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Research Article

Pomegranate as an Anti-Viral Agent and Immune System Stimulant

Valentina Stefanou, Dimitris Timbis, Dionisios Antonopoulos, Spiros Papatheodorou, Giannis Panourgias, Anna Maria Gouti, Maria Makri, Aspasia Andreou, Vladimiros Lougovois

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

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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
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A B S T R A C T

Pomegranate contains various categories of phenolic compounds such as flavanones, flavones, flavonols, flavanols, anthocyanins, isoflavones, phenolic acids, hydrolysable and non-hydrolysable tannins that possess strong antioxidant, antiviral, anti-inflammatory and antibacterial properties. Pomegranate polyphenols as mixture in extracts, juice or as isolated compounds show very good antiviral activity against Herpes Simplex Type 1 and 2 (HSV-1, HSV-2), Influenza Viruses (H1N1, H3N2, H5N1), Human Immunodeficiency Virus-1 (HIV-1, Clades A to G and group O), HIV-2, Human Enterovirus 71 (EV71), Hepatitis C Virus (HCV), adenoviruses, rotaviruses, feline calivirus (FCV-F9), mosquito-borne dengue virus (DENV), Norovirus (MNV-1). Depending on each case polyphenols manifest antiviral activity through various ways such as killing the virus, cause structural damages on the virion and inactivate the virus, inhibit the viral polymerase activity, protein expression and RNA replication or block absorption of the virus on host cells. Inflammation, oxidative stress and sometimes coincident bacterial infection are referred as viral infection consequences that also must be treated. Pomegranate phytochemicals as they possess also antioxidant anti-inflammatory, antibacterial properties, treat the viral infections' consequences too. Moreover, pomegranate is stimulating and balancing immune system setting the host less vulnerable to viruses contributing thus faster to health achievement and maintenance. It is important to be mentioned that no side effects have been observed in the safety tests that have been carried out and in the cases that pomegranate gave results comparable to commercial drugs, it can be considered as an alternative antiviral and immunostimulant agent.

Keywords: Virus, Inflammation, Infection, Pomegranate, Antioxidant, Phytochemicals, Polyphenols, Antibacterial, Immune-System, Homeostasis

Introduction

Viruses are one of the major hazardous agents to humans and animals. Viral infections, acute and recurrent, cause a wide range of diseases that may be mild, severe of even fatal in immunocompromised patients. Viruses, after entering in the living body, redirect its' metabolism in order to

produce large amounts of their own genome and proteins.[1] Polyphenolic compounds are secondary metabolites of plants found in fruits, flowers, honey, tea, seeds, forages, wine, vegetables, food grains and they have been used since ancient years in traditional medicine in order to treat several disorders and diseases, including viral infections. Phenols and bioflavonoids are the main classes that possess antiviral properties against various virus families such as adenoviruses, enteroviruses, rotaviruses, influenza virus, herpes simplex virus, HIV virus, dengue virus, hepatitis C virus. Researches have shown that for the antiviral activity is needed the presence of hydroxyl group and ester groups. Phenolics which have five or more hydroxyl groups and 3,4,5 three methoxy derivatives possess antiviral and anti-rabies properties, while the gallic acid esters and epicatechin show anti-herpetic properties.[1,2]

Pomegranate Phytochemicals

Pomegranate contains in all of its' plant parts several categories of polyphenolic compounds such as flavones, flavanones, flavanols, flavonols, isoflavones, anthocyanins, phenolic acids, hydroxy-benzoic and hydroxy-cinnamic acids, hydrolysable and non-hydrolysable tannins. Each plant part contains different phytochemicals and also same in different concentrations. Pericarp contains in high concentration punicalin, punicalagin, ellagitannins, gallotannins, granatin A and B, catechin, naringin, kaempferol, pelargonidin and luteolin. Juice contains ellagic acid, gallic acid, quercetin, catechin, casuarinin, rutin, ascorbic acid, epigallocatechin gallate, procyanidin, isoquercetin, catechol, cyanidin glucosides. Seed oil contains punicic acid, ellagic acid and fatty acids. Punicafolin, punicalin, luteolin, luteolin glucopyranosides and apigenin are found in the leaves. Punicaflavone, gallic acid, ursolic, asiatic and maslinic acids can be found in the flowers and ellagitannins and piperidine alkaloids are found in the barks and roots. Pomegranate contains also minerals such as calcium, potassium, magnesium, sodium. The constituents' concentration in the plant parts vary due to the plant variety, climate, ripening stage, cultivar and storage conditions.[3-6]

