500 µl	1
500 µL 1x Tris Buffer	Standard solution 1 ng/ml from 500 µl	0.5
500 µL 1x Tris Buffer	Standard solution 0.5 ng/ml from 500 µl	0.25
500 µL 1x Tris Buffer	Standard solution 0.25 ng/ml from 500 µl	0.13
500 µL 1x Tris Buffer	Standard solution 0.13 ng/ml from 500 µl	0.06
500 µL 1x Tris Buffer	Standard solution 0.06 ng/ml from 500 µl	0.03

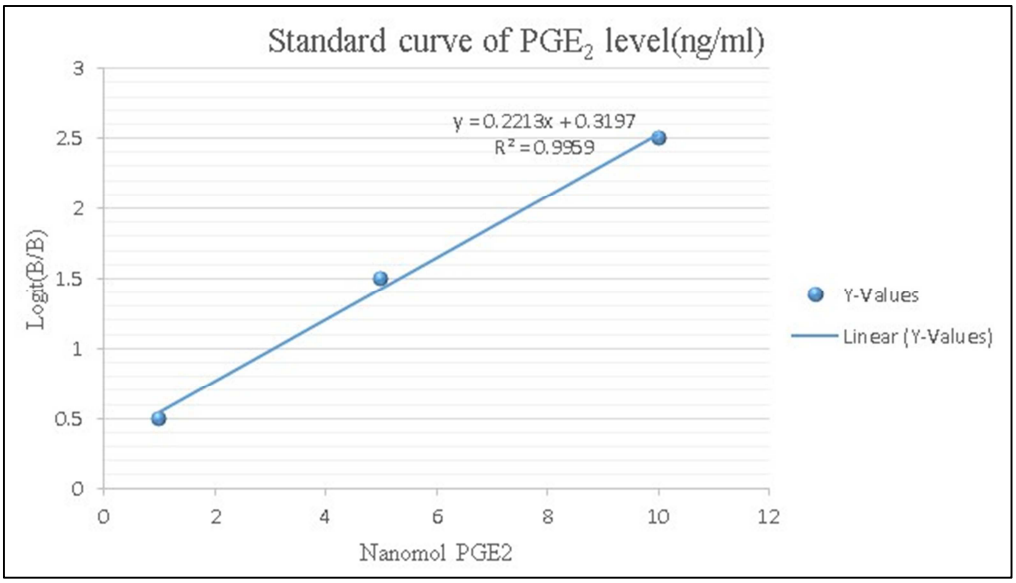

Figure 5. *Standard curve of PGE_2 level (ng/ml). PGE2 levels were measured by adsorption at 405 nm and 420nm by ELISA reader.*

Measurement method:

1) 100 µl of culture medium was added to well NSB and 100 µl to well B0.
2) 100 µl of standard prostaglandin E2 was added to the standard well.
3) 100 µl of each sample was added to the sample well.
4) 150 µl of Prostaglandin E2 Alkaline Phosphatase Detector in all wells except TA well added.
5) 50 µl of prostaglandin E2 antibody was added to all wells except TA and NSB wells.
6) Each plate was covered with a cover plate and incubated at room temperature for 2 hours and placed on an orbital shaker.
7) 2 pNPP tablets were dissolved in 10 ml DEA1x buffer.
8) The plates were placed in the ELISA machine and the wells were washed 5 times with the washing solution.
9) 200 µl of pNPP solution was added to each well.
10) 5 µl of the detector was added to the TA well and the plate surface was covered and placed in the dark on an orbital shaker with a rotational motion of 90 to 60 minutes for placement of pNPP-confirmed discoloration in the ELISA.

Wipe the bottom of the plate with a paper towel to remove fingerprints or dirt. The plate was covered and read at wavelengths between 405 and 420 nm (figure 5).

