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Review Article

A Comprehensive Review of *Punica granatum* (Pomegranate) Properties in Toxicological, Pharmacological, Cellular and Molecular Biology Researches

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Abstract

Punica granatum (Pg), commonly known as pomegranate (Pg), is a member of the monogeneric family, Punicaceae, and is mainly found in Iran which is considered to be its primary centre of origin. Pg and its chemical components possess various pharmacological and toxicological properties including antioxidant, anti-inflammatory (by inhibiting pro-inflammatory cytokines), anti-cancer and anti-angiogenesis activities. They also show inhibitory effects on invasion/motility, cell cycle, apoptosis, and vital enzymes such as cyclooxygenase (COX), lipooxygenase (LOX), cytochrome P450 (CYP450), phospholipase A2 (PLA2), ornithine decarboxylase (ODC), carbonic anhydrase (CA), 17beta-hydroxysteroid dehydrogenase (17β-HSDs) and serine protease (SP). Furthermore, they can stimulate cell differentiation and possess anti-mutagenic effects. Pg can also interfere with several signaling pathways including PI3K/AKT, mTOR, PI3K, Bcl-X, Bax, Bad, MAPK, ERK1/2, P38, JNK, and caspase. However, the exact mechanisms for its pharmacological and toxicological properties remain to be unclear and need further evaluation. These properties strongly suggest a wide range use of Pg for clinical applications. This review will discuss the areas for which Pg has shown therapeutic properties in different mechanisms.

Keywords: *Punica granatum*; Chemical components; Toxicological properties; Signaling pathway; Clinical applications.

Introduction

Punica granatum (Pomegranate) is a small tree which measures between five and eight meters tall and mainly found in Iran, the Himalayas in northern India, China, USA and throughout the Mediterranean region (1). Pg is one of the important endemic plants of Iran, growing in most regions throughout the country, in arid and semiarid regions due to its ability to adapt to adverse ecological conditions. Over 764 cultivars of *Punica granatum* (Pg) have been collected during a germplasm collection and grown in the cities of Saveh and Yazd (Iran), all of which possess specific fruit characteristics including size, color, taste, time of ripening, and disease resistance (2). The Pg can be also divided into several anatomical compartments including seed, juice, peel, leaf, flower, bark, and root with each possessing interesting pharmacological and toxicological activities. The edible fruit is a berry which is about 5-12 cm in diameter with a rounded hexagonal shape, thick reddish skin and around 600 seeds, each surrounded by a water-laden pulp (aril) ranging in color from white to deep red or purple, the aril is the edible part of the fruit. The seeds are embedded in a white,

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spongy, astringent pulp (3). According to the holy Quran, pomegranates grow in the gardens of paradise and the Quran has recited the Pg twice as an example of god's good creations.

The fruit of the Pg has extensively been used as a traditional remedy against acidosis, dysentery, microbial infections, diarrhea, helminth infection, hemorrhage and respiratory pathologies (4). Pg seeds have also been shown to contain the estrogenic compounds, estrone and estradiol (4). Furthermore, the dried pericarp and the juice of the fruit are considered beneficial for treatment of colic, colitis, menorrhagia, oxyuriasis, headache, diuretic, acne, piles, allergic dermatitis, and treatment of oral diseases (5). Recent studies have shown new scientific investigations for the traditional uses of Pg (5).

Chemical contents

Pg contains chemical components in its different compartments, which may possess various pharmacological and toxicological activities (6). These components are summarized in Table 1 (6).

Antioxidant properties

Oxidative stress (OS) produces toxic metabolites (7) which can initiate and promote cancers (8, 9). Consumption of polyphenoles and flavonoids are beneficial for the prevention of cardiovascular, inflammatory, and other diseases by preventing OS that induces lipid peroxidation in arterial macrophages and in lipoproteins (10, 11). The presence of antioxidants has been reported in Pg juice (10). Pg contains some species of flavonoids and anthocyanidins (delphinidin, cyaniding and pelargonidin) in its seed oil and juice (6) and shows antioxidant activity three times greater than green tea extract (12). Pg fruit extracts exhibit scavenging activity against hydroxyl radicals (13) and superoxide anions, which could be related to anthocyanidins (6). The antioxidant action of Pg is observed, not only through its scavenging reactions, but also by its ability to form metal chelates (14). Studies have indicated that methanolic extracts from the peel of Pg has a broad spectrum of antioxidant activities which were evaluated by 1,1-diphenyl 2-picrylhydrazyl (DPPH) free radical scavenging, phosphomolybdenum, Ferric (Fe^{3+}) Reducing Antioxidant Power (FRAP), and Cupric (Cu^{2+}) Reducing Anti-oxidanrt Capacity (CUPRAC) assays (14, 15). Studies have looked at the beneficial effects of pomegranates antioxidant activity *in-vivo* and *in-vitro* and have shown that Pg juice consumption causes a decrease in procarcinogen activation through CYP activity/expression (CYP1A2 and CYP3A) (16), protection of rat gastric mucosa from ethanol or aspirin toxicity (17), protection of neonatal rat brain from hypoxia (18), reduction of hepatic OS (19), reversal of proatherogenic effects which are induced by perturbed shear stress (20), protective effects against UVA- and UVB-induced cell damage and the potential use of Pg polyphenolics in topical applications (21). Other studies have also shown the protective effects of Pg on the cardiovascular system, including reduction of LDL and cholesterol (22, 23), anti-hypertension action by combating OS induced by diabetes and angiotensin II (24), reduction of carotid arterial stenosis and increase of endothelial nitric oxide (NO) syntheses (25, 26); and suggest the Pg as part of a heart-healthy diet through inhibiting of OS mechanism (27).