Anti-Viral Properties of Pomegranate

Influenza viruses are significant human respiratory pathogens that may cause seasonal, endemic infections and also periodic, unpredictable pandemics and are associated with high mortality in infants, elderly people and people with chronic diseases. They possess a segment single stranded RNA-genome with negative orientation and belong to Orthomyxoviridae family. The virus's replication leads to epithelial cell lysis, enhanced mucus production and also due to cytokines secreted by the replication, inflammation and edema. The systemic symptoms of influenza are headaches, myalgia, fever, severe malaise and the respiratory symptoms are rhinitis, coughing and sore throat. Viral spread through bronchiolar tract to the alveoli depending on the immune system of the host may lead to viral pneumonia that can be even fatal or interstitial pneumonitis, a general inflammation of the lung tissue that without treatment may lead to chronic pneumonitis. Hemorrhage and mononuclear infiltration and finally lysis of inte-alveolar space can also be caused. In severe influenza, coincident or secondary bacterial pneumonia are extremely common and they complicate further the histopathological appearance and the consequences on the host. Influenza viruses have also the ability to cause erythrocytes agglutination by binding to sialic acid receptors on the host cell.[7-9]

In a study, the antiviral properties of commercial Wonderful variety pomegranate juice, concentrated liquid extract and 93% pomegranate polyphenol powder extract were evaluated against influenza viruses X31 (H3N2), PR8 (H1N1) and H5N1, a reassortant virus derived from human isolate. After the extract treatment, the virus's titers (numerical expression of the viruses' quantity in given volume of liquid) were determined. The treatment with 800 μg/ ml polyphenol extract at room temperature for 5 minutes leaded into at least 3 log decrease of influenza viruses' H3N2, H1N1 and H5N1 titers. The influenza infectivity decrease has been found to be accompanied with hemagglutinating activity decrease. Electronic microscope analysis data showed that the direct anti-influenza activity of pomegranate polyphenols' powder is mainly because of structural damage that is caused on the virion and more specifically due to small changes that are caused in envelope glycoproteins. Treatment with pomegranate liquid extract 2,5% leaded to significant titers decrease. The results also showed that the acidity of pomegranate liquid extract and juice induced the anti-influenza properties, yet acidity was not a factor in the case of polyphenol powder extract that also was very effective.[2]

Research showed that among the pomegranate polyphenol extract constituents punicalagin, caffeic acid, ellagic acid and luteolin, punicalagin has the strongest antiviral activity against influenza A virus through the viral RNA replication suppression.[10]

It is mentioned that influenza virus invasion in the host cell causes oxidative stress that mediates inflammation response, tissue damage and cell apoptosis. Several studies have shown that oxidative stress often is associated with multiple signaling pathways. The antioxidants are referred to be potential therapeutic options against influenza.[11] Studies refer that superoxide anion produced by macrophages infiltrated into the organs infected by the virus is associated with the development of severe influenza complications.[12] Pomegranate contains in high concentration various bioactive compounds that possess strong antioxidant

properties. Studies showed that the flavonoids pelargonidin, cyanidin, luteolin, luteolin 7-O-glucoside, prodelphinidin, kaempferol, kaempferol 3-O-glucoside, procyanidin, cyaniding -3,5-di-O-glucoside, catechol, pelargonidin -3-O-glucoside, catechin, apigenin, quercetin, quercetin-3,4-dimethyl ether -7-O-α-Larabinofuranosyl (1à6) -β-D-glucopyranoside, the tannins brevifolin, casuarinin, gallic acid, 3,3′, 4′tri-O-methylellagic acid, 3,3′-di-O-methyl ellagic acid, pedunculagin, punigluconin, punicalagin, punicalin, and the alcaloids melatonin and serotonin possess strong antioxidant properties.[13]

In a study, the anti-influenza A (H3N2) activity of pomegranate peel extract has been evaluated using plaque assay, real time PCR and TCID 50% hemagglutination assay. The results showed that the extract reduces influenza A replication in Madin-Darby Canine Kidney (MDCK) cells, a widely used cell line for human influenza isolation and propagation. Exposure of MDCK cells to the extract during viral absorption phase or 24 h post infection reduced remarkably the viral propagation, yet with pretreatment of MDCK cells with the extract 24 h before and not while the infection, no significant antiviral activity has been observed. Pomegranate peel extract showed virucidal activity and inhibited the agglutination by influenza virus of the chicken Red Blood Cells (cRBC). Independent of the virucidal effect of the extract, inhibition of the virus RNA replication in single-cycle growth conditions has also been observed. Punicalagin that also showed antiviral activity against influenza A virus, inhibited the agglutination of chicken red blood cells by influenza and blocked the virus RNA replication. Furthermore, pomegranate peel extract and oseltamivir combination increased the oseltamivir activity due to synergistic action.[14]

Malas variety's pomegranate peel extract in ethanol was found to possess strong antiviral activity against influenza virus A/Puerto Rico/8/34 (H1N1; PR8) and the mechanism of its' antiviral activity by viral replication inhibition has been investigated using time-of-drug-addition assay, hemagglutination inhibition assay, western blot analysis and also by virucidal activity, RNA replication and viral mRNA expression study. The results showed inhibition of viral polymerase activity, viral RNA replication and protein expression yet hemagglutination inhibition has not been observed. The time-of-drug-addition assay results showed that the extract inhibited the virus absorption and early steps of virus replication.[15]

SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is a single standard RNA virus that belongs to a broad viruses' family known as coronaviruses and is responsible for the coronavirus disease 2019 (COVID-19). Possible clinical characteristics of the disease are fever, lung infection, pneumonia, chest pain, breathing difficulty, cough, headache, sputum production, hemoptysis, myalgia or fatigue and diarrhea. It is also referred that the disease may progress into acute cardiac injury, Acute Respiratory Distress Syndrome (ARDS) and acute kidney injury.[16,17]

Molecular docking is a computational procedure that can provide information about the interaction between two molecules such as a biomolecule (DNA or a protein) with a ligand, predict their preferred orientation in order to form a stable complex and also their binding affinity strength of association and it plays important role in the computer aided drug discovery and design.[18,19] Molecular docking study of COVID-19 protease with urolithins, (bioactive metabolite compounds produced by gut microbiota from ellagic acid and ellagitannins.[20,21] has been carried out and was found that there was interaction of Urolithin A with protease (PDB ID:6LU7) and with peptidase (PDB ID:2GTB) with binding energies to be -5.46 kcal/ mol and -6.93 kcal/ mol respectively. Urolithin B interacted with protease (PDB:6LU7) and with peptidase (PDB ID:2GTB) with the binding energies to be -4.67 kcal/ mol and -6.74 kcal/ mol respectively. The target enzymes' most common interacting amino acids with the Urolithins were Glu166, Gln189, His41, His164 and Met165. The study showed that Urolithins have the potent to inhibit remarkably the virus peptidase and protease and prevent the virus's entry into the host cell indicating that Urolithins can probably be used to control the COVID-19 disease.[16]

In another in silico study the effects of pomegranate peel extract compounds on the multistep process of SARS-CoV-2 internalization in host cells have been investigated. More specifically, in the study were determined the interactions and binding affinities among the compounds gallic acid, ellagic acid, punicalin, punicalagin and four selected protein targets with confirmed important role in the virus entry process in the host cell that were SARS-CoV-2 spike glycoprotein, furin, transmembrane serine protease 2 and angiotensin converting enzyme 2. Punicalin and punicalagin were found to form more stable complexes with the amino acid residues at the protein targets' active sites than the positive control did. The binding affinity of punicalagin and punicalin with angiotensin converting enzyme 2 were 7.144 and -7.353 kcal/ mol respectively. Molecular docking results showed hydrogen bond interactions among all the selected compounds and amino acid residues at the SARA-CoV-2 spite glycoprotein active sites with punicalin to demonstrate the strongest interaction with free binding energy of -7.406 kcal/ mol. Moreover, punicalin and punicalagin showed interactions with transmembrane serine protease 2 having binding energy values of -8.168 and -7.358 kcal/ mol respectively. The complexes among all selected compounds and furin were found to be very stable. The complex of furin with gallic acid, ellagic acid and punicalin was formed due to polar interactions with

the aminoacids Ser 368, Thr 365, Pro 256, His 194, Ser 311, Asp 258, Glu 257, Asp 306, Thr 262 and aGly 255.[22]

Herpes Simplex Type 1 (HSV-1) is a virus composed of linear dsDNA, an 100-110 nm diameter icosahedral capsid and a spikey envelope and it belongs to the Alphaherpesvirinae subfamily. The HSV-1 infection pathogenesis is following a circle of primary epithelial cells' infection, latency primarily in neurons and also reactivation. There is a wide range of HPV-1 infection presentations such as herpetic sycosis (HSV folliculitis), orolabial herpes, herpetic whitlow, herpes gladiatorum, eczema herpeticum, Kaposi varicelliform eruption, herpes encephalitis, facial, ocular HSV infections and also chronic or severe HSV infection.[23-26] HSV infections are associated with inflammation and pain.[27]

In research was found that pomegranate rind extract in conjunction with zinc (II) salts (stearate, sulphate, citrate and gluconate) show very strong virucidal activity against HSV-1, while the activities of individual salts and rind extracts were low. All the salts increased the antiviral activity of the extract up to 4-fold. The pomegranate rind extract with the zinc (II) showed comparable and even slightly better activity than acyclovir. Besides, the extract exhibited activity against aciclovir resistant HSV while aciclovir showed no activity. No cytotoxicity has been found in any of the solutions tested.[28]

As HSV infections are often causing inflammation, the treating process should also include anti-inflammatory treatment, and pomegranate except antiviral possesses also anti-inflammatory properties.[29]