3.3. The Effect of AEEA on the Diameter of Mice Joint

In this study, before and after CIA II injection and local swelling of the joint, mice in each cage were examined separately for 30 days and any changes were recorded. The injection and treatment period lasted 21 days. A detailed measurement was performed on the schedule on the first day. The measured joint diameter is less than 0.5 mm. The injection was given on the seventh day. On this day, the joint diameter had reached 1.5 mm. As can be seen in the diagram, from the eighth day of injection, the increasing trend of joint swelling is clearly seen and the joint diameter reaches 3.5 to 4 mm. After ten days, the diameter of the joint is 2.5 mm and on the seventeenth day, maximum swelling is observed. On the 29th day, joint treatment was started. s Injection doses of 50µg / ml were 100 and 200 which were performed in three groups of mice. The fourth group is mice that received a CIA II injection but were not treated. The fifth group is healthy control mice without CIA II injection. (figure 6).

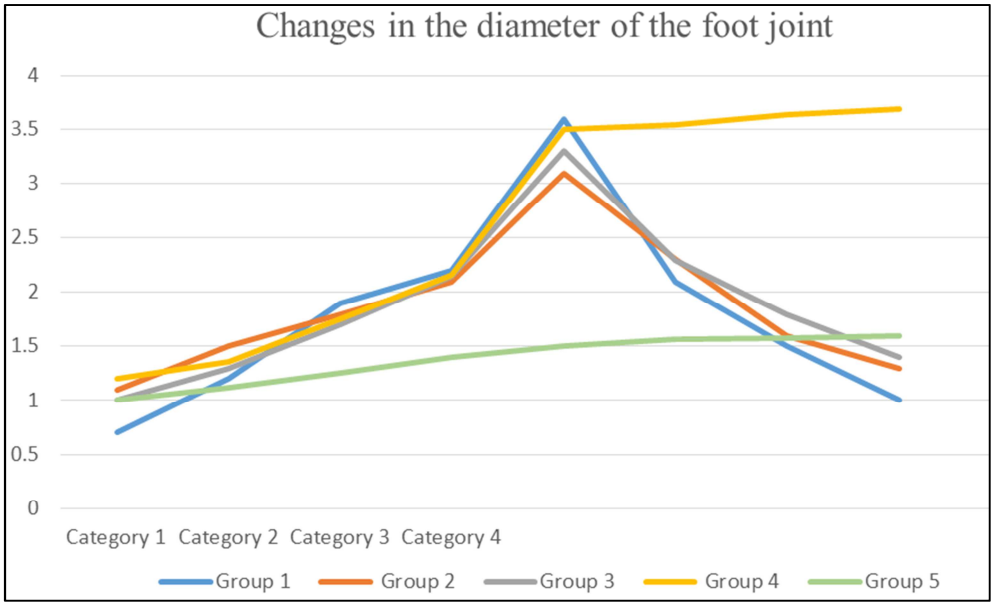

Figure 6. *On day 17 after the CIA II injection, maximum joint swelling was seen. After that, treatment began. Treatment ended on day 29. The first group is related to mice that received a dose of 200 µg/ml the AEEA, the second group received a dose of 100 µg/ml and the third group received a dose of 50 µg/ml. The fourth group of sick mice has no treatment and the fifth group has control mice.*

3.4. Qualitative RNA Assay

In order to evaluate the quality of the extracted RNAs, the samples were injected on 1% agarose gel. Well No. 1 to 7RNA isolated from organ cells and well 8 to 17RNA isolated from cartilage cells. Λ 5 of sample + 1λ of dye 6XRNA loading was injected in the wells and subjected to non-alternating electric field of 90 volts and 15 mA for 45 minutes.

3.5. Quantitative Analysis of the Effect of AEEA on Cytokine Gene Expression by Real-Time PCR

Group 1: Untreated cartilage cells

Group 2: Cartilage cells with AEEA

Group 3: Cartilage cells with 20 ng / ml LPS

Group 4: Cartilage cells with 20 ng / ml LPS with 95 µl of alcoholic extract of Elm after real-time PCR tests for chondrocytes and CT were determined to reduce the expression of cytokines using Paffi formula.

Compared with GAPDH with Cell + LPS + Extract and Cell + LPS, in Cell + LPS + Extract the results obtained in vitro for pro-inflammatory cytokines COX-2 100%, iNOS90 gene, TNF-α 90% and iL-1β 80% reduction Gene expression showed that the results indicate the effect of elm extract on reducing gene expression of pro-inflammatory cytokines (figure 7).