Anti-inflammatory effect

Acute inflammation is a beneficial host response for prevention of tissue injury, but it may also cause immune-associated diseases such as rheumatoid arthritis, inflammatory bowel disease and cancers (28, 29). Interestingly, Pg, Pg has been shown to inhibit inflammation by different mechanisms.

Cyclooxygenase (COX) and lipooxygenase (LOX), which are key enzymes in the conversion of arachidonic acid to prostaglandins and leukotrienes (important inflammatory mediators), respectively, are inhibited by Pg (30, 31). Non-steroidal anti-inflammatory drugs (NSAIDs) have more adverse effects on cardiovascular function by inhibiting COX and suppressing PGI2 (prostacyclin) in comparison to Pg (32). Ahmed et al. have shown that Pg has a significant inhibitory effect on osteoarthritis (OA) by suppressing the expression of matrix metalloproteinases (MMPs) in OA chondrocyte cultures and preventing collagen degradation. It may also inhibit joint destruction in OA patients (33). Pro-inflammatory cytokines

such as IL-1β play an important role in OA pathogenesis (34). IL-1β induces the expression of MMPs, especially MMP-1 and MMP-13, which are associated to the irreversible breakdown of cartilage matrix through digestion of type-II collagen and the consequent release of matrix proteoglycan from the cartilage (34). Furthermore, Pg has shown anti-inflammatory effects in a colitis rat model (35). However, studies have shown the inhibitory effect of Pg on production of pro-inflammatory cytokines (34-36). These studies demonstrate that Pg inhibits the p38-mitogen-activated protein kinase (p38-MAPK) pathway and transcription factor, NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells). Activation of p38-MAPK and NF-kB are associated with increased gene expression of TNF-α, IL-1β, MCP1, iNOS, and COX-2 agents which are critical mediators of inflammation (36). Studies have also shown that administration of 50 mg/kg of Pg extract for 28 days causes a decrease in malondialdehyde (MDA), TNF-α, and IL-1β levels in rats with liver fibroses (37). Also, Shukla *et al*. showed that pretreatment with 13.6 mg/kg of Pg extract decreased the arthritis incidence and lowered IL-6 and IL-1β levels in animal model of rheumatoid arthritis (38).

Carcinogenesis

Pg possesses inhibitory effects on different type of cancers such as prostate (39,40), breast (41), colon (42,43), and lung cancers (44). Different mechanisms have been outlined for pomegranates anti-cancer activities in these studies. Pg inhibits NF-kBand cell viability of prostate cancer cell lines in a dose-dependent manner in the LAPC4 xenograft model, *in-vitro* (45). Pg polyphenols, ellagitannin-rich extract and whole juice extract inhibited gene expression of HSD3B2 (3beta-hydroxysteroid dehydrogenase type 2), AKR1C3 (aldo-ketoreductase family 1 member C3) and SRD5A1 (steroid 5alpha reductase type 1), which are key androgen-synthesizing enzymes in LNCaP, LNCaP-AR, and DU-145 human prostate cancer cells (46). Because Pg inhibits CYP activity/ expression which is necessary for activation of procarcinogens, it may have anti-carcinogenesis effects (16). Some metabolites of pomegranates chemical components such as 3,8-dihydroxy-6H-dibenzo[b, d]pyran-6-one (urolithin A, UA) which is produced from Ellagitannins (ETs) may also possess anti-cancer effects (46). Treatment with (50-150 µg/mL) pomegranate fruit extract (PFE) for 72 h was found to result in a significant inhibition of lung cancer, with dose-dependent arrest of cells in G_0/G_1 phase of the cell cycle, induction of WAF1/p21 and KIP1/p27, decrease in the protein expressions of cyclins D1, D2, and E, decrease in cyclin-dependent kinase (cdk) 2, cdk4 and cdk6 expression, phosphorylation of MAPK proteins, inhibition of PI3K, phosphorylation of Akt at Thr308, NF-kB and IKK (inhibitor of kappa kinase) alpha, degradation and phosphorylation of IκB, Ki-67 and PCNA (44). Also, the levels of Bax and Bcl-2 were altered by PE in PC-3 cell line (47).

Angiogenesis

Angiogenesis is an important process for the development of new blood vessels, which is essential in supplying oxygen and nutrition for tumor growth and progression of cancers (48). Therefore, angiogenesis is a possible target for cancer prevention strategy (49, 50). Fibrocytes are important in angiogenesis, as they lay the requisite intracellular infrastructure of blood vessels (51), secrete extracellular matrix degrading enzymes such as MMP-9 which stimulate endothelial cell invasion, and secrete pro-angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibrocyte growth factor (bFGF), and interleukins (IL) (52). Interestingly, recent study has shown the ability of Pg to inhibit angiogenesis (53). Toi*et et al.* evaluated the anti-angiogenic potential of Pg by measuring vascular endothelial growth factor (VEGF), IL-4, and migration inhibitory factor (MIF) in the conditioned media of estrogen sensitive (MCF-7) or estrogen resistant (MDA-MB-231) human breast cancer cells, and immortalized normal human breast epithelial cells (MCF-10A). VEGF was strongly decreased in MCF-10A and MCF-7, however, MIF was increased in MDA-MB-231, showing significant potential for inhibitory effects of angiogenesis by Pg fractions on human umbilical vein endothelial cells (HUVEC) (53).