In another study pomegranate rind extract co-administrated with zinc (II) topically applied on ex-vivo porcine skin freshly excised showed also strong anti-inflammatory activity.[27] Cycloxigenase (COX), an isoenzyme responsible for the prostanoids' formation is an inflammation mediator involved in cancer related pathological processes. Inhibition of COX provides relief from pain and inflammation.[29] Results showed that topical application of the rind extract reduced remarkably the COX-2 expression. Pomegranate tannins and pomegranate rind extract alone were also found to decrease COX but lesser than the rind extract co-administrated with zinc (II). No anti-inflammatory activity has been observed by the application of Zn (II) salt alone. Pomegranate rind extract penetrated the skin in the viable epidermis and downregulated COX-2. Punicalagin was found in the whole skin in particular lower regions[27] suggesting that effective antiviral against HSV and at the same time anti-inflammatory medicines and ointments could possibly be provided by pomegranate.

Oxidative stress and oxidative damage are pathological consequences of several virus infections. Research showed that there is association between HSV-1 infections of the nervous system (acute and latent) and oxidative damage[30] Pomegranate containing phytochemicals with strong antioxidant properties, can sufficiently treat oxidative stress.[29]

Herpes Simplex Virus 2 (HSV-2) is responsible for genital herpes, a sexual transmitted infection with possible symptoms to be itching, pain and sores, neurological morbidity, meningitis, neonatal herpes simplex encephalitis and also is mentioned to cause cancer.[26,31,32]

Pomegranate pericarp tannin content is found to be very effective against HSV-2 as it is efficiently blocking its' absorption onto cells and also killing the virus. In another study, pomegranate tannins were found to possess anti-HSV-1 and anti-HSV-2 properties as the virus absorption from human adenocarcinoma cells and also African green monkey kidney has been blocked.[5,10]

Recurrent intraoral herpes is a clinical form of HSV infection. Two groups of immunocompromised recurrent intraoral herpes patients aged from 18 to 35 years with lesions affecting keratinized mucosa, used pomegranate peel extract and chlorohexidine mouthwashes for 10 days, 3 times per day. Results showed that erythema and pain were remarkably decreased in both groups with the pomegranate group to have better results in reducing pain and accelerating wound healing than the chlorohexidine group.[33] It is important to be mentioned that chlorohexidine may have side effects such as taste alteration.[34], tooth staining and tartar formation while pomegranate has no side effects and moreover it is effective against dental plaque microorganisms and prevents from tartar.[29,35-37]

In a research, the anti-HSV activity of crude ethanol pomegranate peel extract with total phenolics 282.9 mgGAE/g, total flavonoid content 136.6mg/ g and IC_{50} of DPPH radical 7.7±1.21 µg/ ml compared with butylated hydroxytoluene (BHT) (IC_{50} 25.41±1.89 µg/ ml), has been tested on Vero cell line using MTT (3- [4,5-dimethylthiazol 2yl] 2,5-diphenyltetrazolium bromide) assay. The extract was found to inhibit replication in absorption stage ($p<0.05$). The 50% cytotoxicity concentration (CC_{50}) was found 293.5±10/1 µg/ml and the 50% inhibitory concentration (IC_{50}) was 37.7±6/ 7µg/ ml. In order to measure the antiviral activity and cytotoxicity, selectively index, IS (CC_{50} and IC_{50} ratio) is used. The ideal drug shows cytotoxicity only at very high concentrations and antiviral activity at very low. The highest SI value is, the safer and more effective is the drug. The extract's selectivity index was 7.78 indicating a good anti-HIV activity that probably could be due to absorbtion stage inhibition suggesting that the extract could be considered as a potential anti-HSV agent.[38,39]

Human Immunodeficiency Virus (HIV), genus Lentivirus, belonging to the Orthoretrovirinae subfamily and Retroviridae family, is responsible for the Acquired

Immunodeficiency Syndrome (AIDS) that is human immune system degradation. It is classified into the types HIV-1 and HIV-2 with the type 1 to be the most common and pathogenic strain. Two identical single-standard RNA molecules enclosed in the viral particle core, consist the HIV genome. The HIV-1 type is subdivided into the major group M and the minor groups N,O and P. The group M is subdivided based on genetic sequence data, into subtypes (A to D, F to H, J, K) and it is the most responsible for AIDS pandemic. The first step of HIV replication cycle is binding and entering into the host cell. The HIV protein gets binded onto the primary cellular receptor CD4, a glycoprotein existing on the surface of immune cells and then onto a cellular coreceptor such as the chemokine receptors CXCR4 expressed on T cells and CCR5, expressed on macrophages and also on some T-cells populations.[40-43]

In a study, the inhibitory activity of pomegranate juice against HIV-1 primary clades A to G and group O has been evaluated. The juice has been absorbed onto corn starch and CD4 and CXCR4/ CCR5 were used as cell receptors. The results showed that the pomegranate juice/ corn starch complex is efficiently blocking the virus binding onto the receptors CD4, CXCR4/ CCR5 inhibiting efficiently the viral infection as the pomegranate juice compounds prevented the binding of gp 120 of HIV BaL and gp 120 of HIV IIIB onto CCR5 and CXCR4 respectively. These results indicate that the pomegranate juice/ starch complex could be used in order to provide strong anti-HIV-1 microbicide products.[44,45]

