Pomegranate bioactive constituents target multiple oncogenic and oncosuppressive signaling for cancer prevention and intervention

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Abstract

Cancer remains to be the second highest cause of mortality in our society, falling just short of heart disease. Despite major advancement in cancer therapy over the past decade, momentum has been gaining for an alternative approach of using naturally-occurring and dietary agents for cancer prevention and management. Research on pomegranate (*Punica granatum* L.), a fruit of the Punicaceae family, has shown enormous potential for cancer prevention and intervention. In addition to a rich source of polyphenols, including flavonoids and ellagitannins, in its juice, pomegranate also houses hundreds of other phytochemicals in its pericarp, seed, flower, bark, flowers and leaves, These phytochemicals provide powerful antiproliferative, anti-inflammatory, antioxidant, anti-invasive, antimigratory, anti-angiogenic and anti-metastatic effects without significant toxicity. This makes the use of its various extracts a very attractive strategy to our current battle against cancer. This review article presents a systematic, comprehensive and critical review of research on pomegranate-derived products in both cancer prevention and intervention. It discusses the chemical constituents of pomegranate, the results of both preclinical (*in vitro*, *ex vivo* and *in vivo*) and clinical studies on the anticancer effect of pomegranate phytochemicals and molecular targets in numerous types of cancers, such as breast, gastrointestinal tract (oral, colon, liver and pancreas), gynecological (uterine and ovarian), hematological (lymphoma, leukemia and myeloma), lung, neurological (glioma), urogenital (bladder and prostate), bioavailability, pharmacokinetics and safety of pomegranate constituents. In order to guide the direction of future research, we have also included current limitations and challenges in the field and our post analysis recommendation.

Key words: Pomegranate, *Punica granatum*, extracts, phytochemicals, cancer, prevention, therapy, molecular targets

1. Introduction

Pomegranate (*Punica granatum*, L.), a fruit-bearing deciduous large shrub or small tree, is a native of the Himalayas in northern India to Iran [1]. It has been cultivated in various areas of the Middle East, southeast Asia, European Mediterranean region and northern Africa [2]. However, due to its popularity of consumption, the fruit and its juice, it is now cultivated in various regions of the world, including Afghanistan, China, Greece, India, Iran, Italy, Mexico, Morocco, Russia, Spain, Uzbekistan and the United States [3,4]. Globally, Iran is one of the largest pomegranate-producing countries [5]. In the United States, the primary production sites are the drier regions of Arizona and California [1,2].

The pomegranate shrub is a member of the Punicaceae family which is composed of two species, namely *P. granatum* and *P. protopunica*, of which only *P. granatum* is edible. There are more than 1,000 cultivars of *P. granatum* are known to exist [6]. The shrub grows from 5 to 10 m in height, generating an assortment of leaves, flowers, and fruits amongst thorny branches. Pomegranate fruit (classified as a large berry) ranges in color from light red to a green-yellow or purple. The fruit can be 5-20 cm in diameter, about 200-800 g in weight, grenade-shaped and crowned by the pointed calyx. It contains approximately 200-1,400 white, red or purple arils embedded into a spongy, white membrane enveloped by exocarp. The fruit essentially contains three parts: the seeds (~3% of fruit weight) containing around 20% oil, the juice (~30% of fruit weight) and the peels (pericarp) that include the interior network of membranes [4]. The leaves are glossy, opposite or subopposite, narrow oblong, entire, 3–7 cm long and ~2 cm wide. The flowers are mostly bright red (rarely white or yellow), odorless, and about 3 cm in diameter with three to seven petals.

The pomegranate fruit finds its use for medicinal purposes in ancient cultures for centuries. It prominently features in various major world religions, such as Buddhism, Christianity, Islam, Judaism, and Zoroastrianism [4]. It represents a symbol of life, longevity, health, femininity, fecundity, knowledge, morality, immortality and spirituality [7]. In the Ancient Greek myth of Persephone's abduction, the pomegranate, known as the "fruit of the dead," signifies life and rebirth. This mythology contributed to the inspiration for utilizing pomegranate in the coat of arms of various medical associations, including British Medical Association, Royal College of Midwives, Royal College of Obstetricians and Gynaecologists and Royal College of Physicians [8]. Zoroastrians believe the fruit represents immortality, whereas in China it represents a time-honored symbol of fertility and prosperity [8].

The pomegranate's medicinal value has been recognized in various indigenous and traditional systems of medicine to treat a variety of ailments. The applications of pomegranate are expansive and have notable uses in both Ayruvedic and the Unani systems of medicine. Ayurveda is India's primary form of medicinal system in which pomegranate is regarded as "a pharmacy unto itself" [1]. The bark and roots are believed to possess antihelmintic and vermifuge properties [9], whereas the seeds are known to facilitate urination and prevent urinary disorders [10]. Dried pomegranate peel, bark, leaves and flowers have been used in Indian subcontinent to treat inflammation, diarrhea, nose bleeding, sore throat, hoarseness, periodontitis and ulcers [11,12]. In Unani (a system of medicine practiced in the Middle East and India) and traditional Chinese medicine, pomegranate has been considered a remedy for diabetes [13,14]. In India, Guatemala and Tunisia, water decoction of dried pomegranate peels has been used as astringent and germicide as well as to treat diarrhea, aphthae and ulcers [15-17].

Pomegranate fruits are widely consumed in fresh and beverage forms as juice as well as jam, jelly and wine [18]. Additionally, pomegranate leaves are brewed as tea and dried seeds are used as spice [19]. During the last decade, pomegranate fruit (termed as "super fruit") has been gaining a tremendous reputation as a nutraceutical source as well as functional food for putative health benefits [20] ascribed to the exceptional antioxidant activity of the fruit and juice as compared to other fruits and antioxidant beverages [21-24]. Based on numerous preclinical and clinical studies, pomegranate juice, extracts, and phytoconstituents have been found to be useful for the prevention and/or treatment of cardiovascular disease, hypertension, diabetes, nonalcoholic fatty liver disease, obesity, inflammatory disorders, ulcer, arthritis, microbial infection, dental ailments, acquired immune deficiency syndrome, neurological disorders, erectile dysfunction, male infertility and ultra-violet radiation-induced skin damage [1,4,5,25-33].

The abundance of phytochemicals and numerous biological and medicinal properties coupled with its use in traditional medicine [34] have attracted cancer researchers all over the world to explore the anticancer potential of pomegranate. This fact combined with the prevalence of cancer and the numerous severe side effects of traditional first line treatments make the precedent for investigation of dietary prevention and efficacious natural therapies a prudent approach. There has been a plethora of publications that have focused on anticancer role of pomegranate extracts and phytoconstituents [4,35-41]. However, a large number of articles focus on pomegranate's impact on cancer in an organ-specific system, such as breast cancer [42,43], colon cancer [44,45] and prostate cancer [46,47], rather than comparing its efficacy against cancers of multiple organ systems. Additionally, many studies focused on only a major pomegrarate phytochemical group, e.g., polyphenols [37, 48]. Moreover, there has been limited discussion of the molecular targets of

the various phytochemicals within pomegranate and how that knowledge can be applied to chemopreventive setting.

The purpose of this article is to perform a systematic, comprehensive and critical review of the anticancer and cancer preventive effects of pomegranate phytochemicals with an emphasis on their various molecular targets. The goal is to understand the precise role of pomegranate phytoconstituents in both cancer prevention and intervention. The article analyzes available preclinical *in vitro* and *in vivo* studies as well as clinical reports of the chemopreventive and therapeutic applications of pomegranate in numerous types of cancer. Current limitations, challenges and future directions of research are also discussed.

2. Chemical constituents of pomegranate

The chemical composition of pomegranate is very diverse as different parts of the plant, namely, leaves, root, flowers, seed, peel and arils, contain a wide array of phytochemicals, such as phenols, anthocyanins, tannins, carbohydrates, sterols, fatty acids, vitamins and minerals [4,48]. Approximately half of the total fruit weight corresponds to the peel (also known as pericarp), which contains several important compounds, such as phenolics, flavonoids, proanthocyanidin compounds and ellagitannins [4,28]. The phenolic acids present in pomegranate peel are reprented by caffeic acid, *p*-coumaric acid, chlorogenic acid, gallic acid and ellagic acid (Fig. 1) [50,51]. The peel also contains hydrolyzable tannins or ellagitannins, such as corilagin, granatin A and B, tellimagrandin, pedunculagin, punicalagin, and punicalin (Fig. 1). Punicalagin is unique to pomegranate and it is found in the seeds, peel, leaves and juice [28]. The peel also contains various flavonols, such as kaempferol, quercetin and myricetin as well as flavan-3-ols, such as catechin, epicatechin and epigallactocatechin 3-gallate [52,53]. The pomegranate fruit's edible parts, arils,

contain around 85% water and about 11% total sugars, 1.4% pectin, organic acids, such as citric acid, malic acid and ascorbic acid constitute a small percentage of the composition [48]. Pomegranate juice is a rich source for ellagic acid and its derivatives [54]. Apart from these, arils also contain anthocyanins (sugar derivatives of delphinidin, cyanidin and pelargonidin), namely delphinidin-3-glucoside, delphinidin-3,5-diglucoside, cyanidin-3-glucoside, cyanidin-3,5-diglucoside, pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside [55] (Fig. 2).

Pomegranate seeds carry a high concentration of total lipids, but lack in polyphenol content [56]. Qualitative analysis carried out by two different studies has confirmed the presence of punicic acid (Fig. 3) in seeds [57,58]. Other fatty acids, such as linoleic acid, oleic acid, stearic acid and palmitic acid, were also detected in appreciable amounts ranging from 7 to 3% [58]. Additionally, minor components of oil include triglycerides and glycolipids, such as cerebroside and coumestrol [58]. Pomegranate seeds also contain ellagic acid derivatives, e.g., 3,3′-di-*O*-methyl ellagic acid and 3,3,4′-tri-*O*-methyl ellagic acid, and triterpenoids, e.g., asiatic acid and betulinic acid (Fig. 3). Sterols, namely stigmasterol, sitosterol, cholesterol and sex steroids, such as estrone, testosterone and estriol, were also detected in seeds.

Pomegranate leaves contain various ellagitannins, such as punicalin, punicalagin, punicafolin and corilagin [59]. Other phytochemicals, such as apigenin, strictinin, tercatain and number of other flavone glycosides, namely apigenin-4′-O-β-D-glucopyranoside, luteonin-4′-O-β-D-glucopyranoside, luteonin-3′-O-β-D-glucopyranoside and luteonin-3′-O-β-D-xylopyranoside, have been reported [60] (Fig 4). Pomegranate root is known to contain different alkaloids, namely pelletierine, N-methylpelletierene and pseudopelletierene) and piperidine [61,62]. Alkaloids, such as sedridine, 2-(2′-hydroxypropyl)-Δ^1 piperidine, 2-(2'-propenyl)-Δ^1 piperideine, hygrine and norhygrine, have been reported in the bark [61] (Fig. 5). Additionally,

ellagitannins, including punicalin, punicalagin, punicacortein A, punicacortein B, punicacortein C and punicacortein D, have been reported reported in the bark [59]. A number of phytochemicals were also reported in the flowers of pomegranate. Several phytochemicals belong to hydroxybenzoic acids class, such as gallic acid [63]. Triterpenoids, including urosolic acid, oleanolic acid, masilinic acid and asiatic acid, were also reported as pomegranate flower constituents [64] (Fig. 6).

3. Pomegranate in cancer prevention and therapy

3.1. Literature search methodology

For this work, we have followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) criteria [65], which is recommended for reporting systematic reviews. Relevant articles were first identified via various databases, such as PubMed, ScienceDirect, Scopus, Web of Science, Springerlink, and Google Scholar, from their inception date through March 2020. There was no restriction on year of publication. Articles were included if the following criteria were satisfied: (i) publications in peer-reviewd scientific journals; (ii) English language artiles only; (iii) origical research articles; (iv) *in vitro*, *in vivo* and clinical studies that utilized pomegranate-derived constituents. We excluded items if the following criteria were met: (i) conference abstracts, books, book chapters, and unpublished results; (ii) non-English articles; (iii) reviews, systematic reviews, meta-analysis and letters; (iv) publications that did not involve tumor cell lines or animal tumor models. These exclusion criteria were applied to the article pool after review of article title and abstract to remove unqualified articles. Questionable articles were either included or excluded after consensus discussion among all authors. The authors then proceeded to obtain full-length articles using various methods followed by a review of

reported studies to summarize important study findings. The overview of literature search and selection process is provided in Fig. 7.

3.2. Preclinical studies

3.2.1. Breast cancer

3.2.1.1. In vitro

Pomegranate-derived products have been extensively used for research on breast cancer. Kim et al. [66] conducted a study in which estrogen receptor (ER)-positive (MCF-7) and ER-negative (MB-MDA-231) breast cancer cells were exposed to fermented pomegranate juice polyphenols, fresh pomegranate juice polyphenols and pericarp polyphenols (derived from aqueous extract). It was shown that the fermented juice polyphenols displayed a superior antiproliferative effect to fresh juice polyphenols. Pomegranate seed oil registered a 90% inhibition of cell proliferation and a 75% of cell invasion in MCF-7 cell line as well as a 54% apoptosis in MDA-MB-435 (ER-negative metastatic human breast cancer) cells (Table 1). A follow-up study by Toi et al. [67] showed that the fermented juice polyphenols and seed oil downregulated vascular endothelial growth factor (VEGF), a proangiogenic factor, in MCF-7 cells, where the seed oil upregulated migration-inhibitory factor (MIF) in MDA-MB-231 cells.

Louis Jeune et al. [68] reported that pomegranate extract alone or in combination with genistein (an isoflavone present in soybeans) showed significant concentration- and time-dependent cytotoxic and growth-inhibitory effects in MCF-7 cells through induction of apoptosis. An aqueous pomegranate fruit extract inhibited the proliferation of MDA-MB-231 and SUM 149 cells through induction of apoptosis and suppression of constitutive activation of nuclear factor-κB (NF-κB). The aqueous extract also inhibited NF-κB-dependent reported gene expression associated with

proliferation, invasion and motility with simultaneous decrease in Ras homolog family member A (RhoA) and RhoC protein expression, indicating the ability of the extract in lowering the metastatic potential of aggressive breast cancer phenotypes [69]. Later, a pomegranate juice or a combination of its components (luteolin, ellagic acid and punicic acid) was found to interfere with multiple processes involved in metastasis of breast cancer cells, such as cell growth, adhesion, migration, chemotaxis and epithelial-to-mesenchymal transition (EMT) [70]. An aqueous extract of whole pomegranate fruit inhibited mammosphere formation in two different cell lines, namely neoplastic mammary epithelial HMLER and breast cancer Hs578T. Incubation of mammospheres with the extract reversed them into adherent cultures, indicating promotion of cancer stem cell differentiation. The extract also reduced cell migration, a hallmark of EMT phenotype, and downregulated genes involved in EMT [71]. In MDA-MB-231 cells, a methanolic extract of pomegranate peel resulted in cell death and markedly inhibited cell migration, accompanied by upregulation of the expression of intracellular cell adhesion molecule 1 (ICAM-1), a protein essential for cell adhesion.The methanolic extract also downregulated the expression of matrix metalloproteinase-9 (MMP-9), fibronectin and VEGF, proteins with crucial roles in cancer cell migration [72]. A follow-up study confirmed proapoptotic, antimigratory and anti-invasive effects and identified additional targets, such as vimentin, ZEB1, β-catenin, and E-cadherin, underscoring the antimetastatic potential of pomegranate peel extract [73].

Various ellagitannin-derived compounds, including urolithin B, isolated from pomegranate have been found to exert antiproliferative and antiaromatase activity in ER-positive aromatase-overexpressing MCF-7aro cells. This demonstrates the potential of pomegranate ellagitannin-derived compounds for the prevention of ER-responsive breast cancer [74]. Several conjugated fatty acids, such as α-eleostearic acid, γ-linoleic acid and punicic acid, isolated from pomegranate

seed oil inhibited the proliferation of MCF-7 and MDA-MB-231 cells through modulation of ER activity [75]. A methanolic extract of pomegranate pericarp inhibited the binding of [^{3}H]-estradiol (E_2) to ER and suppressed the growth and proliferation of ER-positive MCF-7 cells. The extract was also found to bind with ER and downregulate the transcription of estrogen-responsive reporter gene transfected into breast cancer cells with concomitant downregulation of estrogen-responsive genes, such as ER-α, progesterone receptor (PR) and pS2 [76]. Later, the same group of researchers found that the extract inhibited the MCF-7 cell proliferation induced by 27-hydroxycholesterol (27HC), an endogenous selective estrogen receptor modulator (SERM) that can act through ER-mediated mechanisms. The extract also downregulated the expression of genes, ER-α, PR, pS2, induced by 27HC [77].