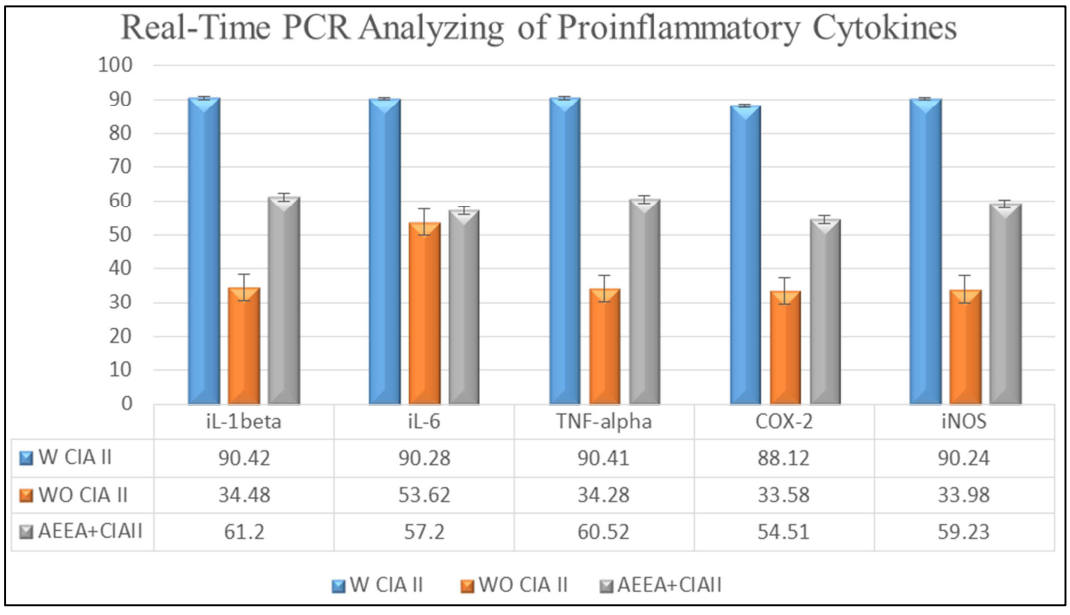

Figure 7. *Quantitative analysis of the effect of AEEA on cytokine gene expression by Real-Time PCR.*

3.6. The Effect of AEEA on Prostaglandin Production in Chondrocytes

Chondrocytes were treated after culture under different conditions as follows:

Group 1: Chondrocyte cells cultured alone for 72 hours had lower levels of PGE2 production than lipopolysaccharide-stimulated cells.

Group 2: Chondrocytes stimulated by 1 ng by 20 ng / ml lipopolysaccharide for 1 hour showed an increase in PGE2 production.

Group 3: Chondrocytes stimulated with 20 ng / ml LPS and treated with 95 µl of AEEA show lower levels of PGE2 production than the group of chondrocytes stimulated with lipopolysaccharide without AEEA (figure 8).

$$B/B\% = \frac{(\text{Absorbance Test} - Average\ NSB) \times 100}{\text{Correct Bo}}$$

According to the above formula, the amount of prostaglandin was equal to 0.89 (formula 1).

3.7. The Effect of AEEA on the Production of Nitric Oxide in Chondrocyte Cells

Chondrocytes were treated after culture under the following conditions:

Group 1: Chondrocyte cells cultured alone for 72 hours had lower NO production levels than cells stimulated with lipopolysaccharide.

Group 2: Chondrocyte cells stimulated for 1 hour by 20 ng / ml lipopolysaccharide showed an increase in NO production.

Group 3: Chondrocytes stimulated with 20 ng / ml LPS and treated with 95 µl of Elm extract showed lower NO production levels than the group of chondrocytes stimulated with lipopolysaccharide without Elm plant.

In this study, the first group of chondrocytes in OD = 0 had zero oxide nitrite and LPS-stimulated chondrocytes were considered as a positive control, which has a maximum nitrite oxide production of 2.2 and chondrocytes stimulated with Lipopolysaccharide with AEEA has OD = 0.044. According to the formula, the amount of nitrite oxide was calculated to be 0.702. (figure 9).

