ECRONICON OPEN ACCESS

EC NUTRITION
Review Article

Anti-Cancer Activity of Pomegranate and its Biophenols; General Review

Maha Abu Hajleh and Emad A S Al-Dujaili*

Department of Pharmacy, Middle East University, Amman, Jordan

***Corresponding Author:** Emad Al-Dujaili, Department of Pharmacy, Middle East University, Amman, Jordan.

Received: November 28, 2016; **Published:** December 07, 2016

Abstract

Cancer is the second leading cause of death and is becoming the leading one in the old age [1]. Vegetable and fruit consumption are inversely associated with reduced cancer incidence and mortality [2]. Antioxidants from fruits have been extensively studied for their free radical scavenging activities to prevent the occurrence of chronic degenerative diseases [3,4]. The fruits which are high in polyphenols are reported to have antioxidant and chemo preventive and/or chemotherapeutic potential. There is a major need for less toxic but yet effective therapies to treat cancer. Pomegranate has been shown to exert anticancer and antioxidant activity, which is generally attributed to its high content of polyphenols due to their effects of neutralizing free radicals [3]. This review provides up to date info on how pomegranate and their biophenols can target a broad spectrum of genes and proteins to suppress cancer growth and progression. Pomegranate induces apoptosis and evokes anti-proliferative, anti-invasive, and anti-metastatic effects. Furthermore, pomegranate blocks the activation of inflammatory pathways. Thus, it was shown that pomegranate juice (PJ) and /or pomegranate extracts (PE) including genistein, significantly inhibit the growth of prostate cancer cells in culture and also inhibit cell proliferation and induce apoptosis in human breast cancer cells (MCF-7), human pancreatic cancer cells, colon cancer (CACO) and in Hepato-cellular carcinoma (HepGII) cell lines [5]. The aim of this review is to investigate the effect of pomegranate and its biophenols in the prevention and potential treatment of various types of cancers.

Keywords: *Pomegranate; Pomegranate Juice; Pancreatic Cancer; Breast Cancer; Colon Cancer; Hepato-Cellular Carcinoma; Lung Cancer; Prostate Cancer; Leukemia*

Abbreviations

Caco: Colon Cancer; Cdk: Cyclin-Dependent Kinase; CML: Human Chronic Myeloid Leukemia; CPD: Cyclobutane Pyrimidine Dimers; COX2: Cyclo-Oxygenase 2; DHT: Dihydrotestosterone; DMBA: 7,12-Dimethylbenzanthracene; EA: Ellagic Acid; Ets: Ellagitannins; Hepgii: Hepato-Cellular Carcinoma; HSD3B2: Hydroxysteroid Dehydrogenase Type II; MCF-7: Human Breast Cancer Cells; NHBE: Normal Human Bronchial Epithelial Cells; NHEK: Normal Human Epidermal Keratinocytes; 8-Ohdg: 8-Dihydro-2′-Deoxyguanosine; ODC: Ornithine Decarboxylase; PC: Pancreatic Cancer; Pca: Prostate Cancer; PE: Pomegranate Extract; PFE: Pomegranate Fruit Extract; PJ: Pomegranate Juice; PGO: Pomegranate Seed Oil; PSA: Prostate Specific Antigen; ROS: Reactive Oxygen Species; TPA: 12-O-Tetradecanoylphorbol 13-Acetate; UV: Ultraviolet

Introduction

Punica granatum is a fruit used in many cultures (the genus name, Punica, is derived from the Roman name for Carthage, where the best pomegranates were known to grow). The pomegranate tree is native to the region of Persia and is now cultivated over the entire Mediterranean area, Asia, and America [5]. The antioxidant activity of flavonoids obtained from pomegranate and its juice (PJ) was observed to be better or close to that of butylated hydroxyanisole and green tea. Pomegranate extract (PE) consists of a mixture of various

phytochemicals, including the punicalagins, a class of tannins unique to pomegranates that have been shown to possess free radical-scavenging properties [7,8]. The antioxidant activity was higher in commercial juices that were extracted from whole pomegranates than in experimental juices that were obtained from the arils only [9].

Antioxidant activities of freeze-dried preparations of pomegranate and its 3 major anthocyanidins (delphinidin, cyanidin, and pelargonidin) were evaluated. Antioxidants are the naturally occurring substances in plants that protect the body from free radicals, which are highly reactive atoms or molecules that influence normal cellular functions. Free radicals are produced naturally as a result of cellular metabolism and in our modern society in the form of pollutants, food additives, pesticides, herbicides, cigarette smoke etc. For example, free radicals can cause cellular damage to cellular components including RNA/DNA, which can potentially lead to cancer. Free radicals can alter cholesterol in an oxidation process in the arteries. Therefore, antioxidants can offer protection against the oxidative stress in our industrialized world, such as pollution, chemicals, viruses and bacteria, and consequently cardiovascular diseases and cancer. Pomegranate has been valued in many cultures for millennia for its therapeutic attributes, including anti-inflammatory, antihypertensive, and anti-diabetic properties [5,10]. Recent studies have shown that pomegranate is a potent anticancer agent that causes the induction of apoptosis and cell cycle arrest in cancer cells, inhibition of multiple signaling pathways in cancer cells, inhibition of tumorigenesis in animal models of various carcinomas [11-14].

Pomegranates can relieve symptoms of many diseases; pomegranate shows antihypertensive, antiviral, antibacterial, and antioxidant properties [15,16]. Pomegranate has been gaining popularity to relief menopausal symptoms, increased risk of breast cancer, heart disease and strokes and side effects of artificial hormone replacement therapy. Pomegranate and certain herbs contain estrogen-like substances that do not have the serious side effects of prescription medications. Pomegranate contains estrone, a natural estrogen which is also produced by the human body. Pomegranate extract improved the menopausal symptoms due to depression and bone loss. Pomegranate is a rich source of hydrolyzable tannins, ellagitannins, catechins, gallocatechins, and anthocyanins and proanthocyanidins (flavonoids). The combination of various types of polyphenols makes the pomegranate antioxidants unique and different from other antioxidants, such as Vitamin A or C, by having a much wider spectrum of action against several and not just one type of free radicals. Polyphenols such as anthocyanins (3- glucosides and 3, 5- glucosides of delphinidin, cyanidin, and pelargonidin) and flavonols are also antioxidants, meaning they help protect cells from damage and may lower inflammation in the body. Pomegranate juice and oil from seeds contain isoflavones. Bark, juice, fruit, root, and rind of the pomegranate tree are used in folk medicine. The bark and roots of the tree have been used in traditional medicine to eliminate tapeworms. In humans and different animal models, it has been found that ellagic acid is metabolized by the colon microflora to form urolithins A and B. Ellagic acid and urolithins can circulate in the blood and accumulate in many target organs, including intestine and prostate, where the effects of pomegranate ellagitannins can be observed.