Hepatitis C virus (HCV), a blood borne human pathogen is an enveloped small positive single-stranded RNA virus and it belongs to the Flaviviridae family and genus Hepacivirus. HCV causes progressive liver damage, chronic hepatitis that often progress into other liver diseases such as liver cirrhosis and hepatocellular carcinoma.[46-48]

In vitro study showed that ellagitannins from crude pomegranate peel extract, punicalin, ellagic acid and punicalagin blocked the HCV NS3/4A protease activity which is essential for the HCV replication. Moreover, punicalin and punicalagin were found to suppress remarkably the HCV replication in cell culture system. It is important to be mentioned that these compounds did not have any adverse effects with the No-Observed Adverse Effect Level (NOAEL) to be set up to the acute dose of 5000 mg/ kg administrated in BALB/c mice. Besides pharmacokinetic studies showed the bio-availability of these compounds.[10,49]

During liver diseases such as Hepatitis C, oxidative stress is induced that is causing hepatic damage and also it is stimulating the process to bother the treatment of the damage. The viral infection results in oxidation of important cell components such as DNA, lipids and proteins while DNA double strand breakage may occur. These disorders are possible to lead to cirrhosis and hepatocellular carcinoma. Pomegranate, containing antioxidant compounds in high concentration is found to improve the antioxidant status of the host and thus it is contributing by its antioxidant properties too (except the antiviral) to the patients' clinical characteristics improvement.[50,51]

Human enterovirus 71 (EV71) belongs to Picornaviridae family, enterovirus genus and is a single-standard positive sense RNA virus responsible for the Hand Foot and Mouth Disease (HFMD) in up to 6 years old children. Usually, HFMD clinical manifestation is mild and self-limiting yet a severe HFMD infection can lead to serious neurological diseases such as encephalitis, poliomyelitis-like paralysis, aseptic meningitis and even death. The antiviral activity of punicalagin against EV 71 has been evaluated in vitro and in vivo. The results of the in vitro study showed that punicalagin decreased the viral cytopathic effect on rhabdomyosarcoma cells with IC_{50} value to be 15 μg/ ml and CC_{50} 300 μg/ ml. As positive control ribavirin, an antiviral agent against EV71 has been used with higher IC_{50} (60 g/ ml). In the in vivo study mice challenged with lethal EV71 dose were treated with punicalagin at doses of 0.4, 1 and 5 mg/ kg body weight and 20%, 40% and 38% of the mice were found to be long term survivors respectively. These results indicate that the proper punicalagin dose is 1 mg/ kg. Further evaluation showed that treatment with 1 mg kg punicalagin delayed for 1 day the paralysis appearance and also decreased the clinical scores in comparation with control group. The symptoms of the EV71 infection were prevented and the viral replication in the muscles were remarkably inhibited in the punicalagin group compared to the control group. After two weeks there was no evidence of the disease in the surviving mice indicating that punicalagin can be considerate as a potent anti-EV71 alternative drug.[52]

Adenoviruses are a non-enveloped virus group, responsible for a wide range of clinical diseases such as fever, common cold or flu-like symptoms, sore throat, pink eye, pneumonia, acute bronchitis (inflammation of the lung's airway) and acute gastroenteritis (inflammation of the intestine or the stomach causing stomach pain, nausea, vomiting and diarrhea). Less common symptoms are bladder infection or inflammation and neurologic diseases that affect the brain and the spinal cord.[53,54]

In a study, the anti-adenovirus activity of crude pomegranate peel ethanolic extract with total phenolics 282.9 mg GAE/g and total flavonoid content 136.6 mg/ g has been evaluated on Helacell line with the MTT (3-[4,5-dimethylthiazol–2-yl]-2,5-diphenyltetrazolium bromide) assay. The 50% inhibitory concentration (IC_{50}) was 18.6±6.7μg/ml and the cytotoxicity concentration (CC_{50}) 165±10.1μg/ml. The extract's selectivity index was 8.89 suggesting that it could be used as a potential anti-adenovirus agent.[55]

In another research the anti-adenovirus properties and

cytotoxicity of crude pomegranate peel extract, its ethyl acetate, n-butanol, hexane and chloroform fractions and also of the main compounds gallic acid, ellagic acid and punicalagin have been evaluated on Hep-2 cell line with the MTT assay. The results showed that the n-butanol fraction showed the highest anti-adenovirus activity with IC_{50} 2.16 μg/ ml, CC_{50} 264.7 μg/ ml and SI of 122.5. The crude extract and the gallic acid showed also good anti-adenoviral activity with IC_{50} of 5.77 μg/ ml and 4.67μM and SI of 49.9 and 10.5 respectively. Both gallic acid, crude extract and n-butanol fraction were found to inhibit the virus replication in post-adsorption phase ($p<0.01$).[56]

