SPER Publications and Solutions Pvt. Ltd.

Original Article

The potential of Tragopogon pratensis to reduce inflammation in osteoarthritis by suppressing of inflammatory factors, prostaglandin E2 and nitric Oxide production

Farbod Taghavi Rad[1], Hossein Maghsoudi[2]*, Saeid Ghorbian[1], Shima Mohammadi[3]

[1] Department of Molecular Biology, Islamic Azad Universty, Ahar Branch, Ahar 5451116714, East Azerbaijan province, Iran, [2] Department of Biology, Payame Noor University (PNU) Tehran 1865865399, Iran, [3] Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan , Isfahan 81746-73441, Iran.

Correspondence: Hossein Maghsoudi, Arabkhari Street, Taghiabad three ways, Varamin highways, Shahr e Ray, Tehran. Iran, Email: hossein_m2002@yahoo.com.

ABSTRACT

Background: Due to the side effects of current therapies for osteoarthritis, alternative medical therapy using the herb *Tragopogon pratensis* L., which in Iranian traditional medicine has been used as a treatment option. Here, we attempted to evaluate the effect of *Tragopogon Pratensis* Alcoholic Extraction (TPAE) on proinflammatory cytokines in bovian synoviocyte and *THP-1*. Methods: To investigate the impact of *Tragopogon Pratensis* Alcoholic Extraction (TPAE) on proinflammatory cytokines suppression in bovine synoviocytes and *THP-1*, we isolated synoviocytes from radiocarpal synovial fluid. In addition, after evaluation of LC50, both cells (5×10^5 cells $well^{-1}$) were incubated at 37°C in a 5% CO2 atmosphere with 90% humidity for 72 hours with TPAE (7.5 μg mL^{-1} as a LC50). One set of cells was activated for 1h with LPS for RT-PCR analysis of *COX-2*, *iNOS*, *IL-1β*, *TNF-α* expression and in parallel, activation of another cells set were done for 24 h. Cells supernatant were analyzed for *PGE-2* and nitrite content. Results: The present study demonstrates that TPAE reduced expression of *COX-2*, *iNOS*, and *TNF-α* in a control group and reduced expression of *COX2* and *iNOS* was significant, as was the reduction in production of *NO* and *PGE2*. Also TPAE decreased the expression of *TNF-α* and *IL-1β* in control group. Conclusions: Our results showed that the anti-inflammatory effect of TPAE was related not only to the synoviocyte cells, but also to the *THP-1* that are active in the synovial membrane.

Keywords: bovine fibroblast-like synoviocyte, *COX-2, IL-1β, iNOS, NO*, osteoarthritis, *PGE2, THP-1, TNF-α*, *Tragopogon pratensis*.

Introduction

Osteoarthritis (OA) as a chronic degenerative joint disease diagnosis with synovial membrane inflammation and erosion of articular cartilage and the subchondral bone. It can occur in the joints of the hands, feet, knees, spine, and other parts of the skeleton.[1] For example, the prevalence of knee osteoarthritis in Iran, among those 15 to 82 years of age is about 16% in published reports. The number of people with arthritis in the United States in 1995 was estimated to be 40 million people.[2] a series of biochemical events play a role in degenerative alteration in the inflamed joints, such as overproduction of proinflammatory cytokine *IL-1β* and tumor necrosis factor alpha (*TNF-α*). Overproduction of these cytokines increases the degradation of the matrix of cartilage by stopping the production of proteoglycans and collagen type II; the mechanism of this is the production of enzymes that destroy the matrix, such as matrix metalloproteinases.[3] This cytokine also increases the expression of cyclooxygenase 2 (*COX-2*) and nitric oxide synthase (*NO*), which are potent proinflammatory enzymes and which cause increased levels of prostaglandin and nitric oxide synthesis [4, 5]. Since the destruction of the cartilage is directly related to the levels of *COX-2*, *IL1-β*, *TNF-α* and *iNOS*, take advantage of compound due to inhibitory effects on expression of mentioned proinflammatory mediators is a promising method to manage catabolic symptoms of osteoarthritis. Because of inefficiency and not preventive

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Farbod Taghavi Rad, Hossein Maghsoudi, Saeid Ghorbian, Shima Mohammadi. The potential of tragopogon pratensis to reduce inflammation in osteoarthritis by suppressing of inflammatory factors, prostaglandin E2 and nitric Oxide production. J Adv Pharm Edu Res 2019;9(S2):132-139.
Source of Support: Nil, Conflict of Interest: None declared.

behavior of conventional treatments in the OA degenerative process, appropriate treatment is in demand.

Among the drugs commonly used for OA are non-steroidal anti-inflammatory drugs (NSAID) and corticosteroids. Particularly, NSAIDs are used in common, however long term usage is related with serious side effects including such as stomach and gastrointestinal ulceration. The requisition of more efficient and fewer adverse effects treatment for OA is still demand for relieving pain and progress in function and quality of life.

Salsify, a medicinal plant with the scientific name of *Tragopogon pratensis* L. is a biennial herbaceous plant which is edible. It is farmed as a vegetable in Northern Europe, and it grows in wet grasslands in the north of Iran and the west of Iran in Kurdistan, Tafresh, Arak and Alborz ranges, and in mountains near Tehran, Isfahan, and Shiraz. The root of the plant is used as an appetizer, expectorant, and for soothing and healing wounds. It aids the body's excretion of toxic materials, and it is effective in the treatment of skin diseases (dermatoses), diabetes, arthritis, providing developmental and liver congestion.[6]

The purpose of this experiment is to assessement the impact of the Trogopogon Pratensis alcoholic extract (TPAE) in reducing the expression of inflammatory cytokine *IL-1β*, *TNF-α*, *iNOS*, *COX-2*, *PGE2*, and *NO* at the molecular level by synoviocyte cells and monocytes / macrophages.

