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Clinical Applications of Pomegranate

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Abstract

Pomegranate, *Punica granatum* L., is an ancient, unique fruit borne on a small, long-living tree in the Mediterranean region, Southeast Asia, and tropical Africa. Pomegranate was mentioned in ancient times in the Old Bible, the Jewish Torah, and mentioned three times in the holy Quran where it was described as one of the paradise fruits. In ayurvedic medicine, pomegranate is used in the treatment of parasitic infection, diarrhea, and ulcers. Recently, pomegranate has been studied in several systems of medicine for its pharmacological actions: anti-inflammatory, antioxidant, and anticarcinogenic. The aim of the chapter is to summarize pomegranate efficacy in many preclinical and clinical studies.

Keywords: pomegranate, ayurvedic medicine, pharmacological activities, preclinical, clinical studies

1. Introduction

Pomegranate, (*Punica granatum* L.), a paradise fruit, has a great value throughout history. It had been mentioned in Judaism, Christianity, and Islamic religions [1]. From ancient times, pomegranate was used in treatment of diarrhea [2], parasitic infections [3], and diabetes mellitus [4]. Greco-Arab and Islamic medicine prescribed pomegranate for sore throat, inflammation, and rheumatism [5]. Various pomegranate activities (anti-inflammatory, antioxidant, and anticancer) encouraged growing number of studies to apply it in solving multiple medical problems [6]. Pomegranate plant is a small tree (**Figure 1**) that is cultivated in the Middle East, Mediterranean region, China, India, California, and Mexico. The fruit (**Figure 2**) is composed of many parts such as seeds, peels (pericarp), pulp, and juice [6].



Figure 1. Pomegranate tree.



Figure 2. Pomegranate fruit.

2. Clinical applications

For its multiple pharmacological potential, pomegranate has been investigated by variable preclinical and clinical studies in a wide variety of health disorders:

2.1. Inflammation

Pomegranate exhibits a potent anti-inflammatory effect through inhibition of cyclooxygenase (COX) and lipoxygenase (important inflammatory mediators) [7].

2.1.1. Gastrointestinal inflammation

2.1.1.1. Gastric inflammation

2.1.1.1.1. Preclinical studies

Helicobacter pylori (*H. pylori*) are major etiological agents in peptic ulcer. Pomegranate methanol extract produced a remarkable anti-*H. pylori* activity with mean diameter of inhibition zone 39 at 100 $\mu\text{g disc}^{-1}$ [8]. This activity is explained by altering bacterial cell surface hydrophobicity and prevention of bacterial adhesion to gastric mucosa [9]. Moreover, pomegranate revealed gastroprotective potential via antioxidant mechanism in aspirin- and ethanol-induced gastric ulceration in animal models [10]. Gastroprotective property of pomegranate is attributed to its constituents (saponin, tannins, and flavonoids) as demonstrated in another study on wistar rats where oral administration (490 and 980 mg/kg body weight) of pomegranate aqueous methanolic extract significantly reduced gastric ulcer index in alcohol-, indomethacin-, and aspirin-induced ulcers [11]. Tannins are high molecular weight phenolic compounds present in many plants, including pomegranate fruit pericarp (peels). These compounds have the capacity to form complexes mainly with proteins [12, 13]. Pomegranate tannins form a protective layer (tannin-protein/tannin-polysaccharide complex), upon damaged epithelial tissues, thus allowing the healing process below to occur naturally through prevention of bleeding and acceleration of ulcer healing [14, 15]. All parts of the pomegranate tree have been used as a source for tannin in the leather industry, changing animal hide into leather. About 10–25% of tannin are present in the trunk bark and were important in leather production in Morocco. In this process, collagen chains in the hide are cross-linked by tannin to give leather. The formation of various complex bonds helps the tannin-protein polymer combination [16, 17]. These facts take our attention to the Islamic advice “To eat pomegranate with its pericarp as it is tanning for the stomach”.