Norovirus belonging to the Caliciviridae family, contains a single standard RNA genome and is the most common cause of non-bacterial gastroenteritis. It is causing inflammation of stomach or intestine with fever, vomiting and diarrhea symptoms. These viruses are non-enveloped and ethanol resistant.[57] In a study, pomegranate juice and pomegranate polyphenols have been evaluated against murine norovirus (MNV-1), MS2 (ssRNA) bacteriophage and feline calicivirus (FCV-F9). The viruses at low titers (~5log_{10}PFU/ ml) and high titers (~7log_{10}PFU/ ml) were mixed and incubated for 1 hour at room temperature with pomegranate juice, 4, 8 and 16mg/ml pomegranate polyphenols and water (control). After incubation, the viral infectivity has been evaluated with standardized plaque assays. It was found that for low titers, the pomegranate juice reduced the titers of MNV-1 MS2 and FCV-F9 by 1.32, 0.32 and 2.56 log_{10}PFU/ ml. For high virus titers there was a titer decrease of 0.06, 0.63 and 1.20 log_{10}PFU/ ml for MNV-1, MS2 and FCV-F9 respectively. Interestingly, after exposure of high and low FCV-F9 titers in all three pomegranate polyphenol solutions the virus was undetectable. At low initial titers MNV-1 exposure at 4,8 and 16mg/ ml pomegranate polyphenols resulted in decrease by1.30, 2.11 and 3.61 log_{10}PFU/ ml respectively. MNV-1 at high initial titers was decreased by 1.56, 1.48 and 1.54 log_{10}PFU/ ml after treatment with 4, 8 and 16 mg/ ml pomegranate polyphenols.[58]

Rotaviruses are double stranded RNA viruses belonging to Reoviridae family. There are 9 rotavirus species (A,B,C,D,E.F,G,H,I,J) with the rotavirus A to be the most common species that is responsible for infections in humans. Rotavirus infection causes dehydrating diarrhea, nausea, vomiting, low grade fever and is responsible for over 600,000 childhood deaths annually. In a study pomegranate extract was found to inhibit sufficiently rotavirus and this is attributed to the high concentration of polyphenolic derivatives and flavonoids that are contained in the pomegranate.[59-61]

Mosquito-borne dengue viruses (DENVs) are single positive-stranded enveloped RNA viruses, traditionally classified into four serotypes (DENV-1,-2,-3,-4), that belong to Flaviviridae family, genus flavivirus and they are responsible for Dengue, an acute febrile disease. Symptoms of Dengue viruses' infections are headache, high fever, painful muscle and bone, swollen glands or rash, pain behind the eyes, sore throat, stomach ache, nausea and vomiting. Severe dengue disease symptoms that may be even fatal, include fluid accumulation with respiratory distress, severe plasma leakage that leads to shock, severe bleeding (dengue hemorrhagic syndrome), low platelet count, severe organ impairment as heart impairment, elevated transaminases ≥1,000 IU/ L or consciousness impairment. It is also referred that there is an increased association between severe dengue and peptic ulcers, diabetes mellitus, bronchial asthma and sickle cell anemia.[62-68]

Dengue-infected 9-year-old patient (NS-1 positive) with 5 days high fever and headache was treated for 5 days with 250 mg of the anti-hyper thermic medicine paracetamol and twice a day with 250 mg amoxicillin. After 5 days treatment the liver function test showed that the value of the liver injury marker SGPT, serum glutamic-pyruvic transaminase (or Alanine Aminotransferase, ALT) was very high (126 U/L), and this is probably, according to several scientific reports, related to the high consumption of the antipyretic and antibiotic drugs. The blood examination showed platelet count decrease to 1.35 Lakhs/ mm^3 and total white blood cell count decrease to 3200/ mm^3. After the hematology data scrutinization, the amoxicillin and paracetamol medications were stopped and only pomegranate juice combinate with sweet lime and green coconut water were administrated to the patient. Two days later of continuous pomegranate-sweet lime juices and coconut water, the blood parameters' values were remarkably improved. Seven days later, the platelets count was 3.1 Lakhs/ mm^3 and the total count of Leucocytes was 6100/ mm^3 instead of 1.35 Lakhs/ mm^3 and 3200/ mm^3 that were in the beginning of the pomegranate-sweet lime-coconut water administration, indicating that this combination can be used in order to restore the leucocyte and platelet count that has been reduced due to dengue infection.[69-71]

