Int. J. Adv. Res. MicroBiol. Immunol.
Volume 2, Issue 1 - 2020, Pg. No. 1-13
Peer Reviewed & Open Access Journal

Research Article

Anti-Inflammatory Properties of Pomegranate

Valentina Stefanou, Spyros Papatheodorou, Aliki Tsakni, Vladimiros Lougovois, Aikaterini Talelli, Giannis Panourgias, Alexandros Dariatos, Ioannis Tsaknis

Department of Food Science and Technology, Faculty of Food Sciences, University of West Attica, Athens, Greece.

I N F O

Corresponding Author:
Valentina Stefanou, Department of Food Science and Technology, Faculty of Food Sciences, University of West Attica, Athens, Greece.
E-mail Id:
stef12valentin@gmail.com
Orcid Id:
https://orcid.org/0000-0003-2856-4206
How to cite this article:
Stefanou V, Papatheodorou S, Tsakni A, Lougovois V, Talelli A, Panourgias G et al. Anti-Inflammatory Properties of Pomegranate. *Int J Adv Res MicroBiol Immunol* 2020; 2(1): 1-13.

Date of Submission: 2020-08-28
Date of Acceptance: 2020-10-15

A B S T R A C T

Pomegranate, containing in high concentration anthocyanins, anthocyanidins, flavones, flavanones, flavanols, flavonols, hydrolysable and non-hydrolysable tannins and alkaloids possesses very strong anti-oxidant, anti-inflammatory and antimicrobial properties. Researches have shown that the expression of pro-inflammatory cytokines IL-6, IL-5, IL-8, IL-10, IL-1β, IL-18, TNFα and IFN-γ and also levels of COX, NF-κB, MPO, NO and MMPs are remarkably downregulated by pomegranate extracts or isolated phytochemicals reducing thus the risk of chronic inflammation, autoimmune and autoinflammatory diseases, tissue damage, eosinophil mediated inflammation, infections and pathogenic processes such as cancer. Certain types of inflammation are caused by free radicals and the antioxidant pomegranate is very effective in eliminating oxidative stress that leads to chronic inflammation and several diseases. Due to antimicrobial properties of pomegranate's phytochemicals, it's found to be beneficial in inflammations where microbes are involved. As pomegranate contains phytochemicals with more than one bioactivity, it can affect more than one of the factors involved in inflammatory incident and process, facilitating the healing process. It is important to be mentioned that pomegranate gave comparable results with commercial drugs in doses used, without side effects and also showed beneficial activity where commercial drugs were harmful, indicating that pomegranate could possibly provide alternative, very effective anti-inflammatory medicines.

Keywords: Pomegranate, Phytochemicals, Tannins, Flavonoids, Anti-Inflammatory, Antioxidant, Antibacterial, Autoimmune, Cytokine, Cell-Lines

Introduction

Inflammation is a complex defense mechanism of the body, where leucocytes migrate to damaged tissues from the vasculature, in order to destroy the agents that could possibly cause tissue injury.[1] Acute inflammation is a response to infection agents as pathogens (bacteria, fungi, viruses), effects of chemicals, radiation, external injuries, wounds, damage through foreign objects. Inflammation plays important role in healing but chronic inflammation can create several diseases and physical disfunctions.[1,2,3,4] Persistent acute inflammation, long term and low-level exposure to an irritant, or genetic factors is possible to lead to chronic inflammation. Chronic inflammation is a threat factor for cancer progression as the release of inflammatory molecules generates highly favorable microenvironment for tumorigenesis, cancer progression and metastasis.[5] Inflammation can also be involved in other chronic diseases

and disfunctions as allergies, obesity, atherosclerosis, periodontitis, hay fever, insulin resistance, diabetes type-2, pulmonary and neurodegenerative diseases (Alzheimer's Disease).[6]

Fruits rich in polyphenols and especially ellagic acid and ellagitannins possess strong antioxidant and anti-inflammatory properties showing several medicinal activities related to their antioxidant and anti-inflammatory properties.[6] Fruits containing tannins in high concentration have been found to be a good alternative strategy in order to prevent cancer and this is due to their anti-inflammatory activity.[7] Pomegranate is very rich in polyphenolic compounds and contains in its' juice, pericarp, seeds, leaves, flowers, bark and roots several phytochemicals in different concentrations, making each plant part proper to treat a wide range of different diseases. Its' anti-inflammatory properties were known and used in traditional medicine since ancient years in several countries to treat inflammation, pain, fevers, parasite infections, diarrhea, ulcerative colitis, diabetes and other health issues.[8,9,10]

Researches have shown that pomegranate can treat efficiently Chronic Inflammatory Diseases (CID) where inflammations can be in joints, skin, lungs and intestinal mucosa, asthma, rheumatoid arthritis, Inflammatory Bowel Disease (IBD), psoriasis, Chronic Obstructive Pulmonary Disease (COBD) and also metabolic and cardiovascular problems.[6]

Pomegranate phytochemicals

Pomegranate contains several bioactive compound categories such as flavonoids (flavonols, flavanols, flavones, flavanones, anthocyanins, anthocyanidins), tannins (hydrolysable and non-hydrolysable), lipids, simple organic acids and alkaloids.

Flavonoids (flavonols, flavanols, flavones, flavanones, anthocyanins), are present in the plant generally as glycosylated forms[11] and they possess strong antioxidant and anti-inflammatory properties.[12] Anthocyanins are the most important and the largest group of flavonoids that exist in pomegranate juice. These compounds and hydrolysable tannins are the most bioactive compounds. Anthocyanins existing in the pomegranate are delphinidin-3-glucoside, delphinidin 3,5-diglucoside, cyanidin 3 rutinoside, cyanidin 3,5-diglucoside, cyanidin 3-hexoside, pelargonidin 3,5-diglucoside, pelargonidin 3-glucoside Some anthocyanidins or aglycones are delphinidin, cyanidin, peonidin, pelargonidin and petunidin. Other flavonoids found in the pomegranate are rutin, kaempferol, kaempferol 3-O-glucoside, kaempferol 3-O rhamnoglycoside, quercetin, dihydrokaempferol-hexoside, catechin, epigallocatechin-3-gallate, epicatechin, apigenin, luteolin, luteolin 7-O-glucoside, naringenin 7-O-rutinoside.[11,13,14]

Important hydrolysable tannins existing in the pomegranate are gallic acid, ellagic acid, gallagic acid, brevifolin carboxylic acid, ellagic acid pentoside, ellagic acid hexoside, ellagic acid deoxy hexoside, granatin A, granatin B, peduncilagin I, pedunculagin II, punicalin a, punicalin β, punicalagin α, punicalagin β, digalloyl-gallagylhexoside, gallagyl hexoside, pomegraniin A, pomegraniin B, punicacortein C, galloyl-HHDP-glucuronide, galloyl-HHDP-hexoside. Non hydrolysable tannins are the catechin and epicatechin polymers.[15,16]

Some of the lipids pomegranate contains are punicic acid which is consisting over 60% of the fatty acids, palmitic acid, arachidic acid, α-eleosteric acid, β-eleosteric acid, oleic acid, linolenic acid, linolelaidic acid, myristic acid, campesterol, estradiol, estrone, β- sitosterol, estriol, testosterone, Asiatic acid, ursolic acid and punicanolic acid.[15]

Important simple organic acids that can be found in pomegranate are citric, ascorbic, chlorogenic, oxalic, tartaric and succinic acids.[15,17,18]

Alcaloids existing in pomegranate plant are hydrine, norhygrine, sedridine, pelletierine and N-methylpelletierine.[17]

Pomegranate contains also minerals such as K, Na, Mg, Mn, Cu, Fe and Zn.