Methods

Cell Culture

Synovial membrane tissue was isolated from the radiocarpal joints of 8 month old Holsteins by digestion of the tissue with type II collagenase (220 U mL^{-1}, Sigma-Aldrich, St. Louis, MO, USA) for 12-18 h at 37°C. (Fig. 1) The secluded cells filtration were done elimination debris and washed 4 times through Hank`s balanced salt solution (GIBCO®, Gran d Island, NY, USA). After centrifugation, cell pellets resuspended in control media containing: Dulbecco's Modified Eagle Medium: Nutrient Mix F-12 (GIBCO®, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (GIBCO®, Grand Island, NY, USA), 50 mg mL^{-1} ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA), and 50 mg mL^{-1} Gentamycin (Daropakhsh, Tehran, IRAN), Penicillin 100 U mL^{-1}, Streptomycin 100 µ mL^{-1} (BIO IDEA, Tehran, IRAN), Amphotericin B 0.25 µg mL^{-1} (Cipla, Mumbai, INDIA), viability of counted synoviocytes were evaluated by MTT assay and hemocytometer-based trypan blue dye exclusion cell quantitation method. Articular synoviocytes were seeded into 12-well plates (TPP Switzerland) (5 × 10^5 cell $well^{-1}$) and incubated at 37C and 5% CO_2 for 5 days.

Human *THP-1* monocyte/macrophage-like cells (Pasteur Institute of Iran) defrosted in the laboratory were propagated in control media containing: RPMI-1640 medium (GIBCO®, Grand Island, NY, USA) supplemented with 2 mM L-glutamine (Sigma-Aldrich, St. Louis, MO, USA), 10% FBS (Sigma-Aldrich, St. Louis, MO, USA), 1.5 g L^{-1} sodium bicarbonate(Sigma-Aldrich), 4.5 g L^{-1} glucose (Sigma-Aldrich, St.Louis, MO,USA),10mM 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES, Sigma-Aldrich ,St. Louis, MO, USA), 1.0 mM sodium pyruvate (Sigma-Aldrich, St. Louis, MO, USA), 0.05 mM β-mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA), 50 mg mL^{-1} Gentamycin (Daropakhsh, Tehran, IRAN), Penicillin 100 U mL^{-1}, Streptomycin 100 µ mL^{-1} (BIO IDEA, Tehran, IRAN), and Amphotericin B 0.25 µg mL^{-1} (Cipla, Mumbai, INDIA). Viability of cell pellet after centrifugation was assessed via trypan-blue exclusion method. Then, viable cells were seeded into 6-well cell culture plates (1 × 10^5 cells $well^{-1}$). Cells were exposed to treatment at the same day.

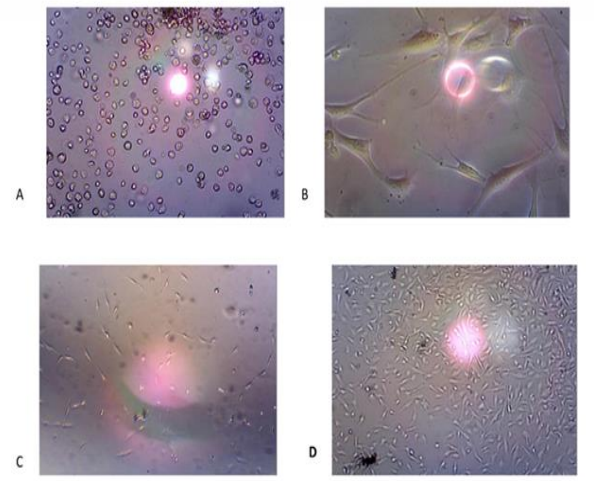

Figure 1: BFLS cell culture process. Figure A (magnification 16×12) showing cells after adding of trypsin and separating from the plate and laying out on separate plates. All cells were seen as spherocytes. Figure B (magnification 16×40) showing that plate after 24h from culturing and all cells are in the from of fibroblast (spindle, pear, long and chaotic) with a core like a cell itself. Figure C (magnification 16×12) the same plate. Figure D (magnification 16×12) same plate after 4 to 5 days from primary culture. All pictures were taken with OLYMPUS contrast phase microscope and labomade JVU 3100 camera in Biotech laboratory of Payame Noor University-branch of Rey.

SDH activity assay

Succinate dehydrogenase is a mitochondrial enzyme complex that participates in both the citric acid cycle and the electron transport chain and it catalyzes the oxidation of succinate to fumarate and carries electrons from FADH to CoQ in eukaryotes and bacteria. This occurs as a central function to maintaining cellular energy metabolism through the Krebs (tricarboxylic acid) cycle and electron transport chain.[7, 8]

Mutation in SDH result in heritable paraganlioma/pheochromo cytoma syndrome and also neurodegenerative disease known as Leigh syndrome. To evaluate SDH activity, product of enzymatic activity were measured at 600 nm. One unit of SDH is the amount of enzyme that generates 1.0 µmole of DCIP per minute at pH 7.2 at 25 °C. (Succinate Dehydrogenase Activity

Colorimetric Assay Kit from Sigma-Aldrich Catalog Number MAK197)

Trypan Blue Exclusion Assay

100μL of THP-1 and chondroblast cells were seeded into 96-wells plates (1 × 10^4 cells/ well) and treated with the following: vehicles control (DMSO), ethanol extract of *Tragopogon pratensis L* at 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 μg mL^{-1}. The cells were treated for 24, 48 and 72 hours and cell growth was photographed under inverted microscope at each time point.