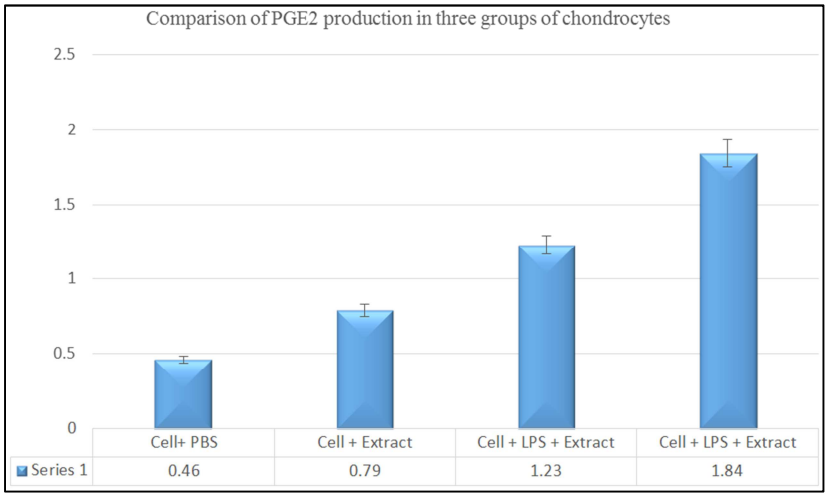

Figure 8. *Comparison of PGE2 production in three groups of chondrocytes, chondrocytes with lipopolysaccharide and lipopolysaccharide-treated cells and AEEA were measured in three replications with *p≤0.05 and 95% SD ± mean accuracy.*

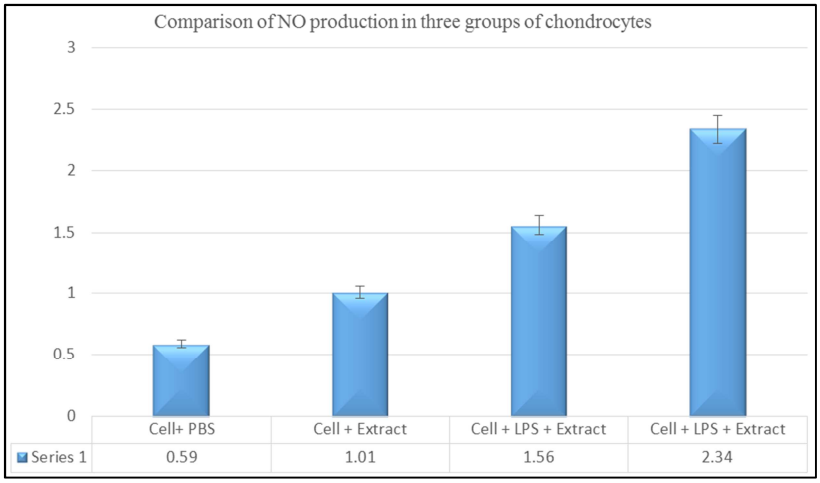

Figure 9. *Comparison of NO production in three groups of chondrocytes, chondrocytes with LPS and LPS-treated cells and AEEA were measured in three replications with *p≤0.05 and 95% SD ± mean accuracy. the amount of nitrite oxide was calculated to be 0.702.*

4. Discussion

Macrophages are the most important and main elements of chronic inflammation [8]. These tissue cells are formed from monocytes in the bloodstream and after they are removed from the bloodstream. These cells are present in most connective tissues and a large number of viscera [8]. Cytokines are polypeptide products of cell types that act as mediators of inflammation and immune responses. The main cytokines of inflammation include: iL-1β, TNF and also a set of chemotactic cytokines called chemokines [9]. The physiology of osteoarthritis shows that with decreasing levels of pro-inflammatory factors such as iL-4, iL-10, iL-13 and 2 TIMPS and growth factors IGF-I, TGF-B, FGFb and BMPS, levels of pro-inflammatory cytokines such as iL-1β And iL-8, 18 - iL, iL-11, iL-17 and aggregates, and PGE2 and nitric oxide.