Different plant varieties and also different climate, mature and storage conditions of even the same variety plants can lead to different constituents' concentrations.[8]

Anti-inflammatory properties

The main compounds that have anti-inflammatory properties in the pomegranate are urolithins. Urolithins are created by ellagitannins and ellagic acid while they are metabolized by gut microbiota. Ellagitannins and ellagic acid are in very high concentrations in the pomegranate extract.[19,20] Pharmacokinetic studies showed that the flavonoids procyanidin which is found in the juice, kaempferol and luteolin in the peel and aperigin in the leaves possess strong anti-inflammatory properties. The tannins that are found to show anti-inflammatory properties are gallic acid, found in flowers, juice, leaves and peel, granatin B and gallagyl-dilacton both existing in the peel. Melatonin is an alkaloid in the pomegranate juice which has anti-inflammatory properties.[21]

Several in vitro and in vivo researches have shown that pomegranate plant parts' extracts show remarkable anti-inflammatory properties in the gastrointestinal tract. Diarrhea that mainly is caused by inflammation was found to be diminished after 100-400 mg/kg aqueous peel extract injection in rat models. Oral administration of 200-400 mg/kg pomegranate rind methanolic extract reduced diarrhea symptoms in the rat models.[22]

The anti-inflammatory activity of pomegranate peel extracts has been evaluated in vitro on Caco-2 (heterogenous human epithelial colorectal adenocarcinoma) cells and ex-vivo on porcine colonic tissue explants. Both tissues were stimulated in order to be inflamed and then were treated with 0, 1.0, 2.5, 5.0, 10 and 25 mg/ml peel extract. The results showed that the 5mg/ml extract consistently shows strong anti-inflammatory activity.[23]

Inflammatory Bowel Disease (IBD), a chronic disease characterized by recurrent intestinal inflammation episodes is linked with an autoimmune reaction to environmental and genetic factors. In research, two groups of IBD patients with high risk of clinical relapse which were in stable therapy for at least 3 months, were administrated twice daily for 12 weeks with 125 ml pomegranate juice and 125 ml of placebo drink. The results showed that there was difference among the two groups in the surrogate mucosal improvement marker fecal neutrophil-derived protein calprotectin with the pomegranate group to show improvement. Besides, after 12-week pomegranate juice consumption there was beneficial change on local and systemic inflammatory markers.[24]

Gastritis is an inflammation of gastric mucosa which can be caused by endogenous or exogenous factors such as microbes, stress, pepsin, acidity, alcohol and non-steroidal anti-inflammatory drugs (NSAIDs).[22] Helicobacter pylori is a bacterium that can cause gastritis and also other problems such as peptic ulcer disease and gastric cancer[25]. Flavonoids, tannins and alkaloids that are contained in pomegranate possess strong antimicrobial activity.[26,27,28,29] In vitro studies showed that pomegranate extracts show significant anti- H. pylori activity. Methanolic peel extract was found to have comparable activity with metronidazole, a commercial antibiotic. Another research showed that methanolic rind extract had the highest anti- H. pylori activity than other plant extracts. Ethanolic pericarp extract showed very strong anti-H. pylori activity too.[22]

In a research, nociceptive mice were administrated with 10, 30 and 100 mg/kg of HPLC characterized pomegranate extract and the results were compared with those of two NSAIDs: diclofenac, which is the most commonly used NSAID and indomethacin that shows gastrointestinal toxicity. Diclofenac is also causing gastric damage as indomethacin but the ulcer depths are shallow and the gastric damage less severe. The inflammatory phase inhibition has been observed from 10 mg/kg pomegranate extract and there was no gastric damage with the systemic administration of pomegranate compared with indomethacin. The extract not only did not cause damage as the above medicines and other NSAIDs but gastroprotective activity has been also observed. In the inflammatory phase, pomegranate showed remarkable ceiling effect in comparation with diclofenac. The pomegranate extract, was found to be rich in hydrolysable tannins and anthocyanins with main phenolics to be ellagic acid free form and glucoside derivatives followed by punicalagin derivatives. The ellagitannin content was 8-fold higher than anthocyanin content. Cyanidin glucosides were the 71% of total anthocyanidins. In the extract there were also in traces other phenolic compounds too as flavan-3-ols and phenolic acids.[9] The pomegranate's gastroprotective activity is attributed to bioactive compounds such as tannins, flavonoids and saponin. It is referred that the tannins, forming complexes with proteins and polysaccharides are creating a layer on the epithelial tissues that acts protectively as it prevents the bleeding and accelerates the healing process.[30] Administration of Wistar rats with 490 and 980 mg/kg pomegranate aqueous methanolic extract containing saponin, tannins and flavonoids was found to decrease remarkably at both doses the ulcer lesion created by indomethacin and aspirin.[31]

In another study has been seen that pomegranate peel extract possess strong anti-inflammatory and anti-gastric ulcerogenic activity. Its' anti-inflammatory properties were found to be comparable or even better than these of the commercial drug indomethacin but in contrast with this drug which shows gastrointestinal toxicity and creates ulcers, the extract did not cause any ulcers and additionally showed strong gastroprotective and anti- ulcerogenic activity.[32]

Proinflammatory cytokines are signaling proteins secreted by immune cells such as macrophages and promote inflammation. Chronic overproduction of cytokines leads to inflammatory diseases.[33] Mitogen-Activated Protein kinases (MAPKs) play important regulatory role in the pro-inflammatory cytokines production and are involved in signaling events leading to inflammation.[34] Researches showed that pomegranate peel polyphenols and also isolated ellagic acid and punicalagin are regulating the MAPKs pathway and thus pro-inflammatory cytokines and inflammatory mediators are decreased.[35]

Pomegranate beverage administration in colitis rats model reduced the pro-inflammatory cytokines in serum and mucosa levels and intestinal inflammation has been decreased. Mammalian Target of Rapamycin (mTOR) is a protein kinase, regulating protein synthesis and cell growth in response to nutrients, energy levels, growth factors and stress.[36] It reconfigures cellular metabolism, cytokine responses, macrophage polarization, antigen presentation and cell migration.[37] Deregulated mTOR signaling is involved in inflammation, diabetes and cancer progression.[38] Pomegranate polyphenols were found to downregulate the mTOR downstream pathway.[7]

Nitric Oxide (NO) acts as a mediator and also a regulator in inflammations. It has cytotoxic activity against pathogenic microbes and parasites, yet it also can cause damages

on host tissues. inducible Nitric Oxide Synthase (iNOS) generates NO. The dysfunctional induction of the iNOS expression can cause several human diseases.[39,40] Pomegranate shows potential NO inhibition in RAW 264.7 macrophages. Administration of pomegranate,100 mg/kg on mice with paw edema decreased remarkably the edema. The isolated active anti-inflammatory hydrolysable tannins punicalin, punicalagin, strictinin A and granatin B were found to inhibit the NO production and iNOS expression in the cells RAW 264.7.[4] Cycloxigenase (COX) is the prostaglandin endoperoxide synthase, an isoenzyme responsible for the prostanoids' formation. COX is one of the various inflammation mediators involved in pathological process related with cancer. COX inhibition provides relief from inflammation and pain.[5] Granatin B had the best inhibitory effect on COX-2 and iNOS and also inhibited more efficiently the paw swelling than the other isolated compounds.[4]

Ulcerative colitis, a subtype of inflammatory bowel disease is an inflammatory disease that occur in the colon mucosal. Using a chronic inflammation mice model the anti-inflammatory activity of ethanolic pomegranate peel extract has been evaluated. 6 groups of Swiss Webster mice were administrated daily with aspirin 43 mg/kg, ellagic acid 26 mg/kg, dextran sodium sulfate 2% b/v, ethanolic pomegranate peel extract 240 mg/kg and 480 mg/kg. In all above groups dextran sodium sulfate 2% has been administrated over 3 cycles except the normal group. The results showed that both the 240 mg/kg and 480 mg/kg ethanolic pomegranate extract doses decreased the colon inflammation. Besides the COX-2 and iNOS expressions were remarkably reduced. The results among the ethanolic pomegranate extracts, pure ellagic and aspirin groups were comparable[10] and this indicates that the pomegranate peel extract could be used as an alternative treatment for ulcerative colitis, especially because there are no side effects in contrast with the ani-inflammatory medicines that are used.[9,10,41,42,43,44]

In a research on colon inflammation, the effects of pomegranate extract and of the gut microbiota derived metabolite Urolithin A have been evaluated. Fisher rats were daily orally administrated by 250 mg/kg pomegranate extract or 15 mg/kg for 25 days. 5% Dextran sodium sulphate that causes inflammatory bowel disease has been administrated for the last 5 days. The results showed that the inflammatory markers cyclooxygenase-2, iNOS and prostaglandin E synthase were reduced in the colonic mucosa by both administrations of pomegranate extract and Urolithin A. Both administrations were found to be beneficial for the gut microbiota too. In the Urolithin A administrated group were observed several downregulated pathways including this of inflammatory response. In the pomegranate extract group but not in the Urolithin A group has been observed decrease of oxidative stress in colon and plasma mucosa. Only Urolithin A preserved the colonic architecture. In health subjects with normal metabolism, Urolithin A is the main effective compound that comes from pomegranate consumption. In inflamed colon as in inflammatory bowel disease, the anti-inflammatory activity is because of the synergic action of ellagic acid and ellagitannin fraction with Urolithin A in minor amount.[45]

Oral administration of pomegranate extract and ellagitannin and ellagic acid enriched pomegranate extract reduced significantly the over-expression of iNOS and COX-2 in murine chronic model of Cronh's disease.[46]