The trypan blue exclusion assay is performed procedure to assess cell viability. After trypsinization, counting of triplicated wells of viable cells were done by hemocytometer. The present experiment was repeated 3 times.

MTT Assays

THP-1 and chondroblast (1 × 10^4 cells/well) cells were seeded into 96-well cell culture plates in 100 mL of complete culture medium. Cells were exposed to treatment with the following: vehicles control (DMSO), ethanol extract of *Tragopogon pratensis.L* at 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μg mL^{-1}. Cells were incubated with serum free medium corresponding negative control. Treated cells were incubated for 24h, 48 h and 72 h. afterward 3h before ending desire time point, 20 μL of MTT solution (5 mg mL^{-1}, PBS) was added to each well and then subjected incubated at 37 °C for 3h. The medium was discarded and each well was replaced with 100 μL of dimethyl sulfoxide (DMSO). The plate was wrapped with the aluminum foil and mildly rotated for 10 min to completely dissolve precipitate. Spectrophotometric MeTPre absorbance at a wavelength of 570 nm was recorded. MeTPre background absorbance at 690 nm was recorded and subtracted from the 570 nm meTPre absorbance.

Treatment Procedure

TP (The root of the plant was brought from arak 34°00'N, 49°40'E of Iran and the alcoholic extract made by Iranian Research Center for Genetic Resources). Incubation of bovine synoviocyte were done to indicating optimal concentration of TP in this experiment. (5 × 10^5 cells/well) for 72 h into six different group. Group number 1: cell, group number 2: cell+ LPS, group number 3: cell+ LPS+ steroid, group number 4: cell+ LPS+ NSAID, group number 5: cell+ LPS+ TPAE, group number 6: cell+ LPS+ PBS.

24h activated cells with lipopolysaccharide (LPS, 20 ng mL^{-1}; Sigma-Aldrich, St. Louis, MO, USA) were harvested and evaluated for viability of the cells. Steroids and NSAIDs were used as a positive control.

Total RNA Isolation

Cells were harvested by trypsin-EDTA 0.05 % (BIO IDEA, Tehran, IRAN) treatment, then Total cellular RNA was extracted with Trizol reagent (Cinnagen, Tehran IRAN) and precipitated with chloroform(Cinnagen, Tehran IRAN). Subsequently, after vigorous agitation and 3 min incubation at room temperature, the upper aqueous phase of centrifuged samples that including RNA was precipitated with isopropyl alcohol (Cinnagen, Tehran IRAN) and resuspended in RNase-free

water (Cinnagen, Tehran IRAN) Total RNA was quantified wit h UV spectrophotometry (Spectrophotometer UV-2100 series, Unico, Spain) and evaluated for RNA concentration and integrity.

Complementary DNA Synthesis

For each sample, 1 μg of total RNA was converted to complementary DNA (cDNA) using Moloney-Murine Leukemia Virus reverse transcriptase from the Advantage 2-step RT-PCR kit (vivantis). The reverse transcription reaction was performed at 42C for 60 min and followed by heating at 94C for 5 min to terminate cDNA synthesis reaction and abolish DNase activity.

Semi Quantitative PCR

Semi quantitative RT-PCR was performed using primers specific to bovine *COX-2* (forward, CTC TTC CTC CTGTGC CTG AT; reverse, CTG AGT ATC TTT GAC TGTGGG AG), bovine *TNF-a* (forward, TAA CAA GCC GGTAGC CCA CG; reverse GCA AGG GCT CTT GAT GGCAGA), bovine *IL-1β.* (forward, TTC TCT CCA GCC AACCTT CAT T; reverse, ATC TGC AGC TGG ATG TTTCCA T), bovine *iNOS* (forward, CGG TGC TGT ATT TCCTTA CGA GGC GAA GAA GG; reverse, GGT GCT GCTTGT TAG CAG GTC AAG TAA AGG GC), and bovine glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) (forward, ATT CCA CCC ACG GCA AGT T; reverse CGCTCC TGG AAG ATG GTG AT) as the housekeeping gene. Primers specific for human *TNF-a* (forward, GAGTGA CAA GCC TGT AGC CCA TGT TGT AGC; reverse, GCA ATG ATC CCA AAG TAG ACC TGC CCA GAC T), human *IL-1β.* (Forward, GAA GTA CCT GAG CTC GCCATG GAA; reverse, CGT GCA GTT CAG TGA TCG TACAGG), and human *GAPDH* (forward, TGA AGG TCGGAG TCA ACG GAT TTG GT; reverse, CAT GTG GGCCAT GAG GTC CAC CAC) as the housekeeping gene were also used. Thermal cycling was performed on the Thermal Cycler (rotor gen-6000, Corbett) using 2 mL cDNA template and reagents from the eva green (Cinnagen, Tehran, IRAN). Electrophoresis on 1.5% (wt vol^{-1}) agarose gels containing ethidium bromide (5 mg mL^{-1}, Sigma-Aldrich) prepared to measure the final yield and specificity of PCR amplification products. The band intensity of the PCR products was quantified using Adobe Photoshop C2 software and normalized to the *GAPDH* housekeeping gene. The experiment is representative of 3 independent experiments (n=3) and the mean ± 1 SD are shown in the figures.