All of these events lead to inflammation associated with inflammation, cartilage cell apoptosis, and cartilage destruction [10]. To study the effects of Elm fruit extract on mice, first CIA II produced localized arthritis in the locomotor organs of mice. After clinical and paraclinical studies for 3 weeks from the clinical point of view, the mice Signs of swelling, redness and unwillingness to move.

NO plays a variety of roles in inflammation, including reducing the use of leukocytes in inflammatory sites. It has a soluble, short-acting gas free radical that is made by a variety of cells. Elecampane, which is introduced in traditional Iranian medicine as an analgesic and anti-inflammatory and is used in the treatment of rheumatoid arthritis, contains significant amounts of flavonoid compounds, terpenoids, etc.

Ebadi, Ismaili et al.'s research showed that some flavonoid and cytosterol compounds have analgesic and anti-inflammatory effects [11]. Flavonosids are one of the inhibitors of nitric oxide synthesizing enzyme and inhibit the production of NO, which increases after formalin injection, so its reduction leads to analgesic activity [12].

Prostaglandins (PGE) are cyclic derivatives of arachidonic acid. COX-1 is present in many tissues. COX-2 is found mainly in inflammatory cells and its products play an important role in tissue damage such as inflammation [13]. Comparing the production of PGE2 and NO in three groups of chondrocytes, chondrocytes with lipopolysaccharide and cells treated with lipopolysaccharide and Elm fruit extract, the results of gene expression in PGE2 were reduced by 89% and NO by 70%. In chondrocytes, expression of cytokine Cox-2 gene was reduced by 89% and iNOS by 79%. The anti-inflammatory effect of flavonoids is exerted by inhibiting inflammatory cytokines such as tumor necrosis factor TNF from activated macrophages in inflammation, and these pro-inflammatory substances increase the synthesis of prostaglandins [14]. iL-1β and TNF-α are primary mediators of disease-producing cytokines in osteoarthritis [15, 16]. Studies have shown that iL-1β and TNF-α have an abnormal increase in synovial joint fluid and cartilage tissue in osteoarthritis patients.

5. Conclusions

According to the results of measuring the expression level of iL-1β gene (in control, patient and positive groups), an increase in gene expression level was observed in the positive group and 76% decrease in the treatment group. This mice was reported to be 89% decrease in TNF-α gene expression. In this study, experiments were performed to investigate the effect of AEEA on inhibiting the production of pro-inflammatory cytokines produced by LPS. The extract had an inhibitory effect on transcription and expression of pro-inflammatory genes. In a study performed on the plant, it was stated that the plant has an anti-inflammatory effect. Induction of COX-2 and iNOS at high levels of PGE2 and NO also results in cartilage attenuation, inhibition of matrix production, and chondrocyte cell death [17]. In contrast, decreased PGE2 and NO production have been reported in relieving osteoarthritis-related pain and inflammation [18]. Our study emphasized that AEEA reduces the expression of pro-inflammatory genes, and has also been shown to reduce NO and PGE2 production in cells. In this study, the effect of placebo (PBS) in two stages without extract and with AEEA on cytokine gene expression by Rest software was also tested and the result showed no effect in both stages (without extract 83% reduction Cholera with 71% reduction). Our study emphasized that AEEA has a down-regulated expression of Cox-2, iNOS, TNF-α genes and is effective in reducing the production of NO and PGE2 in surface cells.

6. Recommendation

This study demonstrated that the consumption of alcoholic extract of Elaeagnus angustifolia L.(AEEA) decreased the expression of iL-1β gene by 76% and the expression of TNF-α gene by 89% in treated mice. Also, the amount of NO and PGE production has diminished significantly, so it is suggested to use a higher concentration of this extract to further reduce the expression of pro-inflammatory genes.

In this research, the concentration of 6 mg/kg of extract was used. The LD50 of the extract was determined to be 19 mg/kg, so it is proposed to carry out this study with 2 times the concentration of the extract, i.e. 12 mg/kg, so that the expression of pro-inflammatory genes reaches 100%.

References

[1] Guyton, A.; Hall, J., (2004). Textbook of Medical Physiology: translating by F. Shadan, Iran: publishing Chehr, 903-1028, 2004.