In a study, the anti-inflammatory activity of pomegranate peel extract rich in hydrolysable tannins as α- and β-punicallin, α- and β- punicalagin and also granatin-B, ellagic acid, gallic acid and their derivatives has been evaluated on bovine mammary epithelial cells BME-UV1. The peel extract at the dosage of 10 mg/ml showed good anti-inflammatory activity decreasing pro-inflammatory cytokine expressions.[47] Tumor Necrosis Factor (TNF) is a cytokine that helps a healthy body to fight off infections, but is also involved in inflammatory diseases and other pathological processes. High levels of TNF indicate or can cause inflammation. In order to treat inflammatory disorders anti-TNF drugs have been developed, yet has been found that they are not effective always and even is possible to cause aggravation of the disease in some rare cases. 48 hours treatment of the BME-UV1 with the pomegranate peel extract caused remarkable decrease in comparation with the control group.[47,48] Interleukin 1 beta (IL-1β), a pro-inflammatory cytokine that is involved in inflammation, pain and autoimmune conditions was also decreased after the pomegranate treatment.[47,49] Interleukin (IL-10) is an anti-inflammatory cytokine that plays important role in immune mediated damage prevention. If the immune system is irregulated, the activity of IL-10 can lead to chronic infections. After the pomegranate treatment of BME-UV1, the IL-10 levels were also reduced.[47,50]

Interleukin (IL-6) is a prototypical cytokine that maintains homeostasis and it is TNF produced immediately when homeostasis is disturbed by tissue injury or infections in order to defense against the emergent stress. Dysregulated expression and synthesis of IL-6 dictates the transition from acute to chronic inflammation that is related with many diseases, so inhibition of it is very important[1,51]. Interleukin IL-1β is protecting the host against infections but if it is dysregulated it is associated with inflammatory damages and auto-inflammatory diseases.[52] The anthocyanin delphinidin was found to eliminate inflammatory inhibiting significantly the IL-1β, IL-6 and COX-2 expression.[53,54]

Adipocytes and macrophages secrete pro-inflammatory markers and cause systemic inflammation that leads to metabolic complications. The anti-inflammatory properties

of pomegranate peel extract and also the isolated phenolics ellagic acid and punicalagin were evaluated on an in vitro coculture system of RAW 264,7 macrophages and 3T3-L1 murine adipocytes and also on the separated cells. The results showed that the pomegranate peel extract showed strong anti-inflammatory activity on the independently cultivated inflamed cells, as CCL-2 secretion decrease has been observed in all cell types. The macrophage TNFα secretion was also decreased and expression and secretion of adipocyte IL-6 were reduced too. Despite the anti-inflammatory activity of the extract on the separated cells, it didn't show the same activity in the coculture. The isolated phytochemicals punicalagin and ellagic acid on the other hand, showed good anti-inflammatory properties on coculture. Punicalagin used in same concentration as it existed in the extract showed good anti-inflammatory activity as it was found to reduce remarkably the IL-6 secretion. Ellagic acid too, reduced CCL-2 adipocyte secretion and CCL-2 and TNFα macrophage secretion. Besides, the IL-6 secretion and expression decreased in the coculture.[55]

In research was found that pomegranate leaf extract can decrease lung inflammation in mice. Ethyl acetate fraction obtained from hydroalcoholic leaves extract was administrated on male Swiss mice with lipopolysaccharide induced acute lung injury, before the lipopolysaccharide installation. The results showed decreased TNF-α and IL-1β expression compared with the control group. In the research was also examined the extracts' effect on NO and cytokine production by lipopolysaccharide stimulated macrophages RAW 264.7 and was found that the pretreatment with 100 mg/kg extract decreased the NO production and cytokine gene expression. The results were similar with those of mice that were administrated with kaempferol, a very strong anti-inflammatory compound and this indicates that the prophylactic treatment with the extract reduces acute lung inflammation. In another research has been found that pomegranate leaves hydroalcoholic extract showed anti-inflammatory activity in rats with acute peritonitis.[56]

Nuclear Factor-κB (NF-κB) is a transcription factor that acts as a main mediator of inflammatory responses by regulating multiple adaptive immune functions. It participates in inflammasome regulation and plays important role in activation, differentiation and survival of inflammatory T cells and innate immune cells. Dysregulated NF-κB activation leads to inflammatory diseases.[57] Research showed that pomegranate juice, extract and also the isolated bioactive compounds delphinidin and penicillin reduced the NF-κB activation in different cells. It was found that with the pomegranate the IL-6, IL-8 and NF-κB target genes expression were inhibited at intestinal cells that have been exposed in pro-inflammatory stimuli.[58]

The pomegranate polyphenols' anti-inflammatory properties have been evaluated on Sprague-Dawley rats with colitis induced by dextran sodium sulphate. The mice were first administrated with pomegranate beverage rich in ellagitannins and ellagic acid and after that colitis was induced by 3 cycles of 3% dextran sodium sulphate exposure followed by recovery period of 2 weeks. It was found that the pomegranate protected against the inflammatory by 50% and ulceration by 66.7% respectively compared with the control group. Besides the expression of the pro-inflammatory cytokines TNF-a and IL-1β has been markedly decreased. COX-2 and iNOS were also significantly reduced.[59]

In a study on murine model of asthma has been found that biodegradable microparticles that were formed by Polylactic-co-Glycolic acid (PLGA) with encapsulate pomegranate extract could be used as supplementary or even alternative therapy due to the pomegranate's anti-inflammatory properties. It was found that the cytokines IL-5, associated with eosinophil mediated inflammation and IL-1β were decreased and thus the recruitment of leucocytes and especially of the eosinophils to bronchoalveolar fluid has been inhibited.[60,61]

Interferon gamma (IFN-γ) is a cytokine that plays important role in innate and adaptive immunity.[62] Overactivity of IFN-γ causes tissue damage, necrosis and inflammation that can lead to pathogenesis and to chronic diseases.[63] Interleukin IL-18 is a pleiotropic immune regulator. Uncontrolled IL-18 plays important pro-inflammatory role as it induces IFN-γ, which when it is overactive it leads to inflammation. IL-18 is involved in inflammation and in various diseases' pathogenesis.[64] Sprague-Dawley rats were administrated daily with pomegranate juice (400 mg/kg) or isolated punicalagin (4 mg/kg) or 5-ASA drug (100 mg/kg). On 11th day colitis was induced and on 18th day TNF-α, IL-1β, IL-18 and NF-κB were determined and compared with the control group. The results showed that the pretreatment with juice and purified punicalagin reduced the disease severity and extent. All of 5-ASA, pomegranate juice, isolated punicalagin decreased remarkably the neutrophil infiltration giving comparable results. In gene expression studies was found that in both pomegranate juice and purified punicalagin groups the levels of IL-18, IL-1β and TNF-α were reduced. The NF-κB levels were decreased by 84% with pomegranate juice and by 64% by purified punicalagin. These results indicate that the juice is biologically more active than the isolated punicalagin and it could probably be used for inflammatory bowel disease treatment.[65]

In research on acute and chronic colitis the anti-inflammatory properties of ellagic acid have been evaluated measuring the inflammatory mediators TNF-α, IL-6 and INF-γ. In the acute colitis model mice were treated for 7 days with 5% dextran

sulfate sodium in order to induce colitis and same time they were administrated with supplement of ellagic acid (2%). Results showed decrease of the disease severity. Reduce of the TNF-α, IL-6 and IFN-γ has been observed. In the chronic colitis model, colitis has been caused with repeated dextran sulfate sodium (1%) administration of 2 cycles of 7-days. This administration has ben interrupted by 7 days periods. Ellagic acid (0.5%) has also been administrated in the mice. Ellagic acid inhibited the disease's progression decreasing the intestinal inflammation and downregulation NOS, COX-2 and the signaling pathways NF-κB, STAT 3 and p38 MAPK.[66]

In a research, pomegranate extract has been administrated in mice that were sensitive in arthritis. Arthritis has been induced by collagen. The mice were administrated with the pomegranate extract before and after the collagen immunization and the results showed delay of the onset and decrease of the incidence of the arthritis. The severity of arthritis was also lower in the extract administrated mice. Decreased joint infiltration by the inflammation cells, less cartilage and bone damage and remarkable decrease of the IL-6 levels in the joints have been observed. It has also been seen in mouse macrophages that with the pomegranate, downstream mediators multiple and signal transduction pathways that are involved in the rheumatoid arthritis pathogenesis have been cancelled.[67]

Mast cells and basophils, multifunctional effector cells, are involved in various inflammatory and immune events and they produce a wide range of mediators and cytokines. In a study on human basophilic cell line KU812, the cells were stimulated in order to cause inflammation and then was measured pomegranate powder extract's inhibitory effect on the pro-inflammatory cytokine gene expression and production by the stimulated cells. It was found that the pomegranate extract decreased remarkably the inflammatory gene expression and production of IL-8 and IL-6 by the stimulated KU812 cells. Besides, decrease of the NF-κB activation has been observed. The powder extract that was used had 86.0% ellagitannins, 3.2 sugars, 1.9% organic acids, 0.8% nitrogen, 2.5% ash and 1.2% moisture. The polyphenols' percentage in the extract was 77% ellagic acid, gallic acid and glucose oligomers of 2-10 units in different combinations, 19% ellagitannins and 4% free ellagic acid.[68]