Joseph et al. [78] isolated galactomannan polysaccharide (PSP001) from the fruit rind of pomegranate which showed *in vitro* cytotoxic effect against Ehrlich ascites carcinoma (EAC), a transplantable mouse carcinoma of possible mammary origin. A standardized extract of pomegranate (Pomella), containing ellagitannins (gallic acid, punicalagin α and punicalagin β), inhibited the proliferation and viability of MMTV-Wnt-1 mouse mammary cancer stem cells, possibly by arresting cell cycle progression in the G_0/G_1 phase and increasing caspase-3 activity in a time- and concentration-dependent manner [79]. Pomegranate juice-derived ellaginatinns, such as gallic acid, ellagic acid, hexahydroxydiphenic acid, punicallins and punicalagins, exhibited antiproliferative activities against BT-549 breast ductal carcinoma cells [80]. Banerjee et al. [81] reported that a pomegranate fruit extract enhanced the action of tamoxifen, a SERM, in both sensitive and tamoxifen-resistant MCF-7 cells through the inhibition of cell viability by inducing cell-death machinery. A methanolic pomegranate fruit peel extract, containing ellagic acid as the most abundant phenolic acid, exhibited antiproliferative effects against MCF-7 cells through

antioxidant and proapoptotic mechanisms [82]. Later, the antiproliferative effects of methanolic peel extract in MCF-7 cells was confirmed by another group [83]. Similary, acetone and methanolic extracts from whole pomegranate peels were reported to possess antiproliferative activities against MCF-7 and MDA-MB-231 cells [84]. A polyphenol-rich extract of pomegranate peel decreased the growth of MCF-7 and doxorubicin-resistant MCF-7 cells (MCF-7/DX) through antioxidant and proapoptotic mechanisms [85]. Polysaccharide (PSP001) isolated from the fruit rind has also been shown to have antitumor activity in MCF-7 breast cancer cells [86]. Punicalagin isolated from pomegranate husk exhibited strong antiproliferative activity against MCF-7 cells and this effect could possibly be due to the ability of the pomegranate constituent to inhibit oxidative DNA adducts [87]. A standardized extract of pomegranate fruit skins (POMX), containing 95% glycone ellagitannins and free ellagic acid, significantly inhibited MCF-7 cell growth in a concentration- and time-dependent manner by inducing cell cycle arrest in G_2/M phase followed by the induction of apoptosis. POMX downregulated genes associated with mitosis, chromosome organization, RNA processing, DNA replication, and DNA repair and upregulated genes involved in regulation of cell proliferation and apoptosis. Furthermore, POMX-mediated downregulation of homologous recombination (HR) genes correlated with increased levels of their predicted miRNAs, such as miRNA-24 (predicted target BRCA1) and miRNA-183 (predicted target RAD50), suggesting that the pomegranate extract may regulate miRNAs involved in DNA repair machinery [88]. A polyphenolic extract prepared from a pomegranate juice concentrate, containing punicalagin A, punicalagin B, delphinidin-3-glucoside, and cynidin-3-glucoside, inhibited the growth of MDA-MB-231 and BT-474 cells, but not non-cancerous MCF-10F and MCF-12F cells. The extract also decreased the expression of specificity protein 1 (sp1), sp3 and sp4 and sp-regulated genes as well as miR-27a and elevated the expression of ZBTB10, a transcriptional

repressor, in BT-474 cells. Additionally, the extract induced the expression of SH-2 containing inositol 5'-phosphatase 1 (SHIP-1) which was accompanied by downregulation of miRNA-155 and inhibition of phosphoinositide 3-kinase (PI3K)-dependent phosphorylation of Akt [89].

In recent years, several investigators studied antibreast cancer effects of pomegranate seeds. Contantini and colleagues [90] characterized conjugated linolenic acids, e.g., punicic acid, as the major components of a hydrophilic fraction (80% aqueous methanol extract) from pomegranate seed oil. In MCF-7 and MDA-MB-231 cells, the hydrophilic seed extract caused a significant decrease in cell viability, increase in the number of cells at G_0/G_1 phase and suppression of VEGF and nine proinflammatory cytokines. An ethanolic or methanolic extract of pomegranate seeds caused inhibition of proliferation of MCF-7 cells though the underlying mechanisms of action were not clearly understood [91,92].

There has been at least one study that evaluated the effects of pomegranate flower extracts on proliferation of breast cancer cells. Ethanolic extracts of flowers of seven pomegranate varieties were investigated against MCF-7 cells. The results indicated cytotoxic activities of extracts of two varieties (GR and ZH), possibly due to antioxidant and anti-inflammatory (inhibition of 5-lipoxygenase, 5-LOX) properties [93].

Several investigators evaluated the efficacy of novel formulations of pomegranate phytoconstituents against breast cancer *in vitro*. Shirode and colleagues [94] prepared formulations of poly(D,L-lactic-*co*-glycolic acid)-poly(ethylene glycol) (PLGA-PEG) nanoparticles (NPs) loaded with pomegranate extract or individual polyphenols, such as punicalagin and ellagic acid. In MCF-7 and Hs578T cells, the pomegranate nanoformulations had a 2- to 12-fold enhanced effects on cell growth inhibition compared to their free counterparts, and the extract-containing nanoprototype was found to be the most potent. Monodispersed silver-NPs (Ag-NPs) of

pomegranate extract developed by green synthesis was found to inhibit the proliferation of MCF-7 cells by reducing DNA synthesis and promoting apoptosis-inducing cell cycle changes [95]. Similarly, platinum NPs (Pt-NPs) biosynthesized using pomegranate crusts produced a superior antiproliferative effect when compared to pomegranate peel in MCF-7 cells by inducing apoptosis through cell cycle arrest at G_0/G_1 phase [96]. In order to maximize the chemotherapeutic efficacy of pomegranate extract, it was incorporated in an optimized solid lipid NPs (SL-NPs) formulation. The pomegranate extract-loaded NPs exhibited better cytotoxic profile in MCF-7 cell line compared to that of the free extract [97].

3.2.1.2. Ex vivo and in vivo

Utilizing the murine mammary gland organ culture model, Kim and colleagues [66] found that the fermented juice polyphenols displayed a 47% inhibition of cancerous lesions induced by mammary carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) (Table 2). Using the same mouse mammary organ culture model, Mehta and Lansky [98] observed that a purified fraction derived from pomegranate fermented juice polyphenols by high-performance chromatography and pomegranate seed oil elicited a 87% reduction in the number of DMBA-induced mammary lesions compared to control. This indicaties the breast cancer preventive potential of pomegranate juice and seed oil.

Banerjee et al. [89] conducted the first *in vivo* study to evaluate antitumorigenic effect of pomegranate in breast cancer. Oral administration of a pomegranate juice extract (0.8 mg gallic acid equivalents/kg/day) for 35 days reduced tumor volume and size in athymic nude mice bearing BT474 tumor cells as xenografts (Table 2). The tumor tissues from the extract-treated animals exhibited higher level of apoptosis, decreased expression of Sp1, Sp3, Sp4, VEGF, survivin, NF-

κB p65, pAkt and pPI3K, and increased expression of ZBTB10 and SHIP-1 compared to control tumors. Additionally, the expression of miRNA-27a and miRNA-155 were significantly reduced in tumors in the extract-treated animals. Pomegranate fruit rind-derived galactomannan polysaccharide (PSP001) alone or in combination with doxorubicin produced a significant reduction in the tumor burden and increased the life span of mice containing EAC ascites or EAC solid tumors compared to corresponding control animals [78]. Bishayee and colleagues [99] evaluated for the first time *in vivo* chemopreventive potential of a novel pomegranate emulsion (PE) that consists of most bioactive constituents present in the whole fruit using the classical preclinical mammary carcinoma model utilizing female Sprague-Dawley rats and DMBA. The animals were orally administered (p.o.) with PE (0.2, 1 or 5.0 g/kg), starting 2 weeks before and 16 weeks following DMBA exposure. PE caused a striking reduction of DMBA-induced mammary tumor incidence and tumor burden accompanied by reversal of histopathological changes in tumor tissue. PE also dose-dependently suppressed intratumor cell proliferation, induced apoptosis, increased Bax expression, decreased Bcl-2 and manifested a proapoptotic shift in Bax/Bcl-2 ratio. The accompanying gene expression study revealed that PE upregulated the expression of BAD, caspase-3 (CASP3), caspase-7 (CASP7), caspase-9 (CASP9), poly (ADP ribose) polymerase (PARP) and cytochrome c (CYT. C) in mammary tumors. In a follow-up study, it has been found that PE downregulated the expression of ER-α and ER-β and lowered the ratio of ER-α to ER-β. PE also decreased the expression, cytoplasmic accumulation and nuclear translocation of β-catenin and suppressed the expression of cell growth regulatory protein cyclin D1, which represents a downstream target of both ER and Wnt/β-catenin signaling [100]. Finally, PE decreased the expression of cyclooxygenase-2 (COX-2) and heat shock protein 90 (HSP90), prevented the degradation of inhibitory κBα (IκBα), hindered the translocation of NF-κB from

cytosol to nucleus and elevated the expression and nuclear translocation of nuclear factor erythroid 2p45 (NF-E2)-related factor (Nrf2) in tumor tissues [101]. In contrast to the aforementioned findings, Lepionka et al. [102] recently reported that oral feeding of pomegranate seed oil increased breast cancer incidence and accelerated tumor growth in female Sprague-Dawley rats subjected to DMBA mammary carcinogenesis, and these results could be due to the influence of seed oil constituents on DMBA metabolism.

3.2.2. Gastrointestinal tract and associated cancers

3.2.2.1. Oral cancer

3.2.2.1.1. *In vitro*

Seeram et al. [103] demonstrated that pomegranate juice (PJ), total pomegranate tannin (TPT) extract, and punicalagin isolated from pomegranate husk have cytotoxic effects on *in vitro* human oral cancer cells (KB and CAL27). A follow-up study confirmed similar cytotoxic effects of pomegranate juice-derived ellagitannins in KB cells [80]. Similarly, Weisburg et al. [104] found that ellagitannin-enriched pomegranate polyphenol extract exerted cytotoxic effect on various oral cancer cells (CAL27, HSC-2 and SCC 1483) via activation of caspase-3 activity, cleavage of PARP, and reduction of glutathione (GSH) levels.

3.2.2.2. Colon cancer

3.2.2.2.1. In vitro

Pomegranate juice (PJ), total pomegranate tannin (TPT) extract, and punicalagin isolated from pomegranate husk have cytotoxic effects against a panel of colon cancer cells (HT-29, HCT116, SW480 and SW620). In HT-29 and HCT116 cell lines, the cytotoxic effects were accompanied by apoptosis [103]. Kasimetty et al. [80] reported that pomegranate juice (PJ)-derived ellagitannins

induced cell cycle arrest at G_0/G_1 and G_2/M stages, and also inhibited proliferation and clonogenic efficiency of HT-29 tumor cells via activation of caspase-3 activity. Banerjee et al. [105] demonstrated that polyphenolic extract from pomegranate juice exerted cytotoxic, anti-inflammatory and antiangiogenesis effects on HT-29 cells via upregulation of caspase-3, PARP cleavage, downregulation of COX-2, vascular cell adhesion molecule 1 (VCAM-1), VEGF, and NF-κB p65, reduction in phosphorylation of phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) and increase in the expression of miRNA-126. Recently, Chen et al. [106] showed that both pomegranate peel-derived ellagitannins alone and ellagitannins in combination with 5-fluorouracil (5-FU) significantly inhibited HT-29 cell proliferation and induced S-phase cell cycle arrest. Concomitant mechanistic studies showed induction of intrinsic apoptosis via dissipation of mitochondrial membrane potential, increased Bax to Bcl-2 ratio, and cleavage of caspase-3 and caspase-9. Galactomannan polysaccharide (PSP001) extracted from pomegranate peel exerted cytotoxic effect on HCT116 cells via apoptosis induction [78]. Subsequent study by the same group [107] observed similar cytotoxic, proapoptotic and additional antimigratory effects on HCT116 cells with PSP001 alone or silver nanoparticles (SNPs) containing PSP001 (SNP@PSP). Larrosa et al. [108] used another *in vitro* colon cancer model (Caco-2) and found that punicalagin extracted from pomegranate peel as well as ellagic acid induced cytotoxicity and S-phase cell cycle arrest, possibly via downregulation of cyclin A and cyclin B1, upregulation of cyclin E, induction of apoptosis, downregulation of Bcl-XL as well as activation of caspase-3 and caspase-9, and release of cytochrome c. The investigation on pomegranate's anticancer properties has expanded from ellagitannin to other fractions of pomegranate extract. Moreira et al. [85] reported that pomegranate peel extract exhibited antiproliferative effect on another colon cancer cell line (LOVO) and its doxorubicin-resistant form (LOVO-DX) via induction of apoptosis, suggesting

pomegranate extract's potential to treat chemotherapy-resistant cancers. Similary, acetone and methanolic extracts from whole pomegranate peels were found to exert cytotoxicity in RKO colorectal cancer cells [84]. Devanesan et al. [109] developed aqueous pomegranate peel extract-derived silver nanoparticles that demonstrated increased cytotoxicity in RKO cells, possible due to programmed cell death via autophagy. Interestingly, Costantini et al. [90] found only a marginal effect of a methanolic extract of pomegranate seed oil against HT29 and HCT 116 colon cancer cells.

3.2.2.2.2. *In vivo*

Based on encouraging *in vitro* results as presented earlier, several groups have also explored the anticancer properties of various pomegranate extracts in several animal models. Kohno et al. [110] demonstrated that dietary pomegranate seed oil (PSO) reduced the incidence and multiplicity of azoxymetahne (AOM)-induced colonic adenocarcinomas in male F344 rats. The associated increase in conjugated linolenic acids (CLA) content of colonic mucosa and liver and the upregulation of peroxisome proliferator-activated receptor gamma (PPARγ) expression in colon mucosa were proposed as the potential mechanism for antitumor property of PSO. Similarly, Boateng et al. [111] used the same chemical carcinogenesis model and found that treatment with pomegranate juice significantly reduced the frequency of aberrant crypt foci (ACF), which represent early preneoplastic lesions of adenocarcinoma, in rats. The investigators also reported increased glutathione S-transferase (GST) activity in the liver of rats subjected to pomegranate juice treatment compared to control animals. Similarly, Waly et al. [112] used AOM-induced tumor model in Sprague-Dawley rats and found that pomegranate peel extract can reverse the tumor-inducing effects of AOM, via increase in total antioxidant concentration (TAC), elevation

in GSH, GST, glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT). To further expand on the study, the same group [113] reported similar reduction of ACF formation in proximal and distal colon of rats following pomegranate peel extract administration with simultaneous reduction of AOM-induced genotoxicity, elevation in GSH/GSSG ratio, increase in malondialdehyde (MDA) level and suppression of myeloperoxidase (MPO) activity. Banerjee et al. [105] studied the effect of pomegranate juice consumption with the same cancer model and reported similar suppression of total ACF and dysplastic ACF formation in colon. Additionally, they found that the mRNA and protein levels of COX-2, inducible nitric oxide synthese (iNOS), NF-κB, VCAM-1, insulin growth factor (IGF), insulin growth factor receptor (IGFR), PI3K, Akt, mammalian target of rapamycin (mTOR) as well as the expression of miR-126 were modulated with pomegranate juice treatment. Bastide et al. [114] reported that pomegranate extract containing 12% ellagic acid reduced mucin-depleted foci (MDF, biomarker for colorectal cancer) formation in rats fed dark cooked meat with nitrite (DCNO, cured meat with known carcinogenic property) and received AOM injection. The extract-mediated protection against colon carcinogenesis was associated with full suppression of fecal excretion of nitrosyl iron. Sadik et al. [115] used another *in vivo* colon cancer model, namely 1,2-dimethylhydrazine dihydrochloride (DMH)-induced tumorigenesis in male Wistar albino rats, and found that treatment with a standardized pomegranate fruit extract enhanced survival of tumor-bearing animals, lowered tumor incidence and reduced carcinoembryonic antigen (CEA, a serum tumor marker) in parallel with a normalization of colonic histopathological characteristics. This study also suggested that changes in expression of various Wnt-target genes, including Wnt5a, frizzled receptor-8 (FRZ-8), β-catenin, T cell factor/lymphoid enhancer binding protein (Tcf4/Lef1), c-myc, cyclin D1, adenomatous polyposis coli (APC), and axin1 in colonic tissues potentially

contributed to the anticarcinogenic effect of pomegranate constituents. Tortora and colleagues [116] investigated the chemopreventive potential of pomegranate mesocarp, a polyphenol-rich by-product of juice production, by using another colorectal cancer model, Apc-mutated Pirc (F344/NTac-Apcam1137) rats. A mesocarp decoction was found to reduce the number and multiplicity of MDF in experimental animals via induction of apoptosis.

3.2.2.3. Liver cancer

3.2.2.3.1. In vitro

There are several studies on pomegranate-derived products in *in vitro* liver cancer. While 24-h treatment with pomegranate seed oil did not change the viability of human liver cancer cells (HepG2 and Huh7) [90], pomegranate fruit rind-derived galactomannan polysaccharide (PSP001) [78] and a novel formulation of PSP001 (SNP@PSP) [Padinjarathil et al. 2018] exerted cytotoxic and antimigratory effects in HepG2 cells. Li et al. [117] detected four polyphenols (gallic acid, ellagic acid, punicalagin A&B and punicalin A&B) in various parts of pomegranate, such as peels, flesh, seeds, juice and leaf. Interestingly, the peel and flesh extracts (containing 83% of total phenolic content of pomegranate) exhibited significantly potent cytotoxicity against HepG2 cells, whereas less pronounced effects were observed with other parts. A polyphenol-rich pomegranate peel extract inhibited the proliferation of HepG2 cells with simultaneous induction of cell cycle arrest at the S-phase and apoptosis, increase in the level of reactive oxygen species (ROSs) and cyt. c, elevation in the activity of caspase-3 and caspase-9, upregulation of the expression of Bax and p53 and downregulation of Bcl-2 expression [118]. Saratale and colleagues [119] developed a cost-effective green synthesis of silver nanoparticles (AgNPs) containing pomegranate leaf extract (PGE). This product (PGE-AgNPs) showed remarkable and greater cytotoxicity against

HepG2 cells in concentration-dependent manner compared to PGE, possibly through free-radical scavenging activity. Similarly, solid lipid nanoparticles containing pomegranate extract exhibited superior cytotoxic activity in HepG2 cells when compared to the free extract [97].