Plant Description (parts of plant and available forms)

Pomegranate grows as a multi-stemmed shrub or large tree, as high as 20 or 30 feet, that produces suckers from the base. It is native to Iran and is cultivated in the Mediterranean, Asia, Africa, and Europe. Deciduous leaves are opposite, or in whorls, approximately 3/8 to 4 inches long. Flowers are 1¼ inch and have a red tubular calyx. The fruit has a leathery skin, usually deep pink or red. The inside of the fruit has white spongy tissue that creates spaces filled with sacs or tart pulp and seed filled with juice called arils (Figure 1). The fruit and seed are used in modern herbal medicine. In some traditional folk remedies, the rind and root or bark may also have been used, but they contain potentially toxic substances and should be avoided. Pomegranate juice is available as a liquid in different types and pomegranate extract is available in pill, capsule, or powder form.

Pomegranate and cancer

Scientific research is demonstrating that pomegranate may be helpful in the prevention and treatment of various types of cancer such as breast cancer, skin cancer, prostate cancer and lung cancer. Because it is high in antioxidants and other nutrients, some people think

that drinking pomegranate juice regularly may help prevent cancer. Pomegranate slows down the reproduction of cancer cells and may hasten their death, also help reduce blood supply to tumors, starving them and making them smaller [18]. Table 1 summarizes the anti-carcinogenic effects of pomegranate-derived products. Pomegranate is a rich source of hydrolyzable tannins or ellagitannins, catechins, gallocatechins, and anthocyanins. The combination of various types of polyphenols makes the pomegranate antioxidants unique and different from other antioxidants, such as Vitamin A or C, by having a much wider spectrum of action against several and not just one type of free radicals [17].

Figure 1: *Pomegranate tree, flower, fruit and juice.*

Organ	Study Model	Pomegranate Formulation	Target/ Mechanism(s)	Reference
Prostate cancer				
	Monolayer cell cultures	Fermented juice polyphenols, extract, and pericarp polyphenols Cold-pressed or CO_2-extracted seed oil, fermented juice polyphenols, and pericarp polyphenols	Inhibits proliferation and invasion, Inhibits secretory phospholipase Inhibits proliferation and invasion Inhibits secretory phospholipase	[27] [24, 25]

		Standardized extract (POMx, POM Wonderful) containing ellagitannins (37-40%) and ellagic acid (3.4%) but no anthocyanins; Juice (POM Wonderful) containing ellagitannins (1 mg/ml), ellagic acid (0.97 mg/ml) and anthocyanins; ellagitannins and ellagic acid	Suppresses androgen receptor expression Inhibits androgen-synthesizing enzymes	[25]
		Acetone extract of pomegranate fruit	Induces apoptosis Increases Bax/ Bcl-2 ratio Increases p21 & p27 Down-regulates cyclins and cdks	[13]
		POMX prepared from skin and arils minus seeds and standardized to ellagitannins (37%)	Induces apoptosis & inhibits cell growth Increases JNK phosphorylation Suppresses AKT/mTOR signaling Decreases IGF-1 mRNA levels	[31]
		Fruit extract (POMX; POM Wonderful) standardized to ellagitannins [punicalagins (37–40%), and ellagic acid (3.4%); Juice concentrate (POM Wonderful) containing punicalagins (1,561 mg/L), ellagic acid (121 mg/L), anthocyanins (387 mL/L), other hydrolysable tannins (417 mg/L)	Inhibits NF-κB activity	[28]
		Juice (POM Wonderful) with flavonoids (anthocyanins, catechins, and phenols) constituting 40% of total polyphenols	Antiproliferative, proapoptotic effects, increase in nitric oxide and reduction in oxidative state in exploratory bioassays	[34]
		Pomegranate juice; ellagitannins extracted from POMX, urolithins	Inhibits CYP enzyme activity	[25, 37]
		Pomegranate juice	Up-regulates anti-invasive mi-RNAs (−335,−205,−200, & −126) Down-regulates pro-invasive mi-RNA (−21 and −373) Reduces pro-inflammatory cytokines (IL-6,-12p40,-1β)	[33]
	Athymic nude mice	Ellagitannin-rich fruit extract (POM Wonderful) standardized to ellagitannins [punicalagin] (37%) and ellagic acid (3.5%); acetone fruit extract	Inhibits tumor growth & multiplicity. Decreases serum PSA levels	[29, 13]

		Fruit extract (POMX; POM Wonderful) standardized to ellagitannins [punicalagins] (37–40%), and ellagic acid (3.4%); juice concentrate (POM Wonderful) containing punicalagins (1,561 mg/L), ellagic acid (121 mg/L), anthocyanins (387 mL/L), and other hydrolysable tannins (417 mg/L)	Delays emergence of androgen independence Decreases NF-κB activity	[28]
	TRAMP mice	Acetone extract of pomegranate fruit	Reduces tumor formation Decreases metastasis Increases survival. Inhibits IGF-I/AKT/mTOR signaling	[29]
	Human trials	Juice (POM Wonderful) with flavonoids (anthocyanins, catechins, and phenols) constituting 40% of total polyphenols; pomegranate extract (POMX)	Increases PSA doubling time Disease stabilization	[34, 35]
	Monolayer cell cultures	Acetone extract of pomegranate fruit	Inhibits UVA-mediated phosporylation of STAT3, AKT, ERK1/2, mTOR & p70S6K. Decreases PCNA & Ki-67 expression. Up-regulates Bax & Bad Down-regulates Bcl-X_L	[38]
		Acetone extract of pomegranate fruit	Inhibits UVB-mediated MAPK phosphorylation; NF-κB/p65 activation	[39]
		Pomegranate extract POMX (POM Wonderful) with 135000 ppm polyphenols with major constituents gallic acid equivalent and ellagitannins	Protects keratinocytes from UV-B-induced oxidative stress and photo-aging Inhibits UV-B-mediated decrease in cell viability and intracellular GSH content; increase in lipid peroxidation & up-regulation of MMPs −1,−2,−7 and −9 Inhibits MAPKs; c-Jun	[27]
Skin cancer				
		Pomegranate fruit extract standardized to ellagitannins [gallic acid & punicalagins] (37.5%) & ellagic acid (2.7%)	Protects fibroblasts from cell death post UV Decreases NF-κB activity	[41]

		Aqueous extracts of pomegranate juice, peel and seed (POM Wonderful)	Facilitates skin repair Stimulates type I procollagen synthesis Inhibits MMP-1 production	[49]
	3-D EpiDerm	Pomegranate extract POMX (POM Wonderful) with 135000 ppm polyphenols with major constituents gallic acid equivalent and ellagitannins; POMx juice contains anthocyanins, ellagitannins and hydrolyzable tannins; POM seed oil	Inhibits UVB-induced CPDs & 8-OHdG formation; PCNA expression Increases p21 Inhibits UVB-induced MMPs-1,-2,-3,-7,-8,-9,-11,-12; c-Jun and c-Fos; tropoelastin expression	[42]
	SKH-1 mice	Acetone extract of pomegranate fruit	Inhibits UVB-induced skin edema, hyperplasia, leukocytic infiltration; lipid peroxidation; CPDs & 8-OHdG formation; PCNA, ODC & COX-2 expressions; MAPK phosporylation; NF-κB/p65 activation, phosporylation of c-Jun; MMPs expression Increases p53 and p21 expressions	[43, 44]
	CD-1 mice SKH-1 mice	Pomegranate seed oil; Acetone extract of pomegranate fruit	Decreases tumor incidence & multiplicity. Inhibits TPA-mediated increase in skin edema and hyperplasia; ODC activity, COX-2 expression; phosphorylation of MAPKs and NF-κB activity	[47, 27]
	Balb/c mice	Pomegranate fruit extract	Delays onset and incidence of tumor. Suppresses MAPKs and NF-κB activity	[48]
	Wistar rats	Methanolic extract of dried pomegranate peel	Accelerates wound healing Increases hydroxyproline content	[50]
	Guinea pigs	Methanolic pomegranate peel extract based-ointment	Accelerates wound healing	[51]
Colon cancer				
	Monolayer cell cultures	Punicalagin, ellagic acid, standardized pomegranate tannin extract (punicalagin 85%, ellagic acid 1.3% and ellagitannins 12%) and pomegranate juice; POMX (POM Wonderful)	Induces apoptosis, cell cycle arrest Inhibits growth	[32, 59]