Invasion and motility

Understanding the mechanisms of tumor

cell invasion and metastasis could prove to be important for preventing tumor cell spread. MMP-1, MMP-2, and MMP-9, are a family of zinc-dependant endoproteinases which are most closely linked with metastasis of cancer cells (54-57). Activity of MMPs are regulated on certain levels including transcriptional control, proenzyme activation, and inhibition of activated MMPs by non specific inhibitors such as α2-macroglobulin (58), and specific endogenous inhibitors such as tissue inhibitors of metalloproteinase (TIMP) (58). TIMPs bind to the active site of MMPs and block access of MMPs to their substrates (58). Caffeic acid phenethyl ester (CAPE), a compound of Pg which is also derived from honey bee propolis has shown dose-dependent decreases in MMP and TIMP-2 mRNA levels in HT1080 human fibrosarcoma cells, as detected by reverse transcriptase-polymerase chain reaction (RT-PCR). Gelatin zymography analysis confirmed this when compared to controls (57). This study shows the role of CAPE as a potent anti-metastatic agent, which can significantly inhibit the metastatic and invasive capacity of malignant cells (57). Pg has shown dose-dependent inhibition effect on NF-kB-dependent reporter gene expression which is associated to proliferation, invasion, and motility in aggressive breast cancer phenotypes. This effect is behind to decrease RhoC and RhoA protein expression, suggests a role for these extracts in lowering the metastatic potential of aggressive breast cancer species (60). Four pure chemicals, ellagic acid, caffeic acid, luteolin, and punicic acid, obtain from the Pg fruit were presented as potential inhibitors of *in-vitro* invasion of human PC-3 prostate cancer cells in an assay employing Matrigel artificial membranes (61).

Cell cycle arrest

The cell cycle is a series of events which takes place in a cell, leading to its division and duplication. It consists of four distinct phases; G_1 phase, S phase (synthesis), G_2 phase (collectively known as interphase) and M phase (mitosis). Multiple checkpoints have been identified to verify whether the processes at each phase of the cell cycle have been accurately completed before progression into the next phase. Cell cycle may be altered following exposure to Pg. Previous studies have suggested several mechanisms for these effects, such as modulation of cell signaling molecules in the cell cycle machinery. *Punica granatum extrac*t (PE) inhibited the proliferation of mouse mammary cancer cell line (WA4), derived from mouse MMTV-Wnt-1 mammary tumors in a time and concentration-dependent manner through an arrest of cell cycle progression in the G_0/G_1 phase (62). Ellagitannins, derived from Pg juice, and their metabolites, urolithins exhibit dose and time-dependent decreases in cell proliferation and clonogenic efficiency of HT-29 cells through cell cycle arrest in the G_0/G_1 and G_2/M stages of the cell cycle followed by induction of apoptosis (43). Moreover, Pg treatment induced a dose-dependent arrest in the G_0/G_1 phase of the cell cycle which was assessed by DNA cell cycle analysis in the lung cancer cell line (A549) (63). Pg pretreatment of normal human epidermal keratinocytes (NHEK) has been found to increase the cell cycle arrest induced by UVA in the G_1 phase of the cell cycle (64). Furthermore, Androgen-independent cell line, DU 145 has shown a significant increase from 11% to 22% in G_2/M cells ($p < 0.05$) by treatment with (35 μg/mL) Pg cold-pressed oil (65). Ellagic acid is a phenolic compound, which may belong to Pg, and induces cell cycle arrest and apoptosis in T24 human bladder cancer cells *in-vitro* through induced G_0/G_1 phase arrest, increased p53 and p21 and decreased cyclin-dependent kinase (Cdk2) gene expression (65). Cdks as the mainly one Cdk4 is a key key molecule in the regulation of cell cycle progression at the G_1-S phase restriction point is inhibited by p16 (INK4a), a tumor suppressor. It has been reported that the N-terminal of different truncated p16 (INK4a) molecules is not crucial for the interaction with Cdk4 (66). However, Ostad *et al*. have previously shown that the C-terminal domain of p16 (INK4a) is adequate in inducing cell cycle arrest, growth inhibition, and CDK4/6 interaction (68).

Apoptosis

Apoptosis is the process of programmed cell death, which is a useful marker for predicting tumor response after anti-cancer treatment. Pg causes apoptosis by different mechanisms. PE