Matrix Metalloproteinases (MMPs) are enzymes that are found in every inflamed human tissue and they are serving several functions in injury, defense, inflammation and repair. MMP 1,3 and 13 are classical inflammation and cartilage degradation markers in arthritic joints. In a research, human osteoarthritis cartilages were treated with pomegranate fruit extract and was found that the IL-1β induced expression of MMP 1,3 and 13 has been inhibited. It is important to be told that the pomegranate fruit extract is not toxic for the human cartilage cells.[69,70,71]

Certain types of inflammation is referred to be mediated by reactive oxygen metabolites that can create or amplify the already existed inflammation.[72] Free radicals as the Reactive Nitrogen Species (RNS) and the Reactive Oxygen Species (ROS) are created in the body by several endogenous systems, pathological states and exposure to harmful conditions. ROS overproduction leads to oxidative stress. It is referred that continued oxidative stress can lead to chronic inflammation.[73] Achieving a good antioxidant status can be protective against cellular injury and disfunction that are observed in inflammatory disorders.[72] Pomegranate is potent antioxidant as it contains in high concentration several phytochemicals that in mixture as they exist in the juice and in the plant parts extracts or isolated show strong antioxidant properties. The flavonoids luteolin, kaempferol, luteolin-7-O-glucoside, kaempferol 3-O-glucoside, prodelfinidin, procyanidin, cyanidin, cyanidin-3,5-di-O-glucoside, cyanidin-3-O-glucoside, catechin, apigenin and quercetin are found to possess very strong antioxidant properties. Tannins with potent antioxidant activity are gallic acid, ellagic acid, ellagitannins, 3,3'-di-O-methyl ellagic acid, 3,3'-4tri-O-methyl ellagic acid, punicalin, punicalagin, punigluconin and pedunculagin. Besides, the alkaloids melatonin and serotonin have strong antioxidant properties.[8,21]

Due to its' antioxidant properties, pomegranate intake improves the plasma antioxidant capacity and has been found that the prevalence of the oxidatively damaged molecules is reduced and same time, increase in the antioxidant-dependent immune responses in patients with chronic inflammatory diseases are observed.[6]

Research on 30 Sprague- Dawley rats with acute inflammation showed that Reactive-Oxygen Species (ROS) levels were remarkably reduced with 100 Ml pomegranate extract per day which has been given to the rats 1 day before and 2 after the surgery.[20]

Paraoxonase 1 (PON-1) is a hydrolytic enzyme that is associated with high density lipoprotein and possesses antioxidant and anti-inflammatory properties. Deficiency of PON-1 leads to oxidative stress and to inflammation, while increase of the PON-1 activity can be beneficial.[74] Research showed that daily administration of 10 ml pomegranate extract in rheumatoid arthritis patients for 12 weeks decreased the Disease Activity Index (DAS28) by 17% and this could be linked to the remarkable decrease in serum oxidative status and to the significant increase of PON-1 activity. It was also found that pomegranate extract addition to serum of rheumatoid arthritis patients decreased the lipid peroxidation that is caused by free radicals by 25%.[75]

Psoriasis is a chronic inflammatory autosomal dermatitis. Pomegranate rind extract and also isolated compounds were found acting by different ways and affecting different parameters to show anti-inflammatory and anti-psoriatic activity. The flavonol kaempferol was found able to block the TNF-induced IL-8 activation in keratinocytes. Besides, kaempferol reduces the TNF generated ROS without affecting the viability of cells, suggesting that this phytochemical can probably be useful in diseases as psoriasis where TNF plays important role. The flavonoid luteolin possesses strong anti-inflammatory properties in keratinocytes as it was found to inhibit the production of the IL-8, IL-6 and Vascular Endothelial Growth Factor (VEGF). VEGF, is an important angiogenesis inducer. In inflammatory diseases as psoriasis and also in various cancers, blocking angiogenesis is possible to lead to treatment, thus VEGF inhibitory is beneficial in those cases.[76,77] Thymidine phosphorylase is an angiogenic enzyme. It is referred that exists in high levels in psoriatic lesions. The isolated phytochemicals punicalin, punicalagin and 2,3(S)-hexahydroxydiphenoyl-D-glucose were found in research to show very strong anti-psoriatic activity as they inhibit the thymidine phosphorylase activity at a range of 89-95%. The pomegranate rind extract in acetone/water (75%) inhibited the activity of the enzyme by 87% as well.[78]

In a study on mouse model of contact dermatitis, topical application of standardized rind extracts on the mouse ear edema at dosages of 5, 2.5 and 1 mg/ear and also ellagic acid at dosages of 0.65, 0.325 and 0.13 mg/ear, equivalent to the extract's ellagic acid content, decreased significantly and dose dependently the ear edema. The rind extract gave maximal inhibition by 79.12% and the ellagic acid by 73.63%. In the same research, diclofenac at dosage of 1mg/ear and triamcinolone at dosage of 0.1 mg/ear inhibited the edema by 37.91% and 73.63% respectively.[79] In the dosages used, pomegranate gave comparable or even better results than the commercial medicines. Myeloperoxidase (MPO) is a toxic enzyme existing in the neutrophils' azurophilic granules and its' function is to use H_2O_2 and generate HClO and other reactive moieties in order to kill pathogens during infections. In sterile inflammation, MPO and the oxidants derived by MPO are pathogenic as they promote inflammation, lead to tissue damages and limit the immune responses. High levels and activity of MPO are observed in autoimmune diseases and decreasing them is beneficial.[80] In the research above, the rind extract decreased the MPO activity by up to 69.68% and ellagic acid by up to 68.79%. Diclofenac and triamcinolone reduced the MPO activity by 80.14% and 76.66% respectively. These all above results show that the topical application of pomegranate rind extract and ellagic acid could be considerate as promising alternative treatment of inflammatory skin disorders.[79]

In a study SKU-1064 human skin fibroblast cells were exposed to UVA and UVB radiation and then the protective and anti-inflammatory effect of pomegranate fruit extract has been evaluated. The activation of the pro-inflammatory transcription factor NF-κB was suppressed and this is probably one of the reasons that the pomegranate extract was found to protect efficiently the fibroblast cells SKU-1064 from cell death.[81]

In a research, pomegranate ointment standardized with 40% ellagic acid was applied on wound and the results showed lower number of inflammatory cells in comparation with the control group.[82]

Neutrophils play important role in inflammation. They release big amounts of ROS which is produced by Myeloperoxidase (MPO) and NADPH-oxidase. Regulation of neutrophils is important for the inflammatory treatment. In a research on colon inflammation the anti-inflammatory activity of punicic acid, a pomegranate seed oil constituent has been evaluated. In an in vitro study has been found that punicic acid inhibited the priming of ROS production that is induced by TNF-α. Punicic acid also showed strong anti-inflammatory activity my MPO release inhibition. In vivo studies showed that the intake of punicic acid and of seed oil reduced the activation of neutrophils and the tissue damage caused by MPO and ROS.[83]

Herpes Simplex Virus (HSV) infections are associated with topical infection and pain. It is referred that pomegranate rind extract and zinc (II) when they are co-administrated to show a strong antiviral activity against HSV. Topical application on ex vivo porcine skin of pomegranate rind extract and total pomegranate tannins with and also without zinc, showed remarkable anti-inflammatory properties downregulating the Cyclooxygenase (COX-2) expression (marker for modulation of arachidonic acid inflammatory pathway), with the rind extract to give better results than the total tannins, indicating that probably it's not the tannins alone responsible for the anti-inflammatory activity of the rind extract. The fact that topical application of both total pomegranate tannins and rind extract gave good anti-inflammatory results, means that they penetrated the skin and regulated the COX-2 expression.[84]

Diabetic patients have increased concentration of circulatory cytokines due to inflammation. Daily consumption of 250 ml pomegranate juice by patients with type 2 diabetes for 12 weeks resulted in significant decrease of IL-6 compared with the control group.[85]

One of the reasons that causes inflammation is protein denaturation. Pomegranate extract was found to inhibit protein denaturation in various concentrations with maximum inhibition (70.12±1.12%) to be observed at 500μg/ml and the half maximal inhibitory concentration (IC50) to be 105.35±1.99 μg/ml. Aspirin which has been used as control, showed maximum inhibition (77.12±1.42%)

at 200 μg/ml. The protein used was albumin that was heated in order to study and compare pomegranate's and aspirin's denaturation inhibitory activities.[86]