2.1.1.2. Intestinal inflammation

2.1.1.2.1. Preclinical studies

Inflammatory response is induced by transduction cascades initiated by many inflammatory mediators, that is, tumor necrosis factor α (TNF- α) and nuclear factor κB (NF- κB). Pomegranate inhibited TNF- α -induced NF- κB activation and COX-2 expression in colon cell line. This effect was highly presented by pomegranate juice compared to single constituents, that is, tannin and punicalagin. This highlights the synergism between all bioactive pomegranate compounds [18]. Prebiotics are food agents that stimulate the growth or activity of beneficial microorganisms. Pomegranate peel extract (6 mg/d for 4 weeks) increased the cecal pool of beneficial bifidobacteria when given to high-fat diet mice. Additionally, it counteracted the high-fat-induced expression of inflammatory markers both in the colon and in the visceral adipose tissue [19]. Through its antioxidative action, pomegranate elagic acid (EA) (10 mg/kg) in colonic-delivering microsphere significantly ameliorated the severity of colonic lesions and reduced myeloperoxidase (MPO) activity and lipid peroxidation. This effect was obtained by orally administering it to rat model of dextran sulfate sodium (DSS)-induced ulcerative colitis [20]. Mast cells are

important inflammatory cells that release histamine. Mast cell stabilizing is an additional anti-inflammatory mechanism of pomegranate where its hydroalcoholic extract significantly lowered DSS-induced elevated histamine level in mice colon tissue [21].

2.1.1.2.2. *Clinical studies*

The only human trial is the ongoing phase I study on the role of pomegranate juice ellagitannins in the modulation of inflammation in inflammatory bowel diseases. This has been registered since December 2016. Available online: <http://www.clinicaltrials.gov>

2.1.2. *Joint inflammation*

2.1.2.1. *Preclinical studies*

A pomegranate compound, delphinidin, attenuated the inflammatory signaling that results in rheumatoid arthritis. This mechanism was mediated by inhibition of the histone acetyl transferase and NF- κ B activation in human rheumatoid arthritis synovial cell line [22]. Pomegranate alleviated features of arthritis in collagen-induced arthritic mice (CIA). This effect was associated with histopathological evidence of reduced inflammatory cells and joint tissue damage. Moreover, pomegranate decreased the interleukin 6 (IL-6) level and suppressed inflammatory signal transduction pathways in mouse macrophages [23].

2.1.2.2. *Clinical studies*

Pomegranate (2 capsules of 250 mg pomegranate extract/day for 8 weeks) improved disease activity, some inflammatory blood biomarkers and oxidative stress (increased glutathione peroxidase) in 30 rheumatoid arthritis patients in a double-blind, placebo-controlled, randomized study [24].

2.1.3. *Respiratory inflammation*

2.1.3.1. *Preclinical studies*

Pomegranate peel aqueous extract attenuated lipopolysaccharide (LPS)-induced lung inflammation in mice. Furthermore, it inhibited the production of human neutrophil reactive oxygen species (ROS) and myeloperoxidase [25]. Synergistic anti-inflammatory effect of pomegranate extract (encapsulated into microparticles) with dexamethasone was demonstrated in asthma model mice. The microparticles attenuated leukocytes' recruitment to bronchoalveolar fluid, particularly eosinophils, reduced cytokines (IL-1 β and IL-5), and reduced protein levels in the lungs. These findings supported the alternative/complementary use of pomegranate in treatment of lung inflammation [26]. Pomegranate (80 μ mol/kg/day) significantly attenuated the expression of inflammatory mediators, apoptosis, and oxidative stress that were induced by acute mice exposure to cigarette smoke (for 3 days). Additionally, on chronic cigarette smoke exposure (1–3 months) pomegranate reduced expression of TNF- α and normalized lung cell architectures. Moreover, pomegranate juice attenuated the damaging effects of cigarette smoke extract on cultured human alveolar cells [27]. Pomegranate juice diminished

inflammatory changes in pulmonary tissue via its antioxidative capacity in a study that was carried out on 27 streptozotocin-induced diabetic rats, which were given either pomegranate or saline for 10 weeks [28].