has been found to induce apoptosis by increasing caspase-3 activity in a mouse mammary cancer cell line (WA4) (62). In addition, Pg extracts and punic acid, an omega-5 long chain poly unsaturated fatty acid derived from Pg, have been shown to induce apoptosis in both an estrogen in sensitive breast cancer cell line (MDA-MB-231) and an estrogen sensitive cell line developed from MDA-MB-231 cells (MDA-ERalpha7) through lipid peroxidation and the PKC (Protein kinase C) signaling pathway (69). They also cause disruption to the cellular mitochondrial membrane (69). The relationship between pg-induced apoptosis in human prostate cancer cells (LAPC4) and the IGF/IGFBP system have been investigated (70). POMx (a highly potent Pg extract prepared from skin and arils, minus the seeds) and IGFBP-3 have been shown to synergistically stimulate apoptosis. Inhibition of cell growth resulted in increased JNK phosphorylation and decreased Akt and mTOR activation (70). Pg treatment of normal human epidermal keratinocytes (NHEK) inhibited UVB-mediated activation of MAPK and NF-kB pathways, as well as other signal transducers and activators of the apoptosis pathway including transcription 3 (STAT3), PKB/AKT, ERK1/2, mTOR, PI3K, Bcl-X(L) (antiapoptotic protein), Bax and Bad (proapoptotic proteins) (64). The role of MAPK signaling pathways and effects of PI3K/AKT, ERK1/2, P38, and JNK on epidermal growth factor (EGF) signaling in proliferation of human mesenchymal stem cells (hMSCs) have been shown *in-vitro* (71). The cell growth is controlled by the interaction of survival and cell growth arrest pathways and the activity of survival pathways such as Akt and ERK1/2 with regard to XIAP (inhibitor of apoptosis) in serum starvation has been investigated and the survival role for ERK in serum starvation has been reported (72). Recently, the Pg inhibition of cell growth, followed by apoptosis of highly aggressive human prostate carcinoma PC3 cells through modulations in the cyclin kinase inhibitor-cyclin-dependent kinase machinery have been shown by Malik *et al.* These events were associated to alterations in the levels of Bax and Bcl-2, shifting the Bax: Bcl-2 ratio in favor of apoptosis (47). Ellagitannins (ETs) and hydrolysable tannins, which are found in Pg and their hydrolyzed product, as well as ellagic acid (EA), have been reported to induce apoptosis in human colon cancer Caco-2 cells through down-regulation of cyclins A and B_1, upregulation of cyclin E, cell-cycle arrest in the S phase, induction of apoptosis via intrinsic pathways (FAS-independent, caspase-8 independent) through bcl-XL down-regulation with mitochondrial release of cytochrome c into the cytosol as well as activation of initiator caspase-9 and effector caspase-3 (73). Induction of Bax and Bak (proapoptotic), down-regulation of Bcl-X(L) and Bcl-2 (anti-apoptotic), induction of WAF1/p21 and KIP1/p27, a decrease in cyclins D1, D2, and E, and a decrease in cdk2, cdk4, and cdk6 expression have been shown to occur in prostate cancer PC3 cells, following Pg treatment (74).

Punica granatum effects on vital enzymes

Enzymes are proteins that catalyze biochemical/chemical reactions. Pg has been shown to inhibit different enzymes including phospholipase A2 (PLA2) (that catalytically hydrolyzes the bond releasing arachidonic acid and lysophospholipids) (75), cyclooxygenase (COX), lipooxygenase (LOX) (30), cytochrome P450 (76) and ornithine decarboxylase (ODC) (77) which plays a role in the urea cycle and catalyzes the decarboxylation of ornithine to polyamines such as putrescine. Polyamines regulate growth processes and stimulate the growth of cancer (78). Carbonic anhydrase (CA) that catalyzes the hydration of carbon dioxide to form bicarbonate (HCO_3^-) is also inhibited (79). CA inhibitors such as Pg have been shown to inhibit cancer cell growth *in-vitro* and *in-vivo* (80). Aromatase is enzyme responsible for a key step in the biosynthesis of estrogens and catalyzes the formation of estrone and estradiol, which is inhibited by Pg (81). One of the possible mechanisms in which Pg can inhibit breast cancer is its inhibitory effect on aromatase and 17 beta-hydroxysteroid dehydrogenase enzymes (17β-HSDs), as well as its anti-estrogenic activity (41). Furthermore, ellagitannins (ET) and urolithin B (UB), which are found in relatively high quantities in Pg, have been shown to most effectively inhibit aromatase activity in a live cell assay (82). Serine protease (SP) is another enzyme which

is inhibited by Pg. SP is enzymes in which one of the amino acids in the active site is serine. Protease plays an essential role in modulating the turnover of extracellular matrix (EC), which provides morphological support for cell growth and differentiation (83). Furthermore, protease has a verity of important functions including angiogenesis, vasculogenesis, apoptosis, and cell migration/invasion (84). Ellagic acid and punicalagin, from Pg, have shown lower inhibitory effects on alpha-secretase (TACE) and other serine proteases such as chymotrypsin, trypsin, and elastase, thus indicating that they are relatively specific inhibitors of beta-secretase (BACE1) (85). Other studies have shown that catechin and epicatechin (epigallocatechin-3-gallate) (86-88), which are present in Pg (89), can inhibit SP.

Cellular differentiation

Cellular differentiation, in developmental biology, is the process by which a less specialized cell becomes a more specialized cell type. Study has shown that Pg stimulates the differentiation of osteoblastic MC3T3-E1 cells and affects the function of these cells (4). Pg seed oil (but not aqueous extracts of fermented juice, peel or seed cake) has been shown to stimulate keratinocyte proliferation in monolayer culture, without effecting fibroblast function, and as a result facilitates skin repair and promotes regeneration of dermis and epidermis (56). flavonoid-rich fractions from fermented Pg juice and aqueous extraction of Pg pericarps are strong promoters of differentiation in human HL-60 promyelocytic leukemia cells, which are detected by nitro blue tetrazolium reducing activity, non specific esterase activity, specific esterase activity, and phagocytic activity, whereas flavonoid-rich fractions from fresh Pg juice only show a relatively mild differentiation-promoting effect. Furthermore, the effect of Pg on differentiation has been observed in breast and prostate cell lines (90).