[2] Choi, H. G. et al. (2021) 'The Fate of Bone Marrow Lesions After Open Wedge High Tibial Osteotomy: A Comparison Between Knees With Primary Osteoarthritis and Subchondral Insufficiency Fractures', The American Journal of Sports Medicine, 49 (6), pp. 1551–1560. doi: 10.1177/03635465211002160.

[3] Jakaite, L., Schetinin, V., Hladůvka, J. et al. Deep learning for early detection of pathological changes in X-ray bone microstructures: case of osteoarthritis. Sci Rep 11, 2294 (2021). https://doi.org/10.1038/s41598-021-81786-4

[4] Loeser, R. F., Goldring, S. R., Scanzello, C. R., & Goldring, M. B. (2012). Osteoarthritis: a disease of the joint as an organ. Arthritis and rheumatism, 64 (6), 1697–1707. https://doi.org/10.1002/art.34453

[5] Hodgens A, Sharman T. Corticosteroids. [Updated 2021 Oct 16]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554612/

[6] Goldring, MB, Otero, M., (2011). Inflammation in Osteoarthritis curr Opin Rheumatol, 23 (5): 471-8.

[7] Mokhberi, M., (2004). The effects of aqueous extract of elm and thyme. Master Thesis of Shahid Beheshti University of Science.

[8] Krzyszczyk P, Schloss R, Palmer A, Berthiaume F. The Role of Macrophages in Acute and Chronic Wound Healing and Interventions to Promote Pro-wound Healing Phenotypes. Front Physiol. 2018 May 1; 9: 419. doi: 10.3389/fphys.2018.00419. PMID: 29765329; PMCID: PMC5938667.

[9] Ramzi, Dr. Davood, (2011). Pathology, ABS, Tehran Publishing: Arian Pajouhesh Noor Danesh, first edition, Pages 41-23.

[10] AU, R., phan p., AUA, (2006). Frondozc. Effect of different chondroitin sulfate eaw material on pro-inflammatory gene expression in chondrocyte and monocytes (Abstract). Int cartilage res soc final program Abstract 2006: 1 (1): 90 2006 by Taylor & Francis Group, page 1-16.

[11] Ebadi, M., (2002). Flavonoisds In: Pharmacodynamic Basis of herbal medicine. CRC press LLC. 2000N. W. Boca Raton. Ne.

[12] Olszanecki R, Gebska A, Kozlovski VI, Gryglewski RJ. Flavonoids and nitric oxide synthase. J Physiol Pharmacol. 2002 Dec; 53 (4 Pt 1): 571-84. PMID: 12512693.

[13] Anthony Assassination, 2013 (Pharmacology of Katzung and Assassination, translated by Dr. Hossein Khalili et al., Arjmand Book Publishing, Second Edition, Pages 226 – 223.

[14] Toker G, Kupeli E, Memisoglu M, Yesilada E., (2004). Flavonoids with antinociceptive and anti-inflammatory activitiesfrom the leaves of Tilia argentea (silver linden). J Ethnopharmacol 2004; 95: 393–97.

[15] Schafer, SJ.; Luyten, FP.; Yanagishita, M.,; Reddi, AH., (1993). Proteoglycan metabolism is age related and modulated by isoforms of plateletderived growth factor in bovine articular cartilage explant cultures. Arch Biochem Biophys 1993; 302 (2): 431–8.

[16] Nambi, G. Does low level laser therapy has effects on inflammatory biomarkers IL-1β, IL-6, TNF-α, and MMP-13 in osteoarthritis of rat models—a systemic review and meta-analysis. Lasers Med Sci 36, 475–484 (2021). https://doi.org/10.1007/s10103-020-03124-w

[17] H. Chen, J. Qin, H. Shi, Q. Li, S. Zhou, L. Chen, Rhoifolin ameliorates osteoarthritis via the Nrf2/NF-κB axis: in vitro and in vivo experiments, Osteoarthritis and Cartilage, 10.1016/j.joca.2022.01.009, (2022).

[18] He, L., Pan, Y., Yu, J., Wang, B., Dai, G. and Ying, X., 2021. Decursin alleviates the aggravation of osteoarthritis via inhibiting PI3K-Akt and NF-kB signal pathway. International Immunopharmacology, 97, p. 107657.