2.2. Cancer

2.2.1. Prostate cancer

2.2.1.1. Preclinical studies

Prostate cancer suppression was exerted by different pomegranate fruit parts (juice, peel, and seed oil) on LNCaP, PC-3, and DU 145 human cancer cell lines. This effect was manifested by inhibition of proliferation, invasion, phospholipase A2 (PLA2) expression, and apoptosis induction [29, 30]. Pomegranate fruit extract inhibited cell growth and induced apoptosis via remodeling of apoptosis regulating proteins in prostate cancer PC-3 cell line. In addition, oral administration of pomegranate fruit extract to mice implanted with CWR22Rnu1 cells significantly suppressed tumor growth and decreased prostate-specific antigen (PSA) in the serum [31, 32]. Oral pomegranate fruit extract (100 mg/kg) for 4 weeks inhibited testosterone-induced prostatic hyperplasia, prostate weight, prostatic acid phosphatase activity, and total glutathione in rats [33].

2.2.1.2. Clinical studies

A two-stage phase-II clinical trial on 46 subjects with recurrent prostate cancer and rising serum prostate-specific antigen (PSA) after surgery or radiotherapy was carried out. The participants consumed daily eight ounces of pomegranate juice (570 mg of total polyphenol gallic acid equivalents) until meeting the disease progression endpoints. About 35% of patients achieved a significant decrease in serum (PSA). There was a significant increase in mean PSA doubling time from baseline of 15–54 months post-treatment. In a parallel in vitro study of patients' serum on LNCaP cell growth, there was a significant reduction in cell proliferation and induction of apoptosis after treatment with pomegranate juice [34].

2.2.2. Breast cancer

Pomegranate constituents have been proved to be antiproliferative, noninvasive [35], apoptotic [36] angiogenesis [37], and tumor growth inhibitors [38]. Pomegranate seed oil and fermented juice polyphenols exhibited antiangiogenesis potential by suppression of vascular endothelial growth factor in MCF-10A and MCF-7 and upregulated migration inhibitory factor (MIF) in MDA-MB-231 breast cancer cell lines [38].

2.2.3. Colon cancer

Pomegranate juice derived ellagitannins and their intestinal bacterial metabolites, urolithins, exhibited dose- and time-dependent decreases in cell proliferation, and clonogenic efficiency of HT-29 cells. The half maximal inhibitory concentration, IC₅₀ values, ranged from 56.7 μ M for urolithin A to 74.8 μ M for urolithin C [39].

2.2.4. Hepatocellular carcinoma

Oxidative stress is a precipitating factor of hepatocellular carcinoma (HCC), one of the most lethal cancers. Pomegranate emulsion (1 or 10 g/kg) was given 4 weeks before dietary carcinogen diethylnitrosamine (DENa)-induced rat hepatocarcinogenesis and 18 weeks thereafter. Pomegranate revealed chemopreventive activity manifested by reduced incidence, number, multiplicity, size, and volume of hepatic nodules. This effect was mediated by pomegranate antioxidant activity and inhibition of nuclear factor-kappaB (NF- κ B) (a potent stimulant of Wnt/ β catenin signaling which is involved in cell proliferation, cell survival, and apoptosis) [40, 41].

2.2.5. Bladder cancer

Transitional cell carcinoma results in most of the bladder tumors [42]. The tumor suppressor gene p53 which is essential for cell cycle arrest and apoptosis [43] was believed to be inactivated in more than 50% of carcinogenesis of bladder cancers [44]. Polyphenols in pomegranate rind extract was shown to inhibit bladder cancer cell EJ proliferation via p53/miR-34a axis [45].

2.3. Cardio vascular disorders

2.3.1. Preclinical studies

Pomegranate protected against cardiovascular injury initiated by cigarette smoking in rats through its antioxidative property [46]. Moreover, antioxidative and anti-inflammatory effects of pomegranate extract reduced the size of atherosclerotic plaques in the aortic sinus and reduced the proportion of coronary arteries with occlusive atherosclerotic plaques when it was given orally in a dose of 307.5 μ l/L of drinking water/day for 2 weeks to mice model of coronary heart disease [47]. Furthermore, pomegranate extract supplementation (625 mg/day) for 10 days to pigs prevented hyperlipemia-induced coronary endothelial dysfunction via a stimulation of the Akt/endothelial nitric oxide-synthase pathway [48].