Anti-mutagenicity

A mutagen is a physical orchemical agent that alters the genetic material of an organism, usually DNA, permanently and thus increases the frequency of mutations above the natural background level Mutagenicity is the capacity of a chemical or physical agent to cause such permanent change. It has been shown that Pg peel fractions, especially methanol, has anti-mutagenic activities as was detected by the Ames Salmonella/microsome assay against sodium azide (NaN3), methyl methane sulphonate (MMS), 2-aminofluorene (2-AF), and benzo(a)pyrene (B(a)P) induced mutagenicity in Salmonella typhimurium (TA97a, TA98, TA100 and TA102) tester strains (15). Methanolic extract of Pg (15 mg/plate) shows the highest anti-mutagenic activity in TA 100 cells (91).

Clinical application

Considering the mentioned properties, Pg has the potential to be used in many clinical applications. Studies have shown that Pg inhibits prostate cancer cell growth, induces apoptosis in PC-3 cells (highly aggressive prostate carcinoma cells), suppresses invasion of PC-3 cells and decreases proliferation of DU-145 prostate cancer cells *in-vitro* (52). Treatment of HT-29 colon cancer cells has been indicated by Pg juice through decreasing COX-2 expression and inhibiting inflammatory cell signaling processes which may cause cancer initiation and progression (92). Furthermore, COX-2 is involved in the proliferative response of human periodontal fibroblast (HPLF) cells to Emdogain (EMD) (93). There has been a correlation between inducible nitric oxide (iNOS) synthase and COX-2 expression in human colorectal adenocarcinoma. In fact, a possible link between advanced stages of this disease and higher expression of iNOS and COX-2 has been shown by Habibollahi *et al.* (94).

Pg has been shown to inhibit breast cancer cell lines MCF-7 and MB-MDA-231 by hindering angiogenesis, tumor growth, invasiveness, proliferation, and induction of apoptosis (53, 95-97). An inhibitory effect of Pg has been shown on lung and skin cancer models (44, 98). Moreover, Wongwattanasathien *et al.* have shown the inhibitory effect of Pg on HL-60 human leukemia cells through inhibition of proliferation and differentiation of these cell lines (91). Pg has been shown to have anti-atherosclerotic effects through protection of

endothelial function, destructive effects on reactive oxygen species such as NO by its antioxidant properties (99), increase MPM uptake of oxidized LDL, and decrease of lipid peroxidation and cholesterol levels (100). Furthermore, Pg has been shown to have anti-hyperlipidemia activity through activation of peroxisome proliferators activated receptor (PPAR-α), which can decrease cardiac uptake and circulating lipids (101). Furthermore, Pg decreases cholesterol levels by decreasing absorption and increasing fecal excretion of cholesterol, as well as effecting cholesterol metabolism through HMG-CoA reductase and sterol O-acyltransfrase (102). It has also been demonstrated that Pg has anti-hypertensive effects, with inhibited serum angiotensin converting enzyme (ACE) and decreased systolic blood pressure being observed in hypertensive patients (103). Reduced myocardial ischemia and improved myocardial perfusion were also caused by Pg (104). In an animal model of diabetes, Pg resulted in lower serum C-peptides, a pro-insulin metabolite marker for endogenously secreted insulin, by 23 percent compared to baseline levels in diabetic patients (105). Pg has also shown effectiveness in controlling oral inflammation (106) as well as bacterial (107) and fungal counts in periodontal diseases (108), and Candida associated denture stomatitis (109). Pg has been found to have antibacterial properties against oral bacteria (106, 109), including methicilin-resistance *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA) (110). Kasai *et al*. have also shown the protective properties of Pg against UV-induced damage and skin pigmentation, compared to the placebo (111). Azadzoi *et al.* found anti-erectile dysfunction properties for Pg, measured by intracavernous blood flow and penile erection in a rabbit model of arteriogenic erectile dysfunction (112). Therefore, it could be used in male infertility to improve epidermal sperm concentration, sperm motility, decrease the number of abnormal sperms, and increase spermatogenic cell density (113). Pg hasneuroprotective properties in neonatal hypoxia-ischemic brain injury (18, 114) and preventive effects on Alzheimer's disease, as shown in animal models (improved learning of water maze tasks) (115). Anti-obesity effects (116) have also been described for Pg. COX-2 has been involved in spatial memory retention and may impair the memory by injecting it intra-hippocampal, in rats (117). The inhibitory effect of Pg on COX-2 could be valuable in inhibiting memory impairment.

Conclusion

In this review, we gathered all the published studies on Pg without date elimination. However attempts were made to explain the new data. Iran is considered to be the primary origin of Pg. Pg juice, fruit, and extracts have been used extensively in the folk medicine of ancient cultures for various medicinal properties (118). Pg has been shown to possess phytochemicals which may hold pharmacological and toxicological properties (6). Nevertheless, the exact effects and involved mechanisms for the pharmacological and toxicological effects of many of these chemicals remain to be cleared. Nowadays, the use of herbal products or medicinal plants, because of their safety and efficiency in the prevention and/or treatment of several chronic diseases, are being extensively investigated worldwide.