3.2.2.3.2. In vivo

Bishayee and co-investigators [120] examined, for the first time, the mechanism-based chemopreventive potential of a novel pomegranate emulsion (PE) containing numerous phytochemicals, including caffeic acid, corilagin, ellagic acid, ferulic acid, gallic acid, punicalagins, protocatechuic acid, and γ-tocopherol, against diethylnitrosamine (DENA)-initiated and phenobarbital-promoted rat hepatocarcinogenesis that resembles human hepatocellular carcinoma (HCC). Oral administration of PE (1 or 10 g/kg), initiated 4 weeks before DENA treatment and continued for 18 weeks, exhibited a striking chemopreventive effect as evidenced by reduced incidence, number, multiplicity, size and volume of hepatic nodules, precursors of HCC. Both doses of PE also reduced the number and area of γ-glutamyl transpeptidase (GGT)-positive hepatic foci coupled with attenuation of lipid peroxidation (thiobarbituric acid-reactive substances, TBARS) and protein oxidation (protein carbonyls) in DENA-challenged rats. Based on mechanistic studies, PE elevated gene expression of hepatic antioxidant and carcinogen detoxifying enzymes and elevated hepatic Nrf2 at transcriptional and translational levels. In a subsequent study, PE was found to reverse DENA-mediated inflammatory cascades by abrogation of hepatic expression of iNOS, 3-nitrotyrosine (3-NT), heat shock protein 70 (HSP70), HSP90, COX-2 and NF-κB as well as by hindering nuclear translocation of NF-κB during experimental hepatocarcinogenesis [121]. Finally, PE has been found to inhibit cell proliferation, as evidenced by reduced expression of proliferating cell nuclear antigen (PCNA), altered cell cycle progression

(cyclin D1), and induced apoptosis by upregulating Bax and downregulating Bcl-2 under the same experimental conditions [122]. PE also dose-dependently reduced hepatic β-catenin and elevated glycogen synthase kinase-3β (GSK-3β) at protein and mRNA levels, indicating that pomegranate bioactive constituents exert chemoprevention of HCC through antiproliferative and proapoptotic mechanisms by modulating Wnt/β-catenin signaling pathway. Subsequently, El-Ashmawy et al. [123] confirmed the liver cancer preventive effect of pomegranate constituents and several mechanisms of action as previously reported by Bishayee and colleagues [120-122]. In their study, treatment of DENA-exposed rats with pomegranate hull extract (PHE) for 10 weeks (4 weeks before and 6 week after DENA exposure) increased survival of hepatocarcinogen-treated animals, decreased liver tumor size, reduced the expression of Bcl-2, cyclin D1 and β-catenin and elevated hepatic GSH level. All the aforementioned studies suggest that pomegranate phytochemicals target Nrf2, NF-κB and Wnt/β-catenin signaling to confer chemoprevention of HCC.

3.2.2.4. Pancreatic cancer

3.2.2.4.1. In vitro

There is at least one study that investigated the effect on pomegranate on pancreatic cancer cells. Nair et al. [124] reported that a standardized whole fruit extract of pomegranate had antiproliferative effect and altered cell cycle progression arrest via increasing the proportion of PANC-1 pancreatic cancer cells that lack CD44 and CD24 expression, which are associated with increased tumor-initiating activity. Similar results were achieved in another pancreatic cancer (AsPC-1) cell line. Isolated pomegranate phytochemicals, ellagic acid, luteolin and ursolic acid, were found to be only modestly effective in suppressing cell proliferation, suggesting synergistic effects of pomegranate phytochemicals.

3.2.3. Gynecological cancers

3.2.3.1. Uterine cancers

3.2.3.1.1. In vitro

In one of the earliest studies, a polyphenol-rich whole pomegranate fruit extract showed very little effect on the growth of HeLa human cervical cancer cells [125]. Similarly, a lack of effect was observed with a methanolic extract of pomegranate pericarp against HeLa and HEC-1A human endometrial cancer cells; however, a growth-inhibitory effect was observed in SiHa human cervical cancer cells with very high concentration [76]. Interestingly, pomegranate peel and flesh extracts significantly inhibited the proliferation of HeLa cells whereas the extracts of seeds, leaves as well as juice showed less pronounced effects [117]. Later, Fazio et al. [84] observed antiproliferative effects of acetone and methanolic extracts of pomegranate peels against HeLa and Ishikawa (human endometrial adenocarcinoma) cells. The HeLa cells treated with the methanolic extract exhibited higher caspase-3, caspase-7 and caspase-9 activity, PARP cleavage and apoptosis. Ellagic acid, a polyphenol extracted from pomegranate peel, showed inhibition of invasive ability and promotion of apoptotic characteristics of HeLa cells via increased expression of insulin-like growth factor binding protein 7 (IGFP7) and simultaneous inhibition of Akt/mTOR signaling [126]. Pomegranate husk-derived punicalagin exhibited antiproliferative activities against three cervical cell lines, namely HeLa, SiHa and CaSki, in a concentration-dependent manner, with CaSki cell line being the most sensitive [87]. The antiproliferative effect of punicalagin in HeLa cells was confirmed by a subsequent study that also showed inhibition of cancer cell migration, downregulation of MMP-2 and MMP-9, upregulation of TIMP-2 and TIMP-

3, cell cycle arrest in the G_1 phase, induction of apoptosis by upregulation of Bax, downregulation of Bcl-2 and β-catenin and its downstream proteins, cyclin D1 and c-myc [127].

3.2.3.2. Ovarian cancer

3.2.3.2.1. In vitro

As one of the most lethal gynecologic malignancies, there is great interest in finding novel treatments for ovarian cancer in the face of emerging chemotherapy resistance. While a methanolic extract of pomegranate pericarp failed to exhibit any cytotoxicity against SKOV3 human ovary cancer cells [76], other studies documented antiproliferative effects of a methanolic peel extract [83] and pomegranate juice-derived ellagitannins [80] in the same cell line without an understanding of the mechanisms of action. Additionally, pomegranate seed extract was found to inhibit the growth of SKOV3 cells with an unknown mechanism [92]. Finally, pomegranate fruit juice and its components, ellagic acid and luteolin, were found to suppress the proliferation and migration of another human ovarian cancer cell line, namely A2780, in a concentration-dependent manner via downregulation of the expression of MMP-2 and MMP-9 [128].

3.2.3.2.2. In vivo

Liu et al. [128] expanded their *in vitro* study to confirm the anticancer effects of pomegranate juice and its phytoconstituents using an *in vivo* ovarian cancer model. In this study, ES-2 human ovarian adenocarcinoma cells were injected to the right hind leg of female nude mice. Two weeks following ES-2 cell injection, the tumor-bearing animals were treated with pomegranate fruit juice (20 mL/kg), ellagic acid (50 mg/kg) or luteolin (50 mg/kg) for approximately 24 days. All three treatments inhibited tumor growth and suppressed the expression of intratumor MMP-2 and MMP-9 without any effect on body or spleen weight of experimental animals compared to control.

3.2.4. Hematological cancers

3.2.4.1. Lymphoma

3.2.4.1.1. *In vitro*

Various pomegranate constituents demonstrated anticarcinogenic effects in diverse hematologic cancers through a variety of mechanisms. Settheetham and Ishida [129] conducted one of the earliest *in vitro* studies to measure the effect of pomegranate extract on cancer cells. The results indicated that boiled-water extract of pomegranate peel inhibited the growth of human Burkitt's lymphoma cell lines, Raji and P3HR-1, via apoptotic DNA fragmentation. Similarly, pomegranate fruit rind-derived galactomannan polysaccharide (PSP001) was found to exhibit significant cytotoxicity against Dalton's ascites lymphoma, a murine lymphoid cancer cell line, through apoptosis induction [78].

3.2.4.2. Leukemia

3.2.4.2.1. In vitro

Kawaii and Lansky [130] evaluated flavonoid-rich fractions from fresh and fermented pomegranate juice and from an aqueous extract of pomegranate pericarp extract as potential differentiation-inducing agents in HL-60 human acute promyelocytic leukemia (APL) cells. The fractions from fermented juice and pericarp extracts were found to exert strong differentiation-promoting effects with proportional inhibitory effects on HL-60 cell proliferation. Antiproliferative activity was also observed when Jurkat (peripheral blood T-cell leukemia), SUP-B14 (acute lymphoplastic leukemia, ALL), MOLT-3 (ALL), CCRF-CEM (ALL), HL-60 (APL),

THP-1 (acute monocytic leukemia, AMCL), K562 (chronic myloid leukemia, CML) and KG1a (acute myelogenous leukemia, AML) cell lines were exposed to 6.25-12.5% of pomegranate juice extracts for up to 48 h. The extract-treated cell lines were arrested in G_0/G_1 phase and showed an increase in apoptotic activity [131]. A follow-up study by the same group illustrated the cytotoxic effect of acetonitrile fraction obtained from pomegranate juice against CCRF-CEM (ALL), MOLT-3 (ALL), HL-60 (APL), and THP-1 (AMCL) cells. Additionally, the cells were arrested in S phase, exhibited caspase-3 activation and decreased adenosine triphosphate (ATP) levels (indicative of metabolically active cells) and underwent apoptosis after exposure to the acetonitrile fraction. Accompanying liquid chromatography mass spectrometry analysis revealed that the acetonitrile fraction contained ellagitannins, ellagic acid, and hydroxycinnamic acid derivatives, but depleted in anthocyanins [132]. A pomegranate pericarp extract (PPE) has been found to contain punicalagin anomers (punicalagin A and B), ellagic acid-hexoside and ellagic acid. Self-assembled NPs made of PPE and gelatin (PPE-gelatin NPs) were found to be less effective than PPE in inducing the early stage of apoptosis in HL-60 cells. However, both PPE-gelatin NPs and PPE had similar effects in inducing the late stage of apoptosis and necrosis [133]. In another study, K562 human CML cell line was exposed to PSP001 polysaccharide (isolated from pomegranate fruit rind), resulting in a concentration- and time-dependent increase in cytotoxic activity [86]. In the same leukemia cells (K562), a pomegranate peel extract promoted growth inhibition via G_2/M phase arrest and apoptosis induction through the upregulation of caspase-9, caspase-3, caspase-7, cyt. c, p21 and p53 and Akt, with simultaneous downregulation of Bcl-2 and Bax [134].

3.2.4.3. Myeloma

3.2.4.3.1. In vitro

Antiproliferative activity was also encountered in U266 human multiple myeloma (MM) cells treated with 1-500 µg/mL of extracts of pomegranate leaves, stems, and flowers for up to 72 h. The leaf and stem extracts triggered apoptosis with an increase in the loss of mitochondrial membrane potential, whereas the flower extract resulted in slight decrease in MMP [135]. Finally, pomegranate juice resulted in antiproliferative effect, G_0/G_1 phase arrest as well as increase in PPARγ mRNA expression in three MM cell lines, namely U266, KMS26 and MM1S. Interestingly, pomegranate juice or bortezomib (BTZ), a proteasome inhibitor used as a first line drug to treat MM, exhibited better cytotoxic effect than a combination treatment of U266 cell line. However, pre-treatment with pomegranate juice inhibited the cytotoxic effect of BTZ. On the other hand, treatment of U266 cells with pomegranate juice after BTZ exposure improved the cytotoxic effect [136].

3.2.5. Lung and respiratory tract cancers

3.2.5.1. In vitro

A pomegranate fruit (edible portion) extract was tested against an *in vitro* model of human non-small cell lung cancer (A549 cells). Treatment with the extract at 50-150 µg/mL for 72 h was found to decrease the viability of A549 cells with concurrent arrest of cells at G_0/G_1 phase, induction of $p21^{WAF1}$ and $p27^{KIP1}$ and decrease in the expression of Ki-67, PCNA, cyclin D1, cyclin D2, cyclin E, cyclin-dependent kinase 2 (cdk2), cdk4 and cdk6. Additional findings involve inhibition of phosphorylation of MAPK and Akt, decrease in the expression of PI3K, NF-κB and IKKα, degradation and phosphorylation of IκBα and inhibition of NF-κB-DNA binding activity [137]. Subsequently, other investigators confirmed the antiproliferative activity of methanolic extracts of pomegranate peel and seeds against the same lung cancer cell line [83,92]. The

anticancer potential of a polysaccharide (PSP001) isolated from rinds of fully ripened fruits of pomegranate was investigated using KB human nasopharyngeal carcinoma cells with positive results [86]. Moreover, silver nanoparticles (SNPs) containing PSP001 (SNP@PSP) exhibited a time- and concentration-dependent cytotoxic behavior in A549 cells via caspase-mediated apoptosis induction. SNP@PSP also showed antimigratory potential based on wound healing assay [107].

The anticancer effect of an extract of pomegranate leaf (containing punicalagin and ellagic acid) have been examined in A549, H1299 (human non-small cell carcinoma) and LL2 (mouse Lewis lung carcinoma) cell lines *in vitro*. In all three cell lines, the extract suppressed proliferation and colony formation. In H1299 cells, the extract arrested cell cycle progression in G_2/M phase and induced apoptosis by decreasing ROS accumulation and mitochondrial transmembrane potential ($\Delta\Psi m$). Further, the extract blocked H1299 cell migration and invasion in parallel with reduction of MMP-2 and MMP-9 expression [138]. Punicalagin isolated and purified from the pith and carpellary membrane of pomegranate fruit was incubated with A549 and Hep-2 human larynx epithelial cancer cells and demonstrated low cytotoxicity, while still demonstrating antioxidant effects mediated through chelation of metal ions, restitution of lipid oxidation products, and decreased levels of ROS and reactive nitrogen species [139]. Punicalagin extracted from pomegranate husk powder exhibited strong antiproliferative activity against A-549 as well as H1299 cells. The mechanism of antiproliferative effect was proposed to be antioxidant effects, including diminished free radicals and decreased oxidized DNA adducts [87]. The same research group extended their earlier study to document antiproliferative and antimutagenic activities of punicalagin and ellagic acid against A549 and H1299 cells [140].

3.2.5.2. In vivo

Based on encouraging *in vitro* results, several investigators evaluated the preventive and therapeutic actions of pomegranate-derived products using various *in vivo* models on lung cancer. In a mouse xenograft model with A549 tumors, pomegranate fruit extract (0.1 and 0.2%, w/v) in drinking water resulted in significant inhibition of tumor growth and increased the survival of animals with tumor volumes equal to or less than 1200 mm^3 [137]. However, the investigators did not provide any mechanisms of such antitumor effects. In a second study, benzo(a)pyrene [B(a)P] or N-nitroso-tris-chloroethylurea (NTCU) was used to induce lung tumors in A/J mice. The animals had access to drinking water containing pomegranate fruit extract one week before the carcinogenic challenge. Long-term consumption of pomegranate extract reduced lung tumor multiplicity in both models. Accompanying studies revealed several underlying mechanisms of action, such as inhibition of activation NF-κB and IκBα kinase (IKK), degradation and phosphorylation of IκBα, phosphorylation of mitogen-activated protein kinases (e.g., extracellular signal-regulated kinase 1/2, c-Jun NH_2-terminal kinase 1/2, and p38), phosphatidylinositol 3-kinase (p85 and p110), phosphorylation of Akt, activation of mammalian target of rapamycin (mTOR) signaling, phosphorylation of c-met, and suppression of the markers of cell proliferation (Ki-67 and PCNA) and angiogenesis (iNOS, CD31, and VEGF) [141]. Finally, Husari and coinvestators [142] used cigarette smoke (known to cause oxidative stress, DNA damage and lung cancer) to induce lung nodules and tumors in AJ mice. The animals drank pomegranate juice, starting one week prior to cigarette smoke (delivered through a smoke generator) and continued for 5 months, which reduced nodule incidence, nodule multiplicity, mean nodular size, tumor incidence and tumor multiplicity. Pomegranate juice supplementation also significantly inhibited intranodular mitotic activity, measured using anti-phosphohistone-H3 (PHH3) antibody, and

reduced hypoxia-inducible factor-1α (HIF-1α) expression. All these studies underscore the value of pomegranate juice consumption in preventing and/or treating human lung cancer.