Quantitative REAL-TIME PCR

Real-time PCR was carried out by similar primer sequences as mentioned above. Briefly, cDNA template (2 mL) was mixed with the fluorescent dye SYBR Green reagent from supermix kit (Cinnagen, Tehran, IRAN) in a total reaction volume of 25 mL. These real-time PCR mixtures were subjected to precise thermal cycling on the iQ5 Multicolor Real-Time PCR Detection System (corbett).

Nitrite Determination Assay

NO production assay was performed by MeTPring the amount of the stable metabolite, nitrite, using resuspended sodium nitrite in distilled water as standard sample. The equal volume of culture supernatant (100 L) was react with Griess reagent (sigma-aldrich) (one part 0.1% naphthylethylenediamine and one part 1% sulfanilamide in 5% H3PO4) in a 96-well flat bottom plate for 10 min in dark and room temperature. Mounts of nitrite were evaluated by meCCTring absorbance at 540 nm using Eliza reader (Sco DIAGNOSTIC). Levels of nitrite were normalized to standard values.

PGE2 High Sensitivity IMMUNOASSAY

A commercial PGF2 immunoassay (Invitrogen, human prostaglandin E2, ELISA kit) was used, to calculate amount of PGF2 secretion from cellular supernatant. Supernatant samples of standard PGF2 were run in parallel. Briefly, 100 mL of each supernatant sample was assayed in triplicate on a 96-well microplate coated with a goat anti-mouse polyclonal antibody. Fifty microliters of *PGE2* high sensitivity conjugate was added to each sample well. Next, 50 mL of *PGE2* antibody solution was added to each sample well. The microplate was incubated for 18-24 h at 2-8°C. After incubation, the wells were removed and washed 3 times with PGF2 wash buffer. After the last wash is aspirated, 200 mL of pNPP substrate was added to each well of microplate. After 1h incubation in 37C, the reaction was terminated by adding 50 mL of stop solution. The optical density of the samples at an absorbance of 405 nm (with wavelength correction set between 570 nm and 590 nm) was determined instantly using the ELISA reader (Sco DIAGNOSTIC).

Statistical Analysis

Data are presented as the mean ± 1 SD of percent of activated control samples. Multiple comparisons by one-way analysis of variance (ANOVA) and SPSS 22.0 software (SPSS Inc., Chicago, IL, USA), student t-test, and REST v.2000 were used for analyzing the data. The null hypothesis states that proinflammatory gene expression or production will not be different between control and TP-treated samples. In the statistical approach of ANOVA, we calculate the variance between groups of samples as well as the variance of samples within each individual group. We then compare those values. We now use these values to calculate the F statistic, the ratio of the two variances – the between-groups variance to the within-groups variances. It is used under the assumption that the different groups have similar standard deviations (they have similar variability about their mean values).

Results

The impact of TPAE on expression of Cytokine in synoviocyte

Synoviocytes cultured in 6 groups for 72 h (as described in Experimental Section) showed low levels of *TNF-* α and *iL-1*β expression in comparison with LPS activated synoviocyte. 1 h activated synoviocyte with 20 ng mL^{-1} LPS showed improvement in expression of *TNF-*α and *iL-1*β. To reported 36.3% suppression in expression of *TNF-* α and 29.7% reduction of *iL-1*β expression, we compared TPAE with activated controls. Treatment with PBS had no effect on gene expression. Treatment with corticosteroid as a control showed approximately 100% suppression of *TNF-*α and *iL-1*β. (Fig. 2, Table 1)

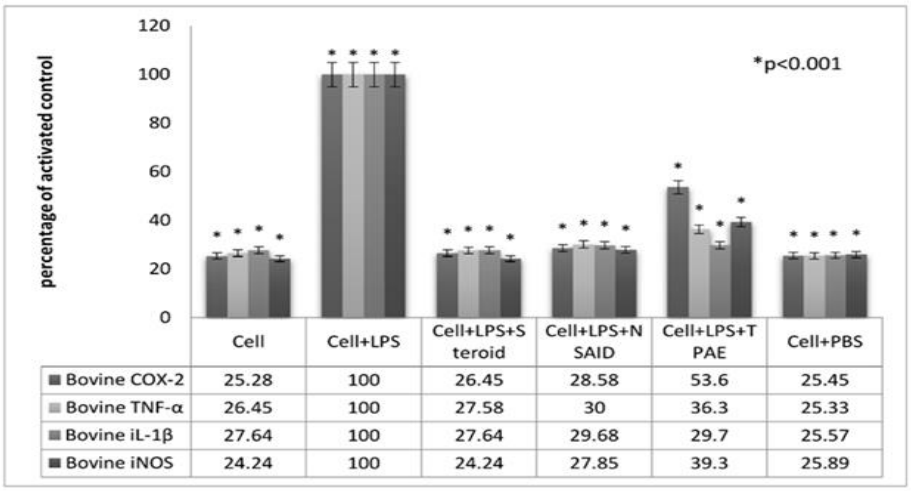

Figure 2: the effect of TPAE on proinflammatory gene expression in synoviocyte using real-time PCR. Bovine fibroblast-like synoviocyte was incubated with TPAE for 72h and activated with LPS for 24h. Quantification of normalized TNF-a, IL-1b, COX-2 and iNOS expression are shown. Statistical significances between activated control and other groups were analyzed using the t-Student test (mean±SD, n=3).