Cigarette smoke causes and increases oxidative stress in the lungs. The effect of the antioxidant pomegranate juice on mice exposed acutely and chronically in cigarette smoke has been evaluated. 4 groups of C57BL/6J mice, the control, cigarette smoke, cigarette smoke and pomegranate juice and pomegranate juice group were used. Acute exposure to smoke was done in 3 days and chronic exposure results were achieved by 5 times per week exposure for 1 and 3 months. The pomegranate juice groups were administrated with 80 μmol/kg juice daily and other groups were receiving distillated water. The results showed that the acute exposure in cigarette smoke increased remarkably the expression of TNF-α, IL-1β, IL-6 in comparation with the control group. The oxidative stress and apoptosis were also increased markedly. The chronic exposure to cigarette smoke resulted in TNF-α expression increase and to emphysematous changes in lung sessions compared with the control group. The pomegranate juice administration normalized the lung architecture and was found to attenuate the increase of TNF-α expression. In an invitro research on cultured human alveolar cells (A549), the ability of pomegranate juice to reduce smoke damaging effect has been evaluated. The cells after a 48 hours pretreatment with pomegranate juice (0.5μM) or vehicle, were exposed to cigarette smoke which had be extracted from filters and then the cell viability has been assessed. It was found that the cigarette smoke extract decreased the cells' proliferation and also triggered the dearth of cells. With the pomegranate juice pretreatment, attenuation of the harmful cigarette smoke extract's effects has been observed.[87]

The pomegranate leaves' hydralcoholic extract is mentioned to have anti-inflammatory properties. In research, male BALB/c mice were administrated with galloyl-hexahydroxy-diphenoyl (HHDP)- glucose, a compound isolated from pomegranate leaves which was found to have very good activity against Acute Lung Injury (ALI), a disorder of acute inflammation, indicating that it could probably be a good alternative way to treat ALI and also other inflammations.[88]

In research were evaluated the oral anti-inflammatory activities of the fruit, rind, leaves and flower aqueous-ethanolic 50% extracts at doses of 150, 250 and 500 mg/kg body weight of Wistar albino rats. Was found that in oral pretreatment with dried extracts there was remarkable and dose dependent inhibition of edema in comparison with control groups. The rind extract showed the best activity with dosage 500 mg/kg as it inhibited by 82.14% the inflammation. The 50% aqueous-ethanolic flower extract inhibited inflammation by 71.42% and the leaf extract by 67.85%, both in 500 mg/kg too. That means that the pomegranate is a potent anti-inflammatory agent.[89]

Oral administration of 250 and 500 mg/kg aqueous and methanolic pomegranate peel extracts on mice with paw edema resulted in remarkable inhibition of the edema, dose dependently. At dose of 500 mg/kg the methanolic extract inhibited by 80.72% the edema and the aqueous extract by 51.94% in comparation with the control group, with this significant anti-inflammatory activity to be explained by the phenolic compounds' high concentration. The edema inhibition ability of the 500 mg/kg methanolic extract was comparable with diclofenac's (50 mg/kg) ability.[90] Administration of 200 mg/kg of pomegranate aqueous-methanolic rind extract in mice with inflammation and algesia induced by hot plate, reduced the inflammation and pain, downregulating oxidative stress markers, NF-κB, IL-6, IL-1β and TNF-α. Besides, markedly decrease in paw swelling has been observed.[91]

Pomegranate Safety

It has been seen in experiments of pomegranate's anti-inflammatory properties evaluation and also in experiments on pomegranate's safety, that the pomegranate extracts and juice are safe. Administration of 1420 mg/day fruit extract tablets in humans did not cause any side effects. Research showed pomegranate fruit extract 600 mg/Kg body weight daily which is more than the doses used in experiments that were carried out to evaluate the pomegranate's anti-inflammatory properties, did not cause adverse effects.[8,41,42,92,93,94]

Discussion

Inflammation is a complex defense mechanism of the body that can be caused by several parameters such as pathogens, trauma, continuous oxidative stress, expose to harmful or irritant agents, protein denaturation and other. Upon exposure to irritant agents, the immune cells such as macrophages are secreting proinflammatory cytokines, signaling proteins that promote inflammation. Chronic overproduction of these cytokines leads to inflammatory diseases and for this reason it is important to be downregulated. Pomegranate contains phytochemicals such as flavonols, flavones, flavanols, flavanones, anthocyanidins, anthocyanins, hydrolysable and non-hydrolysable tannins, simple organic acids and alkaloids that as a mixture in the juice or extracts where synergic action may occur and also as isolated compounds, show very strong anti-oxidant and anti-inflammatory properties. Ellagitannins metabolized by gut microbiota create urolithins, that also possess very strong anti-inflammatory properties. In vivo, in vitro and ex vivo researches showed that the pomegranate possesses significant anti-inflammatory activity.

Pomegranate extracts and isolated phytochemicals were found to inhibit the expression of the cytokines IL-6, IL-10, 1L-5, IL-8, IL-1β, IL-18, TNFα, IFN-γ that if they are overexpressed there is high risk of chronic inflammation and infection, auto-inflammatory and autoimmune diseases, eosinophil mediated inflammation, tissue damage and pathogenic processes such as cancer. Besides pomegranate reduces NF-κB that if is unregulated it leads to inflammatory diseases and inhibits MMPs, classical inflammation marker in arthritic joints, Nitric Oxide (NO) a mediator and regulator in inflammatory that can cause tissue damages. Pomegranate also reduces MPO which in high levels limits the immune responses and causes inflammation, tissue damages and autoimmune diseases. COX, inflammatory mediator related with cancer is also decreased by pomegranate. There are certain types of inflammation which are caused due to free radicals such as ROS and RNS. The polyphenol rich pomegranate is potent antioxidant and reduces significantly free radicals improving the antioxidant status and eliminating oxidative stress that can lead to chronic inflammation and to several diseases. Besides, pomegranate increases the anti-oxidant dependent-immune responses. Various pomegranate constituents as flavonoids, tannins and alkaloids have antimicrobial properties and for this reason pomegranate can be beneficial in infections where the inflammation is caused or aggregated by microbes, such as gastritis. One of the reasons inflammation is caused, is proteins' denaturation that was found to be sufficiently inhibited by pomegranate.

In the doses used in each experiment pomegranate gave comparable and in some cases even better results than commercial anti-inflammatory medicines and it is very important to be mentioned that the pomegranate in contrast with the drugs did not have side effects and also in some cases was beneficial and protective where the medicines were harmful. For example, NSAIDs show gastrotoxicity while pomegranate is gastroprotective due to tannins, saponin and flavonoids that it contains. Anti-TNF medicines in some cases aggregate than inhibit the disease while pomegranate is without side effects reducing sufficiently the TNF levels. Pomegranate, affects several times more than one of the inflammatory-involved factors and shows remarkable anti-inflammatory activity in treating various diseases as asthma, arthritis, rheumatoid arthritis, psoriasis, contact dermatitis, lung inflammation, edema, diabetes, acute and chronic colitis, ulcerative colitis, gastritis, diarrhea, Inflammatory Bowel Disease and Cronh's disease. Besides pomegranate was found very effective in treating the inflammation due to UVA and UVB radiation, decreasing cigarette smoke effects and also in wound and ulcers healing reducing the infection.

Has been observed that in some cases better results are given by extracts (mixture of phytochemicals) and in others by isolated compounds. Further clinical studies can give more information about the isolated compounds or mixture of them, the synergic action if it occurs and the biochemical pathways the phytochemicals are involved to achieve anti-inflammatory activity.

Conclusion

Pomegranate contains many phytochemicals, each one with different bioactivities. As there are compounds with more than one of antioxidant, antimicrobial and anti-inflammatory properties, treatment with such mixture of bioactive compounds is approaching the health issue overall, affecting more than one parameter that affect or create the problem. That's an advantage compared with commercial drugs which approach mostly unilaterally the problem and also have side effects while pomegranate not only is safe but also may be beneficial to issues that commercial medicine is harmful. This indicates that pomegranate could be considered as a very promising alternative treatment for inflammatory diseases.