		Pomegranate fruit extract standardized to ellagitannins [punicalagin α and β] (25%) and ellagic acid (3.5%)	Inhibits Wnt signaling	[62]
		Ellagic acid; urolithins	Inhibits migration and adhesion Inhibits activation of NF-κB & MAPKs Down-regulates COX-2, PGE_2, PAI-1 and IL-8	[53]
		Pomegranate juice (POM Wonderful) (punicalagin 1.74 g/L), pomegranate tannin extract and punicalagins	Suppresses TNFα-induced COX-2 expression, AKT & NF-κB activity	[60]
	F344/Ducrj rats	Pomegranate seed oil	Inhibits the incidence and multiplicity of azoxymethane-induced colonic adenocarcinomas Increases PPAR expression	[63]
	TNBS mouse model	Ellagic acid	Attenuates morphologic alterations associated with cellular injury Maintains glandular architecture Decreases inflammatory cells infiltrate Represses COX-2 and iNOS. Inhibits MAPKs and NF-κB signaling	[54]
		Punicic acid	Down-regulates neutrophil hyper-activation Decreases ROS-induced tissue damage	[55]
	DSS mouse model	Pomegranate extract standardized to punicalagins (35%), punicalin (13%), ellagic acid glucoside (4.5%) and ellagic acid (8.9%); urolithin A	Decreases inflammation markers (iNOS, COX-2, PTGES and PGE_2)	[56]
		Pomegranate flower extract; ellagic acid rich fraction from pomegranate extract	Attenuates oxidative stress and subsequent colonic inflammation	[58]
Lung cancer				
	Monolayer cell cultures	Aqueous extract of pomegranate peel	Anti-oxidant Inhibits myeloperoxidase activity	[11]
		Acetone extract of pomegranate fruit	Decreases cell viability Induces p21 and p27 protein expressions. Down-regulates cyclins/cdks. Decreases PCNA & Ki67 expression. Inhibits MAPKs; PI3K/AKT pathway, NF-κB activity	[65]

	Athymic nude mice	Acetone extract of pomegranate fruit	Inhibits tumor growth	[65]
	A/J mice	Acetone extract of pomegranate fruit	Decreases tumor incidence & multiplicity. Inhibits phosphorylation of MAPKs; PI3K/AKT/mTOR pathway; NF-κB/p65 activation Inhibits c-Met phosphorylation Decreases Ki-67 & PCNA; iNOS, CD31 & VEGF	[14]
Breast cancer				
	Monolayer cell cultures	Urolithins	Inhibits aromatase activity Inhibits proliferation	[66]
		Punicic acid; Cold-pressed or CO_2-extracted seed oil, fermented juice polyphenols, and aqueous pericarp extract	Inhibits proliferation Induces apoptosis dependent on lipid peroxidation and the PKC pathway	[12, 13]
		Pomegranate fruit extract	Suppresses NF-κB Decreases RhoC and RhoA	[70]
		Pomegranate seed oil; fermented juice polyphenols	Down-regulates VEGF and MIF	[71]
	Organ culture	Fermented juice polyphenols	Reduces DMBA-induced lesions	[13, 68]
Blood cancer				
	Monolayer cell cultures	PSP001 polysaccharide from pomegranate rind	Inhibits leukemia cell growth	[35]
		Pomegranate juice; pomegranate ellagitannin nanoparticles	Induces apoptosis; cell cycle arrest	[81, 82]
		Pomegranate ellagitannin nanoparticles	Promotes differentiation in promyelocytic leukemia cells	[72]

Table 1: *Summary of the anti-carcinogenic effects of pomegranate-derived products.*

The fruit can be divided into (i) the seeds which constitute ~3% of the fruit weight, (ii) the juice which is roughly 30% and finally (iii) the peel which includes the interior network of membranes present inside the fruit [19]. The seed oil contains conjugated linolenic acid as the predominant fatty acid, with punicic acid. Other components of the oil include sterols, steroids, and cerebrosides [20]. The antioxidant activity of the pomegranate juice is significantly greater than the well-known anti-oxidants products of red wine and green tea, and is attributed to its polyphenolic content [7]. In the peel, mesocarp and arils which include anthocyanins, gallotannins, ellagitannins, gallagyl esters, hydroxybenzoic acids, hydroxycinnamic acids and dihydroflavonol (Figure 2); of these, cyanidin–pentoside–hexoside, valoneic acid bilactone, brevifolin carboxylic acid, vanillic acid 4-glucoside and dihydrokaempferol-hexoside have only been reported recently. The ellagitannins are the predominant phenols and the concentration of punicalagins in whole fruit extract amounts to 55% of total weight in 1g capsule [15].

In test tubes, pomegranate extracts made from juice, rind, and oil slow down the reproduction of cancer cells and may hasten their death. Some extracts also help reduce blood supply to tumors, starving them and making them smaller [5]. Most studies have focused

on breast and prostate cancer cells. In another study, pomegranate juice extract given to mice slowed down the growth of lung tumors. However, most of these studies have been in test tubes or in animals, not humans.

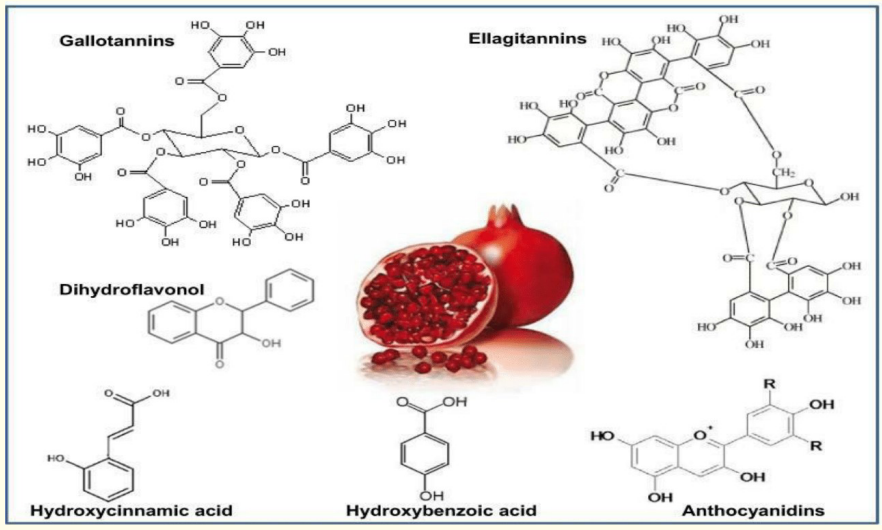

Figure 2: *Structures of most important antioxidants bio/polyphenols present in pomegranate whole fruit.*