2.3.2. Clinical studies

Natural pomegranate juice (150 ml/day) succeeded to significantly lower systolic and diastolic blood pressure 4–6 h post-consumption in 13 hypertensive patients [49]. Furthermore, a 1 year consumption of pomegranate juice by 10 atherosclerotic patients with carotid artery stenosis significantly reduced common carotid intima-media thickness (IMT), systolic blood pressure, and serum lipid peroxidation. Whereas after 3 years of pomegranate consumption, no additional beneficial effects occurred except for further reduction of serum lipid peroxidation by up to 16% [50].

2.4. Metabolic disorders

2.4.1. Preclinical studies

High level of low-density lipoprotein (LDL) is a risk factor for cardiovascular disease. The esterase paraoxonase1 (PON1) prevents oxidation of LDL. Decreased levels of PON1 increase the incidence of cardiovascular disease. Pomegranate juice (12.5 mL/L of juice in 1 l of water/day for 4 months) significantly induced PON1 gene expression and activity when given daily

to streptozotocin-induced diabetic mice fed with a high-fat diet. Furthermore, pomegranate reduced blood glucose level and body weight [51]. Metabolic syndrome includes common clinical disorders such as obesity, hypertension, dislipidemia, and diabetes. Pomegranate juice and fruit extract induced a significant decrease in vascular inflammation markers; thrombospondin (TSP), and cytokine TGF β 1 and increase in plasma nitrate, nitrite levels, and nitric oxide-synthase expression (important factors for arterial function enhancement) in a metabolic syndrome rat model [52]. Pomegranate extract (300 mg/kg/day for 8 weeks) reduced the levels of high-fat diet-induced elevated serum interleukin 6 (IL6) and corticosterone in rats [53]. Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver diseases in the world [54]. The pathogenesis of NAFLD includes the increased accumulation of triglyceride in hepatocytes, which progresses to nonalcoholic steatohepatitis (NASH) due to oxidative stress. In high-fat, high-sugar-diet-fed rats, pomegranate juice (60 \pm 5 ml/day for 7 weeks) exhibited a significant modulation in hepatic steatosis, ballooning, lobular and portal inflammation, as well as significant attenuation of hepatic pro-inflammatory and pro-fibrotic gene expression. It significantly decreased plasma levels of alanine, aspartate aminotransferase, insulin, triglycerides, and glucose with respect to control [55]. A study comparing the anti-diabetogenic effect of glibenclamide (5 mg/kg) and pomegranate juice (1 ml/day) was carried out on 40 streptozotocin (STZ)-nicotinamide (NAD)-induced type 2 diabetes mellitus rats for 21 days. Pomegranate juice (1 mL/day) showed significant repair and restoration signs in islets of Langerhans. Additionally, it significantly lowered the level of plasma total cholesterol, triglyceride, and inflammatory biomarkers, which were actively raised in diabetic rats [56].

2.4.2. Clinical studies

Concentrated pomegranate juice (50 g daily for 4 weeks) exerted a significant increase in total and high-density lipoprotein cholesterol from baseline levels in 40 type 2 diabetic patients. Only serum interleukin-6 (IL-6) was significantly reduced among other tested inflammatory markers. There was about 75% increase in mean value of serum total antioxidant capacity (TAC) [57]. In a double-blinded, randomized crossover controlled study, daily 500 mL of pomegranate juice was introduced to 30 individuals with a metabolic syndrome for a week. Systolic and diastolic blood pressure as well as high sensitivity C-reactive protein was significantly reduced. However, pomegranate consumption significantly increased the level of triglyceride and low-density lipoprotein cholesterol which is attributed by the authors to the more lipogenic effect of fructose than glucose after hepatic metabolism into triglycerides [58]. On the other hand, administration of 400 mg of pomegranate seed oil capsules twice daily for 4 weeks to 25 dyslipidemic patients insignificantly reduced serum of TNF- α level [59].