Sources of Funding: No Sources of Funding

Conflicts of Interest: None

References

1. Gabay C. Interleukin-6 and chronic inflammation. *Arthritis Research & Therapy* 2006; 8(Suppl 2): S3; (DOI:10.1186/ar1917).
2. Ahmed A. An overview of inflammation: Mechanism and consequences. *Frontiers in Biology* 2011; 6(4): 274-281. (DOI 10.1007/s11515-011-1123-9)
3. Kumar R, Clermont G, Vodovotz Y, Chowd C. The dynamics of acute inflammation. *Journal of Theoretical Biology* 2004; 230(2004): 145-155. (DOI: 10.1016/j.jtbi.2004.04.044).
4. Lee CJ, Chen LG, Liang WL, Wang CC.Food Chemistry 2010; 118(2); 315-322. (DOI: 10.1016/j.foodchem.2009.04.123).
5. Gandhi J, Khera L, Gaur N, Paul C, Kaul R. Role of Modulator of Inflammation Cyclooxygena se-2 in Gammaherpesvirus Mediated Tumorigenesis. *Fronties in Microbiology* 2017; 8(538). (DOI: 10.3389/fmicb.2017.00538).
6. Danesi F, Ferguson L. Could Pomegranate Juice Help in the Control of Inflammatory Diseases. *Nutrients* 2017; 9(958). (DOI:10.3390/nu9090958).
7. Kim H, Banerjee N, Ivanov I, Pfent CM, Prudhomme KR, Bisson WH, Dashwood RH, Talcott ST, Mertens-Talcott SU. *Mol Nutr Food Res* 2016; 60(9): 1912-1923. (DOI:10.1002/mnfr.201501008).

8. Stefanou V, Papatheodorou S, Vougiouklaki D, Antonopoulos D, Lougovois V, Tsaknis I, Houhoula D. Medicinal Properties of Antioxidant Pomegranate in Cardiovascular Health. *International Journal of Preventive Cardiology* 2020; 1(1): 10-19.
9. Gonza´lez-Trujano ME, Pellicer F, Mena P, Moreno DA, Garcı´a-Viguera C. Antinociceptive and anti-inflammatory activities of a pomegranate (Punica granatum L.) extract rich in ellagitannins. *Int J Food Sci Nutr* 2015; 66(4): 395-399. (DOI: 10.3109/09637486.2015.1024208).
10. Kusmardi K, Hermando D, Estuningytas A, Tedjo A, Priosoeryanto B. The potency of Indonesia's pomegranate peel ethanol extract (Punica Granatum Linn) as anti-inflammatory agent in mice colon induced by dextran sodium sulfate: focus on cyclooxygenase -2 and INOS expressions. *Asian Journal of Pharmaceutical and clinical research* 2017; 10(12): 370-375. (DOI: 10.22159/ajpcr.2017.v10i12.21390).
11. Zhao X, Yuan Z, Fang Y, Yin Y, Feng L. Flavonols and Flavones Changes in Pomegranate (Punicagranatum L.) Fruit Peel during Fruit Development. *J Agr Sci Tech* 2014; 16: 1649-1659.
12. Maleki SJ, Crespo JF, Cabanillas B. Anti-inflammatory effects of flavonoids. *Food Chemistry* 2019; 299: 125124; (DOI: 10.1016/j.foodchem.2019.125124).
13. Gardeli C, Varela K, Krokida E, Mallouchos A. Investigation of Anthocyanins Stability from Pomegranate Juice (Punica Granatum L. Cv Ermioni) under a Simulated Digestion Process. *Medicines* 2019; 6(90). (DOI:10.3390/medicines6030090).
14. Lantzouraki D, Sinanoglou V, Zoumpoulakis P, Glamo˘clija J, Ciri´c A, Sokovi M, Heropoulos G, Proestos C. Antiradical-antimicrobial activity and phenolic profile of pomegranate (Punica granatum L.) juices from different cultivars: A comparative study. *RSC Advances* 2015; 5(4): 2602-2614. (DOI:10.1039/c4ra11795f).
15. Bar-Ya'akov I, Tian L, Amir R, Holland D. Primary Metabolites, Anthocyanins, and Hydrolyzable Tannins in the Pomegranate Fruit. 2019; 10: 620. (DOI: 10.3389/fpls.2019.00620).
16. Hollebeeck S, Larondelle Y, Schneider Y.-J, During A. The Use of Pomegranate (Punica granatum L.) Phenolic Compounds as Potential Natural Prevention Against IBDs. *Inflammatory Bowel Disease* 2012; Chapter 15; (DOI: 10.5772/27532).
17. Rahimi HR, Arastoo M, Ostad S.N. A Comprehensive Review of Punica granatum (Pomegranate) Properties in Toxicological, Pharmacological, Cellular and Molecular Biology Researches. *Iranian Journal of Pharmaceutical Research* 2012; 11(2): 385-400.
18. Lantzouraki D, Sinanoglou V, Zoumpoulakis P, Proestos C. Comparison of the Antioxidant and Antiradical Activity of Pomegranate (Punica Granatum L.) by Ultrasound-Assisted and Classical Extraction. *Analytical Letters* 2015; 49(7): 969-978. (D0I: 10.1080/00032719.2015.1038550).
19. Gonza´lez-Sarrı´as A, Larrosa M, Toma´s-Barbera´n F. A, Dolara P, Espı´n J. C. NF-kB-dependent anti-inflammatory activity of urolithins, gut microbiota ellagic acid-derived metabolites, in human colonic fibroblasts. *British Journal of Nutrition* 2010; 104(04): 503-512. (DOI: 10.1017/S0007114510000826).
20. Asgary S, Javanmard S, Zarfeshany A. Potent health effects of pomegranate. *Advanced Biomedical Research* 2014; 3(1): 100. DOI: 10.4103/2277-9175.129371.
21. Wang RE, Ding Y, Liu R, Yiang L, Du L. Pomegranate: Constituents, Bioactivities and Pharmakokinetics. *Fruit, Vegetable and Cereal Science and Biotechnology* 4 2010; (Special Issue 2) no2(2010): 77-87. Global Science Books.
22. Colombo E, Sangiovanni E, Dell'Agli M. A Review on the Anti-Inflammatory Activity of Pomegranate in the Gastrointestinal Tract. E*vidence-based Complementary and Alternative Medicine* 2013: 1-11. (DOI: 10.1155/2013/247145).
23. Mastrogiovanni F, Mukhopadhya A, Lacetera N, Ryan M. T, Romani A, Bernini R, Sweeney T. Anti-Inflammatory Effects of Pomegranate Peel Extracts on In Vitro Human Intestinal Caco-2 Cells and Ex Vivo Porcine Colonic Tissue Explants. *Nutrients* 2019; 11(3): 548. (DOI: 10.3390/nu11030548).
24. Scaioli E, Belluzzi A, Ricciardiello L, Del Rio D, Rotondo E, Mena P, Derlindati E, Danesi F. Pomegranate juice to reduce fecal calprotectin levels in inflammatory bowel disease patients with a high risk of clinical relapse: Study protocol for a randomized controlled trial. *Trials* 2019; 20(327). (DOI: 10.1186/s13063-019-3321-8).
25. Kusters JK, van Vliet AHM, Kuipers EJ. Pathogenesis of Helicobacter pylori Infection. *Clinical Microbiology Reviews* 2006; 19(3): 449-490. (DOI:10.1128/CMR.00054-05).
26. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents* 2005; 26(2005): 343-356. (DOI:10.1016/j.ijantimicag.2005.09.002).
27. Scalbert A. Antimicrobial properties of tannins. *Phytochemistry* 1991; 30(12): 3875-3883. (DOI: 10.1016/0031-9422(91)83426-L).
28. Cushnie TPT, Cushnie B, Lamb AJ. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents* 2014; 44(2014): 377-386. (DOI: 1 0.1016/j.ijantimicag.2014.06.001).
29. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern

Plants. *Frontiers in Microbiology* 2019; 10: 911. (DOI:10.3389/fmicb.2019.00911).
30. Elnawasany S. Clinical Applications of Pomegranate. *Intech Open* 2018; Chapter 7: 127-148. (DOI: 10.5772/intechopen.75962).
31. Alam MS, Alam MA, Ahmad S, Najmi AK, Asif M, Jahangir T. Protective effects of Punica granatum in experimentally-induced gastric ulcers. *Toxicology Mechanisms and Methods* 2010; 20(9): 572-578. (DOI: 10.3109/15376516.2010.508079).
32. Labib RM, El-Ahmady SH. Antinociceptive, anti-gastric ulcerogenic and anti-inflammatory activities of standardized egyptian pomegranate peel extract. *Journal of Applied Pharmaceutical Science* 2015; 5(1): 48-51. (DOI: 10.7324/JAPS.2015.50109).
33. Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. *Frontiers in Immunology* 2014; 5(491): 1-12. (DOI: 10.3389/fimmu.2014.00491).
34. Manzoor Z, Koh YS. Mitogen-activated Protein Kinases in Inflammation. *Journal of Bacteriology and Virology* 2012; 42(3): 189-195. (DOI: 10.4167/jbv.2012.42.3.189).
35. Du L, Li J, Zhang X, Wang L, Zhang W, Yang M, Hou C. Pomegranate peel polyphenols inhibits inflammation in LPS-induced RAW264.7 macrophages via the suppression of TLR4/NF-κB pathway activation. *Food & Nutrition Research* 2019; 63(0): 3392. (DOI: 10.29219/fnr.v63.3392).
36. Zarogoulidis P, Lampaki S; Turner F, Huang H, Kakolyris S, Syrigos K, Zarogoulidis K. mTOR pathway: A current, up-to-date mini-review (Review). *Oncology letters* 2014; 8: 2367-2370. (DOI: 10.3892/ol.2014.2608).
37. Weichhart T, Hengstschläger M, Linke M. Regulation of innate immune cell function by Mtor. *Nature Reviews Immunology* 2015; 15(10): 599-614. (DOI:10.1038/nri3901).
38. Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. *Cell* 2017; 168(6): 960-976. (DOI: 10.1016/j.cell.2017.02.004)
39. Korhonen R, Lahti A, Kankaanranta H, Moilanen E. Nitric Oxide Production and Signaling in Inflammation. *Current Drug Targets - Inflammation & Allergy* 2005; 4(4): 471-479. (DOI: 10.2174/1568010054526359).
40. Pautz A, Art J, Hahn S, Nowag S, Voss C, Kleinert H. Regulation of the expression of inducible nitric oxide synthase. *Nitric Oxide* 2010; 23(2:) 75-93. (DOI: 10.1016/j.niox.2010.04.007).
41. Patel C, Dadhaniya P, Hingorani L, Soni MG. Safety assessment of pomegranate fruit extract: Acute and subchronic toxicity studies. *Food and Chemical Toxicology* 2008; 46(8): 2728-2735. (DOI: 10.1016/j.fct.2008.04.035)
42. Vidal A, Fallarero A, Peña BR, Medina ME, Gra B, Rivera FY et al. Studies on the toxicity of Punica granatum L. (Punicaceae) whole fruit extracts. *Journal of Ethnopharmacology* 2003; 89(2-3): 295-300. (DOI: 10.1016/j.jep.2003.09.001).
43. Teslim OA, Vyvienne M'K, Olatokunbo OM, Oluwafisayo AJ, Mlenzana NB, Shamila M et al. Side effects of non-steroidal anti-inflammatory drugs: The experience of patients with musculoskeletal disorders. *American Journal of Health Research* 2014; 2(4): 106-112. (DOI: 10.11648/j.ajhr.20140204.11).
44. Kasturi J, Palla PR, Bakshi V, Boggula N. Non-steroidal anti-inflammatory drugs: an overview. *Journal of Drug Delivery and Therapeutics* 2019; 9(1-s): 442-448. (DOI: 10.22270/jddt.v9i1-s.2287).
45. Larrosa M, González-Sarrías A, Yáñez-Gascón M. J, Selma MV, Azorín-Ortuño M, Toti S et al. Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on phenolic metabolism. *Journal of Nutritional Biochemistry* 2010; 21(8): 717-725. (DOI:10.1016/j.jnutbio.2009.04.012).
46. Rosillo MA, Sánchez-Hidalgo M, Cárdeno A, Aparicio-Soto M, Sánchez-Fidalgo S, Villegas et al. Dietary supplementation of an ellagic acid-enriched pomegranate extract attenuates chronic colonic inflammation in rats. *Pharmacological Research* 2012; 66(3): 235-242. (DOI: 10.1016/j.phrs.2012.05.006).
47. Mastrogiovanni F, Bernini R, Basiricò L, Bernabucci U, Campo M, A Romani A et al. Antioxidant and anti-inflammatory effects of pomegranate peel extracts on bovine mammary epithelial cells BME-UV1. *Natural Product Research* 2018: 1-5.
48. Perše M, Unkovič A. The Role of TNF in the Pathogenesis of Inflammatory Bowel Disease. *Biological Therapy for Inflammatory Bowel Disease* 2020. (DOI: 10.5772/intechopen.84375).
49. Ren K,Torres R. Role of interleukin-1β during pain and inflammation. *Brain Research Reviews* 2009; 60(1): 57-64. (DOI: 10.1016/j.brainresrev.2008.12.020).
50. Howes A, Gabrysova´ L, O'Garra A. Role of IL-10 and the IL-10 Receptor in Immune Responses. *Reference Module in Biomedical Sciences* 2014. (DOI: 10.1016/B978-0-12-801238-3.00014-3).
51. Tanaka T, Narazaki M, Kishimoto T. Interleukin (IL-6) Immunotherapy. *Cold Spring Harbor Perspectives in Biology* 2017; 10(8): a028456. (DOI: 10.1101/cshperspect.a028456).
52. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunological*

Reviews 2017; 281(1): 8-27. (DOI: 10.1111/imr.12621).
53. Noda Y, Kaneyuki T, Mori A, Packer L. Antioxidant Activities of Pomegranate Fruit Extract and Its Anthocyanidins: Delphinidin, Cyanidin, and Pelargonidin. *Journal of Agricultural and Food Chemistry* 2002; 50(1): 166-171. (DOI: 10.1021/jf0108765).
54. Baradaran Rahimi V, Ghadiri M, Ramezani M, Askari VR. Anti-inflammatory and anti-cancer activities of pomegranate and its constituent, ellagic acid: Evidence from cellular, animal, and clinical studies. *Phytotherapy Reseaerch* 2020. (DOI: 10.1002/ptr.6565).
55. Winand J, Schneider YJ. The anti-inflammatory effect of a pomegranate husk extract on inflamed adipocytes and macrophages cultivated independently, but not on the inflammatory vicious cycle between adipocytes and macrophages. *Food & Function* 2014; 5(2): 310-318. (DOI: 10.1039/c3fo60443h).
56. Rodrigues Pinheiro AJMC, Gonçalves JS, Dourado AWA, de Sousa EM, Brito NM, Silva LK et al. Punica granatum L. Leaf Extract Attenuates Lung Inflammation in Mice with Acute Lung Injury. *Journal of Immunology Research* 2018; 1-11. (DOI: 10.1155/2018/6879183).
57. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduction and Targeted Therapy* 2017; 17023. (DOI: 10.1038/sigtrans.2017.23).
58. Shaheen HM, Alsenosy A. Nuclear Factor Kappa B Inhibition as a Therapeutic Target of Nutraceuticals in Arthritis, Osteoarthritis, and Related Inflammation. *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases (Second Edition)* 2019; 437-453. (DOI: 10.1016/B978-0-12-813820-5.00025-8).
59. Kim H, Banerjee N, Sirven MA, Minamoto Y, Markel ME, Suchodolski JS et al. Pomegranate polyphenolics reduce inflammation and ulceration in intestinal colitis—involvement of the miR-145/p70S6K1/HIF1α axis in vivo and in vitro. *Journal of Nutritional Biochemistry* 2017; 43: 107-115. (DOI: 10.1016/j.jnutbio.2017.02.005).
60. de Oliveira JFF, Garreto DV, da Silva MCP, Fortes TS, de Oliveira RB, Nascimento FRF et al. Therapeutic potential of biodegradable microparticles containing Punica granatum L. (pomegranate) in murine model of asthma. *Inflammation Research* 2013; 62(11): 971-980. (DOI 10.1007/s00011-013-0659-3).
61. Kouro T, Takatsu K. IL-5- and eosinophil-mediated inflammation: from discovery to therapy. *The Japanese Society for Immunology* 2009; 21(12): 1303-1309. (DOI: 10.1093/intimm/dxp102).
62. Kelchtermans H, Billiau A, Matthys P. How interferon-g keeps autoimmune diseases in check. *Trends in Immunology* 2008; 29(10): 4-79-486. (DOI: 10.1016/j.it.2008.07.002).
63. Kak G, Raza M, Tiwari BK. Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases. Biomolecular Concepts 2018; 9(1): 64-79. (DOI: 10.1515/bmc-2018-0007).
64. Lee J, Cho D, H. Park. IL-18 and Cutaneous Inflammatory Diseases. *International Journal of Molecular Sciences* 2015; 16(12): 29357-29369. (DOI: 10.3390/ijms161226172).
65. Shah TA, Parikh M, Patel KV, Patel KG, Joshi CG, Gandhi T.R. Evaluation of the effect of Punica granatum juice and punicalaginon NF-κB modulation in inflammatory bowel disease. *Molecular and Cellular Biochemistry* 2016; 419(1-2): 65-74. (DOI: 10.1007/s11010-016-2750-x).
66. Marín M, María Giner R, Ríos JL, Carmen Recio M. Intestinal anti-inflammatory activity of ellagic acid in the acute and Chronic dextrane sulfate sodium models of mice colitis. *Journal of Ethnopharmacology* 2013; 150(3): 925-934. (DOI: 10.1016/j.jep.2013.09.030).
67. Shukla M, Gupta K, Rasheed Z, Khan KA, Haqqi TM. Consumption of hydrolyzable tannins-rich pomegranate extract suppresses inflammation and joint damage in rheumatoid arthritis. *Nutrition* 2008; 24(7-8): 733-743. (DOI: 10.1016/j.nut.2008.03.013).
68. Rasheed Z, Akhtar N, Anbazhagan AN, Ramamurthy S, Shukla M, Haqqi TM. Polyphenol-rich pomegranate fruit extract (POMx) suppresses PMACI-induced expression of pro-inflammatory cytokines by inhibiting the activation of MAP Kinases and NF-κB in human KU812 cells. *Journal of Inflammation* 2009; 6(1): 1-12. (DOI: 10.1186/1476-9255-6-1).
69. Rasheed Z. Intake of Pomegranate Prevents the Onset of Osteoarthritis: Molecular Evidences. *International Journal of Health Sciences* 2016; 10(2). (DOI: 10.12816/0048807).
70. Mu¨ller-Quernheim J. MMPs are regulatory enzymes in pathways of inflammatory disorders, tissue injury, malignancies and remodelling of the lung. *European Raspiratory Journal* 2011; 38(1): 12-14. (DOI: 10.1183/09031936.00079311).
71. Manicone A, McGuire J. Matrix metalloproteinases as modulators of inflammation. *Seminars in Cell & Developmental Biology* 2008; 19(1): 34-41. (DOI: 10.1016/j.semcdb.2007.07.003).
72. Conner EM, Grisham MB. Inflammation, Free Radicals, and Antioxidants. *Nutrition* 1996; 12(4): 274-277. (DOI: 10.1016/s0899-9007(96)00000-8).
73. Biswas S, Das R, Banerjee ER. Role of free radicals in human inflammatory diseases. *AIMS Biophysics* 2017; 4(4): 596-614. (DOI: 10.3934/biophy.2017.4.596).
74. Litvinov D, Mahini H, Garelnabi M. Antioxidant and AntiInflammatory Role of Paraoxonase 1: Implication in Arteriosclerosis Diseases. *North American Journal of Medical Sciences* 2012; 4(11): 523-532. (DOI: 10.4103/1947-2714.103310).