Research using both mouse mammary organ culture and human breast cancer cells *in vitro* has demonstrated anticancer effects of pomegranate extracts have shown that a topically-applied pomegranate fruit extract can block skin tumor formation in mice. Another study demonstrated significant antitumor activity of pomegranate-derived materials against human prostate cancer. Yet another study shows the extracts of pomegranate can promote differentiation--the ability of cancer cells to revert to their normal counterparts. Antioxidant activities of freeze-dried preparations of pomegranate and its 3 major anthocyanidins (delphinidin, cyanidin, and pelargonidin) have anticancer effects. Pomegranate extract exhibited scavenging activity against OH and O^{-2}. The anthocyanidins were found to inhibit a Fenton reagent $^{-}$OH generating system possibly by chelating with ferrous ion. Also, anthocyanidins scavenged O^{-2} in a dose-dependent manner. Anthocyanidins inhibited H_2O_{2-}induced lipid peroxidation in the rat brain homogenates and ID_{50} values of delphinidin, cyanidin, and pelargonidin.

The activity of punicalagin, ellagic acid and pomegranate tannin as anti-tumor, these compounds have the ability to decrease the viable cell number of human oral, prostate and colon tumor cells [21]; however superior activity was obtained with pure pomegranate juice. Pomegranates are the richest source of a natural substance called ellagic acid. In humans and different animal models; it has been found that ellagic acid is metabolized by the colon microflora to form urolithins A and B. Ellagic acid and urolithins can circulate in the blood and reach and accumulate in many target organs, including intestine and prostate, where the effects of pomegranate ellagitannins are observed. Thus, ellagic acid inhibits cancer formation and is believed to inhibit cancer mutation by latching onto DNA-masking sensitive sites on the genetic material that might otherwise be occupied by harmful chemicals [22]. Ellagic acid is particularly effective in the inhibition of lung cancer caused by tobacco.

Pomegranate also contains other polyphenols, such as anthocyanins (3- glucosides and 3, 5- glucosides of delphinidin, cyanidin, and pelargonidin) and flavonols. During the juice processing, the whole fruit is pressed and ellagitannins are released into pomegranate juice. Pomegranate is a potent anticancer agent that causes the induction of apoptosis and cell cycle arrest in cancer cells, inhibition of multiple signaling pathways in cancer cells, inhibition of tumorigenesis in animal models of various carcinomas [23,24]. The pharmaco-

logical properties of pomegranate extracts have been analyzed and the constituents of *P. granatum* include gallocatechins, delphinidin, cyanidin, gallic acid, ellagic acid, pelargonidin and sitosterol, and phytochemicals, including the punicalagins, a class of tannins unique to pomegranates, which have been shown to possess free radical-scavenging properties, ellagitannins, and other flavonoids like quercetin, kaempferol, and luteolin glycosides. The polyphenols in *P. granatum* inhibits eicosanoid enzymes, including cyclooxygenase and lipoxygenase. Flavonoids and tannins present in this fruit inhibit cancer cell growth both *in vitro* and *in vivo*. Furthermore, the pomegranate seed oil contains a very high amount of steroids, including esterone, campestrol, estriol, testosterone, stigmasterol. Many of these compounds have cancer chemopreventive properties [25-27], in addition to antimicrobial, anti-parasitic, antiviral and anticancer properties. Pomegranate consumption possesses multiple biological actions and may be helpful in the prevention and therapy of cancer.

Effect of pomegranate on different types of cancer

Prostate cancer

Prostate cancer is the second leading cause of cancer deaths in many Western countries and represents the most common cancer in man. It was estimated for 2014 that in the US, 233,000 men would develop prostate cancer and about 30,000 could die from prostate cancer [67]. Nowadays there is a lack in the treatment of this disease except for the surgery and radiation approach applicable for prostate cancer in early stage. Among all natural compounds studied for the prevention and/or treatment of prostate cancer, pomegranate has been proven to possess relevant *in vitro* and *in vivo* beneficial effects.

Pomegranate contains high levels of antioxidants and phytochemicals with anti-inflammatory properties; the polyphenol-rich fractions of pomegranate fruit suppressed proliferation and invasion and inhibited secretory phospholipase expression in prostate cancer cells. The juice and oil from pomegranate inhibited proliferation and induced apoptosis in androgen dependent and independent prostate cancer cell lines [28]. Remarkably, pomegranate did not cause cytotoxicity in normal prostate epithelial cells. Consumption of pomegranate may retard prostate cancer progression and may prolong the survival of prostate cancer cells.

Human studies: In a human study, men who had surgery or radiation for prostate cancer lengthened the amount of time it took for their PSA levels to double by drinking 8 oz. of pomegranate juice each day. Men whose PSA levels double in a short period of time are more at risk for death from prostate cancer. Those who drank pomegranate juice increased the time it took for their PSA levels to double from about 15 months to 54 months which was a significant increase.

The beneficial effect of pomegranate juice in prostate cancer was reported a prolongation of the PSA doubling time upon consumption of juice, in patients suffering from the disease. The study showed that treatment with pomegranate juice was associated with statistically significant prolongation of PSA doubling time in these patients from a mean of 15 months at baseline to 54 months post treatment [22]. The anti-cancer effect of pomegranate in prostate, colon and other tissues has been attributed to the localization of the bioactive metabolites (ellagitannins or their metabolites ellagic acid and urolithins) at higher levels in these organs. The prevention of procarcinogen activation mediated through the inhibition of CYP enzyme activity may play an important role in pomegranate juice's effect on tumor promotion, and progression.

***In vitro* studies:** The effect in inhibiting *in vitro* prostate cancer cell invasion across matrigel membranes was observed when Ellagic acid, caffeic acid, luteolin, and punicic acid, constituents of pomegranate were combined [68]. The polyphenol-rich fractions of the pomegranate fruit suppressed proliferation and invasion and inhibited secretory phospholipase expression in prostate cancer cells. The juice and oil from pomegranate inhibited proliferation and induced apoptosis in androgen dependent and independent prostate cancer cell lines. The pomegranate extract inhibited cell growth and caused apoptosis of highly aggressive human prostate cancer cells. Consumption of pomegranate may retard prostate cancer progression and may prolong the survival of prostate cancer cells. Moreover, pomegranate juice slow or even reverse the increase in PSA (prostate-specific antigen) in blood of prostate cancer patients, and may allow men treated for prostate cancer to outlive their risk of dying from their cancer and keeping their PSA levels stable.