Pomegranate Immune - Stimulant

Homeostasis is any process that living beings are using in order to achieve maintain fairly stable conditions that are needed for survival. The immune system contributes to homeostasis as it is preparing the body to fight off infections and also enhance the healing process in case there is any harm and thus, a strong immune system is a prerequisite for health maintenance.[72,73] Lymphocytes are white blood cells and they are part of the immune system. Two main lymphocyte types exist: B cells which produce antibodies that attack invading viruses, bacteria and toxins, and T cells that destroy the body's cells which have been taken over by viruses or became cancerous.[74]

In an in vitro study human lymphocyte cells isolated from peripheral blood (80 µl suspension, 10^6 cells/ ml) were incubated for 72 h at 32°C with 20 µl pomegranate extract with the final extract concentrations to be 0.1, 0.25 and 0.5 µl/ ml. The stimulation index of the pomegranate was found to be 1.2 times higher than the index of a commercial immune-stimulant agent at the same concentration as the values of the % stimulation index for 100 ppm pomegranate extract and commercial immune stimulant were 1245 and 1024 respectively, indicating that pomegranate is a potent immune-stimulant agent.[75]

In an in vivo study, BALB mice that have been starved in order to mimic an emergency condition were divided into 3 groups and they were administrated with high calorie biscuit with either pomegranate extract (equal to 400 g/ day consumed by a 70 kg man) or commercial immune-stimulant or no immune-stimulant (control group). Immunoglobulin G is the most common antibody and a sensitive immune marker which represents a specific, due to infections, humoral immune response. Total serum immunoglobulin G has been determined in week 0, 2, 4 and 8 in order to evaluate the humoral immunity stimulation. Until week 4 there was not significant Immunoglobulin G value difference among the groups but at the 8th week there was a remarkable increase at both pomegranate extract and commercial immune-stimulant group. The increase was comparable among those groups indicating that pomegranate can also be used in order to improve immunity status in an emergency situation.[75]

Macrophages are a diverse phenotype of phagocytic cells derived from parent monocytes in the peripheral blood and bone marrow precursors. They play essential role in the maintenance and the defense of host tissues. Phagocytosis is the first step of macrophages' response to invading microorganisms such as viruses and bacteria. Immunological tests showed that pomegranate beverage with spirulina and echinacea with total phenolics 2.294 ± 0.64 mg gallic acid /ml, total flavonoids 2.084 ± 0.55 mg catechin/ ml and antioxidant activity 94.54 ± 0.18% caused remarkable increase in phagocytosis.[76,77] M1 and M2 are two macrophage phenotypes that play different roles in immune system. M1 phenotype produces Reactive Oxygen Intermediates (ROI), reactive oxygen species (ROS) or Nitric Oxide (NO) to attack viruses and bacteria. M2 is anti-inflammatory phenotype and resolves inflammation that favors the oxidative metabolism. ROS and NO overproduction by the M1 macrophages lead to oxidative stress that is related to inflammation and several diseases and thus macrophage polarization may be crucial for the tissue fate. In healthy subject macrophages remain in balance state. Administration of mice with pomegranate juice rich in gallic acid and ellagic acid lead into inhibition of the M2 to M1 macrophage phenotype shifting, favoring thus the anti-inflammatory M2 phenotype.[78-83] In an in vitro study on J774A1 macrophage-like cell line was found that pomegranate juice and polyphenols reduced dose dependently the macrophage response to M1 pro-inflammatory activation, as there was remarkable decrease of IL-6 and TNF-α secretion in response to stimulation by Lipopolysaccharide and INF-γ. Moreover, dose dependently, punicalagin and pomegranate juice promoted the macrophages M2 anti-inflammatory phenotype.[84]

During aging process the M1 macrophage phenotype is increasing and M2 macrophages are reduced. Administration of old mice with dietary pomegranate juice led to inhibition of the M2 to M1 shifting.[84,85]

In a research, Holstein calves were administrated with pomegranate extract rich in polyphenols in order to study the effects of the extract on the general health and the immunocompetence of the calves in their first 70 days of age. Pomegranate extract polyphenols enhanced the mitogen induced cytokine production and improved the total immunoglobulin G responses to vaccination, benefiting the calves' immune competence and health.[86]

In another study, immunocompromised mice were administrated for 28 days with pomegranate peel polysaccharides (100, 200 and 400 mg/ (kg d)) and was found that the immune organ index of the mice has been increased. Significant increase of the hepatic antioxidant capacity has also been observed. The antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase were increased remarkably indicating that pomegranate peel polysaccharides can possibly be used as an effective immunopotentiating therapy, as an alternative immunostimulant agent or as a mean to reduce chemotherapy induced immunosuppression.[87]