75. Balbir-Gurman A, Fuhrman B, Braun-Moscovici Y, Markovits D, Aviram M. Consumption of pomegranate decreases serum oxidative stress and reduces disease activity in patients with active rheumatoid arthritis: a pilot study. *The Israel Medical Association Journal* 2011; 13(8): 474-479
76. Garcı´a-Pe´rez ME, Kasangana PB, Stevanovic T. Bioactive Polyphenols for Diabetes and Inflammation in Psoriasis Disease. *Studies in Nature Products Chemistry* 2017; 231-268. (DOI: 10.1016/B978-0-444-63931-8.00006-0).
77. Shaik- Dasthagirisaheb YB, Varvara G, Murmura G, Saggini A, Potalivo G, Caraffa A et al. Vascular Endothelial Growth Factor (VEGF), Mast Cells and Inflammation. I*nternational Journal of Immunopathology and Pharmacology 2*013; 26(2): 327-335. (DOI: 10.1177/039463201302600206).
78. Jacob J, Gopalan R, Lakshmanaperumalsamy P, Ramanaiah I, Damaji B, Gopala KS et al. Evaluation of Anti-psoriatic Potential of the Fruit Rind of Punica granatum L. *Pharmacognosy Journal* 2019; 11(3): 466-468. (DOI: 10.5530/pj.2019.11.73).
79. Mo J, Panichayupakaranant P, Kaewnopparat N, Songkro S, Reanmongkol W. Topical Anti-inflammatory Potential of Standardized Pomegranate Rind Extract and Ellagic Acid in Contact Dermatitis. *Phytotherapy Research* 2013; 28(4): 629-632. (DOI: 10.1002/ptr.5039).
80. Strzepa A, Pritchard KA, Dittel BN. Myeloperoxidase: A new player in autoimmunity. *Cellular Immunology* 2017; 317: 1-8. (DOI: 10.1016/j.cellimm.2017.05.002).
81. Pacheco-Palencia LA, Noratto G, Hingorani L, Talcott ST, Mertens-Talcott SU. Protective Effects of Standardized Pomegranate (Punica granatum L.) Polyphenolic Extract in Ultraviolet-Irradiated Human Skin Fibroblasts. Journal of Agricultural and Food Chemistry 2008; 56(18): 8434-8441; (DOI: 10.1021/jf8005307)
82. Lukiswanto B, Miranti A, Sudjarwo S, Primarizky H, Yuniarti W. Evaluation of wound healing potential of pomegranate (Punica granatum) whole fruit extract on skin burn wound in rats (Rattus norvegicus). *Journal of Advanced Veterinary and Animal Research* 2019; 6(2): 202-207. (DOI: 10.5455/javar.2019.f333).
83. Boussetta T, Raad H, Lette´ron P, Gougerot-Pocidalo M.-A, Marie J-C, Driss F et al. Punicic Acid a Conjugated Linolenic Acid Inhibits TNFa- Induced Neutrophil Hyperactivation and Protects from Experimental Colon Inflammation in Rats. *PloS ONE* 2009; 4(7): e6458. (DOI: 10.1371/journal.pone.0006458).
84. Houston DMJ, Bugert J, Denyer SP, Heard CM. Anti-inflammatory activity of Punica granatum L. (Pomegranate) rind extracts applied topically to ex vivo skin. *European Journal of Pharmaceutics and Biopharmaceutics* 2017; 112: 30-37. (DOI: 10.1016/j.ejpb.2016.11.014).
85. Sohrabb G, Nasrollahzadeh J, Zand H, Amiri Z, Tohidi M, Kimiagar M. Effects of Pomegranate Juice Consumption on Inflammatory Markers in Patients With Type 2 Diabetes: A Randomized, Placebo-Controlled Trial. *J Res Med Sci* 2014; 19(3): 215-20.
86. Pereira WD, Geetha RV, Thangavelu L. Anti-inflammatory activity of Punica granatum extract on oral microbes -In vitro. *International Journal of Research in Pharmaceutical Sciences* 2019; 10(2): 1019-1022. (DOI: 10.26452/ijrps.v10i2.375).
87. Husari A, Hashem Y, Bitar H, Dbaibo G, Zaatari G, Sabban M. Antioxidant activity of pomegranate juice reduces emphysematous changes and injury secondary to cigarette smoke in an animal model and human alveolar cells. *International Journal of Chronic Obstructive Pulmonary Disease* 2016; 227. (DOI: 10.2147/COPD.S97027)
88. Pinheiro AJMCR, Mendes ARS, de J Neves MDF, Prado CM, Bittencourt-Mernak MI, Santana FPR et al. Galloyl-Hexahydroxydiphenoyl (HHDP)-Glucose Isolated From Punica granatum L. Leaves Protects Against Lipopolysaccharide (LPS)-Induced Acute Lung Injury in BALB/c Mice. *Frontiers in Immunology* 2019; 10. (DOI: 10.3389/fimmu.2019.01978).
89. Bagri P, Ali M, Aeri V, Sultana S, Bhowmik M. Evaluation of anti-inflammatory and analgesic activity of Punica Granatum Linn. *International Journal of Drug Development & Research* 2010; 2(4): 698-702.
90. Douaouri N El H, Djebli N. In vivo anti-inflammatory activity and chemical composition of Algerian pomegranate (Punica granatum L.). *International Journal of Biosciences* 2018; 12(2): 76-90. (DOI: 10.12692/ijb/12.2.76-90).
91. Karwasra R, Singh S, Sharma D, Sharma S, Sharma N, Khanna K. Pomegranate supplementation attenuates inflammation, joint dysfunction via inhibition of NF-κB signaling pathway in experimental models of rheumatoid arthritis. *Journal of Food Biochemistry* 2019; e12959. (DOI: 10.1111/jfbc.12959).
92. Vidal A, Fallarero A, Peña BR, Medina ME, Gra B, Rivera F et al. Studies on the toxicity of Punica granatum L. (Punicaceae) whole fruit extracts. *Journal of Ethnopharmacology* 2003; 89(2-3): 295-300. (DOI: 10.1016/j.jep.2003.09.001).
93. Basu A, Penugonda K. Pomegranate juice: a heart-healthy fruit juice. *Nutrition Reviews* 2008; 67(1): 49-56 (DOI: 10.1111/j.1753-4887.2008.00133.x).
94. Vlachojannis C, Zimmermann BF, Chrubasik-Hausmann S. Efficacy and Safety of Pomegranate Medicinal Products for Cancer. *Evidence-Based Complementary and Alternative Medicine* 2015; 1-15. (DOI: 10.1155/2015/258598).