A consistent suppression of both androgen-synthesizing enzymes and androgen receptor expression was demonstrated with pomegranate treatment. It was inferred that pomegranate exerted its inhibitory effect against prostate cancer through down-regulation of genes involved in androgen synthesis [28]. However, the mechanism of the down-regulation was not fully understood and further studies are needed to determine how the alteration of cell proliferation and apoptosis is related to the expression of androgen synthesizing enzymes and androgen receptor. Constitutive NF-κB signaling was observed in androgen-independent prostate cancer, and is frequently used as a biochemical indicator for tumor recurrence after surgery. Pomegranate extract inhibited NF-κB signaling, both in *in vitro* and *in vivo* in prostate cancer models.

Induction of apoptosis by pomegranate, *in vitr*o, was shown to be dependent on inhibition of the NF-κB activity.

Pomegranate juice inhibits critical cellular processes involved in invasion and metastasis, micro-RNA (mi-RNA) expression and production of pro-inflammatory cytokines. It reduced the levels of secreted pro-inflammatory cytokines/ chemokines known to promote tumor growth suggesting that the inhibitory effect of pomegranate on prostate cancer cell metastasis is in part mediated through reducing inflammation. PE inhibits the growth of cancer cells through cell cycle arrest and stimulation of apoptosis possibly by reducing the expression level of androgen biosynthesis genes such as the 3β-hydroxysteroid dehydrogenase type II (HSD3B2) and steroid 5α reductase type I (SRD5A1) genes in LNCaP cells. More recently, PE was shown to reduce the production of testosterone and dihydrotestosterone (DHT) in LNCaP and 22RV1 cells. Therefore, PE may have chemopreventive as well as chemotherapeutic effects against prostate cancer in humans.

Animal studies: *In vivo* studies on SCID mice implanted with LAPC4 prostate cancer cells exhibit constitutive NF-κB activity on emergence of the androgen-independent state. Mice were administered pomegranate extract made from skins of fruits and standardized to ellagitannins, such that it contained 37 - 40% punicalagins and 3.4% free ellagic acid. Pomegranate fed group displayed significantly delayed growth and the pomegranate extract prevented the regrowth of prostate cancer cells and was associated with low serum levels and decreased NF-κB activity [34].

The fruit extract of pomegranate fruit was found to contain six anthocyanins namely pelargonidin 3-glucoside, cyanidin 3-glucoside, delphinidin 3-glucoside, pelargonidin 3,5-diglucoside, cyaniding 3,5-diglucoside, and delphinidin 3,5-diglucoside; ellagitannins and hydrolysable tannins [47-49]. This extract was shown to inhibit *in vivo* the growth and viability of prostate cancer cells through modulation of the cki-cyclin-cdk network, with up-regulation of p21 and p27 during G1-phase arrest, independent of p53. This correlated with down-regulation of the cyclins D1, D2, and E and cyclin-dependent kinases 2, 4, and 6, operative in the G1 phase of the cell cycle [30-33]. When nude mice implanted with androgen-sensitive prostate cancer cells were administered PE (0.1% and 0.2%; wt/vol) in drinking water and tumor growth was compared with the untreated controls, pomegranate treatment resulted in significant inhibition in tumor growth and volume and was associated with decreased serum PSA levels [29].

The inhibition of LAPC-4 prostate cancer xenograft growth in the SCID mouse model administered an ellagitannin-enriched pomegranate extract orally was significant [28]. Pomegranate juice and seed oil inhibited proliferation and induced apoptosis in androgen dependent and independent prostate cancer cell lines, pomegranate did not cause cytotoxicity in normal prostate epithelial cells. Pomegranate derivatives inhibited the growth of prostate cancer xenografts in nude mice [28]. There is now good evidence that ellagitannins, abundant in pomegranate, contribute significantly towards its reported biological properties.

The efficacy of pomegranate against prostate cancer, using transgenic TRAMP mouse model showed that co-treatment of prostate cancer cells with pomegranate extract and IGFBP-3, a protein which decreases during progression of prostate cancer, resulted in synergistic stimulation of apoptosis and additive inhibition of cell growth, associated with increased JNK phosphorylation, and suppression of AKT/ mTOR signaling [36,37].

Breast cancer

Breast cancer is the most common form of cancer (other than skin) and the leading cause of cancer mortality among women, next to lung cancer, in the United States. Each year, 182,000 women are diagnosed with breast cancer, and more than 43,000 die. In addition, 1,600 men are diagnosed with breast cancer, with 400 fatalities [11,12]. Breast cancer, like many other cancers, tends to spread throughout the body without any symptom.

***In vitro* studies:** Mouse mammary organ culture and human breast cancer cells *in vitro* has demonstrated anticancer effects of pomegranate extracts, and pomegranate seed may cause breast cancer cells to self-destruct, and exert suppressive effects on human breast cancer cells *in vitro*. This could have important implications for breast cancer treatment and the safety of estrogen replacement therapy. Pomegranate seed oil triggers apoptosis, a self-destruct mechanism, in breast cancer cells. In addition, pomegranate juice can be toxic to most estrogen-dependent breast cancer cells, while leaving normal breast cells largely unaffected. Pomegranate fruit rich in Flavonoid-polyphenol fractions have been shown to exert anti-proliferative, anti-invasive, anti-eicosanoid and pro-apoptotic actions in breast and prostate cancer cells and other solid malignancies.

The effects of pomegranate extracts on the inhibition of growth and proliferation of human MCF-7 breast cancer cells were determined by using LDH and MTS bioassays [69,11]. Pomegranate extracts inhibited cell growth and decreased cell survival through induction of cell death in both a time- and dose-dependent manner. It was demonstrated that pomegranate extracts caused DNA strand breakage in tumor cells and apoptotic cell death in several tumor cells and arrest cell cycle progression. Many cancer chemotherapeutic drugs with DNA damage capability are known to induce accumulation of p53 in the cells, indicating cell cycle arrest or programmed cell death (apoptosis). Thus, pomegranate extracts inhibited cell growth and decreased cell survival through induction of cell death (extensive cell death was observed in proliferating human breast cancer cells after treatments with pomegranate extracts).

Pomegranate seed oil was found induce cancer cell line death (apoptosis) ranging from 75 - 90% inhibition of proliferation of (MCF-7) at 100 µg/mL and 54% apoptosis at 50 µg/mL. In another test, pomegranate fermented juice polyphenols effected 47% inhibition of cancerous lesion formation, suggesting that pomegranate products possess breast cancer preventive potential greater than that previously reported. Pomegranate-derived compounds including ellagic acid, gallagic acid, and urolithins A and B (and their acetylated, methylated, and sulfated analogues) were examined for their ability to inhibit aromatase activity *in vitro*. Urolithin B was found to be the most effective in inhibiting testosterone-induced breast cancer cell proliferation and suppressing aromatase activity. Pomegranate components processed into fermented juice, aqueous pericarp and seed oil extracts blocked endogenous active estrogen biosynthesis and inhibited the steroid-converting enzyme, 17-beta-hydroxysteroid dehydrogenase-1. The seed oil was found to be the most potent followed by fermented juice.