2.5. Infections

2.5.1. Bacterial and fungal infection

2.5.1.1. Preclinical studies

Antimicrobial activity of pomegranate has been widely investigated in many studies. *Escherichia coli* (*E. coli* O157:H7) is associated with many disorders: diarrhea, hemorrhagic colitis, thrombocytopenic purpura, and hemolytic uremic syndrome. Pomegranate ethanolic

extract was shown to be bacteriostatic and bacteriocidal against *E. coli* with minimal inhibitory concentration (MICs) from 0.49 to 1.95 mg/ml and minimal bactericidal concentration (MBCs) from 1.95 to 3.91 mg/ml [60]. Tuberculosis is an infectious disease with a time long emergence of drug resistance. Pomegranate *juice*, and *peel extracts* prepared with methanol/water, and its polyphenolic byproducts namely caffeic acid, ellagic acid, epigallocatechin-3-gallate (EGCG) and quercetin, were examined against drug-resistant clinical isolates of *Mycobacterium tuberculosis* and β -lactamase producing *Klebsiella pneumoniae*. The peel extracts exerted higher antimycobacterial activity (MIC 64–1024 $\mu\text{g}/\text{mL}$) than the juice (MIC 256 - > 1024 $\mu\text{g}/\text{mL}$). EGCG and quercetin showed more antitubercular and antibacterial activity than caffeic acid and ellagic acid [61]. Biofilm is a protective layer made of extracellular polymeric substances where the pathogen hides with subsequent modulation of its virulence and pathogenicity. Pomegranate methanolic extract was believed to counteract the formation of biofilms by *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *E. coli*, and *Candida albicans*. Moreover, pomegranate extract disrupted the preformed biofilms with inhibition of germ tube formation, a virulence trait, in *C. albicans*. Further studies revealed the ability of ellagic acid to inhibit the growth of all species in suspension at higher concentrations (>75 $\mu\text{g ml}^{-1}$) and biofilm formation at lower concentrations (<40 $\mu\text{g ml}^{-1}$) [62]. Besides single antifungal activity, pomegranate extract showed a synergistic effect with other antimicrobial agents. Punicalagin synergism with fluconazole against *C. albicans* and *C. parapsilosis* was demonstrated in an in vitro study with a twofold decrease of MIC for fluconazole [63]. Pomegranate methanolic extract showed synergistic effect with five antibiotics: chloramphenicol, gentamicin, ampicillin, tetracycline, and oxacillin against methicillin-resistant *S. aureus* strains (MRSA) and methicillin-sensitive *S. aureus* (MSSA). Most potent synergism was noticed when pomegranate was combined with ampicillin. This combination increased the post-antibiotic effect (PAE) of ampicillin from 3 to 7 h. as well as it reduced cell viability by 99.9 and 72.5% in MSSA and MRSA populations, respectively [64]. Methanol extract of pomegranate showed a synergistic action with ciprofloxacin against extended-spectrum β -lactamase (ESBL) producing *E. coli* and metallo- β -lactamase (MBL) producing *Pseudomonas aeruginosa*; that effect was attributed to bacterial efflux pump inhibitor (EPI) activity of the pomegranate polyphenolic constituents [65]. In an ongoing study of our work where we are testing the antifungal activity of some medicinal herbs against clinical isolates of *C. albicans* strain, pomegranate methanolic extract showed an inhibitory zone of 12 mm and synergistically augmented the action of fluconazole by increasing the inhibition zone from 25 to 35 mm after combination. The potent antifungal action of pomegranate led some researchers [66] to design a new antifungal peptide, pomegranin, with an N-terminal sequence from fresh pomegranate peels by ion exchange chromatography. Pomegranin suppressed mycelial growth in the fungi *Botrytis cinerea* and *Fusarium oxysporum* with half maximal inhibitory concentration (IC₅₀) of 2 and 6.1 μM , respectively.

2.5.2. Virus infection

2.5.2.1. Preclinical studies

Pomegranate showed antiviral action against many viruses: influenza, human immunodeficiency virus (HIV), herpes simplex virus (HSV), and adenoviruses in multiple studies. Of pomegranate polyphenol extract (PPE) constituents (ellagic acid, caffeic acid, luteolin, and