Pomegranate Safety

Several studies that have been carried out in order to test the safety of the different constituents derived from pomegranate showed that in the examined dosages no side effects were observed. In a research where 86 overweight human subjects were administrated for 28 days with 1420 mg/ day pomegranate fruit extract in tablet no side effects were observed, nor adverse changes in individuals' blood or urine were reported.[88] Animal studies showed that pomegranate component at concentrations that have been used in traditional medicine has no side effects.[89] Study showed that 90 days administration with pomegranate extract 600 mg/ kg body weight did not cause any side effects as there were not any histopathology findings or treatment related gross.[3,90] In another study where Wistar rats were administrated with pomegranate seed oil for 28 days was found that the no observable adverse effect level (NOAEL) is 4.3 g pomegranate seed oil/ kg body weight/ day.[91]

Discussion

Pomegranate is a rich source of various bioactive compounds that possess more than one bioactivity such as antioxidant, antiviral, antibacterial and anti-inflammatory activities. Pomegranate extracts, juice and isolated constituents were found to be very effective on several viruses such as Influenza viruses (H3N2, H1N1, H5N1), Herpes Simplex Virus Type 1 (HSV-1), Herpes Simplex Virus Type 2 (HSV-2), Human Immunodefficiency Virus-1 (HIV-1, Clades A to G and group O), HIV-2, Hepatitis C Virus (HCV), Human Enterovirus 71 (EV71), rotaviruses, adenoviruses, norovirus (MNV-1), feline calivirus (FCV-F9), mosquito-borne dengue virus (DENV).

The antiviral activity of pomegranate is manifested by various ways and depending on each case, pomegranate polyphenols are found to be virucidal, cause structural damages on the virion inactivating the virus, inhibit the viral polymerase activity, the protein expression and viral RNA replication or block the binding/ absorption of the virus on host cells.

Inflammation and oxidative stress are in several viral infections referred as pathological consequences that also should be treated as they are can cause various diseases and disorders if they remain untreated. Pomegranate is a potent antioxidant and anti-inflammatory agent and thus it can sufficiently treat oxidative stress and inflammation caused by the viral infection. It is mentioned that in severe influenza coincident or secondary bacterial pneumonia may occur. Pomegranate is a strong antibacterial agent[3] and can treat the bacterial infection contributing also by this way to the health achievement.

A living organism can be considered as an equilibrium of biochemical parameters and functions and its' maintenance and survival depends on that equilibrium maintenance. As in every system, also in living beings, this equilibrium maintenance in fact depends on the superiority of the "forces" that keep all these parameters into a functional unit over the forces, inner or external that lead to the unity's destruction.

As much important is to discuss about the antiviral properties of an agent, that much and even more important in some cases is also to discuss about the ability of the organism to defend against harmful and destructional agents that threat its' unity existence and maintenance. After all, as strong are the forces that keep all the biochemical parameters and functions into a functional unit, so difficult is for destructive agents and forces to threat the organism's existence.

Immune system is contributing to this maintenance, to homeostasis. Researches have shown that pomegranate is stimulating and also balancing the immune system. More specifically pomegranate is found to improve the immunity status in emergency situation, improve the general health even in immunocompromised patients and increase the stimulation index giving results even better than commercial immune stimulant.

In a healthy immune system, M1 macrophage phenotype that produces ROI, ROS and NO and the anti-inflammatory M2 phenotype are balanced. During aging and pathological processes, the balance is disturbed in favor of the M1 phenotype, responsible for the inflammatory responses. Pomegranate promotes the M1 to the M2 shifting.

Even if almost in every health issue approach the focus is set on the health threatening factors for example the viruses, in fact the point is the health achievement and maintenance itself, that of course can be reached by removing health threatening factors, yet most times this is a unilateral approach, as the results of the viral activity, such as infections, inflammation, oxidative stress are remaining and need also to be treated and moreover the immune system strengthening is also important as a good immune system can defend more efficiently against health threatening factors.

Pomegranate containing constituents with multiple bioactivities, that also can treat the consequences of the viral infection while it is strengthening the immune system making the host less vulnerable to viruses and in several cases gives comparable or even better results (as antiviral and immune stimulant agent) without any side effects, could possibly be considered as an alternative antiviral medicine.

Further research will provide us with useful information about the antiviral and immune stimulating activity of the pomegranate's bioactive compounds and about the biochemical pathways they are involved either as isolated compounds or as a mixture in the extracts and the juice, where synergic action may occur.

Conclusion

Pomegranate as it contains in high concentrations bioactive compounds with more than one bioactivity, antiviral, antioxidant, antibacterial and anti-inflammatory, it contributes by multiple ways in health achieving, preventing or treating overall the health issue caused by the virus and not only by removing the factor that has created it. Pomegranate polyphenols show very good antiviral activity against several viruses and also with their antioxidant, anti-inflammatory and antibacterial properties can treat oxidative stress, inflammation and co-incident bacterial infections that are several times results of the viral infections while the immune system is getting strengthened and stimulated. As the antiviral activity of pomegranate was found in several cases to be comparable to the commercial drugs' activity, pomegranate can be considered as an alternative antiviral agent which also offers also various other benefits to the health without side effects.

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