The seed oil inhibited cell proliferation and induced apoptosis in estrogen sensitive and insensitive breast cancer cell lines, dependent on lipid peroxidation and the PKC pathway [62]. Pomegranate seed containing linolenic acid isomers were found to modulate estrogen receptor activity in a concentration dependent manner [70]. In fact, both the seed oil and fermented juice polyphenols have been shown to retard oxidation and prostaglandin synthesis, and inhibit breast cancer cell proliferation and invasion, and promote apoptosis [11]. In mouse, mammary organ cultures treated with pomegranate fermented juice and seed oil before exposure to DMBA, the seed oil was considerably more potent than the juice in causing reduction in the number of DMBA induced lesions [12]. Pomegranate extract in combination with genistein was more effective than the individual treatment in inhibiting growth of breast cancer cells and induction of apoptosis [14]. The decrease in proliferation, invasion, and motility in aggressive breast cancer phenotypes with pomegranate fruit extract treatment was associated with suppressed NF-κB gene expression and a decrease in RhoC and RhoA protein expression [69]. PGO triggers apoptosis in breast cancer cells. In the murine mammary gland organ culture, fermented juice polyphenols were effective in inhibiting DMBA-induced cancerous lesion formation [68]. Punicic acid, the omega-5 long chain polyunsaturated fatty acid present in the seed oil inhibited proliferation and induced apoptosis.

Polyphenolic fractions from pomegranate fruit were assessed *in vitro* for their possible chemopreventive activity or as adjuvant in a therapeutic setting against human breast cancer cells [11]. Polyphenols inhibited aromatase and 17-β-hydroxysteroid dehydrogenase type 1 activity by 60 - 80%. When human breast cancer cell lines MCF-7 and MB-MDA-231 cells were treated with fermented pomegranate juice and fresh pomegranate juice, polyphenols from fermented juice showed about twice the antiproliferative effect as compared to polyphenols from fresh pomegranate juice. The proapoptotic effect of pomegranate extracts was also investigated on human breast cancer cells in combination with genistein [14], a phytoestrogen isoflavone able to induce apoptosis in ER+ breast cancer cells [71]. Apoptosis induction and cell-growth inhibition of the combination was significantly higher than that of single compounds [14]. These results suggest that the association of genistein and pomegranate could be useful in association with anticancer drugs used for breast tumor.

Tamoxifen is often used against ER+ breast cancer and acts as an ER modulator in breast tissues. Pomegranate fruit extracts additively enhanced tamoxifen-induced inhibition of mitogenic action of estrogen, tamoxifen-induced inhibition of cell cycle, and tamoxifen-induced apoptosis in human breast cancer cells [72]. Furthermore, pomegranate fruit extract restored sensitivity to tamoxifen in tamoxifen-resistant breast tumor cells [72]. The extract exerted a cytotoxic effect also in quiescent WA4 cells, with a dose- and time-dependent activation of caspase-3 that suggests apoptotic cell death. The cytotoxic effect of the extract has been attributed to its components, such as ellagic acid, ursolic acid, and luteolin. Among these phenolic compounds, ursolic acid exerted the most potent inhibitory effect on cell viability and proliferation. The WA4 cell line is characterized by a majority of stem cells [73]. Therefore, the cytotoxic effects of pomegranate extract on WA4 cells were particularly relevant due to the role of stem cells in primary and secondary breast cancer onset.

Colon cancer

The chemopreventive activity of pomegranate on colon carcinogenesis has been found to be also related to its antioxidant activity. Pomegranate peel extract reduced the incidence of azoxymethane-induced genotoxicity and azoxymethane-induced premalignant lesions by blocking azoxymethane-induced impairment of biochemical indicators of oxidative stress in colonic tissue homogenates [74,75].

***In vitro* studies:** Chemopreventive effects of pomegranate juice derived ellagitannins and their intestinal bacterial metabolites urolithins have been studied in HT-29 human colon cancer cells [50]. Both ellagitannins and urolithins inhibited CYP1 activity, suppressed cell proliferation and decreased efficiency of HT-29 colon cancer cells. Inhibition of cell proliferation was mediated through cell cycle arrest in the G0/G1 and G2/M stages of the cell cycle followed by induction of apoptosis. This indicated that not only ellagic acid and punicalagins but also other ellagitannins present in pomegranate juice can potentially contribute to colon cancer chemoprevention [50].

These results suggest that polyphenols in pomegranate can play an important role in the modulation of inflammatory cell signaling in colon cancer cells. Pomegranate juice significantly suppressed TNFα-induced COX-2 protein expression, AKT activation and NF-κB binding activity in these cells [57]. Interestingly, ellagic acid alone was ineffective in suppressing NF-κB binding activity further suggesting that the interactions between polyphenols such as anthocyanins and flavonols present in the juice may be responsible for the enhanced anti-proliferative activity [57]. Thus, pomegranate juice possesses higher and diverse antioxidant activity than punicalagin and ellagic acid [22].

Ellagic acid was shown to induce apoptosis in colon cancer cells via stimulation of the intrinsic apoptotic pathway [76]. Induction of Fas-independent apoptosis in Caco-2 colon cancer cells was associated with down-regulation of cyclins A and B1 and upregulation of cyclin E and cell-cycle arrest in S phase. Remarkably, normal colon cells were resistant to ellagic acid/punicalagin induced apoptosis [76]. The standardized ellagitannin extracts obtained from pomegranate and berries have been shown to inhibit Wnt signaling, emphasizing further the inhibitory potential of ellagitannin-rich foods against colon carcinogenesis [51]. Administration of pomegranate seed oil in the diet significantly inhibited the incidence and multiplicity of colonic adenocarcinomas. The inhibition of tumor incidence was associated with increased expression of peroxisome proliferator-activated receptor (PPAR) gamma protein in the non-tumor mucosa [51]. These findings suggest beneficial effects of pomegranate against the development of colonic tumors in mice.

Animal studies: Pomegranate seed oil rich in linolenic acid suppresses chemically induced colon carcinogenesis in rats administered for 32 days, and significantly inhibited the incidence and multiplicity of azoxymethane-induced colonic adenocarcinomas, associated with increased expression of peroxisome proliferator-activated receptor gamma protein in the normal mucosa [51].

Inflammation plays a key role in the development of colon cancer, and several anti-inflammatory agents have shown promise in the prevention of colon cancer. The effects of pomegranate juice [57] on inflammatory cell signaling proteins in HT-29 human colon cancer cell line were evident in suppressing TNFα-induced (COX)-2 protein expression and reducing phosphorylation of the NF-κB/p65 subunit and its binding to the NF-κB response element. PJ also abolished TNFα-induced AKT activation, needed for NF-κB activity [57]. These results suggest that polyphenolic constituents in the pomegranate can play an important role in the modulation of inflammatory signals in colon cancer cells. Ellagitannins, derived from PG polyphenols and their metabolites, urolithins exhibit dose and time-dependent decrease in cell proliferation and clonogenic efficiency of HT-29 cells through cell cycle arrest in the G0/G1 and G2/M stages of the cell cycle followed by induction of apoptosis. Ellagitannins (ETs) and its hydrolysed product, ellagic acid (EA) have been reported to induce apoptosis in human colon cancer Caco-2 cells through down-regulation of cyclins A and B1, up regulation of cyclin E, cell-cycle arrest in the S phase and induction of apoptosis.