3.2.6. Neurological cancers

3.2.6.1. In vitro

Limited studies have been conducted on the association between pomegranate phytochemicals and antitumorigenic effects in the nervous system. In U87MG human glioma cells, pomegranate-derived punicalagin (1-30 μg/mL) admistered for 24 h had demonstrated reduced cell viability in association with increased cyclin E, and decreased cyclin A and cyclin B levels. The treatment with punicalagin also triggered apoptosis as evidenced by PARP cleavage, activation of caspase-9 and decrease in Bcl-2 level. The treatment also increased microtubule-associated protein light chain 3 II (LC3-II) cleavage and caused green fluorescence-LC3 fusion protein (GFP-LC3-II)-stained punctate pattern in U87MG cells, indicating autophagy. Additionally, punicalagin increased the extent of AMPK and p27 phosphorylation which correlated with the induction of autophagic cell death [143]. Ferreira et al. [144] prepared pomegranate seed oil nanoemulsion containing ketoprofen using pullulan as a polymeric stabilizer and evaluated its antitumor activity against C6 rat malignant glioma cells. The nanoemulsion suppressed C6 cell growth; however, the associated mechanisms were not studied. In an extended work, the same research group found that 3% pomegranate seed oil had slightly better cytotoxic profile compared to a nanoemulsion containing the same pomegranate product in C6 cells [145].

3.2.7. Skin cancer

3.2.7.1. In vitro

Very limited information is available regarding *in vitro* effect of pomegranate in skin cancer. Various ellaginatinns, including gallic acid, ellagic acid, hexahydroxydiphenic acid, punicallins and punicalagins, from pomegranate juice displayed moderate antiproliferative activity in SK-MEL human malignant melanoma cells [80]. Pomegranate peel-derived galactonmamman polysaccharide (PSP001) exhibited cytotoxicity against another human malignant melanoma (A375) cells, possibly via induction of apoptosis [78].

3.2.7.2. In vivo

Pomegranate fruit and seeds have been used for research to explore anticancer potential in various *in vivo* skin cancer models. Hora et al. [146] reported for the first time a decrease in skin tumor (papilloma) incidence and multiplicity in female CD-1 mice topically treated with DMBA and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and subjected to 5% pomegranate seed oil treatment. Mechanistically, this is thought to be attributed to the observed decrease in TPA-induced epidermal ornithine decarboxylase (ODC), a marker for skin cancer promotion. Afaq and colleagues [147] investigated the inhibitory effect of pomegranate fruit extract, containing anthocyanins, ellagitannis and hydrolyzable tannins, on DMBA-initiated and TPA-promoted skin tumor formation in CD-1 mice. The results indicated that topical application of the extract displayed reduced tumor incidence, total number of tumors and tumor multiplicity. The extract also afforded significant inhibition of TPA-mediated increase in skin edema and hyperplasia, epidermal ODC activity, expression of ODC and COX-2, phosphorylation of ERK1/2, JNK1/2 and p38, activation of NF-κB and IKKα, and phosphorylation and degradation of Iκβα [147]. Later, George and coinvestigators [148] confirmed the antiskin tumor effect and associated mechanisms (e.g., interference with MAPK and NF-κB signaling) of oral pomegranate fruit extract

in the same two-stage (DMBA/TPA) mouse skin tumorigenesis model. Additional findings indicated induction of apoptosis and inhibition of proliferation (based on PCNA expression) and DNA strand breaks in the skin tissue/tumor tissue of experimental animals. A synergic effect was observed when the animals received topical application of diallyl sulfide (DAS), a component of garlic, in addition to oral pomegranate juice.

3.2.8. Urogenital cancers

3.2.8.1. Bladder cancer

3.2.8.1.1. In vitro

Whole fruits or peels of pomegranate cultivars from different geographic locations were subjected to several extraction methods. These extracts evaluated for polyphenolic content, antioxidant capacity and tested for antiproliferative activity against T24 human bladder cancer cells. The Soxhlet extract of peels from fruits of Israeli origin was found to be the most efficacious with the highest ellagic acid content (responsible for antioxidant activity), whereas the aqueous extract from Israeli whole fruit was the least efficacious with lowest ellagic acid content [149]. Pomegranate rind extract containing 13% (w/w) ellagic acid (100 ug) was incubated with EJ human bladder carcinoma cells for 48 h and was found to have antiproliferative and proapoptotic effects. These effects were mediated via increased activity of caspase-3, induction of p53, increased miR-34a levels leading to inhibition of c-myc and CD44 [150]. In another study, T24 and J82 urinary bladder urothelial carcinoma cell lines were exposed to pomegranate fruit ethanolic extract at various concentrations (5-200 µl/mL) for 12-72 h. The results demonstrated that the extract was more effective in suppressing the proliferation of T24 cells than J82 cells. Apoptosis induction in T24 cells was mediated via activation of procaspase-3, procaspase-8,

procaspase-9, procaspase-12, increase in Bax/Bcl-2 ratio, increased expression of CCAAT-enhancer-binding protein homologous protein (CHOP) and binding immunoglobulin protein (BiP), therefore activating endoplasmic reticulum stress, mitochondrial damage and death receptor pathways [151]. The same research group later reported that 20 proteins, involved in cell proliferation, apoptosis, cytoskeleton regulation, proteasome activity, and aerobic glycolysis, were differentially expressed in ethanolic extract-treated T24 cells. Additional signaling studies revealed that the extract inhibited T24 cells proliferation through restriction of PTEN/Akt/mTOR pathway via profilin 1 (Pfn1) upregulation and evoked apoptosis through inhibition of X-linked inhibitor of apoptosis protein (XIAP) and Diablo over-expression [152]. In an extended study, it has been found that an ethanolic extract of pomegranate peels exhibited better growth-inhibitory activity against T24 and J82 cells than that of pulp. The ethyl acetate fraction of the peel extract demonstrated the highest anticancer activity attributed to apoptosis [153].

3.2.8.1.2. In vivo

Few investigators confirmed bladder cancer-inhibitory effects of pomegranate products *in vivo*. Zhou et al. [150] reported that pomegranate rind extract suppressed the growth of xenografted EJ tumor in mice with concomitant upregulation of the expression of miR-34a. Using another xenograft model, Chang et al. [153] showed that oral administration of ethylacetate fraction of ethanolic extract of pomegranate peel decreased the volume and weight of T24 tumors in mice and evoked intratumor apoptosis.

3.2.8.2. Prostate cancer

3.2.8.2.1. In vitro

Prostate cancer has one of the highest incidences and mortality rates, and certainly has been one of the biggest forefronts in pomegranate research. Albrecht et al. [154] launched the first *in vitro* study on the effects of cold-pressed pomegranate seed oil, supercritical CO_2-extracted seed oil, polyphenol extract of fermented pomegranate juice, and polyphenol extract of pomegranate pericarp on prostate cancer cells (LNCaP, PC-3 and DU-145). These investigators observed antiproliferative, proapoptotic and anti-invasive effects of various pomegranate-derived products via induction of cell cycle arrest at G_2/M checkpoint, apoptosis, increased caspases-3 activity, and upregulation of $p21^{WAF1}$ and mitogen-activated protein kinase-activated protein kinase 2 (MAPK-APK2) expression, along with downregulation of growth arrest and DNA-damage-inducible protein 45α (GADD45α) and c-myc gene expression. Lansky et al. [155] extended the previous study and tested the same pomegranate extracts in various combination and reported synergistic antiproliferative and anti-invasive effects, possibly via downregulation of phospholipase A2 (PLA2) expression in DU-145 and PC-3 cells. Malik et al. [156] reported similar antiproliferative and proapoptotic effects with pomegranate fruit extract on PC-3 cells, and further proposed possible mechanisms, including upregulation of Bax, Bak, $p21^{WAF1}$ and $p27^{KIP1}$, along with downregulation of Bcl-XL, Bcl-2, cyclin D1, cyclin D2, cyclin E, and cdk2, cdk4, cdk6 expression. Seeram et al. [103] also observed antiproliferative effect of pomegranate juice, total pomegranate tannin extract, punicalagin and ellagic acid in two other types of prostate cancer cells (RWPE-1 and 22Rv1), possibly via the inhibition of lipid peroxidation. Hong et al. [157] observed antiproliferative and proapoptotic effects of a standardized pomegranate extract (POMx), pomegranate juice, punicalagin and ellagic acid against DU-145, LNCaP and androgen receptor (AR) expressing LNCaP (LNCaP-AR) cells. Subsequent mechanistic study showed downregulation of 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2), aldo-keto reductase

family 1 member C3 (AKR1C3), steroid 5α reductase type 1 (SRD5A1) and AR by all pomegranate-derived products. POMx also significantly inhibited the proliferation of LNCaP cells under both normoxic and hypoxic conditions. Under hypoxic environment, POMx exhibited antiangiogenic effect via downregulation of HIF-1α and VEGF [158]. Using DU-145 cells, Rettig et al. [159] compared the anticancer effect POMx with that of pomegranate juice and found that POMx was more effective in exhibiting cytotoxicity via induction of apoptosis and inhibition of NF-κB. In addition to reporting the antiproliferative and proapoptotic effects of POMx on LAPC4 cells via upregulation of c-Jun N-JNK phosphorylation, downregulation of insulin-like growth factor-1 (*igf*-1) mRNA expression, and decreased Akt and mTOR activation, Koyama et al. [160] found that co-treatment with POMx and IGF binding protein-3 resulted in an intriguing synergistic stimulation of apoptosis and additive inhibition of cell growth.

Wang and colleagues [161,162] conducted several studies to explore the potential of pomegranate juice in the prevention and treatment of prostate cancer metastasis. In the first study, in addition to causing cell death of hormone-refractory prostate cancer cells, pomegranate juice increased cell adhesion and decreased cell migration. Mechanistic results revealed upregulation of genes related to cell adhesion, such as ICAM-1 and E-cadherin and downregulated genes involved in cell migration, such as hyaluronan-mediated motility receptor (HMMR) and collagen type Iα1 (COL1A1). Interestingly, anti-invasive microRNAs, such as miR-126, miR-200, miR-205 and miR-335, were upregulated, and at the same time pro-invasive microRNAs, such as miR-21 and miR-373, were downregulated. Additionally, pomegranate juice suppressed pro-inflammatory cytokines, such as IL-6, IL-1β and IL-12P40, and chemokine, namely regulated on activation, normal T cell expressed and secreted (RANTES) and also inhibited the ability of the chemokine stromal-derived growth factor 1α (SDF1α) to chemoattract prostate cancer cells [161]. In an

attempt to further identify the active phytochemicals associated with the effects of pomegranate juice reported in the preceding study, the same research group [162] published a secondary study to document that a specific combination of pomegranate juice components (luteolin, ellagic acid, caffeic acid and punicic acid) inhibited the growth of hormone-dependent and hormone-refractory prostate cancer cells and suppressed their migration and chemotaxis towards SDF1α, a chemokine with important role in prostate cancer metastasis to the bone. The investigators again reported modulatory effects of pomegranate juice components on another extensive list of target genes, miRNAs and proteins related to cell cycle control, adhesion, migration and metastasis.

Several independent investigations documented encouraging effects of pomegranate peels against *in vitro* prostate cancer models. In one study, a methanolic extract of pomegranate peel was found to inhibit the proliferation of PC-3 prostate adenocarcinoma cells [83]. Another study reported that an ethanolic extract of pomegranate peel displayed similar antiproliferative effect on PC-3 cells when compared to anticancer drug taxol as a positive control with elevated nucleosome production in apoptotic cells [163]. Finally, Deng et al. [164] reported that pomegranate peel extract (containing punicalagin and ellagic acid) exerted antiproliferative, proapoptotic, anti-invasive and antimigratory effects on a panel of prostate cancer cells (PC-3, DU-145 and TRAMP-C1) with associated dissipation of mitochondrial membrane potential, accumulation of reactive oxygen species, increased Bax to Bcl-2 ratio, elevated caspase-3 activity, downregulation of MMP-2 and MMP-9 and upregulation of TIMP-2.

Following the early success with pomegranate seed-derived products as antiprostate cancer agents, various investigators conducted additional *in vitro* experiments with mixed results. An ethanolic seed extract displayed marginal inhibitory effect on the growth of PC-3 cells [163]. Likewise, it has been found that a hydrophilic fraction (80% aqueous methanolic extract) of

pomegranate seed oil had minimal effect on the viability of DU-145 cells [90]. On the contrary, a methanolic extract of pomegranate seed exerted antiproliferative activity against PC-3 cells even at low concentrations [92]. Interestingly, a pomegranate whole seed ethanolic extract (containing punicic acid, α-linoleic acid and α-linolenic acid) showed a promising antiproliferative activity against hormone-dependent LNCaP cells with an IC_{50} value 3-fold lower than that of positive control vinblastine [91].

Recently, *in vitro* cytotoxic profile of pomegranate extract-loaded solid lipid nanoparticles was investigated in PC-3 cells with results showing nanoencapsulation of the extract enhanced its anticancer efficacy compared to the free extract [97].

3.2.8.2.2. In vivo

Based on encouraging *in vitro* results, several laboratories extended anticancer investigation on pomegranate using various *in vivo* models of prostate cancer. Parenteral administration of pomegranate pericarp polyphenols and supercritically extracted seed oil significantly inhibited the growth of xenografted PC-3 tumor in athymic BALB/c male homozygous nude mice. However, there were no significant differences in survival of animals between experimental and control groups [154]. Malik et al. [156] showed that pomegranate fruit extract in drinking water suppressed tumor growth, increased survival of tumor-bearing animals and lowered serum prostate-specific antigen (PSA) levels in athymic nude male mice injected with androgen-sensitive CWR22R*v*1 human prostate carcinoma cells. Seeram et al. [165] also reported significant inhibition of tumor growth with the oral administration of ellagitannins-enriched extract of pomegranate fruit skin in

severe combined immunodeficient (SCID) mice injected with LAPC4 cells. Subsequently, the same group [158,159] studied antitumor effects and associated mechanisms of action of POMx on the same xenograft animal model. Rettig et al. [159] reported inhibition of growth of androgen-independent LAPC4 xenografts, intratumor antiproliferative and proapoptotic activities, abrogation of NF-κB activity in tumor samples together with suppression of serum PSA level. Sartippour et al. [158] found that POMx reduced xenograft size and tumor vessel density via downregulation of HIF-1α, suggesting antiangiogenesis as an additional anticancer mechanism of ellagitannin-rich pomegranate extract. Wang et al. [166] conducted another investigation on the effects of treatment with a combination of pomegranate juice components (luteolin, ellagic acid and punicic acid) on male SCID mice bearing PC-3M-luc xenograft or *Pten*$^{-/-}$*; K-ras*^{G12D} allograft tumors. Intraperitoneal (i.p.) injection of pomegranate juice phytochemicals inhibited the growth, angiogenesis and metastasis of PC-3M-luc tumors via suppression of C-X-C motif chemokine 12 (CXCL12/SDF1-α)/C-X-C chemokine receptor type 4 (CXCR4) and Akt signaling axis. In *Pten-/-; K-ras*^{G12D} tumor model, similar effects were observed with tumor growth and metastasis.

Adhami et al. [167] evaluated the effects of pomegranate fruit extract against prostate cancer development utilizing a classical chemoprevention protocol of transgenic adenocarcinoma mouse prostate (TRAMP) model. The animals received 0.1 and 0.2% fruit extract in the drinking water for 6, 14 and 28 weeks. The investigators observed that continuous supplementation of fruit extract conferred significant survival advantage, reduced palpable tumor incidence and hindered metastasis to lungs, liver and lymph nodes over water-fed TRAMP mice. Mechanistically, pomegranate extract resulted in concurrent and considerable inhibition of IGF-I/Akt/mTOR pathway in both prostate and tumor tissues.

3.3. Clinical studies

With encouraging preclinical results in various cancers, pomegranate constituents attracted several investigators to conduct clinical studies to gather valuable clinical data, predominantly in the field of breast, colon and prostate cancers.

3.3.1. Breast cancer

Interest in pomegranate and breast cancer has been growing over the past decade as evident by the rapid rise of preclinical studies demonstrating pomegranate's efficacy in breast cancer cells and animal models. Kapoor et al. [168] conducted the first clinical study on the effects of pomegranate juice (POM Wonderful 100% juice) on various biomarkers associated with breast cancer risk. Sixty-four healthy postmenoposal women randomly assigned to drink 8 ounces of either 100% commercial pomegranate juice (intervention group) or apple juice (control group) for a period of 3 weeks. Interestingly, the investigators reported that there was no significant decline in serum sex hormones or sex hormone binding globulin (SHBG) in the intervention group compared to control (Table 3). Nevertheless, subgroup analyses revealed significant decline in estrone and testosterone levels in 38 normal weight females (intervention group) compared to control, warranting the need for future investigations to determine the effects of pomegranate in reducing breast cancer risk in normal vs overweight/obese populations.