punicalagin), punicalagin had the highest affect against influenza A virus through suppression of viral RNA replication and agglutination of chicken RBCs. In addition, pomegranate polyphenol extract augmented the anti-influenza effect of oseltamivir when given together [67]. Pomegranate juice prevented HIV-1 binding to CD4 and blocked viral entry [68]. Moreover, agents present in pomegranate juice (polyphenols, beta-sitosterol, sugars, and ellagic acid) and fulvic acid were demonstrated as envelope virus neutralizing compounds that neutralize the viral infectivity by binding to the envelope lipid or sugar moieties [69]. Adenoviruses are a group of non-enveloped viruses that give rise to in a wide range of illnesses. Pomegranate peel ethanol extract exhibited anti-adenovirus activity on HeLa cell line where the half maximal inhibitory concentration (IC₅₀) and 50% cytotoxicity concentration (CC₅₀) of the extract were 165 ± 10.1 and 18.6 ± 6.7 $\mu\text{g/ml}$, respectively. The selectivity index (SI), the ratio of CC₅₀ and IC₅₀, was 8.89 [70]. Moreover, pomegranate tannins were shown to have anti-HSV-1, HSV-2 effect via blocking of virus adsorption to African green monkey kidney and human adenocarcinoma cells [71]. Hepatitis C virus (HCV) is the leading cause of end-stage liver disease. Ellagitannins from pomegranate peel crude extract, punicalagin, punicalin, and ellagic acid, specifically blocked the HCV NS3/4A protease activity in an in vitro study. Furthermore, punicalagin and punicalin significantly suppressed HCV replication in cell culture system. Moreover, these compounds were well tolerated ex vivo and “no-observed adverse effect level” (NOAEL) was established up to an acute dose of 5000 mg/kg in BALB/c mice. Additionally, these components were bio-available by pharmacokinetics study [72].

2.5.3. Parasitic infection

2.5.3.1. Preclinical studies

From ancient times, pomegranate was described as an antihelminthic agent. Malarial infection represents a public health and economic burden in tropical and subtropical regions of the world [73]. Pomegranate gallagic acid and punicalagin exerted an antiplasmodial activity against *Plasmodium falciparum* D6 and W2 clones with IC₅₀ values of 10.9, 10.6, 7.5, and 8.8 μM , respectively [74].

Schistosomiasis is a morbid widely distributed tropical disease [75]. Blood flukes of the genus *Schistosoma* pass a complex life cycle including multiple morphologically distinct phenotypes in definitive human and intermediate snail hosts [76]. In vitro and in vivo studies were designed to evaluate pomegranate impact on *Schistosoma mansoni* (*S. mansoni*), one of the three major species infecting humans. Pomegranate peels and leaves extracts significantly affected both adult *S. mansoni* worms and schistosomules with 100% death rate, after 24 h of exposure to plant extracts. Oral administration of the pomegranate extract to mice at a dose of 800 mg/kg, 45 days post-infection and on three consecutive days yielded a high percentage of dead adult worms (77.30 and 72.2) with either leaves or peels extract, respectively. In addition, reduction in tissue egg load, liver, and intestinal ova counts was observed. This antiparasitic effect was confirmed by electron microscopic examination that revealed ultrastructural alterations in the tegument and the male genital systems of the worms. Bone marrow examination of pomegranate-treated *S. mansoni*-infected mice showed eosinophilic degranulation that indicates reduced *S. mansoni* activity [77].

2.6. Central nervous system disorders

2.6.1. Cognitive disorders

2.6.1.1. Preclinical studies

Cognitive disorders affect learning, memory, perception, and problem-solving. These disorders include amnesia, dementia, and delirium. Pomegranate ellagic acid (30 and 100 mg/kg) ameliorated scopolamine- (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.)-induced amnesia in mice. Furthermore, chronic administration of ellagic acid (30 mg/kg) improved the memory deficit induced by diazepam (1 mg/kg) in rats [78]. Memory impairment, a feature of Alzheimer's disease (AD), is initiated by neuroinflammation and impairments in synaptic plasticity. These disorders are induced by the effect of extracellular amyloid-beta ($A\beta$) deposits called senile plaques. The generation of $A\beta$ is dependent on the proteolytic processing of amyloid precursor protein (APP) [79]. Pomegranate is believed to slow the rate of neurodegeneration in Alzheimer's disease. At a cellular level, pomegranate compound, punicalagin, was examined for its memory protective anti-inflammatory effect on lipopolysaccharide (LPS)-induced neuroinflammation in astrocytes and microglial BV-2 cells. In a dose of 1.5 mg/kg punicalagin attenuated LPS (250 μ g/kg daily 7 times) induced memory impairment and blocked the LPS-induced expression of inflammatory proteins via suppression of NF- κ B activation [80]. In addition, freeze-dried pomegranate (25–200 μ g/ml) in a dose-dependent manner reduced COX-2-dependent prostaglandin E2 (PGE2) production in SK-N-SH cells stimulated with IL-1 β [81]. The neuroprotective action of pomegranate was obscured in an animal study in which dietary supplementation of 4% pomegranate extract to APP^{sw}/Tg2576 mice for 15 months ameliorated the loss of synaptic structure proteins, inhibited neuroinflammatory activity, and enhanced autophagy (degradation and recycling of cellular components). Moreover, it reduced β -site cleavage of APP [82]. Along with figs and dates, pomegranate dietary intake attenuated the levels of inflammatory cytokines in APP^{sw}/Tg2576 mice a model of Alzheimer disease, as well as delayed the formation of senile plaques [83].