The information presented in this review article which was obtained from *in-vitro*, *in-vivo* and clinical trial investigations has shown some of the pharmacological and toxicological mechanisms and properties of PE. These properties include anti-oxidative, anti-inflammatory, anticancer, anti-angiogenesis, and inhibitory effect on invasion/motility, cell cycle arrest, apoptosis, stimulation of cell differentiation and anti-mutagenic effects, and inhibitory effects on vital enzymes such as COX, LOX, CYP450, PLA_2, ODC, CA, 17β-HSDs and SP. Existence of these pharmacological and toxicological mechanisms and properties and interference of several signaling pathways including PI3K/AKT, mTOR, PI3K, Bcl-X, Bax, Bad, MAPK, ERK1/2, P38, JNK, and caspase relation to Pg, suggest that Pg can be extensively used as a possible therapy for prevention and treatment of several types of diseases including prostate cancer, colon

Table 1. Phytochemicals present in *Punina granatum* (pomegranate).

Pomegranate Phytochemicals	Formula	Molecular weight (MW)	Plant Part
Ellagitannins and Gallotannins			
2,3-(S)-HHDP-D-glucose[a]	$C_{20}H_{18}O_{14}$	482.35	Bark, peel
Castalagin	$C_{41}H_{26}O_{26}$	934.63	Bark
Casuariin	$C_{34}H_{24}O_{22}$	784.54	Bark
Casuarinin		936.65	Bark, pericarp
Corilagin	$C_{27}H_{22}O_{18}$	634.45	Fruit, leaves, pericarp
Cyclic 2,4:3,6-bis(4,4′,5,5′,6,6′-hexahydroxy [1,1′-biphenyl]- 2,2′-dicarboxylate) 1-(3,4,5-trihydroxybenzoate) b-D-Glucose	$C_{41}H_{28}O_{26}$	936.65	leaves
Granatin A	$C_{34}H_{24}O_{23}$	800.54	Pericarp
Granatin B	$C_{34}H_{28}O_{27}$	952.64	Peel
Pedunculagin	$C_{34}H_{24}O_{22}$	784.52	Bark, pericarp
Punicacortein A	$C_{27}H_{22}O_{18}$	634.45	Bark
Punicacortein B	$C_{27}H_{22}O_{18}$	634.45	Bark
Punicafolin	$C_{41}H_{30}O_{26}$	938.66	Leaves
Punigluconin	$C_{34}H_{26}O_{23}$	802.56	Bark
Strictinin	$C_{27}H_{22}O_{18}$	634.45	Leaves
Tellimagrandin I	$C_{34}H_{26}O_{22}$	786.56	Leaves, pericarp
Tercatain	$C_{34}H_{26}O_{22}$	786.56	Leaves
2-O-galloyl-4,6(S,S) gallagoyl-D-glucose	$C_{41}H_{26}O_{26}$	934.63	Bark
5-O-galloyl-punicacortein D	$C_{54}H_{34}O_{34}$	1222.8	Leaves
Punicacortein C	$C_{47}H_{26}O_{30}$	1070.7	Bark
Punicacortein D	$C_{47}H_{26}O_{30}$	1070.7	Bark, heartwood
Punicalin	$C_{34}H_{22}O_{22}$	782.53	Bark, pericarp
Punicalagin	$C_{48}H_{28}O_{30}$	1084.7	Bark, pericarp, peel
Terminalin/gallayldilacton	$C_{28}H_{20}O_{16}$	602.37	Pericarp
Ellagic Acid Derivatives			
Ellagic acid	$C_{14}H_{6}O_{8}$	302.19	Fruit, pericarp, bark
Ellagic acid, 3,3′-di-O-methyl	$C_{16}H_{10}O_{8}$	330.25	Seed
Ellagic acid, 3,3′, 4′-tri-O-methyl	$C_{17}H_{12}O_{8}$	344.27	Seed
Ellagic acid, 3′-O-methyl-3, 4-methylene	$C_{16}H_{8}O_{8}$	328.23	Heartwood
Eschweilenol C	$C_{20}H_{16}O_{12}$	448.33	Heartwood
Diellagic acid rhamnosyl(1-4) glucoside	$C_{40}H_{30}O_{24}$	894.65	Heartwood
Catechin and Procyanidins			
(-)-Catechin	$C_{15}H_{14}O_{6}$	290.27	Juice
Catechin-(4,8)-gallocatechin	$C_{30}H_{26}O_{13}$	594.52	Peel
Gallocatechin	$C_{15}H_{14}O_{7}$	306.27	Peel
Gallocatechin-(4,8)-catechin	$C_{30}H_{26}O_{13}$	594.52	Peel
Gallocatechin-(4,8)-gallocatechin	$C_{30}H_{26}O_{14}$	610.52	Peel
Procyanidin B1	$C_{30}H_{26}O_{12}$	578.52	Juice
Procyanidin B2	$C_{30}H_{26}O_{12}$	578.52	Juice
Anthocyanins and Anthocyanidins			
Cyanidin	$C_{15}H_{11}O_{6}$	287.24	Peel

Table 1. (Continued)