The impact of TPAE on expression of COX-2 gene and production of PGE2 in synoviocyte

Compared to activated synoviocytes, the sixth group of incubated bovine synoviocytes for 72h (as described in Experimental Section) expressed low levels of *COX-2*. As well as, secretion of PGF2 from synoviocytes was low in the cellular supernatant. Expression of cox-2 from activated synoviocytes

with 20ng mL^{-1} LPS was reported in high level, as well as significantly increased amount of secreted PGF2 was observed. Activated controls levels in comparison with TPAE treatment cells indicated increases downregulated *COX-2* expression up to 53.6%. (Fig. 2 and table 1) activated synoviocytes that pretreatment with TPAE reported reduction in PGF2 levels up to 52% compared with activated control. (Fig. 3) Treatment with PBS has no effect on gene expression. Treatment with corticosteroid and NSAID as a control has shown approximately 100% suppression of *COX-2*. (Fig. 2, Table 1)

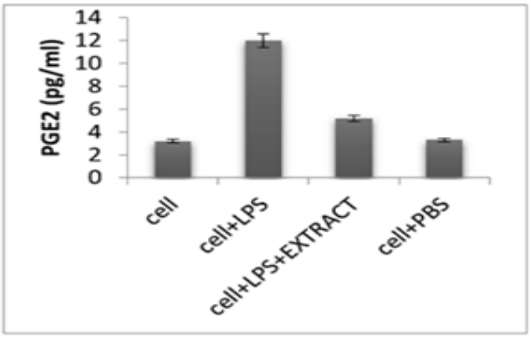

Figure 3: The effect of TPAE on PGE2 levels in synoviocytes. BFLS were incubated with TPAE for 72h and activated with LPS for 24h. Mean PGE2 levels released into the cellular supernatant are shown as percent of activated control. Statistical significances between activated control and other groups were analyzed using the t-Student test (mean±1 SD, n=3).

The impact of TPAE on expression of INOS gene and production of nitrite in synoviocyte

As comparing activated synoviocytes, incubated synoviocytes control media alone and TPAE alone showed low levels of iNOS expression and nitric production. Pretreated activated synoviocyte with TPAE displayed 72% decrease in NO levels in comparison with activated control cells (Fig. 4) Activated Synoviocyte pretreated with TPAE displayed considerable increase downregulation expression of iNOS up to 39.3%. (Fig. 2 and table 1) Treatment with PBS has no effect on gene expression. Treatment with corticosteroid and NSAID as a control showed approximately 100% suppression of *INOS*. (Fig. 2 and table 1)

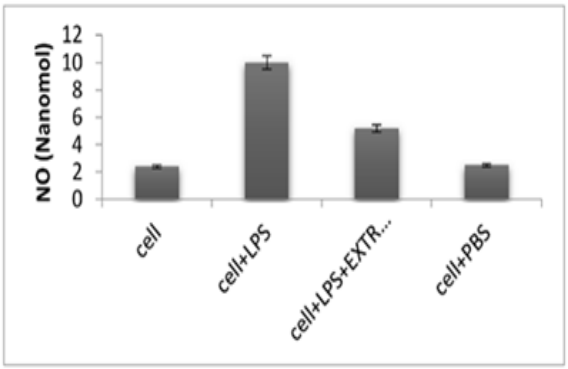

Figure 4: The effect of TPAE on nitrite levels in synoviocytes. BFLS were incubated with TPAE for 72h and activated with LPS for 24h. Mean nitrite levels released into the cellular supernatant are shown as percent of activated control. Statistical significances between activated control and other groups were analyzed using the t-Student test (mean± SD, n=3).

Table 1. The effect of TPAE on proinflammatory gene expression in Synoviocytes using semiquantitative RT-PCR analysis. Bovine fibroblast- like synoviocyte (BFLS) were incubated with TPAE for 72 h and activated with LPS for 1 h. Normalized gene expression is shown as percent of activated control (cell+ LPS). ** p<0.001

Gene	Cell	Cell+LPS	Cell+LPS+ Steroid	Cell+LPS+ NSAID	Cell+LPS+ TPAE	Cell+PBS
Bovine COX-2	25.28±0.1137**	100	26.45±0.7147**	28.58±0.4947**	53.6±0.7**	25.45±0.1137**
Bovine TNF-a	26.45±0.2237**	100	27.58±0.7147**	30±0.5947**	36.3±0.392**	25.33±0.1137**
Bovine IL-1B	27.64±0.3337**	100	28.12±0.7147**	29±0.6947**	29.7±0.1**	25.57±0.1137**
Bovine iNOS	24.24±0.55**	100	26.24±0.7147**	28.85±0.8947**	39.3±0.1137**	25.89±0.1137**