2.6.2. Ischemic stroke

2.6.2.1. Preclinical studies

Ischemic stroke is one of the neurodegenerative diseases. An in vitro study utilized serum glucose deprivation (SGD) as a model for ischemia-induced brain injury in PC12 cells. Pretreatment with different pomegranate extracts, namely, pulp hydroalcoholic extract (PHE), pulp aqueous extract (PAE), and pomegranate for 2 h significantly and concentration-dependently, increased cell viability and decreased DNA damage initiated by SGD insult [84].

2.6.3. Multiple sclerosis

2.6.3.1. Preclinical studies

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system and is associated with demyelination, neurodegeneration, and sensitivity to oxidative stress.

Pomegranate seed oil (PSO) in nanodroplet formulation induced more significant beneficial effects in the mice model of multiple sclerosis (MS) than natural pomegranate seed oil. This effect was evident by dramatic alleviation of lipid demyelination and oxidation in mice brains [85].

2.6.4. Neonatal hypoxic-ischemic brain injury

2.6.4.1. Preclinical studies

Neonatal hypoxic-ischemic (HI) brain injury is a fatal condition that affects preterm very low birth-weight infants. After administration to pregnant mice, pomegranate juice revealed antioxidant-driven neuroprotective effect in experimentally induced HI brain injured neonatal offsprings [86–87].

2.7. Miscellaneous disorders

2.7.1. Skin disorders

2.7.1.1. Preclinical studies

Prolonged human exposure to sun's ultraviolet (UV) radiation, especially its UV-B, causes many adverse effects. Pomegranate fruit extract was proved to be a photo-chemo preventive agent on human epidermal keratinocytes. It alleviated ultraviolet A and B radiation-induced cell damage in a dose- and time-dependent manner [88, 89].

2.7.1.2. Clinical studies

Oral ellagic acid-rich pomegranate extract either in high (200 mg/d ellagic acid) or low doses (100 mg/d ellagic acid) improved ultraviolet-induced skin pigmentation of 26 subjects in 4 weeks double-blind placebo-controlled trial [90].

2.7.2. Male infertility and erectile dysfunction

2.7.2.1. Preclinical studies

Pomegranate juice improved epididymal sperm concentration, spermatogenic cell density, diameter of seminiferous tubules, and sperm motility. It decreased the number of abnormal sperms compared to control rat animals. Moreover, pomegranate juice resulted in improvement of antioxidant enzyme activity in both rat plasma and sperm [91]. Pomegranate juice significantly increased intracavernous blood flow and smooth muscle relaxation in a rabbit model of arteriogenic erectile dysfunction [92].

2.7.2.2. Clinical studies

In a randomized, double-blind, placebo-controlled, 10-week crossover trial, pomegranate juice (1.5 mmol polyphenols daily) showed insignificant improvement when introduced to 53 men with mild-to-moderate erectile dysfunction [93].

2.7.3. Dental disorders

2.7.3.1. Preclinical studies

Bacterial and fungal co-infection initiates oral diseases. Pomegranate phytotherapeutic gel was shown to be superior to miconazole in attenuation of microbial adherence with three and four associated organisms: *Streptococci* strains (*mutans* ATCC 25175, *sanguis* ATCC 10577 and *mitis* ATCC 9811) and *C. albicans* [94]. Elagic acid exerted a moderate inhibitory effect at 12.5 mg/mL with inhibition to adherence <50% against different strains of *Streptococcus mutans* bacteria that induced dental caries [95].