Pomegranate Phytochemicals	Formula	Molecular weight (MW)	Plant Part
Cyanidin-3-glucoside	$C_{21}H_{21}O_{11}$	449.38	Juice
Cyanidin-3,5-diglucoside	$C_{27}H_{31}O_{16}$	611.52	Juice
(Continued)			
Cyanidin-3-rutinoside	$C_{27}H_{31}O_{15}$	595.53	Juice
Delphinidin	$C_{15}H_{11}O_{7}$	303.24	Juice
Delphinidin-3-glucoside	$C_{2}1H_{21}O_{12}$	465.38	Juice
Delphinidin 3, 5-diglucoside	$C_{27}H_{31}O_{17}$	627.52	Juice
Pelargonidin 3-glucoside	$C_{21}H_{21}O_{10}$	433.38	Juice
Pelargonidin 3,5-diglucoside	$C_{27}H_{31}O_{15}$	595.53	Juice
Flavonols			
Apigenin-4′-O-*β*-D-glucoside	$C_{21}H_{20}O_{11}$	448.32	Leaves
Kaempferol	$C_{15}H_{10}O_{6}$	286.24	Peel, fruit
Luteolin	$C_{15}H_{10}O_{6}$	286.24	Peel, fruit
Luteolin-3′-O-*β*-D-glucoside	$C_{21}H_{20}O_{10}$	432.11	Leaves
Luteolin-4′-O-*β*-D-glucoside	$C_{21}H_{20}O_{10}$	432.11	Leaves
Luteolin-3′-O-*β*-D-Xyloside	$C_{21}H_{18}O_{10}$	418.09	Leaves
Myricetin	$C_{15}H_{10}O_{8}$	318.04	Fruit
Quercetin	$C_{15}H_{10}O_{7}$	302.04	Peel, fruit
Quercimeritrin	$C_{21}H_{20}O_{12}$	464.38	Fruit
Quercetin-3-O-rutinoside	$C_{27}H_{30}O_{16}$	610.52	Fruit
Quercetin-3,4′-dimethyl ether 7-O-*α*-L-arabinofuranosyl-(1-6)-*β*-D-glucoside	$C_{28}H_{32}O_{16}$	624.54	Bark, peel
Eriodictyol-7-O-*α*-Larabinofuranosyl (1-6)-*β*-D-glucoside	$C_{26}H_{30}O_{15}$	582.51	Leaves
Naringenin 4′-methylether 7-O-*α*-L-arabinofuranosyl (1-6)-*β*-D-glucoside	$C_{27}H_{32}O_{14}$	580.53	Leaves
Organic Acids			
Caffeic acid	$C_{9}H_{8}O_{4}$	180.16	Juice
Chlorogenic acid	$C_{16}H_{18}O_{9}$	345.31	Juice
Cinnamic acid	$C_{9}H_{8}O_{2}$	148.16	Juice
Citric acid	$C_{6}H_{8}O_{7}$	192.12	Juice
o-Coumaric acid	$C_{9}H_{8}O_{3}$	164.16	Juice
p-Coumaric acid	$C_{9}H_{8}O_{3}$	164.16	Juice
Ferulic acid	$C_{10}H_{10}O_{4}$	194.18	Juice
Gallic acid	$C_{7}H_{6}O_{5}$	170.12	Juice
L-Malic acid	$C_{4}H_{6}O_{5}$	134.09	Juice
Oxalic acid	$C_{2}H_{2}O_{4}$	90.03	Juice
Protocatechuic acid	$C_{7}H_{6}O_{4}$	154.12	Juice
Quinic acid	$C_{7}H_{12}O_{6}$	192.17	Juice
Succinic acid	$C_{4}H_{6}O_{4}$	118.09	Juice
Organic Acids			
Caffeic acid	$C_{9}H8O_{4}$	180.16	Juice

Table 1. (Continued)

Pomegranate Phytochemicals	Formula	Molecular weight (MW)	Plant Part
Chlorogenic acid	$C_{16}H_{18}O_9$	345.31	Juice
Cinnamic acid	$C_9H_8O_2$	148.16	Juice
Citric acid	$C_6H_8O_7$	192.12	Juice
o-Coumaric acid	$C_9H_8O_3$	164.16	Juice
p-Coumaric acid	$C_9H_8O_3$	164.16	Juice
Ferulic acid	$C_{10}H_{10}O_4$	194.18	Juice
Gallic acid	$C_7H_6O_5$	170.12	Juice
L-Malic acid	$C_4H_6O_5$	134.09	Juice
Oxalic acid	$C_2H_2O_4$	90.03	Juice
Protocatechuic acid	$C_7H_6O_4$	154.12	Juice
(Continued)			
Quinic acid	$C_7H_{12}O_6$	192.17	Juice
Succinic acid	$C_4H_6O_4$	118.09	Juice
Tartaric acid	$C_4H_6O_6$	150.09	Juice
Simple Gallyol Derivatives			
Brevifolin	$C_{12}H_8O_6$	248.19	Leaves
Brevifolin carboxylic acid	$C_{13}H_8O_8$	292.2	Leaves
Brevifolin carboxylic acid-10-monosulphate	$C_{13}H_7KO_{10}S$	394.25	Leaves
1,2,3-Tri-O-galloyl-*β*-D-glucose	$C_{27}H_{24}O_{18}$	448.32	Leaves
1,2,4-Tri-O-galloyl-*β*-D-glucose	$C_{27}H24O18$	286.24	Leaves
1,2,6-Tri-O-galloyl-*β*-D-glucose	$C_{27}H_{24}O_{18}$	286.24	Leaves
1,4,6-Tri-O-galloyl-*β*-D-glucose	$C_{27}H_{24}O_{18}$	432.11	Leaves
1,3,4-Tri-O-galloyl-*β*-D-glucose	$C_{27}H_{24}O_{18}$	432.11	Leaves
1,2, 4, 6-Tetra-O-galloyl-*β*-D-glucose	$C_{34}H_{28}O_{22}$	418.09	Leaves
1,2,3,4, 6-Pent-O-galloyl-*β*-D-glucose	$C_{41}H_{32}O_{26}$	318.04	Leaves
Methyl gallate	$C_8H_8O_5$	302.04	Heratwood
3,4,8,9,10-pentahydroxy-dibenzo[b,d]pyran-6-one	$C_{13}H_8O_7$	464.38	Leaves
Fatty Acids and Triglycerides			
Eicosenoic acid	$C_{20}H_{40}O_2$	312.53	Seed oil
Linoleic acid	$C_{18}H_{32}O_2$	280.45	Seed oil
Linolenic acid	$C_{18}H_{30}O2$	278.43	Seed oil
Oleic acid	$C_{18}H_{34}O2$	282.46	Seed oil
Palmitic acid	$C_{16}H_{32}O_2$	256.42	Seed oil
Punicic acid	$C_{18}H_{30}O_2$	278.43	Seed oil
Stearic acid	$C_{18}H_{36}O_2$	284.48	Seed oil
Tri-O-punicylglycerol	$C_{57}H_{92}O_6$	873.34	Seeds
Di-O-punicyl-O-octadeca-8Z-11Z-13E-trienylglycerol	$C_{57}H_{92}O_6$	873.34	Seeds
1-O-trans, cis, trans, octadecatrienol glycerol	$C_{21}H_{36}O_4$	352.51	Seed oil
1-O-isopentyl-3-O-octadec-2-enoyl glycerol	$C_{26}H_{50}O_4$	426.67	Seed oil
Sterols and Terpenoids			