The impression of TPAE on Cytokine Gene Expression in Human THP-1 Cells

72 h incubated human THP-1 cells are shown low level expression of *TNF- α* and *iL-1β* in comparison with LPS-activated cells. The cells that activated with 20 ng mL^{-1} of LPS displayed a considerable upregulated in expression of *TNF- α* and *iL-1β*. Activated THP-1 cells that exposed with TPAE, *TNF-α* was decreased by 39.91% compared with activated control cells.(Fig. 5 and table 2) pre-treatment with TPAE compared with activated control cells showed 33.01% suppression in the expression of *iL-1β*. Treatment with PBS had no effect on gene expression. Treatment with corticosteroid as a control showed approximately 100% suppression of *TNF-α* and *iL-1β*. (Fig. 5, Table 2)

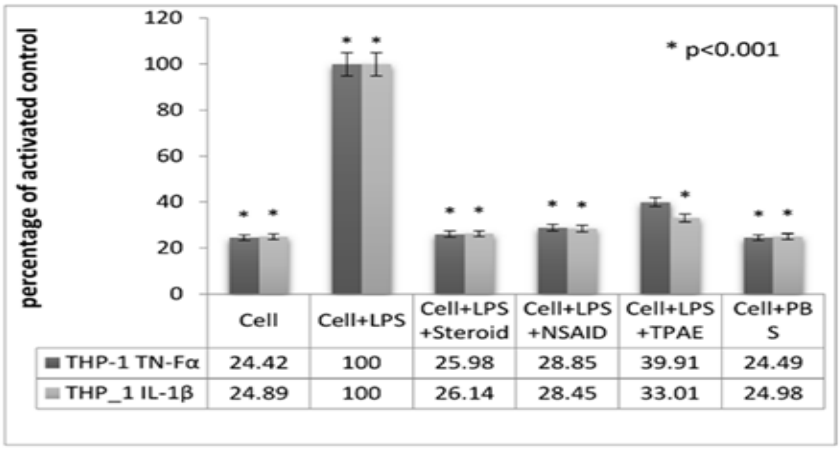

Figure 5: The effect of TPAE on cytokine gene expression in *THP-1* cells using real-time PCR. *THP-1* cells were incubated with TPAE for 72h and activated with LPS for 24h. Quantification of normalized *TNF-α* and *IL-1β* expression is shown. Statistical significances between activated control and other groups were analyzed using the t-Student test (mean±1 SD, n=3).

Table 2: The effect of TPAE on proinflammatory gene expression in THP-1 cells using semiquantitative RT-PCR analysis. THP-1 cells were incubated with TPAE for 72 h and activated with LPS for 1 h. Normalized gene expression is shown as percent of activated control (Cell+ LPS). **P < 0.001.

Gene	Cell	Cell+LPS	Cell+LPS+Steroid	Cell+LPS+NSAID	Cell+LPS+TPAE	Cell+PBS
THP-1 TNF-α	24.42±0.22**	100	25.98±0.71**	28.85±013**	39.91±0.39**	24.49
THP-1 IL-1β	24.89±0.34**	100	26.14±0.82**	28.45±0.23**	33.01±0.1**	24.98

Discussion

Arthritis is a debilitating progressive disease, and factors such as age, genetics, trauma, obesity, and increased biomechanical stress on the joints may affect the course of the disease. Increased bone density in the joint may lead to a cascade of catabolic processes at the molecular level. Inflammatory cytokines can cause devastating effects, primarily in the articular cartilage. These substances impact not only cell aging and apoptosis, but also synthesis of major elements of cartilage such as proteoglycans and collagen. Moreover, the inflammatory elements result in improved synthesis and release of proteolytic enzyme such as MMP and ADAMTS family of matrix metalloproteinases, leading to the breakdown of articular cartilage. In addition to its effect on cartilage, there are effects on the synovial cells and articular tissues around the joint leading to inflammation. Inflammatory cytokines can also increase their synthesis process and can propel the inflammatory process.[9] The observations made in OA show that *IL1-β* and *TNF-α* activate intracellular signals such as *P53MAPK, JNK, NF-ΚBC* that lead to catabolic response and eventually cause cartilage damage.[10]

IL1-β plays a role as a cytokine involved in the pathogenesis of OA and leads to an inflammatory and catabolic response in the cartilage. Chondrocytes exposed to *IL1-β* and *TNF-α* are quickly driven to the induction of apoptosis. Activation of *TNF-α* and *IL1-β* and other inflammatory cytokines leads to inhibition of the signaling pathway involved in the path of growth of *TGF-β* and inhibition of *SAMAD7* protein and inhibits the synthesis of *TGF-β* type II receptor in cartilage cells . Furthermore, the secretion of enzymes and mediators involved in the pathophysiology of OA includes *iNOS*, which leads to the production *of NO*, phospholipase A2, cyclooxygenase2 (*COX-2*), and prostaglandin2 synthetase (which produces prostaglandin E2 (*PGE2*)).[11, 12]

TNF-α is secreted from the same cells in the joint that are responsible for synthesis of *IL1-β* and increased expression of it in similar tissues such as synovial fluid, synovial membrane, cartilage and the subchondral bone layer has been reported. *TNF -α* is important in the pathogenesis of OA.[13-16]

IL1-β and *TNF-α* secretion are usually simultaneously increased; in addition, the impact on the signaling pathway of cell and tissue catabolism is associated with increased joint inflammation, reduced productivity of the respiratory chain, and lower ATP in cartilage mitochondria, resulting in decreased mitochondrial membrane potential and also induced synthesis of *PGE2, iNOS, NO, COX-2* .[11, 12, 17, 18]

In this article, we acclaim that TPAE reduces *TNF-α* levels by 81%, and as a result was expected to decrease inflammation and reduce the synthesis of *PGE2, iNOS, NO, COX-2* that was not

reported before. Of course, the need to do more research on this issue seems clear.