3.3.2. Colon cancer

Nuñez-Sánchez et al. [169] has been the pioneer in advancing the field of pomegranate clinical studies in colorectal cancer. In 2014, they investigated whether ellagic acid derivatives and urolithins (produced by gut microbiota from ellagitannins and ellagic acid) in colon tissues from colorectal cancer (CRC) patients receiving oral pomegranate extract (PE, 900 mg/day for 15 days)

with different punicalagin:ellagic acid ratio, such low (PE-1) and high (PE-2). Results showed significant levels of ellagic acid derivatives and urolithins in human colon tissue of CRC patients following consumption of pomegranate products [169]. Nuñez-Sánchez et al. [170] subsequently reported that PE displayed a moderate, but statistically insignificant, modulatory effect on the expression of specific colon tissue microRNAs (miR-646, miR-1249, miR-135b-5p, miR-135b-3p, miR-92b-5p, miR-765, miR-496, miR-181c-3p, and miR-18a-3p) in CRC patient who received 900 mg per day of PE-1 or PE-2. In another study, the same PE regimen in CRC patients (as indicated earlier) was significantly associated with a counterbalance effect in the expression of several genes, such as cluster of differentiation 44 (*CD44*), β-catenin (*CTNNB1*), cyclin-dependent kinase inhibitor 1A (CDKN1A), epidermal growth factor receptor (*EGFR*) and thymidylate synthase (*TYMS*) [Nuñez-Sánchez et al., 2017]. The investigators also reported that the observed changes were not associated with the presence of urolithins or ellagic acid in colon tissues [171]. Recently, González-Sarrías et al. [172] found that the previously described regimen with PE-1 or PE-2 decreased the plasma levels of lipopolysaccharide-binding protein (LBP), a marker of metabolic endotoxemia related to CRC progression, in newly diagnosed CRC patients.

3.3.3. Prostate cancer

Pomegranate-derived products have been subjected to substantial clinical investigation in prostate cancer. The earliest study (an open-label, single-center clinical trial) was conducted by Pantuck et al. [173] who reported that oral consumption of 8 ounces of pomegranate juice (Pom Wonderful, Los Angeles, CA, USA), containing 570 mg total polyphenol gallic acid equivalents, daily up to 54 months significant prolonged the prostate-specific antigen (PSA) doubling time (PSADT) in men with a rising PSA level following primary therapy. Additionally, the researchers

incubated LNCaP prostate cancer cells with the serum of prostate cancer patients before and after oral pomegranate juice treatment and reported antiproliferative and proapoptotic effects. There was also a significant reduction in oxidative state and sensitivity to oxidation of serum lipid following pomegranate juice consumption [173]. The same group of investigators later conducted a double-blind, placebo-controlled and multi-institutional investigation with expanded population size. Interesting, in contrary to the earlier observation, this study concluded that there was no significant prolongation of PSADT in prostate cancer patients after treatment with a pomegranate liquid extract (Pom Wonderful). However, they noted a specific subtype of patient, specifically men with manganese superoxide dismutase (MnSOD) AA genotype, had significant prolongation of PSADT after pomegranate extract treatment, suggesting that a certain patient population might have increased benefit from the antiproliferative effects of pomegranate [174]. Paller et al. [175] conducted a randomized, double-blind, multi-center phase II study on the effects of pomegranate fruit extract (POMx, Pom Wonderful) in men with recurrent prostate cancer and observed that there was at least 6 months of prolongation in PSADT with 3 g of POMx per day for 6-18 months, along with 13% of patients demonstrating reclination of PSA levels. In an attempt to identify a mechanism of action of pomegranate constituent in prostate cancer, Freedland et al. [176] also used POMx to study its effect on tissue 8-hydroxy-2′-deoxyguanosine (8-OHdG), an oxidative stress biomarker, Ki-67 (a marker for proliferation), cancer pS6 kinase (a marker of mTOR activity), and NF-κB (a measure of inflammation). The dietary intervention with POMx before radical prostectomy did not significantly lower the level of 8-OHdG compared to placebo group. The changes in other markers as well as serum PSA levels were also similar between two groups.

Based on a clinical study conducted in Spain, Gonzáles-Sarrías et al. [177] reported detection of pomegranate polyphenol metabolites (e.g., ellagic acid metabolites and urolithins) in prostate

tissue of prostate cancer patient after pomegranate juice intake (200 mL/day for 3 days), suggesting the metabolites found in the tissue may be the active ingredient responsible for pomegranate's potential therapeutic effects in prostate cancer. However, no significant changes in the expression of three proliferation biomarkers, namely *CDKN1A*, *MKi-67* and *c-Myc*, were noticed in prostate tissue samples following pomegranate juice consumption. Stenner-Liewen et al. [178] conducted a phase IIb, double-blind, randomized, placebo-controlled clinical trial in Switzerland to evaluate the effect of pomegranate juice (Biotta AG, Egnach, Switzerland) intake in patients with prostate cancer. Interestingly, they concluded that there was no difference between the treatment and placebo group with regards to PSA kinetics and pain scores. Finally, Thomas et al. [179] used a whole food supplement blend containing pomegranate whole fruit powder, turmeric powder, broccoli powder and green tea extract to conduct a double-blind, randomized placebo-controlled trial in the United Kingdom. They found that the intervention for 6 months demonstrated significant suppression of rising PSA levels in prostate cancer patients. The therapeutic potential of pomegranate in prostate cancer continues to be controversial, warrantying the need for additional clinical trials with long-term study design and appropriate biomarkers of disease progression.

4. Bioavailability and pharmacokinetics of pomegranate constituents

Various research studies have been conducted to determine the bioavailability and pharmacokinetics of pomegranate in both preclinical and clinical settings. These studies focused mostly on the metabolites of ellagitannins, punicalagin and ellagic acid.

When rats were provided with a diet containing 6% punicalagin (derived from pomegranate husk) for 37 days, their plasma became saturated on the 7th day of the study with a mean

concentration of 29 μg/mL of punicalagin. Three isomers of punicalagin were found in the plasma. The researchers also detected five punicalagin metabolites, such as two ellagic acid derivatives, gallagic acid, 3,8-dihyroxy-6H-dibezo[*b,d*]pyran-6-one glucuronide and 3,8,10-trihyroxy-6H-dibezo[*b,d*]pyran-6-one, in the kidney and liver [180]. In another study by the same group, rats were fed 6% punicalagin for 37 days to evaluate the bioavailability and metabolism of this pomegranate phytochemical. In plasma, the main detectable entities were punicalagin as well as glucuronides of methyl ether derivatives of ellagic acid. Only 3-6% of ingested punicalagin was detected in the pure or metabolic form in urine and feces. The main urinary metabolites were aglycones or glucuronic conjugates of dihydroxy and trihydroxy-6H-dibenzo[*b,d*]pyran-6-one. The feces contained hydrolyzed metabolites of punicalagin, including aglycones, gallagic acid, ellagic acid, and punicalin, along with microflora ellagic acid derivative, such as 3,8-dihyroxy-6H-dibezo[*b,d*]pyran- 6-one. Additionally, this study noted two stages of metabolism of punicalagin in rats. In the first stage, hydrolyzed and conjugated punicalagin were the main metabolites. In the second stage, the gut microflora metabolites predominated and were present in the feces as well as their conjugated products in the plasma [181].

A later study used pigs that were fed with ellagitannins over a period of 177 days to determine the bioavailability and metabolism of these compounds. The results demonstrated that ellagitannins released ellagic acid in the jejunum and subsequently intestinal flora metabolized ellagic acid sequentially to produce tetrahydroxy- (urolithin D), trihydroxy- (urolithin C), dihydroxy- (urolithin A), and monohydroxy- (urolithin B) dibenzopyran-6-one metabolites which were absorbed from the intestinal walls. The study also explains why urolithins persist for elevated periods of time and equates this fact to enterohepatic circulation. Moreover, the presence of ellagic acid metabolites in bile and urine and its absence in intestinal tissues indicates its absorption in the

stomach. In addition, there was no organ accumulation of ellagitannins in lung, liver, heart, kidney, muscle, or adipose tissues in pigs [182].

Seeram et al. [183] conducted one of the first clinical studies to investigate the bioavailability of ellagitannins and ellagic acid. One human subject consumed 180 mL pomegranate juice that contained 318 mg punicalagings (major pomegranate ellagitannins) and 25 mg ellagic acid. While no intact ellagitannins were found in the plasma, ellagic acid was detected and reached a maximum concentration of 31.9 ng/mL at 1 h following consumption. Interestingly, ellagic acid was no longer detected in the subject's plasma after 4 h. In a follow-up study, the same research group [184] explored the pharmacokinetics of ellagitannins in 18 healthy volunteers who consumed 180 mL of pomegranate juice that contained punicalagins (387 mg/L) and ellagic acid (12 mg/L) one time and blood and urine samples were taken at different time intervals. Ellagic acid was detected in plasma samples of all human subjects with a maximum concentration (C_{max}) of 0.06±0.01 mmol/L, area under the concentration-time curve (AUC) of 0.17±0.02 (μmol·h)·L^{-1}, a time of maximum concentration (t_{max}) of 0.98±0.06 h and elimination half-life ($T_{1/2E}$) of 0.71±0.08 h. The major ellagic acid metabolites found in plasma and urine were dimethylellagic acid glucuronide and urolithin derivatives. Although ellagic acid was no longer detected in the plasma 5 h after ingestion, urolithin metabolites were still found in the urine 48 h after ingestion. Therefore, urolithins might contribute to long-term therapeutic effects of pomegranate juice consumption beyond the effects exerted by ellagitannins and ellagic acid. The same investigators [185] expanded the previous studies and compared bioavailability of polyphenols from various pomegranate products. Sixteen healthy volunteers sequentially consumed, with one-week washout period between treatments, pomegranate juice, a polyphenol liquid extract preparation and polyphenol powder extract, containing 857, 776 and 755 mg of polyphenols as gallic acid

equivalents, respectively. The bioavailability based on plasma ellagic acid levels over a 6 h period did not exhibit statistical differences in AUC for three products. Similar results were observed with the level of urinary urolithon-A glucuronide. However, the t_{max} was delayed for the powder extract (2.58±0.42 h) compared to juice (0.65±0.23 h) and liquid extract (0.94±0.06 h).

Cerdá and colleagues [186,187] conducted several clinical studies to evaluate the bioavailability and metabolism of pomegranate juice ellagitannins. In one study, six healthy participants (four men and two women) drank 1 L of pomegranate juice containing 5.58 g/L of polyphenols, including 4.37 g/L of punicalagin isomers, for 5 days. Intact punicalagin and ellagic acid present in the juice did not show in the plasma or the urine of participants. However, the plasma contained three microbial ellagitannin metabolites, the main metabolite being a derivative of urolithin A. The urine contained six different metabolites and the main metabolite was also the same derivative of urolithin A [186]. In another study, fecal samples donated by six volunteers were incubated with ellagic acid and punicalagin under anaerobic conditions. Urolithin A was produced from both ellagic acid and punicalagin after fermentation in all the fecal cultures after 24 h. Urolithin A was produced with varying production rates and concentration in various fecal samples, indicating different composition of fecal microflora in individuals [187].

The absorption and metabolism of pomegranate polyphenols were examined on 11 healthy human subjects fed 800 mg of standardized pomegranate extract in a capsule with 330.4 mg of punicalagins and 21.6 mg of ellagic acid. The plasma samples were examined and five major metabolites, namely urolithin A, hydroxy-urolithin A, urolithin A-glucuronide, urolithin B and dimethyl ellagic acid-glucuronide were detected. Like the aforementioned study, the levels of these metabolites varied between subjects and between time intervals. The pharmacokinetic analysis revealed a C_{max} of 31.9 ng/ml at one hour and a t_{max} of 33.8±12.7 ng/mL at 1 h [188]. Very recently,

García-Villalba and colleagues [189] identified four previously unknown urolithins, namely 4,8,9,10-tetrahydroxy urolithin (urolithin M6R), 4,8,10-trihydroxy urolithin (urolithin M7R), 4,8,9-trihydroxy urolithin (urolithin CR), and 4,8-dihydroxy urolithin (urolithin AR), in human feces following daily intake of a pomegranate extract (160-640 mg phenolics). The new metabolites were identified only in 19% of the subjects. Moreover, phase II conjugates of the novel urolithins were detected in urine, confirming their absorption, circulation, and urinary excretion. The endogenous production of these "R" urolithins can be considered an additional metabolic feature for volunteer stratification.

Based on the results obtained from the aforementioned studies, major pomegranate phytochemicals are absorbed from the gastrointestinal tract and become bioavailable. The main metabolic products of ellagitannins appear to be urolithins which are formed by gut microflora and the difference in gut microflora also explain the contrasting concentrations and the rate of formation in individuals. Nevertheless, further research needs to be conducted regarding the effects of different compositions of microflora on urolithin formation. Moreover, long-term analysis needs to be carried out to determine the effects of prolonged metabolite formation. The metabolites of ellagitannins may be responsible for the long-term therapeutic effects of pomegranate, including its anticancer potential. However, more studies must be conducted to validate this premise.

5. Toxicity studies

Pomegranate is a fruit that has been consumed as well as used for its medicinal properties since ancient times. Due to this fact, pomegranate has been generally regarded as safe for utilization in humans. Numerous studies using micriorganisms, animals and human have been conducted regarding the toxicity of various pomegranate products, such as pomegranate juice,

whole pomegranate extract, pomegranate seed oil, pomegranate emulsion and pomegranate ellagitannins [190].

5.1. In vitro

Vidal and colleagues [62] performed one of the earliest studies to evaluate the toxicity of a hydroalcoholic extract of whole pomegranate fruit used in Cuban traditional medicine. Using a chick embryo model, the investigators showed that the extract at a level below 0.1 mg per embryo did not exhibit any toxicity. The genotoxicity of a pomegranate whole fruit hydroalcoholic extract was assessed using various *in vitro* assays to detect DNA damage at different expression levels. The results indicated genotoxic effects of the extract based on point reverse-mutation and mitotic gene-conversion test in microorganisms as well as sister chromatid exchange and chromosomal aberration assays in Chinese hamster ovary cells [191]. Meerts and colleagues [192] conducted a series of *in vitro* experiments to evaluate the toxicity of pomegranate seed oil (PSO) and showed no mutagenicity based on the Ames test as well as chromosomal aberration test in human lymphocytes. Similarly, Heilman et al. [193] indicated that urolithin A, a predominant urolithin isoform found in the plasma and urine following the comsumption of ellagitannis and ellagic acid, was not genotoxic.

5.2. In vivo

In OF-1 mouse model, the median lethal dose (LD_{50}) of a pomegranate fruit extract following intraperitoneal administration to both sexes was found to be 731 mg/kg body weight. In male

Wistar rats, a repeated intranasal administration, at a dose of 0.4 or 1.2 mg/kg, produced no toxic effects as evidenced from food intake, weight gain, behavioral observations as well as biochemical and histopathological indices [62]. Patel et al. [194] systematically examined the acute and subchronic toxicity of whole pomegranate fruit extract on mouse and rat models. The extract was standardized to contain 70% polyphenols, including 30% punicalagins, 5% ellagic acid and 0.3% gallic acid. In the acute toxicity study, the oral LD_{50} of the extract was found to be greater than 5 g/kg body weight and the intraperitoneal LD_{50} of the extract was 187.5 mg/kg (Swiss albino mice) and 217.5 mg/kg (Wistar rats). In the subchronic study, oral administration of the extract up to 600 mg/kg body weight/day for 90 days in rats did not lead to any adverse effects based on general observations, ophthalmic examinations, growth patterns, food consumption, organ weights and histopathological assessments. Based on the experimental results, the no observed-adverse-effect level (NOAEL) of the extract was determined as 600 mg/kg body weight/day. Considering the fact that a 6 oz of pomegranate juice contains 266-337 mg of punicalagin, 32-40 times lower than the highest dose tested in this study, it can be concluded that human consumption of pomegranate juice should have no toxicity. Interestingly, another study indicated genotoxic effect of a whole fruit extract based on bone marrow micronucleus assay in Balb/C mice [191]. However, an ethanolic extract of pomegranate fruit or leaf did not exhibit any mutagenic effects in Swiss mice [195].

The acute oral toxicity study in female Wistar rats revealed devoid of toxic effects of PSO at 2,000 mg/kg body weight. Dietary administration of PSO at 10,000, 50,000 or 150,000 ppm over a 28-day period elicited no adverse effects or mortality. However, in the 150,000-ppm group, both male and female rats experienced elevated hepatic enzymes (alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase) in plasma and increased liver to body weight

ratio. These effects were ascribed to very high fatty acid level in diet rich in PSO without any toxicological relevance. The NOEL in this study was 50,000 ppm PSO (4.3 g/PSO/kg/day) [192].

Oral administration of a pomegranate emulsion (10 g/kg body weight), three times a week, for 22 weeks in male Sprague-Dawley rats was devoid of any toxic manifestation as evidenced from growth pattern as well as general observations related to food and water intake and behavioral changes. Additionally, assessment of cardiac performance by transthoracic echocardiography did not reveal any cardiotoxicity in rats [120]. The same extract at 5 g/kg body weight (p.o., three times a week) for 18 weeks did not exhibit any toxicity in female Sprague-Dawley rats [99] as observed previously in male counterparts [120].

Various pomegranate phytoconstituents and metabolites have been subjected to safety assessment by different laboratories. A study conducted by Cerdá et al. [180] found no adverse effects of a diet containing 6% punicalagin in rats over the course of 37 days. The dose of punicalagin was increased weekly and the average daily intake of punicalagin was 4.8 g/kg of body weight per day. The authors concluded that this was comparable to a 70 kg human consuming 194 L per day of pomegranate juice. No histological abnormalities were noted in the liver or the kidney. Similarly, oral administration of ellagitannins in Sprague-Dawley rats over a time period of 96 days did not result in adverse effects or significant differences in eating patterns, growth rates, blood parameter values, and histopathological characteristics of liver and kidney [196]. Heilman et al. [193] described the safety profile of direct oral administration of urolithin A to Wistar rats. A diet containing urolithin up to 5% for 28 or 90 days did not show any alteration in growth pattern as well as blood chemistry and hematology and urinalysis results and also did not indicate any target organ toxicities. The NOAEL was found to be the highest dose tested (i.e., 5% urolithin in diet) or 3,451 and 3,826 mg/kg body weight/day in male and female rats, respectively.