Table 1. (Continued)

Pomegranate Phytochemicals	Formula	Molecular weight (MW)	Plant Part
Asiatic acid	$C_{30}H4_8O_5$	488.7	Juice
Betulinic acid	$C_{30}H_{48}O_3$	456.70	Seed
Cholesterol	$C_{27}H_{46}O$	386.65	Seed oil
Daucosterol	$C_{35}H_{60}O_6$	576.85	Seed
Estrone	$C_{18}H_{22}O_2$	270.37	Seed oil
Estradiol	$C_{18}H_{24}O_2$	272.38	Seed oil
Estriol	$C_{18}H_{24}O_3$	288.38	Seed oil
Friedooleanan-3-one	$C_{30}H_{50}O$	426.72	Bark
β-Sitosterol	$C_{29}H_{50}O$	414.71	Seed oil, leaves, stem
Stigmasterol	$C_{29}H_{48}O$	412.69	Seed oil
Testosterone	$C_{19}H_{28}O_2$	288.42	Seed oil
Ursolic acid	$C_{30}H_{48}O_3$	456.70	Seed
Alkaloids			
Hygrine	$C_8H_{15}NO$	141.21	Root bark
(Continued)			
Norhygrine	$C_7H_{13}NO$	127.18	Root bark
Pelletierine	$C_8H_{15}NO$	141.21	Bark
N-methyl pelletierine	$C_9H_{17}NO$	155.24	Bark
Sedridine	C8H17NO	143.23	Bark
Pseudopelletierine	$C_9H_{15}NO$	153.22	Bark
Nor-pseudopelletierine	$C_8H_{13}NO$	139.19	Bark
2,3,4,5-tetrahydro-6-propenyl-pyridine	$C_8H_{13}N$	123.20	Bark
3,4,5,6-tetrahydro-a-methyl-2-pyridine ethanol	$C_8H_{15}NO$	141.21	Bark
1-(2,5-dyihydroxy-phenyl)-pyridium chloride	$C_{11}H_{10}ClNO_2$	223.66	Leaves
Other Compounds			
Coniferyl 9-O-[*β*-D-apiofuranosyl-(1-6)]-O-*β*-D-glucopyranoside	$C_{21}H_{30}O_{12}$	474.46	Seed
Pseudopelletierine	$C_9H_{15}NO$	153.22	Bark
Nor-pseudopelletierine	$C_8H_{13}NO$	139.19	Bark
2,3,4,5-tetrahydro-6-propenyl-pyridine	C_8H13N	123.20	Bark
3,4,5,6-tetrahydro-a-methyl-2-pyridine ethanol	$C_8H_{15}NO$	141.21	Bark
1-(2,5-dyihydroxy-phenyl)-pyridium chloride	$C_{11}H_{10}ClNO_2$	223.66	Leaves
Other Compounds			
Coniferyl 9-O-[*β*-D-apiofuranosyl-(1-6)]-O-*β*-D-glucopyranoside	$C_{21}H_{30}O_{12}$	474.46	Seed
Sinapyl 9-O-[*β*-D-apiofuranosyl-(1-6)]-O-*β*-D-glucopyranoside	$C_{18}H_{36}O_2$	284.48	Seed
Phenylethylrutinoside	$C_{57}H_{92}O_6$	873.34	Seeds
Icariside D1	$C_{57}H_{92}O_6$	873.34	Seeds
Mannitol	$C_{21}H_{36}O_4$	352.51	Bark

a HHDP =hexahydroxydiphenoyl.

cancer, breast cancer, lung cancer, skin cancer, leukemia, anti-atherosclerosis, hyperlipidemia, hypertension, myocardial ischemia, myocardial perfusion, diabetes, oral inflammation, infection, anti-erectile dysfunction, male infertility, neonatal hypoxia-ischemic brain injury, alzheimer and obesity.

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