The effect of phenolic compounds in *Tragopogon pratensis* (TP) to address the uncontrolled cell growth that leads to cancer has been demonstrated. [19] These compounds include Gallic acid, Ferulic acid, Rutin, Resveratrol, Sinapic acid and Caffeic acid. Gallic acid is a free radical that induces apoptosis, and has an important role in uncontrolled cell growth and the fight against cancer. Therefore, this is a major factor.[19-21] In research on the treatment of osteoarthritis with NSAIDs, researchers have shown that NSAID medications, especially selective *COX-2* inhibitors, have the greatest impact on preventing the expression of *COX-2*.[22] In this study, we showed that the expression of *COX-2* was reduced 88% by TPAE.

We approve the role of TPAE in downregulate expression of COX2 and iNOS genes, resulting in a reduction of *PGE2* and nitrite production in the cellular supernatant. Our ex vivo investigation claim that TPAE can reduce the expression and production of such inflammatory cytokines in multiple cell types. Localization of immune cells in the synovial tissue macrophage/monocyte play critical role in pathogenesis of arthritic conditions as known. Chondrocyte as an articular cartilage cellular component with the capacity to modulate the phenotype, have a potential for play pivotal role in the development of joint diseases. as mentioned above, the TPAE effect on both cells, we assert that anti-inflammatory of TPAE are not constrained to chondrocyte, but also have an impact of macrophage/monocytes that are in related with a synovial membrane. In this regard, since the capacity of TPAE to decrease proinflammatory targets in several tissue cell type, suggests its possible candidate as substitute or complement to common NSAID use to OA treatment, however our ex vivo statics can inferred to describes all the in vivo effects seen in OA patients remains to be extra examinations

Conclusion

In studies about OA pathophysiology it has been determined that increasing the expression of inflammatory cytokine in these diseases, including *iL-1β* and *TNF-α , PGE2, NO, iNOS, COX-2,* activates pathways of catabolic breakdown of articular cartilage, thus inducing apoptosis and activation of the immune system. The best way to prevent symptoms is to reduce the synthesis of this cytokine. Nowadays chemical drug for reducing inflammation and joint pain available for arthritic patients are associated with a high rate of side effects. To reduce the side effects of chemical drugs, medicinal plants can be used as effective drugs with low side effects for the treatment of this disease. One of these plants is Salsify or TP. Previous studies were conducted on TP that showed the effect of healing and rheumatism treatment and liver congestion.

In our research, we evaluate the impact of TPAE on the expression of inflammatory cytokine in the inflamed cells with LPS20 into two levels of cartilage and monocytes / macrophage cells and our tests showed that TPAE in small quantities has effects on *iL -1β* and in very high quantities on *TNF-α, PGE2, NO, iNOS, COX-2* expression and reduces a lot of expression in cartilage and macrophage\monocyte cells.

We suggest that there may be a future for this plant to be used as a drug for reducing inflammatory cytokine expression and reducing inflammation, joint pain, and swelling associated with osteoarthritis.

Abbreviations

TPAE: *Tragopogon pratensis* Alcoholic Extraction
TNF: Tumor Necrosis Factor
IL: Interleukin
COX: Cyclooxygenase
NO: Nitric Oxide
PGE2: prostaglandin E2
iNOS: induced nitric Oxide
OA: Osteoarthritis
NSAID: non-steroidal anti-inflammatory drugs
DMSO: Dimethyl sulfoxide

Declarations

Acknowledgements

Not applicable.

Funding

No funding was received for this work.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Authors' contributions

HM participated in research design. Both HM and FTR conducted the experiments. HM performed data analysis. Both FTR and SG wrote or contributed to the writing of the manuscript. SHM helped on editing the article. All authors read and approved the final manuscript.

Ethics approval

All experimental procedures were approved by the animal research ethics committee of Payame Noor University (PNU) of Tehran.

Consent for publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

References

1. Au, RY, TK Al-Talib, AY Au, PV Phan, and CG Frondoza. 2007. Avocado soybean unsaponifiables (ASU) suppress TNF-α, IL-1β, COX-2, iNOS gene expression, and prostaglandin E 2 and nitric oxide production in articular chondrocytes and monocyte/macrophages. *Osteoarthritis and cartilage* 15 (11):1249-1255.
2. Davatchi, Fereydoun, Ahmad-Reza Jamshidi, Arash Tehrani Banihashemi, Jaleh Gholami, Mohammad Hossein

Forouzanfar, Massoomeh Akhlaghi, Mojgan Barghamdi, Elham Noorolahzadeh, Ali-Reza Khabazi, and Mansoor Salesi. 2008. WHO-ILAR COPCORD study (stage 1, urban study) in Iran. *The Journal of rheumatology* 35 (7):1384-1390.