Lung cancer

Animal studies: Pomegranate juice extract given to mice slowed down the growth of lung tumors. Pomegranate inhibited lung tumor genesis through targeting multiple signaling pathways and merits consideration for development as a potential chemo preventive agent against human lung cancer. The peel extract attenuated lipopolysaccharide-induced lung inflammation in mice. The effect of oral consumption of pomegranate fruit extract, on mice implanted with human lung carcinoma A549 xenografts showed that pomegranate treatment selectively decreased the viability of A549 cells but had minimal effect on normal bronchial cells. Pomegranate treatment arrested cells in G0-G1 phase of the cell cycle with induction of p21 and p27, decrease in cyclins D1, D2 and E and cdks 2, 4 and 6 protein expressions. This was associated with inhibition of MAPKinase phosphorylation down-regulation of the PI3K/AKT pathway and NF-κB activity, with decrease in the protein expression of proliferation markers Ki-67 and PCNA.

The anticancer effect of PFE on lung cell cultures was confirmed in athymic mice implanted with adenocarcinomic human alveolar basal epithelial cells, and found to be associated with the inhibition of several markers of cell proliferation and angiogenesis including phosphorylation of MAPKs and activation of NF-κB, Ki-67 [60]. These findings indicate pomegranate fruit extract to be a promising chemotherapeutic agent in non-small cell lung cancer. *In vivo* evidence of the antiangiogenic effect of pomegranate fruit extract (PFE) was reported in two A/J mice lung tumors models. In the first one, lung tumor was inducted by benzo[a]pyrene and the second one by N-nitroso-tris-chloroethylurea. The inducible form of NO (iNOS) is a common marker of angiogenesis, which resulted in a decrease in cell number from both lung cancer mice models treated with pomegranate fruit extract.

The effect of PFE was tested in mice implanted with A549 cells [59] the appearance of tumors was observed in animals receiving water as early as 15 days post cell inoculation. This latency period was prolonged to 19 days in animals receiving PFE in drinking fluid. In mice that received water, the average tumor volume of 1,200 mm^3 was reached in 55 ± 2 days after tumor cell inoculation. At this time point, the average tumor volumes in the 0.1 and 0.2% PFE-fed groups were 621 and 540 mm^3, respectively [64]. The average tumor volume of 1,200 mm^3 was achieved in 67 ± 4 days and 79 ± 3 days after tumor cell inoculation in the 0.1% PFE-fed group, and the 0.2% PFE-fed group respectively. These observations indicated that PFE could be a useful chemopreventive /chemotherapeutic agent against human lung cancer [61]. The effect of oral consumption of human achievable doses of PFE using two mouse lung tumor protocols (Benzo(a) pyrene [B(a)P] and N-nitroso-tris-chloroethylurea (NTCU)) were used to induce lung tumors, and PFE was given in drinking water to A/J mice. Mice treated with PFE and exposed to B(a)P and NTCU had statistically significant lower lung tumor multiplicities than mice treated with carcinogens only [61]. Tumor reduction was 53.9% and 61.6% in the B(a)P + PFE group, respectively, compared with control group.

***In-vitro* studies:** The antiproliferative activity of pomegranate fruit extract was tested both in adenocarcinomic human alveolar basal epithelial cells (A549) and in normal human bronchial epithelial cells showing a minimal effect in normal cells and a decrease in cell viability up to 47% at the highest tested pomegranate concentration on A549 cells [59]. In addition, PFE treatment induced a strong cell-cycle arrest in G1 phase, with a 72% cell in G1 at the highest tested concentration (150 μg/mL). The cell-cycle block induced by pomegranate fruit extract was associated with a marked and dose-dependent induction of protein responsible for the transition from G1 to S phase, such as WAF1/p21 and KIP1/p27. Accordingly, pomegranate fruit extract treatment has been found to down regulate cyclins D1, D2, and E and also cdk2, cdk4, and cdk6, all involved in cell-cycle regulation of G1 phase. Moreover, pomegranate fruit extract downregulated (Ki-67 and PCNA) and inhibited (MAPK, PI3K/AKT, and NF-κB/p65) different proliferation markers [59].

The effect of pomegranate peel extract was evaluated on human neutrophil ROS production, *in vitro*, and on lipopolysaccharide-induced lung inflammation, in mice [63]. *In vitro* studies showed that the extract had no effect on superoxide anion generation, suggesting that it does not directly inhibit NADPH oxidase activity and activation pathways, or scavenge superoxide anions. However, the extract inhibited myeloperoxidase activity which may be responsible for the anti-inflammatory effect [58].

PFE has no effect on normal human bronchial epithelial cells (NHBE) but resulted in a significant decrease in the viability of human lung carcinoma A549 cells [59]. PFE treatment of A549 cells also resulted in dose-dependent arrest of cells in G0/G1 phase of the cell cycle, which was associated with induction of WAF1/p21 and KIP1/p27 and accompanied by decrease in the expression of downstream cell cycle regulatory proteins. PFE treatment also resulted in inhibition of several signaling pathways, including MAPK PI3K)/Akt, and NF-κB. It seems that pomegranate inhibited lung tumorigenesis through targeting multiple signaling pathways and merits consideration for development as a potential chemopreventive agent against human lung cancer. Ellagic acid inhibits cancer formation and is believed to inhibit cancer mutation by latching onto DNA-masking sensitive sites on the genetic material that might otherwise be occupied by harmful chemicals. Ellagic acid might be particularly effective in the inhibition of tobacco-induced lung cancer.

Pancreatic cancer

Pancreatic cancer is regarded to be one of the most lethal forms of cancer. Patients with pancreatic cancer face a grim prognosis, as it is highly aggressive and resistant to chemotherapeutics.

***In vitro* studies:** Pomegranate, being rich in phytochemicals, has been of great interest for the treatment of cancer. A low concentration (≤ 40 μg/mL) of PE caused the percentage of PC-1 cells in the G0/G1 phase of the cell cycle to significantly increase in a concentration-dependent manner, with an accompanying significant decrease in the percentage of cells in G2 [77]. This indicates that PE caused a cell cycle arrest in PC-1 cells. Chemotherapeutic drugs were largely ineffective against pancreatic cancer. The reasons for this were not understood, but may result from the presence of multidrug-resistant cancer stem cells which are readily able to repopulate tumors once differentiated cancer cells are eradicated by the drug. Combination therapy of [DLys6]-LHRH and curcumin might be more effective in treating pancreatic cancer than the drug alone [78]. Compared to a clinical achievable concentration of paclitaxel, PE caused a more profound decrease in cell proliferation of > 90% inhibition compared to a maximal inhibition of 60% with paclitaxel, and this inhibition occurred more quickly than that with paclitaxel. The phytochemicals responsible for the inhibition of the proliferation of PC-1 cells were ursolic acid, luteolin and ellagitannins. Pomegranate extract treatment increased the proportion of cells lacking CD44 and CD24 expression, which are associated with increased tumor-initiating ability, demonstrating that PE altered cell phenotype. PE was more effective in inhibiting the proliferation of PC-1 cells than the clinically used dose of paclitaxel. It seems that PE effectively inhibits the growth and viability of human pancreatic cancer cells by inducing cell cycle arrest, and reduces the tumor-initiating phenotype of the cancer cells.