5.3. Clinical studies

Several clinical studies evaluated the safety aspects of pomegranate-derived products in humans. Pomegranate juice (8 oz) consumption by men with rising PSA level following primary therapy did not produce any serious adverse effects and the treatment was well tolerated [173]. A randomized, multicenter, double-blind, phase II study evaluated the effects of pomegranate extract on men with elevated PSA levels. The researchers did not find any toxic effects of the extract. However, diarrhea was reported as a side effect which occurred in 1.9% and 13.5% of participants taking 1 and 3 g dose, respectively [175]. A follow-up study also reported a lack of serious adverse events in pomegranate extract-treated group as well as in placebo group. Both group experienced nausea and diarrhea [176]. The safety and tolerability of short-term or long-tern consumption of pomegranate juice or extract have been confirmed by other investigators [178,179,197-199]. Heber et. al. [200] tested the safety of pomegranate ellagitannin-enriched polyphenol extract (POMx) in overweight individuals in their pilot clinical studies. Participants consumed 0.710 mg, or 1,420 mg of POMx in a capsular form and there were no significant toxic effects reported in any test subjects.

5.4. Assessment of safety profile of pomegranate

The multitude of animal studies suggests that there should be minor to no adverse or toxic effects of pomegranate when used in humans. Minor side effects were found in human clinical trials, such as nausea and diarrhea. In addition, most of the side effects in the animal studies were attributed to components other than the pomegranate compounds. However, given the fact that the

aforementioned study by Sanchez-Lamar et al. [191] concluded that whole pomegranate does have mutagenic effects at high concentrations, additional studies need to be conducted on the individual components of pomegranate to determine which components have the greatest mutagenic effects. Further human clinical studies, specifically regarding the toxicity of prolonged use of pomegranate, need to be conducted in order to fully conclude the safety of pomegranate use in humans.

6. Conclusion and future directions

Known as "a phytochemical reservoir of heuristic medicinal value" [4], pomegranate has acquired a prominent role in traditional medicines for the treatment of various disorders. During the last few decades, pomegranate constituents have been extensively studied for their ability to prevent and treat cancer, establishing its role as a chemopreventative and chemotherapeutic agent with high potential. Nevertheless, the full potential pomegranate in cancer prevention and therapy as well as its molecular targets in cancer are not completely understood. This work analyzed available preclinical *in vitro* and *in vivo* results as well as clinical studies and presented a systematic review of available evidences on anticancer effects of pomegranate-derived constituents with an emphasis on their various molecular targets.

We have found that pomegranate constituents exhibit cytotoxic and antiproliferative effects against numerous cancer cells. In addition, pomegranate-based products suppressed the development and reduced the growth of chemically-induced or xenografted tumors in rodents. The anticancer effects of pomegranate phytochemicals could be mediated through antioxidant, anti-inflammatory, immune-modulatory, cell death-inducing (apoptosis and autophagy), anti-invasive, antimigratory and antiangiogenic effects as well as modulation of various oncogenic and

oncosuppressive signaling pathways (Fig. 8). Based on studies presented here, it is highly likely that numerous pomegranate phytochemicals exhibited synergistic effect in inhibiting the proliferation of tumor cells and/or suppressing the development and growth of tumors. Emerging evidence suggests that bioactive plant phytochemicals exert cancer preventive and anticancer effects when they are used in combination rather than individually. Hence, it plausible that pomegranate phytochemicals may confer the observed chemopreventive and anticancer activities via promotion of multifactorial effects utilizing chemical synergy. Interestingly, pomegranates phytochemicals not only act synergistically with its own phytochemicals, but also with other phytochemicals and even with other established chemotherapeutic drugs to confer superior anticancer action to that of any single compound, providing strong evidence of pomegranate's powerful potential as chemotherapy adjunct. In clinical trial, there was clear evidence that oral ingestion of pomegranate extract result in the accumulation of its active metabolite in various target organs in prostate and colorectal cancer. Furthermore, several cancer-related molecular markers were shown to be modulated by oral intake of pomegranate extract, supporting the preclinical findings. However, due to various limitations in clinical studies, many studies have also reported no significant differences between pomegranate-treated and placebo groups. Yet subsequent subgroup analysis has reported significant effect with pomegranate therapy, thus suggesting that certain population of cancer patient may benefit more from pomegranate's therapeutic activity. This warrant the need for more *in vivo* and clinical data before definite conclusions can be made.

Moreover, we have identified several limitations within the current field pomegranate research. First, the poor bioavailability of pomegranate phytochemicals may limit therapeutic outcome. Several clinical studies reported that the poor bioavailability of pomegranate phytochemicals after

ingestion of pomegranate supplement can contribute to the disconnection between the observed preclinical and clinical effects. Secondly, most of the *in vitro* studies are carried out using breast, prostate and gastrointestinal tract cancer cells, suggesting the need to diversify and investigate the effects of pomegranate on various other types of cancer. Lastly, few clinical studies are conducted with limited sample size and cancer endpoints; moreover, some preclinical results (anticancer effects and mechanisms) failed to be replicated in clinical trials. Despite the limitations, pomegranate remains to have high potential as a source of as chemotherapeutic and chemopreventive drugs. Based on various safety assessment data, pomegranate products seem to be nontoxic *in vitro*, in *vivo,* and clinically. This suggest that pomegranate research remains extremely valuable as an alternative to traditional chemotherapy to improve efficacy and reduce severity of side effects.

Based on the current limitations we have identified, we recommend future investigations to explore the anticancer synergistic effects of pomegranate phytochemicals, compare the effects of pomegranate against cancer across multiple organ systems, identify additional anticancer molecular targets of pomegranate to explain the various anticancer effects of pomegranate, conduct randomized clinical trials with expanded sample size, and analyze cancer endpoints and biomarkers. Improving the bioavailability of pomegranate phytochemicals should also be investgated by using novel formulations and advanced drug delivery systems, such as micelles, liposomes, nanoparticles and nano-emulsions to further potentiate the observed preclinical effects of pomegranate in clinical trials. Additionally, more research should be directed to evaluation of anticancer potential of metabolic products of pomegranate phytochemicals as well as agents derived by colonic microbial metabolism. Based on the overwhelming evidence presented and analyzed in this review, pomegranate constituents evidently remain extremely promising as both

chemopreventative and chemotherapeutic agents. This warrants the need for future investigators to further optimize previous studies to establish the full therapeutic potential of this complex fruit to combat a complex disease, such as cancer.

Conflict of interest

Authors declare no conflicts of interest.

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Legends to figures:

Fig. 1. Polyphenols present in pomegranate fruit.

Fig. 2. Anthocyanins present in pomegranate fruit.

Fig. 3. Lipids and sterols present in pomegranate seeds.

Fig. 4. Phytochemicals present in pomegranate leaves.

Fig. 5. Alkaloids present in pomegranate root.

Fig. 6. Phytochemicals present in pomegranate flowers.

Fig. 7. PRISMA flow chart describing the process of literature search and study selection related to pomegranate in cancer research. The total number of *in vitro*, *ex vivo, in vivo* and clinical studies (160) is greater than the number of studies included in our work (151) because several publications reported results from more than one study type (e.g., *in vitro* and *in vivo* or *in vitro* and *ex vivo*).

Fig. 8. Major anticancer mechanisms and molecular targets of pomegranate-derived constituents based on *in vitro* and *in vivo* studies.

Table 1.
In vitro and effects of pomegranate extracts, fractions and pure compounds on various cancers.

Materials tested	Cell lines used	Anticancer effects	Mechanisms	Conc. & time	References
Breast cancer					
Fermented juice polyphenols, fresh juice polyphenols, pericarp polyphenols, seed oil	MCF-7, MDA-MB-231, MDA-MB-435	Inhibited cell proliferation and invasion	↑Apoptosis; ↓VEGF; ↑MIF	50-350 μg/mL; 4 h	Kim et al. [66]; Toi et al. [67]
Pomegranate extract (with or without genistein)	MCF-7	Caused cytotoxicity and reduced cell survival	↑Apoptosis	1-6 μg/mL; 24, 72 h	Louis Jeune et al. [68]
Aqueous fruit extract	MDA-MB-231, SUM 149	Suppressed proliferation, invasion and motility	↑Apoptosis; ↓NF-κB p65; ↓NF-κB p50; ↓RhoA; ↓RhoC	50-200 μg/mL; 1-5 days	Khan et al. [69]
Pomegranate juice (PJ) or luteolin (L)+ellagic acid (E)+punicic acid (P)	MCF-7, MDA-MB-231	Inhibited cell growth, stimulated adhesion, suppressed migration and chemotaxis	↑E-cadherin; ↓HMMR; ↓TWIST; ↓IL-8; ↓RANTES; ↓PDGFB	PJ: 1, 5% L+E+P: 1-8 μg/mL; 12-72 h	Rocha et al. [70]
Aqueous fruit extract	HMLER; Hs578T	Inhibited formation and promoted differentiation of mammosphere; inhibited cell migration and increased adhesion	↓TWIST1; ↓HMMR; ↓PI3KCA; ↓AKT1; ↓JNK1; ↓JNK2; ↓ALDH1; ↑CLDN4	5, 10 μg/mL; 48 h	Nallanthighal et al. [71]
Methanolic extract of fruit peel	MDA-MB-231	Inhibited cell migration and invasion	↑Apoptosis; ↑ICAM-1; ↓MMP-9; ↓fibronectin; ↓VEGF; ↓vimentin; ↓ZEB1; ↓β-catenin;	12.5-1,000 μg/mL; 24-72 h	Ahmadiankia et al. [72]; Bagheri et al. [73]

			↑E-cadherin		
Pomegranate ellagitannin-derived compounds	MCF-7aro	Inhibited cell proliferation	↓Aromatase	1-5 µmol/L; 48 h	Adams et al. [74]
Conjugated fatty acids from seed oil	MCF-7; MDA-MB-231	Exhibited antiproliferative effects	↓ERα; ↓ERβ (MCF-7)	50-100 µM; 5 days	Tran et al. [75]
Methanolic extract of pericarp	MCF-7	Reduced cell viability and proliferation	↓E2 binding to ER; ↓pS2; ↓ERα; ↓PR	10-320 µg/mL; 48 h	Sreeja et al. [76]
Methanolic extract of pericarp	MCF-7	Inhibited 27HC-induced cell proliferation	↓pS2; ↓ERα; ↓PR; ↓NCOR1	100 µg/mL; 48 h	Vini et al. [77]
Polysaccharide from fruit rind (PSP001)	EAC	Exhibited cytotoxic activity		0.001-1,000 µg/mL; 24, 48 h	Joseph et al. [78].
Pomegranate extract	MMTV-Wnt-1 cells	Inhibited cell proliferation and viability	⊥G_0/G_1; ↑caspase-3	10-200 µg/mL; 24-96 h	Dai et al. [79]
Pomegranate juice-derived ellagitannins	BT-549	Inhibited cell proliferation		IC_{50}=47-198 µM; 48 h	Kasimsetty et al. [80]
Pomegranate extract (with or without tamoxifen)	MCF-7	Inhibited cell viability	↑Apoptosis; ↑Bax; ↓Bcl-2; ⊥G_0/G_1	300 µM; 48 h	Banerjee et al. [81]
Methanolic extract of fruit peel	MCF-7	Reduced cell proliferation	↑Apoptosis; ↑Bax; ↓Bcl-2; antioxidant activity	25-300 µg/mL; 24-72 h	Dikmen et al. [82]
Methanolic extract of fruit peel	MCF-7	Exhibited antiproliferative effect		5-1,000 µg/mL; 72 h	Modaeinama et al. [83]

Acetone and methanolic extracts of fruit peels	MCF-7; MDA-MB-231	Reduced tumor cell viability	Antioxidant activity	5-960 µg/mL; 72 h	Fazio et al. [84]
Polyphenol-rich extract of fruit peel	MCF-7; MCF-7/DX	Inhibited cell growth	↑Apoptosis; antioxidant activity	1-75 µg/mL; 24 h	Moreira et al. [85]
Polysaccharide from fruit rind (PSP001)	MCF-7	Exerted cytotoxic effect	Antioxidant and immunomodulatory activities	0.001-1,000 µg/mL; 24-72 h	Joseph et al. [86]
Punicalagin from pomegranate husk	MCF-7	Displayed antiproliferative activity	↓Oxidative DNA damage	12.5-200 µg/mL; 72 h	Aqil et al. [87]
Extract of pomegranate fruit skins (POMX)	MCF-7	Inhibited cell growth	↑Apoptosis; ⊥G_2/M; ↓DNA repair; ↓HR genes; ↑miRNA-24; ↑miRNA-132; ↑miRNA-183	20-100 µg/mL; 24-96 h	Shirode et al. [88]
Polyphenolic extract of pomegranate juice	MDA-MB-231, BT-474	Inhibited proliferation	↑Apoptosis; ↓sp1; ↓sp3; ↓sp4 ↓survivin; ↓VEGF; ↓VEGFR-1; ↓NF-κB; ↓miR-27a; ↓miRNA-155; ↑ZBTB10; ↑SHIP-1; ↓pPI3K; ↓pAkt	2.5-50 µg/mL; 48 h	Banerjee et al. [89]
Hydrophilic fraction of seed oil	MCF-7; MDA-MB-231	Decreased cell viability	↑G_0/G_1; ↓VEGF; ↓IL-2; ↓IL-6; ↓IL-12; ↓IL-17; ↓CXCL10; ↓MIP-1α; ↓MIP-1β; ↓MCP-1; ↓TNF-α	0.12-0.6 µL 24 h	Costantini et al. [90]
Ethanolic extract of seeds	MCF-7	Exerted antiproliferative activity	Antioxidant activity	5-100 µg/mL; 48 h	Lucci et al. [91]
Methanolic extract of seeds	MCF-7	Registered growth inhibition		5-1,000 µg/mL;	Seidi et al. [92]

				72 h	
Ethanolic flower extracts	MCF-7	Exhibited cytotoxic activities	Antioxidant activity; ↓5-LOX	IC50 = 33-35 mg/L; 48 h	Bekir et al. [93]
PLGA-PEG NPs containing pomegranate extract, punicalagin or ellagic acid	MCF-7; Hs578T	Reduced cell growth		1-10 µg/mL; 96 h	Shirode et al. [94]
Ag-NPs of pomegranate extract	MCF-7	Inhibited proliferation	↑Apoptosis	2.5-50 µg/mL; 1-3 days	Sahin et al. [95]
Ag-NPs of pomegranate extract	MCF-7	Inhibited proliferation	↑Apoptosis	2.5-50 µg/mL; 1-3 days	Sahin et al. [96]
SL-NPs of pomegranate extract	MCF-7	Exhibited cytotoxicity		1-500 µg/mL; 24 h	Badawi et al. [97]
Gastrointestinal tract and associated cancers					
Pomegranate juice, tannin extract, ellagic acid and punicalagin	KB, CAL27 (oral)	Displayed antiproliferative activity		12.5-100 µg/mL; 48 h	Seeram et al. [103]
Pomegranate juice-derived ellagitannins	KB (oral)	Inhibited proliferation		IC_{50}=153-332 µM; 48 h	Kasimsetty et al. [80]
Pomegranate polyphenolic extract	CAL27, HSC-2, SCC 1483 (oral)	Displayed cytotoxic effects	↑Caspase-3; ↑PARP cleavage; ↓GSH	25-250 g/mL; 24 h	Weisburg et al. [104]

Pomegranate juice, tannin extract, ellagic acid and punicalagin	HT-29, HCT116, SW480, SW620 (colon)	Displayed antiproliferative activity	↑Apoptosis (HT-39 & HCT116)	2.5-100 µg/mL; 48 h	Seeram et al. [103]
Pomegranate juice-derived ellagitannins	HT-29 (colon)	Inhibited cell proliferation and clonogenic efficiency	↑Caspase-3; $\perp G_0/G_1$; $\perp G_2/M$	IC_{50}=123-462 µM; 24-72 h	Kasimsetty et al. [80]
Pomegranate extract	HT-29 (colon)	Reduced cell viability	↑Caspase-3; ↑PARP cleavage; ↓COX-2; ↓VCAM-1; ↓NF-κB p65; ↓VEGF; ↓pAkt; ↑miR-126	5- 25 µg/mL; 24-72 h	Banerjee et al. [105]
Peel-derived ellagitannins (PET); PET+5-FU	HT-29 (colon)	Exhibited cytotoxicity	⊥S; dissipation of mitochondrial membrane potential; ↑Bax:Bcl-2; ↑caspase-3; ↑caspase-9	80 µg/mL; 80 µg/mL PET+40 µg/mL 5-FU; 48 h	Chen et al. [106]
Galactomannan polysaccharide (PSP001) from peel	HCT116 (colon)	Induced cancer cell cytotoxicity	↑Apoptosis	0.001-1.000 µg/mL; 72 h	Joseph et al. [78]
PSP001; SNP@PSP	HCT116 (colon)	Displayed cytotoxic and antimigratory effects	↑Apoptosis; ↑caspase-2; ↑caspase-3; ↑caspase-8; ↑caspase-9	0.01-200 µg/mL; 24-72 h	Padinjarathil et al. [107]
Punicalagin extracted from peel	Caco-2 (colon)	Produced cytotoxic effect	↑Apoptosis; ⊥S; ↓cyclin A; cyclin B1; ↑cyclin E; ↓Bcl-XL; ↑ caspase-9; ↑caspase-3; ↑cyt. c	1-100 µmol/L; 72 h	Larrosa et al. [108]
Pomegranate peel extract	LOVO; LOVO DX (colon)	Displayed antiproliferative activity	↑Apoptosis	1-75 µg/mL; 48 h	Moreira et al. [85]