2.7.3.2. Clinical studies

In a human study, pomegranate hydroalcoholic extract was superior to chlorhexidine (standard and positive control) in decreasing the colony forming unit (CFU)/ml by 84 and 79%, respectively, of dental plaque microorganisms [96]. Pomegranate along with *Centella asiatica* extracts significantly improved clinical signs of chronic periodontitis and IL-1beta level when it was applied with biodegradable chips on periodontal disease in 20 patients with remaining probing pocket depths after conventional periodontal therapy [97]. Pomegranate gel was compared to miconazol gel (3 times daily for 15 days) in 60 patients suffering from denture stomatitis. The patients were randomly distributed into two groups of 30 patients each. Clinical response was statistically better in miconazol group ($P < 0.01$) with similar fungal negativity in both groups. Clinical response and fungal negativity was achieved in 21 and 23 patients of pomegranate group as compared to 27 and 25 subjects who received miconazol, respectively. Side effects were only reported from all miconazol-treated patients. The authors explained the better miconazol clinical response by the bigger number of subjects with good oral hygiene score in the miconazol group and the longer duration of miconazol (sticky formulation) in the mouth than pomegranate gel that was washed away on mixing with saliva [98].

3. Pharmacokinetic studies

Pomegranate ellagitannins release ellagic acid in the gut, and this compound is poorly absorbed in the small intestine, while it is largely metabolized by human gut microflora into urolithins, such as urolithins A and B and urolithin-8-methyl ether in the large intestine [99]. Pomegranate anthocyanins (the 3-glucosides and 3, 5-diglucosides of delphinidin, cyanidin, and pelargonidin) are stable in the stomach. While in the neutral pH of the small and large intestines, anthocyanins become less stable and are converted into a variety of metabolites [100–102].

The maximum plasma concentration (C_{max}) of ellagic acid was 33 ng/mL and time of maximum concentration (T_{max}) was 1 h [103]. A pharmacokinetic study on 18 healthy volunteers proved the rapid absorption and plasma clearance of ellagitannins as well as long persistence

(48 h) of urinary excreted urolithin metabolites after 180 ml of pomegranate juice consumption. Prolonged stay of urolithins in the human body is responsible for the health benefits of chronic pomegranate consumption [104]. A 1 liter pomegranate juice containing 4.37 g/L punicalagins and 0.49 g/L anthocyanins was introduced to six healthy individuals for 5 days; urolithin A, urolithin B, and a third unidentified minor metabolite were detected in plasma as well as in urine analysis at 24 h besides an aglycone metabolite corresponding to each of three plasma metabolites. Maximum excretion rates occurred 3–4 days after juice ingestion. The concentrations of urinary metabolites varied significantly in the subjects which may be attributed to colonic microflora variability and the site of ellagitannins metabolism [105]. A crossover pharmacokinetic study reported that higher free ellagic acid EA intake does not enhance its bioavailability in healthy volunteers who consumed two pomegranate extracts of 130 mg punicalagin+524 mg ellagic acid or 279 mg punicalagin+25 mg ellagic acid. The study showed high inter-individual variability; C_{max} ranged from 12 to 360 nM that may be attributed to the ellagitannin pH and protein environment [106].

4. Safety

Pomegranate is safe when it is used in normal doses [107]. The median lethal dose, LD₅₀ of the whole fruit extract, was 731 mg/kg after intra-peritoneal administration to OF-1 mice [108]. Standardized pomegranate extract of 30% punicalagins showed acute oral LD₅₀ in wistar rats and in Swiss albino mice it was more than 5000 mg/kg. Subchronic no-observed adverse effect level (NOAEL) was 600 mg/kg body weight/day [109]. Pomegranate ellagitannin-enriched polyphenol extract in a daily dose of 1420 mg (870 mg of gallic acid equivalents,) for 28 days showed no adverse effects in 64 overweight subjects [110].

5. Conclusion

Pomegranate's uncountable beneficial pharmacological properties encourage more and more studies to discover other secrets for solving mankind health problems.

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