3. Goldring, Mary B. 2000. The role of the chondrocyte in osteoarthritis. *Arthritis & Rheumatology* 43 (9):1916-1926.
4. Goldring, Steven R, and Mary B, Goldring. 2004. The role of cytokines in cartilage matrix degeneration in osteoarthritis. *Clinical Orthopaedics and Related Research* 427:27-36.
5. Mastbergen, S. C., F. P. J. G. Lafeber, and J. W. J. Bijlsma. 2002. Selective COX-2 inhibition prevents proinflammatory cytokine-induced cartilage damage. *Rheumatology* 41 (7):801-808.
6. A, Zargari. 1997. *Medical Plants*. sixth Aufl.: Tehran University Publications.
7. Kim, Hyung J, and Dennis, R Winge. 2013. Emerging concepts in the flavinylation of succinate dehydrogenase. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1827 (5):627-636.
8. Rutter, Jared, Dennis R. Winge, and Joshua D. Schiffman. 2010. Succinate dehydrogenase–assembly, regulation and role in human disease. *Mitochondrion* 10 (4):393-401.
9. Wojdasiewicz, Piotr, Łukasz A. Poniatowski, and Dariusz, Szukiewicz. 2014. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. *Mediators of inflammation* 2014.
10. Mengshol, John A., Matthew P. Vincenti, and Constance E. Brinckerhoff. 2001. IL-1 induces collagenase-3 (MMP-13) promoter activity in stably transfected chondrocytic cells: requirement for Runx-2 and activation by p38 MAPK and JNK pathways. *Nucleic acids research* 29 (21):4361-4372.
11. El Mansouri, Fatima Ezzahra, Nadir Chabane, Nadia Zayed, Mohit Kapoor, Mohamed Benderdour, Johanne Martel-Pelletier, Jean-Pierre Pelletier, Nicolas Duval, and Hassan Fahmi. 2011. Contribution of H3K4 methylation by SET-1A to interleukin-1–induced cyclooxygenase 2 and inducible nitric oxide synthase expression in human osteoarthritis chondrocytes. *Arthritis & Rheumatology* 63 (1):168-179.
12. Hardy, Medora M, Seibert, Karen, Manning, Pamela T, Currie Mark G, Woerner, B Mark, Dorothy, Edwards, Koki, Alane, and Tripp, Catherine S. 2002. Cyclooxygenase 2-dependent prostaglandin E2 modulates cartilage proteoglycan degradation in human osteoarthritis explants. *Arthritis & Rheumatology* 46 (7):1789-1803.
13. Melchiorri, Cinzia, Riccardo, Meliconi, Luigi, Frizziero, Tania, Silvestri, Lia, Pulsatelli, Ilaria, Mazzetti, Rosa Maria, Borzì, Mariagrazia Uguccioni, and Andrea, Facchini. 1998. Enhanced and coordinated in vivo expression of inflammatory cytokines and nitric oxide synthase by chondrocytes from patients with osteoarthritis. *Arthritis & Rheumatology* 41 (12):2165-2174.
14. Massicotte, F., D. Lajeunesse, M. Benderdour, J-P. Pelletier, G. Hilal, N. Duval, and J. Martel-Pelletier. 2002. Can altered production of interleukin-1β, interleukin-6, transforming growth factor-β and prostaglandin E 2 by isolated human subchondral osteoblasts identify two subgroups of osteoarthritic patients. *Osteoarthritis and cartilage* 10 (6):491-500.
15. Farahat, M Nabil, Ghada Yanni, Robin Poston, and Gabriel S Panayi. 1993. Cytokine expression in synovial membranes of patients with rheumatoid arthritis and osteoarthritis. *Annals of the rheumatic diseases* 52 (12):870-875.
16. Sohn, Dong Hyun, Jeremy Sokolove, Orr Sharpe, Jennifer C. Erhart, Piyanka E. Chandra, Lauren J. Lahey, Tamsin M. Lindstrom. 2012. Plasma proteins present in osteoarthritic synovial fluid can stimulate cytokine production via Toll-like receptor 4. *Arthritis research & therapy* 14 (1):R7.
17. Martel-Pelletier, Johanne, Jean-Pierre Pelletier, and Hassan Fahmi. 2003. Cyclooxygenase-2 and prostaglandins in articular tissues. In *Seminars in arthritis and rheumatism*: Elsevier.
18. Jones, SW, SMV Brockbank, KM Clements, N Le Good, D Campbell, SJ Read, MRC Needham, and P Newham. 2009. Mitogen-activated protein kinase-activated protein kinase 2 (MK2) modulates key biological pathways associated with OA disease pathology. *Osteoarthritis and cartilage* 17 (1):124-131.
19. Sohi, Kiranjit K., Nidhi Mittal, Manjinder K. Hundal, and Krishan L. Khanduja. 2003. Gallic acid, an antioxidant, exhibits antiapoptotic potential in normal human lymphocytes: a Bcl-2 independent mechanism. Journal of nutritional science and vitaminology 49 (4):221-227.
20. Kucekova, Zdenka, Jiri Mlcek, Petr Humpolicek, Otakar Rop, Pavel Valasek, and Petr Saha. 2011. Phenolic compounds from Allium schoenoprasum, Tragopogon pratensis and Rumex acetosa and their antiproliferative effects. *Molecules* 16 (11):9207-9217.
21. Salucci, M., L. A. Stivala, G. Maiani, R. Bugianesi, and V. Vannini. 2002. Flavonoids uptake and their effect on cell cycle of human colon adenocarcinoma cells (Caco2). *British journal of cancer* 86 (10):1645-1651.
22. Crofford, Leslie J. 2013. Use of NSAIDs in treating patients with arthritis. *Arthritis research & therapy* 15 (3):S2.