Acetone and methanolic extracts of fruit peels	RKO (colorectum)	Exhibited antiproliferative activity		5-960 µg/mL; 72 h	Fazio et al. [84]
Peel derived silver nanoparticles	RKO (ATCC CRL-2577) (colorectum)	Exerted cytotoxic effect	↑Autophagy	0.3-100 µg/mL; 2-5 days	Devanesan et al. [109]
Methanolic extract of pomegranate seed oil	HT29, HCT 116 (colon)	Displayed marginal effect		0.12-0.60 µL; 24 h	Costantini et al. [90]
Methanolic extract of pomegranate seed oil	HepG2; Huh7 (liver)	Displayed marginal effect		0.12-0.60 µL; 24 h	Costantini et al. [90]
Galactomannan polysaccharide (PSP001) from peel	HepG2 (liver)	Registered cytotoxicity	↑Apoptosis	0.001-1.000 µg/mL; 72 h	Joseph et al. [78]
PSP001; SNP@PSP	HepG2 (liver)	Exhibited cytotoxicity and antimigratory effects	↑Apoptosis; ↑caspase2; ↑caspase-3; ↑caspase-8; ↑caspase-9	0.01-200 µg/mL; 24-72 h	Padinjarathil et al. [107]
Pomegranate juice and extracts of peel, flesh, seeds and leaf	HepG2 (liver)	Inhibited proliferation		20-80 µg/mL; 48 h	Li et al. [117]
Polyphenol-rich ethanolic peel extract	HepG2 (liver)	Reduced cell viability	↑Apoptosis; ⊥S; ↑ROS; ↑caspase-3; ↑caspase-9; ↑cyt. c; ↑Bax; ↑p53; ↓Bcl-2	100-300 µg/mL; 24 h	Song et al. [118]
Silver nanoparticles of aqueous leaf extract	HepG2 (liver)	Induced cell mortality	Antioxidant activity	5-200 µg/mL; 24 h	Saratale et al. [119]
SL-NPs of pomegranate extract	HepG2 (liver)	Exhibited cytotoxicity		1-500 µg/mL; 24 h	Badawi et al. [97]
Pomegranate whole fruit extract; ellagic	PANC-1,	Inhibited proliferation	↑G_0/G_1; ↓G_2; ↓CD44; ↓CD24	50–250 µg/mL;	Nair et al. [124]

acid, luteolin and ursolic acid	AsPC-1 (pancreas)			24-96 h	
Gynecological cancers					
Polyphenol-rich fruit extract	HeLa (cervix)	Exerted marginal effect on cell viability		50 µg GAE/mL; 72 h	McDougall et al. [125]
Methanolic extract of pericarp	HeLa, SiHa (cervix); HEC-1A (endometrium)	Registered growth-inhibitory effects (SiHa only)		20-320 µg/mL; 48 h	Sreeja et al. [76]
Pomegranate juice and extracts of peel, flesh, seeds and leaf	HeLa (cervix)	Inhibited proliferation		20-80 µg/mL; 48 h	Li et al. [117]
Acetone and methanolic extracts of fruit peels	HeLa (cervix); Ishikawa (endometrium)	Exhibited antiproliferative activity	↑Apoptosis; ↑caspase-3; ↑caspase-7; ↑caspase-9; ↑PARP cleavage (HeLa only)	5-960 µg/mL; 72 h	Fazio et al. [84]
Ellagic acid from pomegranate peel extract	HeLa (cervix)	Suppressed cell invasion	↑Apoptosis; ↑IGFBP7; ↓Akt; ↓mTOR	2.5 - 10.0 uM; 24 h	Guo et al. [126]
Punicalagin from pomegranate husk	HeLa, CaSki, SiHa (cervix)	Displayed antiproliferative activity	Antioxidant activity	12.5–200 µg/ml; 72 h	Aqil et al. [87]
Punicalagin	HeLa (cervix)	Suppressed proliferation and migration	↑Apoptosis; ↓Bcl-2; ↑Bax; ⊥G1; ↓β-catenin; ↓cyclin D1; ↓c-myc; ↓MMP-2; ↓MMP-9; ↑TIMP-2; ↑TIMP-3	12.5- 200 µM; 24-48 h	Tang et al. [127]

Methanolic extract of pericarp	SKOV3 (ovary)	Exhibited marginal cytotoxicity		20-320 µg/mL; 48 h	Sreeja et al. [76]
Pomegranate peel extract	SKOV3 (ovary)	Exerted cytotoxicity		5 -1,000 µg/ml; 72 h	Modaeinama et al. [83]
Pomegranate juice-derived ellagitannins	SKOV3 (ovary)	Inhibited cell proliferation		IC_{50}=44-222 µM; 48 h	Kasimsetty et al. [80]
Methanolic extract of pomegranate seeds	SKOV3 (ovary)	Inhibited cell growth		5-1,000 µg/mL; 72 h	Seidi et al. [92]
Pomegranate fruit juice (PFJ), ellagic acid, and luteolin	A2780 (ovary)	Displayed antiproliferative and antimigratory activities	↓MMP-2; ↓MMP-9	5%, 10% (PFJ), 5-15 mg/mL (ellagic acid & luteolin); 12-48 h	Liu et al. [128]
Hematological cancers					
Aqueous extract of peels	Raji, P3HR-1 (Burkitt's lymphoma)	Reduced cell viability	↑Apoptosis	1.3-6.3 µl/mL; 24, 48 h	Settheetham and Ishida [129]
Galactomannan polysaccharide (PSP001) from peel	Dalton's lymphoma	Exerted cytotoxicity	↑Apoptosis	0.001-1,000 µg/ml; 24, 48 h	Joseph et al. [78]
Fresh and fermented pomegranate juice; pomegranate pericarp extract	HL-60 (APL)	Displayed antiproliferative activity	↑Differentiation	100-400 µg/mL; 3 days	Kawaii & Lansky [130]

Pomegranate juice extract	Jurkat (T-cell leukemia); SUP-B14 (ALL); MOLT-3 (ALL); CCRF-CEM (ALL); HL-60 (APL); THP-1 (AMCL); K562 (CML); KG1a (AML)	Displayed antiproliferative activity	↑Apoptosis; ⊥G_0/G_1	6.25%, 12.5% (v/v); 24, 48 h	Dahlawi et al. [131]
Acetonitrile fraction from pomegranate juice	CCRF-CEM (ALL); MOLT-3 (ALL); HL-60 (APL); THP-1 (AMCL)	Reduced cell viability	↑Apoptosis; ↓ATP; ⊥S; ↑caspase-3	6.25-25% (v/v); 48 h	Dahlawi et al. [132]
Pomegranate peel extract (PPE) and PPE-gelatin NPs	HL-60 (PML)	Exhibited differential effects on early and late stage apoptosis		0.0156-0.125 µg/mL; 22 h	Li et al. [133]
Galactomannan polysaccharide (PSP001) from peel	K562 (CML)	Exerted cytotoxicity		0.001-1,000 µg/mL; 24-72 h	Joseph et al. [86]
Pomegranate peels extract	K562 (CML)	Displayed antiproliferative activity	↑Apoptosis; ⊥G_2/M; ↑caspase-9; ↑caspase-3; ↑caspase-7; ↑cyt. c; ↑$p21^{WAF1}$; ↑p53; ↓Bcl-2; ↓Bax; ↑Akt	100-800 µg/mL; 72 h	Asmaa et al. [134]
Extracts of pomegranate leaves, stems, flowers	U266 (MM)	Decreased proliferation	↑Apoptosis; ⊥G_2/M phase; ⊥S; ↓MMP	1-500 µg/mL; 48, 72 h	Kiraz et al. [135]

Pomegranate juice with or without BTZ	U266, KMS26, MM1S (MM)	Inhibited proliferation	$\perp G_0/G_1$; ↑PPARγ	2-12% (v/v); 24 h	Tibullo et al. [136]
Lung and respiratory tract cancers					
Pomegranate fruit extract	A549	Reduced cell viability	$\perp G_0/G_1$; ↑$p21^{WAF1}$; ↑$p27^{KIP1}$; ↓Ki-67; ↓PCNA; ↓cyclin D1; ↓cyclin D2; ↓cyclin E; ↓cdk2; ↓cdk4; ↓pMAPK; ↓PI3K; ↓pAkt; ↓NF-κB; ↓IKKα; ↓IκBα; ↓NF-κB-DNA binding	50-150 μg/mL; 2 h	Khan et al. [137]
Methanolic extract of peel	A549	Displayed antiproliferative activity		5 -1,000 μg/mL; 72 h	Modaeinama et al. [83]
Methanolic extract of seeds	A549	Exerted cytotoxicity		5- 1,000 μg/mL; 72 h	Seidi et al. [92]
PSP001 polysaccharide	K562	Inhibited cell growth	Antioxidant activity	0.001-1,000 μg/mL; 72 h	Joseph et al. [86]
SNP@PSP	A549	Exhibited cytotoxic and antimigratory effects	↑Apoptosis; ↑caspase-2; ↑caspase-3; ↑caspase-8; ↑caspase-9	0.01-200 μg/mL; 24-72 h	Padinjarathil et al. [107]
Leaf extract	A549, H1299, LL2	Inhibited proliferation and colony formation; suppressed migration and invasion	↑Apoptosis; $\perp G_2/M$; ↓ROS; ↓$\Delta\Psi m$; ↓MMP-2; ↓MMP-9	6.25-200 μg/mL; 24-72 h	Li et al. [138]

Punicalagin	A549, Hep-2	Exerted cytotoxicity	Antioxidant effects	10-100 µM;	Kulkarni et al. [139]
Punicalagin	A549, H1299	Displayed antiproliferative activity	Antioxidant effects	12.5-200 µg/mL; 72 h	Aquil et al. [87]
Punicalagin, ellagic acid	A549, H1299	Displayed antiproliferative activity	Antimutagenic effects	12.5-200 µg/mL; 48 h	Zahin et al. [140]
Neurological cancers					
Punicalagin	U87MG (human glioma)	Inhibited cell viability	↑Apoptosis; ↑cyclin E; ↓cyclin A; ↓cyclin B; ↑PARP cleavage; ↑caspase-9; ↑autophagy; ↑pAMPK; ↑pp27	1-30 µg/mL; 24 h	Wang et al. [143]
Nanoemulsion of seed oil with ketoprofen	C6 (rat glioma)	Suppressed cell growth		50, 100 µM; 72 h	Ferreira et al. [144]
Pomegranate seed oil or nanoemulsions of seed oil	C6 (rat glioma)	Reduced cell viability		2.61-8.7 % v/v; 24, 48 h	Ferreira et al. [145]
Skin cancer					
Pomegranate juice-derived ellagitannins	SK-ELM	Inhibited cell proliferation		IC_{50}=70.5-197 µM; 48 h	Kasimsetty et al. [80]
Galactonmannan polysaccharide (PSP001) from peel	A375	Exerted cytotoxicity	↑Apoptosis	0.001-1,000 µg/mL; 24-72 h	Joseph et al. [78]
Urogenital cancers					

Bladder cancer					
Ethyl acetate and Soxhlet extract of whole fruit or peel	T24	Inhibited cell proliferation	Antioxidant activity	50 μg/mL; 48 h	Masci et al. [149]
Pomegranate rind extract	EJ	Displayed antiproliferative activity	↑Caspase-3; ↑cleaved PARP; ↑p53; ↓c-Jun; ↑miR-34a; ↓c-myc; ↓CD44	25-200 μg/mL; 24-72 h	Zhou et al. [150]
Ethanolic extract of pomegranate juice	T24, J82	Exhibited cytotoxic activity	↑Apoptosis; ⊥S phase; ↑cyclin A; ↓cdk1; ↑procaspase-3; ↑procaspase-8; ↑procaspase-9; ↑procaspase-12; ↑Bax:Bcl-2; ↑CHOP; ↑BiP	2.5-200 μg/mL 12-72 h	Lee et al. [151]
Ethanolic extract of pomegranate juice	T24, J82	Displayed antiproliferative activity; reduced cell migration	↑PTEN; ↓pAkt; ↓mTOR; ↓XIAP; ↑Pfn1; ↑Diablo	50 μg/mL; 24-72 h	Wu et al. [152]
Ethanolic extract of pomegranate peels and pulp	T24, J82	Showed growth-inhibitory activity	↑Apoptosis; ⊥sub-G_1; ↑caspase-3; ↑caspase-8; ↑caspase-9; ↑caspase-12; ↑DR4; ↑DR5; ↑Bax; ↓Bcl-2; ↑Bip; ↑VCP	5-200 μg/mL	Chang et al. [153]
Prostate cancer					
Fermented juice polyphenols, pericarp polyphenols and cold-pressed seed oil	LNCaP, PC-3, DU-145	Displayed antiproliferative and anti-invasive activity	↑Apoptosis; ↑caspase-3; ⊥G_2/M; ↑$p21^{WAF1}$; ↓GADD45α; ↓c-myc; ↑MAPK-APK2	ED_{50}= 20-100 μg/mL; 96-168 h	Albrecht et al. [154]

Fermented juice polyphenols, pericarp polyphenols and seed oil	DU-145, PC-3	Inhibited cell proliferation and invasion	↓PLA2	12.5-100 μg/mL; 0.5-96 h	Lansky et al. [155]
Fruit extract	PC-3	Inhibited cell viability	↑Apoptosis; ↑Bax; ↑Bak; ↓Bcl-XL; ↓Bcl-2; ↑$p21^{WAF1}$; ↑$p27^{KIP1}$; ↓cyclin D1; ↓cyclin D2; ↓cyclin E; ↓cdk2; ↓cdk4; ↓cdk6	10-100 μg/mL; 48 h	Malik et al. [156]
Pomegranate juice, total pomegranate tannin extract, punicalagin and ellagic acid	RWPE-1, 22Rv1	Displayed antiproliferative activity	Antioxidant activity	12.5-100 μg/mL; 48 h	Seeram et al. [103]
Fruit extract (POMx), juice, punicalagins and ellagic acid	LNCaP, LNCaP-AR, DU-145	Exhibited antiproliferative activity	↑Apoptosis; ↓HSD3B2; ↓AKR1C3; ↓SRD5A1; ↓AR	3.125-50 μg/mL; 72 h	Hong et al. [157]
POMx	LNCaP	Displayed antiproliferative and antiangiogenic effects	↓HIF-1α; ↓VEGF	1-5 μg/mL; 6 h	Sartippour et al. [158]
POMx	DU-145	Inhibited cell viability	↑Apoptosis; ↓NF-κB	Dilution factor: 50-2,000; 24, 48 h	Rettig et. al. [159]
POMx	LAPC4	Inhibited cell growth	↑Apoptosis; ↑pJNK; ↓pAkt; ↓pmTOR; ↓Igf1	10 μg/mL; 72 h	Koyama et al. [160]

Pomegranate juice	DU145, LNCaP, PC-3	Inhibited cell growth increased cell adhesion and reduced cell migration	↑ICAM-1; ↑E-cadherin; ↓HMMR; ↓COL1A1; ↑miR-335; ↑miR-205; ↑ miR-200; ↑ miR-126; ↓miR-21; ↓miR-373; ↓IL-6; ↓IL-12p40; ↓ IL-1β; ↓RANTES	1%, 5%; 12-72 h	Wang et al. [161]
Combination of juice components (luteolin, ellagic acid, caffeic acid and punicic acid)	DU145, LNCaP, PC-3	Inhibited cell growth, increased cell adhesion and reduced cell migration and chemotaxis	↑E-cadherin; ↓HMMR; ↓TWIST; ↓FSCN1; ↓CDK6; ↓COL1A1; ↓Bcl-2; ↓NEXN; ↓EZH2; ↓CDC25B; ↓ZEB1; ↓ROCK2; ↓CCNB2; ↓CCNB1; ↓DTL; ↓CCNE2; ↑PTEN; ↑CDKN1A; ↑CDKN2A; ↑CLDN1; ↑CDKN2B; ↑miR144; ↑miR-133b; ↑miR-1; ↑miR-122; ↑miR-34c; ↑miR-200c; ↑miR-127; ↑miR-335; ↑miR-124; ↑miR-181a; ↑miR-7; ↑miR-215; ↑miR-15a; ↑Let-7d; ↓miR-20a; ↓miR-21; ↓miR-9; ↓miR-29b; ↓miR-181b	2 or 4 μg/mL for each constituent; 12-72 h	Wang et al. [162]
Methanolic peel extract	PC-3	Displayed antiproliferative activity		5 -1,000 μg/mL; 72 h	Modaeinama et al. [83]

Ethanolic peel extract	PC3	Displayed antiproliferative effect	↑Apoptosis	10- 600 µg/mL; 24 h	Sepehr et al. [163]
Ethanolic peel extract	PC-3, DU-145, TRAMP-C1	Registered antiproliferative, proapoptotic, anti-invasive and antimigratory effects	↓Mitochondrial membrane potential; ↑ROS; ↑Bax; ↓Bcl-2; ↑caspase-3; ↓MMP-2; ↓MMP-9; ↑TIMP2	12.5-200 µg/mL; 24-72 h	Deng et al. [164]
Ethanolic seed extract	PC-3	Produced marginal antiproliferative effect		10-600 µg/mL; 24 h	Sepehr et al. [163]
Aqueous methahnolic extract of seed oil	DU-145	Displayed minimal effects of cell viability		0.12-0.60 µL; 24 h	Costantini et al. [90]
Methanolic seed extract	PC-3	Displayed antiproliferative and cytotoxic activity		5- 1,000 µg/ml; 72 h	Seidi et al. [92]
Ethanolic seed extract	LNCaP	Inhibited cell proliferation		5-100 µg/ml; 48 h	Lucci et al. [91]
SL-NPs of pomegranate extract	PC-3	Exhibited cytotoxic profile		1-500 µg/mL; 24 h	Badawi et al. [97]

Table 2.
Ex vivo and in vivo effects of pomegranate extracts, fractions and pure compounds on various cancers.

Materials tested	Animal models used	Effects	Mechanisms	Dose & route	Frequency & duration	References
Breast cancer						
Fermented juice polyphenols, chromatographic peak (Peak B) of fermented juice polyphenols, seed oil	DMBA-induced mouse mammary gland lesions (*ex vivo*)	Reduced the number of lesions		1, 10 μg/mL	10 days	Kim et al. [66]; Mehta and Lansky [67]
Polyphenolic extract of pomegranate juice	Female athymic BALB/c nude mice with xenografted BT474 tumors	Decreased tumor volume and weight	↑Apoptosis; ↓Sp1; ↓Sp3; ↓Sp4; ↓VEGF; ↓survivin; ↓NF-κB p65; ↓pAkt; ↓pPI3K; ↑ZBTB10; ↑SHIP-1; ↓miRNA-27a; ↓miRNA-155	0.8 mg gallic acid equivalents/kg/day; p.o.	Once a day; 35 days	Banerjee et al. [89]
Galactonmannan polysaccharide (PSP001) from pomegranate fruit rind with or without doxorubicin	Female BALB/c mice with EAC tumors (ascites and solid tumors)	Reduced tumor volume and increased survival		100 mg/kg; i.p.	Once a day; 1-14days	Joseph et al., [78]
Pomegranate emulsion	DMBA-induced mammary	Reduced tumor incidence, total	↑Apoptosis; ↓proliferation;	0.2-5.0 g/kg; p.o.	3 times/week; 18 weeks	Bishayee et al. [99]; Mandal &

	tumorigenesis in female Sprague-Dawley rats	tumor burden and average tumor weight	↑Bax; ↓Bcl-2; ↑Bax:Bcl-2; ↑BAD; ↑CASP3; ↑CASP7; ↑CASP9; ↑PARP; ↑CYT. C; ↓ER-α; ↓ER-β; ↓ERα:ERβ; ↓β-catenin; ↓cyclin D1; ↓COX-2; ↓HSP90; ↓NF-κB; ↓IκBα; ↑Nrf2			Bishayee [100]; Mandal et al. [101]
Pomegranate seed oil	DMBA-induced mammary tumorigenesis in female Sprague-Dawley rats	Increased cancer incidence, tumor multiplicity, total tumor mass and mean tumor mass	Modification of DMBA metabolism	0.15 ml/day; p.o.	21 weeks	Lepionka et al. [102]
Gastrointestinal tract and associated cancers						
Pomegranate seed oil (PGO)	AOM-induced colon carcinogenesis in male F344 rats	Reduced tumor incidence and multiplicity	↑CLA content; ↑PPARγ	0.01% - 1% PGO by weight; diet	32 weeks	Kohno et al. [110]
Pomegranate Juice	AOM-induced colon carcinogenesis in male Fisher 344 rats	Reduced the number of ACF	↑GST	20% fruit juice in drinking water	13 weeks	Boateng et al. [111]
Pomegranate peel extract	AOM induced colon carcinogenesis in	inhibited ACF formation	↑TAC; ↑GSH; ↑GST; ↑GPx; ↑GR; ↑SOD; ↑CAT	1.5 mL/day; p.o.	Daily for 13 weeks	Waly et al. [112]

	male Sprague-Dawley Rats					
Pomegranate peel extract	AOM induced colon carcinogenesis in male Sprague-Dawley rats	Reduced ACF formation	↓MDA; ↑GSH/GSSG; ↓myeloperoxidase	1.5 mL/day; p.o.	Daily for 16 weeks	Waly et al. [113]
Pomegranate juice	AOM induced colon carcinogenesis in male Sprague-Dawley rats	Suppressed ACF and dysplastic ACF formation	↓COX-2; ↓iNOS; ↓VCAM-1; ↓NF-κB; ↓pNF-κB; ↓IGF; ↓IGFR; ↓p-PI3K; ↓pAkt; ↓pmTOR; ↑miR-126	57.2 mL/day; drinking water	70 days	Banerjee et al. [105]
Pomegranate extract	AOM-initiated and DCNO-promoted colon carcinogenesis in male F344 rats	Decreased the number of MDF		0.6% w/w in DCNO; diet	100 days	Bastide et al. [114]
Pomegranate extract	DMH-induced colon carcinogenesis in male Wistar albino rats	Enhanced survival rate and reduced tumor incidence	↓CEA; ↓Wnt5a; ↓FRZ-8; ↓β-catenin; ↓Lef1; ↓Tcf4; ↓c-myc; ↓cyclin D1; ↑Axin1; ↑APC	3% (wt/wt); diet	30 weeks	Sadik et al. [115]
Pomegranate mesocarp decoction	Male Pirc (F344/NTac-Apc^{am1137}) rats	Reduced the number and multiplicity of MDF	↑Apoptosis	10,000 ppm (50 mg/kg); diet	6 weeks	Tortora et al. [116]
Pomegranate emulsion	DENA-intiated and PB-promoted	Reduced incidence, number,	↓TBARS; ↓protein carbonyls; ↑Gsta2;	1, 10 g/kg; p.o.	3 times/week for 18 weeks	Bishayee et al. [120];

	hepatocarcinogenes is in rats	multiplicity, size and volume of hepatic nodules and number and area of GGT-positive foci	↑Gsta5; ↑Gstm1; ↑Gstm7; ↑Gstt1; ↑Nqo1; ↑Ugt1a1; ↑Ugt2b17; ↑Nrf2; ↓iNOS; ↓3-NT; ↓HSP70; ↓HSP90; ↓COX-2; ↓NF-κB			Bishayee et al. [121]
Pomegranate emulsion	DENA-intiated and PB-promoted hepatocarcinogenes is in rats	Inhibited hepatic cell proliferation	↑Apoptosis; ↓cyclin D1; ↑Bax; ↓Bcl-2; ↓Bax:Bcl-2; ↓β-catenin; ↑GSK-3β	1, 10 g/kg; p.o.	3 times/week for 18 weeks	Bhatia et al. [122]
Pomegranate hull extract	DENA-induced hepatocarcinogenes is in rats	Improved survival rate reduced size of hepatic foci	↓Bcl-2; ↓cyclin D1; ↓β-catenin; ↓GSH	6 g/kg; p.o.	3 times/week for 10 weeks	El-Ashmawy et al. [123]
Gynecological cancers						
Pomegranate fruit juice (PFJ), ellagic acid, luteolin	Female nude mice injected with ES-2 cells	Reduced tumor volume and weight	↓MMP-2; ↓MMP-9	20 mL/kg (PFJ), 50 mg/kg (ellagic acid & luteoin)	~24 days	Liu et al. [128]
Lung cancer						
Pomegranate fruit extract	Male athymic(nu/nu) nude mice injected with A549 cells	Induced tumor growth inhibition and improved survival of tumor-bearing animals	N/A	0.1%, 0.2% w/v; dw	~64 days	Khan et al. [137]
Pomegranate fruit extract	Female A/J mice gavaged with B(a)P	Reduced tumor multiplicities	↓NF-κB p65; ↑IκBα; ↓IKKα; ↓pERK1/2; ↓pJNK1/2; ↓pp38;	0.2% w/v; dw	~77-240 days	Khan et al. [141]

	or topically treated with NTCU		↓PI3K; ↓pAkt; ↓pmTOR; ↓pc-met; ↓Ki-67; ↓PCNA; ↓iNOS; ↓CD31; ↓VEGF			
Pomegranate juice	Male A/J mice exposed to cigarette smoke	Decreased the number and growth of lung nodules and tumors	↓PHH3; ↓HIF-1α	80 μmol/kg/day; dw	9 months	Husari et al. [142]
Skin cancer						
Pomegranate seed oil	DMBA-initiated and TPA-promoted skin carcinogenesis in female CD-1 mice	Decreased tumor incidence and multiplicity	↓ODC	100 µL of 5% oil; topical	Twice/week for 19 weeks	Hora et al. [146]
Pomegranate fruit extract	DMBA-initiated and TPA-promoted skin carcinogenesis in female CD-1 mice	Reduced incidence, total number and multiplicity of tumor	↓ODC; ↓COX-2; ↓pERK1/2; ↓pJNK1/2; ↓pp38; ↓NF-κB p65; ↓IKKα; ↓pIκBα	2 mg/mouse; topical	Twice/week; 29 weeks	Afaq et al. [147]
Pomegranate fruit extract with or without DAS	DMBA-initiated and TPA-promoted skin carcinogenesis in male Balb/c mice	Delayed tumor onset and incidence; Demonstrated regression in tumor volume	↑Apoptosis; ↓pERK1/2; ↓pJNK1; ↓NF-κB p65; ↓IKKα; ↓pIκBα	10%; dw	18 weeks	George et al. [148]
Urogenital cancers						
Bladder cancer						

Pomegranate rind extract	EJ cells xenografted in male athymic BALB/C nude mice	Suppressed tumor growth (volume)	↑miR-34a	100 mg/kg; p.o.	Once daily; 4 weeks	Zhou et al. [150]
Ethylacetate layer of ethanolic peel extract	T24 cells xenografted in male BALB/cAnN-Foxn1 nude mice	Reduced tumor volume and weight	↑Apoptosis	2-100 mg/kg; p.o.	once daily; 10 weeks	Chang et al. [153]
Prostate cancer						
Supercritical CO2-extracted seed oil, pericarp polyphenols	PC-3 xenograft in athymic BALB/c male homozygous nude mice	Inhibited tumor growth		2 μg/g; s.c.	35 days	Albrecht et al. [154]
Fruit extract	CWR22Rv1 xenograft in athymic nude male mice	Suppressed tumor volume and improved survival of animals with tumor	↓Serum PSA levels	0.1%, 0.2% wt/vol; d.w.	31-39 days	Malik et al. [156]
Ellagitannin-rich pomegranate extract	LAPC4 xenograft in SCID mice	Decreased tumor volume		0.8 mg/mouse; p.o.	5 days/week; 4 weeks	Seeram et al. [165]
POMx	LAPC4 xenograft in SCID mice	Reduced tumor growth	↑Apoptosis; ↓NF-κB; ↓pIκBα; ↓PSA	0.8 mg/mouse; p.o.	5 days/ week; 3 weeks	Rettig et al. [159]
POMx	LAPC4 xenograft in SCID mice	Reduced tumor volume and intratumor blood vessel density	↓HIF-1α	0.8 mg/mouse; p.o.	5 days/ week; 4 weeks	Sartippour et al. [158]

Combination of luteolin, ellagic acid and punicic acid	PC-3M-luc xenograft and $Pten^{-/-}$; $K\text{-}ras^{G12D}$ allograft tumors in male SCID mice	Inhibited tumor growth and metastasis	↓CXCR4; ↓Gα13; ↓PI3K; ↓pAkt	64 µg/component/day; i.p.	5 days/week; 8 weeks	Wang et al. [166]
Fruit extract	TRAMP mice	Increased survival and inhibited tumorigenesis and metastasis	↓Raptor; ↓Rictor; ↓IGF-1; ↓PI3K; ↓pAkt; ↓pmTOR	0.1, 0.2% wt/vol; d.w.	6-28 weeks	Adhami et al. [167]

Table 3
Clinical trials of several of pomegranate products in cancer patients.

Phase/Study type; ClinicalTrials.gov identifier	Materials tested	No. of patients	Effects	Dose & route	Frequency & duration	References
Breast cancer						
Randomized clinical trial	Pomegranate juice (POM Wonderful 100% juice)	64	Significant decline in estrone and testosterone in subgroup of females with normal weight	8 fluid oz/day; p.o.	4 oz in the morning and 4 oz in the evening; 3 weeks	Kapoor et al. [168]
Colon cancer						
Phase I-II; NCT01916239	Pomegranate extracts (PE-1: 2 mg/g punicalin, 72 mg/g punicalagin and 294 mg/g ellagic derivatives; PE-2: 5.4 mg/g punicalin, 155 mg/g punicalagin and 28 mg/g ellagic derivatives	26	Increased levels of ellagic acid derivatives and urolithin found in colon tissue of CRC patient	Two 450 mg capsules; p.o.	Daily; 12-18 days	Nuñez-Sánchez et al. [169]
Phase I-II; NCT01916239	Pomegranate extracts (PE-1: 2 mg/g punicalin, 72 mg/g punicalagin and 294 mg/g ellagic acid derivatives;	45	Moderate (but statistically insignificant) modulation in miR-646, miR-1249, miR-135b-5p, miR-135b-3p, miR-92b-5p, miR-765,	Two 450 mg capsules; p.o.	Daily; 12-14 days	Nuñez-Sánchez et al. [170]

	PE-2: 5.4 mg/g punicalin, 155 mg/g punicalagin and 28 mg/g ellagic acid derivatives		miR-496, miR-181c-3p, and miR-18a-3p expression, which are linked to common cancer pathways in CRC and Jak-STAT signaling pathway			
Phase I-II; NCT01916239	Pomegranate extracts PE-1: 2 mg/g punicalin, 72 mg/g punicalagin and 294 mg/g ellagic acid derivatives; PE-2: 5.4 mg/g punicalin, 155 mg/g punicalagin and 28 mg/g ellagic acid derivatives	45	Counteracting effect on *CD44*, *CTNNB1*, *CDKN1A*, *EGFR* and *TYMS* gene expression.	Two 450 mg capsules; p.o.	Daily; An average of 13.6±7.5 days	Nuñez-Sánchez et al. [171]
Phase I-II; NCT01916239	Pomegranate extracts PE-1: 2 mg/g punicalin, 72 mg/g punicalagin and 294 mg/g ellagic acid derivatives; PE-2: 5.4 mg/g punicalin, 155 mg/g punicalagin and 28 mg/g ellagic acid derivatives	57	Decrease in plasma level of LBP	Two 450 mg capsules; p.o.	Daily; An average of 14.4±7.6 days	González-Sarrías et al. [172]
Prostate cancer						

Phase II	Pomegranate juice, equivalent to 570 mg total polyphenol gallic acid equivalents	46	Prolongation of PSA doubling time	8 oz; p.o.	Daily; 15-54 months	Pantuck et al. [173]
Randomized, double-blind, placebo-controlled study; NCT00336934	Pomegranate liquid extract with 1.6 mmol of total polyphenol	183	Significant prolongation of PSADT for men with *MnSOD* AA genotype; No significant prolongation of PSADT for other patient population	8 oz; p.o.	Daily; 12 months	Pantuck et al. [174]
Phase II	Pomegranate extract (capsule with 1 g powder)	101	At least 6 months prolongation of PSADT, declining PSA levels in 13% of patients	1 or 3 g of polyphenol extract; p.o.	Daily; 6-18 months	Paller et al. [175]
Phase II; NCT00719030	Pomegranate extract (capsule with 1 g powder)	70	No change in tissue 8-OHdG, Ki67, cancer pS6 kinase, NF-κB and serum PSA levels	2 g of POMx powder; p.o.	One capsule twice daily; Up to 4 weeks	Freedland et al. [176]
	Pomegranate juice with 279 mg of ellagic acid-type phenolics	63	Upon intake of pomegranate juice, main metabolites such as urolithin glucuronides and dimethyl ellagic acid were detected in prostate tissue.	200 mL; p.o.	Daily; 3 days	Gonzalez-Sarrias et al. [177]

Phase IIb	Pomegranate juice (2294 mg/L polyphenol gallic acid)	102	No differences detected with regard to PSA kinetics and pain scores	500 mL/day; 250 mL/day	500 mL: Every day for 4 weeks; 250 mL: After the first 500 mL every day for 4 weeks, received 250 mL daily for 4 weeks	Stenner-Liewen et al. [178]
Randomized, double-blind, placebo-controlled study	Whole food supplement containing 100 mg of pomegranate whole fruit powder	199	Significant suppression of median percentage rise in PSA	One tablet; p.o.	Three times a day; 6 months	Thomas et al. [179]