Skin cancer and Photocarcinogenesis

Skin cancer is the most common form of cancer in the United States, with more than three million skin cancers diagnosed annually. Excessive exposure to solar UVB and to a lesser extent UVA radiations is the major cause of a variety of cutaneous disorders including skin

cancers. The results of promoting sun safety measures alone to prevent skin cancers have been less than successful and novel strategies are needed for the prevention of skin cancer. To this effect, polyphenol rich dietary compounds are being explored as an alternative approach in the fight against skin cancer. The oxidant/antioxidant imbalance induced by ultraviolet (UV) results in the generation of reactive oxygen species (ROS) that cause cellular damage. UV radiation is known to produce a variety of adverse effects that include sunburns, photo-aging, immuno-suppression, photo-dermatosis, DNA mutations which may then lead to cancer [45]. The endogenous antioxidant capacity is a major determinant of the skin response to UV-induced oxidative stress.

Cell culture studies were followed by evaluation of the effects of pomegranate-derived products; juice, extract and oil against UV-B-mediated damage, using the reconstituted human skin (EpiDerm) model [41], EpiDerm pretreated with pomegranate derivatives, was harvested post-UVB exposure, and markers of DNA damage and photoaging were re-assessed. A decrease in UV-B-induced cyclobutane pyrimidine dimers (CPD), and 8-dihydro-2′-deoxyguanosine (8-OHdG) in pomegranate treated skin suggested an augmented DNA repair system. In addition, pomegranate inhibited protein oxidation and decreased the protein expression of proliferating cell nuclear antigen (PCNA). Notably, pomegranate-derived products inhibited UVB-induced increase in the expression of MMPs 1, 2, 3, 7, 9 and 12 and AP-1 constituents, c-Fos and c-Jun. It was observed that all three derivatives possessed similar efficacy in protecting against UVB-induced damage [89]. The polysaccharide fraction isolated from the rind of pomegranate possesses free radical scavenging, anti-glycation, and tyrosinase inhibition properties [46], and ellagic acid rich pomegranate extract inhibited tyrosinase activity in mushrooms [41].

***In vitro* studies:** *In vivo* studies on SKH-1 mouse model showed that oral feeding of pomegranate fruit extract produced substantial protection from the adverse effects of UV-B radiation via modulation of photo-carcinogenesis [42]. Pomegranate extract consumption inhibited UV-B-induced edema, hyperplasia and leucocytic infiltration in the murine skin. This was associated with decrease in the expression of the inflammatory marker COX-2 and inhibition of ornithine decarboxylase (ODC) activity, a rate-limiting enzyme in the biosynthesis of polyamines, which play an important role in the regulation of cell transformation and development of cancer. A decrease in hydrogen peroxide generation and lipid peroxidation was observed in the extract-treated group. DNA damage caused by UV-B triggers p53 accumulation leading to cell cycle arrest allowing more time for the repair or elimination of damaged cells by apoptosis [38].

Pomegranate treatment inhibited UV-B-mediated nuclear translocation of NF-κB, known to be a crucial factor in immuno-inflammatory responses implicated in photo carcinogenesis. Pomegranate effectively inhibited UV-B-induced epidermal hyperplasia and inflammation as evidenced by decreased leucocytic infiltration, protein oxidation and lipid peroxidation, and decreased the expression of MMPs 2, 3 and 9 in murine skin [43].

Continuous oral administration of the extract to brown guinea pigs for 35 days was shown to inhibit UV-induced skin pigmentation. This was associated with a decrease in the number of DOPA-positive melanocytes in the epidermis suggesting that decreased skin pigmentation was associated with inhibition of proliferation of melanocytes and melanin synthesis by tyrosinases present in these cells [44]. A double-blind, placebo-controlled human clinical trial was conducted by the same group where women were administered supplements of high and low dose ellagic acid (200 mg/d and 100 mg/d) extracted from pomegranate for 4 weeks and subjected to ultraviolet irradiation. It was shown that ellagic acid ingested orally had a skin whitening effect even at the lower dose in these subjects. Furthermore, it was determined that the inhibitory effect of the extract on UV induced pigmentation in these subjects was possibly through the same mechanism that was elucidated in the above rodent model [45]. This observation was validated in another human trial where topical and oral administration of pomegranate augmented the protective effect of sunscreens and produced photoprotection from UVB [19].

***In vitro* studies:** In normal human epidermal keratinocytes exposed to UVB, pretreatment with pomegranate fruit extract inhibited UVB-dependent activation of NF-κB and UVB-mediated phosphorylation of ERK1/2, JNK1/2 and p38 protein [43], an important group of MAPK pathway signaling proteins that regulate cell proliferation, differentiation, and survival. It was noted that PFE protected human skin fibroblasts from cell death following UV exposure through a reduced activation of the proinflammatory transcription factor NF-κB, down regulation of proapoptotic caspase-3, and an increased G0/G1 phase associated with DNA repair. However, only higher polyphenolic

concentrations (0.5 - 10g/L) were able to significantly decrease UV-induced reactive oxygen species levels and increase intracellular antioxidant capacity [41]. Pomegranate exhibited protective effects also against UVA. Pretreatment of normal human epidermal keratinocytes with pomegranate fruit extract actually reduced many cellular pathways including phosphorylation of STAT3, AKT, ERK1/2, mTOR, and p70S6K [38]. The ability of PFE to enhance the repair of UVB-mediated DNA damage recorded in hairless mice [43] contributes to its skin photoprotective activity.

Animal studies: Oral feeding of pomegranate fruit extract inhibited skin edema, hyperplasia, infiltration of leukocytes, lipid peroxidation, and hydrogen peroxide generation in the murine skin following UVB exposure. Moreover, it protected against UVB-induced DNA damage and increased p53 and cyclin kinase inhibitor p21 protein levels [43]. Further studies showed the ability of pomegranate to prevent skin cancer, particularly, topical application of pomegranate fruit extract (2 mg/mouse) resulted in a significant inhibition of several markers of skin tumor promotion including skin edema and hyperplasia, epidermal ornithine decarboxylase activity, and protein expression of ornithine decarboxylase and COX-2 [46]. The chemopreventive effect of pomegranate in skin cancer has been found to be potentiated by diallyl sulfide, a component of garlic. Using a two-stage mouse skin tumorigenesis model, pomegranate fruit extract and diallyl sulfide synergistically delayed tumor onset and incidence associated with apoptosis induction and decreased expression of proteins involved in MAPK pathway [79].

Pomegranate seed oil (PGO) has been investigated for possible skin cancer chemopreventive efficacy in mice [38-41,45-46] and found that Skin tumors incidence that were artificially initiated in female mice was 100% in control mice compared to 93% in mice pretreated with 5% PGO prior to the oncogenic application [45]. The effect of PGO on TPA-stimulated ornithine decarboxylase (ODC) activity, an important event in skin cancer promotion, showed a 17% reduction in ODC activity. These initial observations suggested that PGO is a safe and effective chemopreventive agent against skin cancer [45]. The antitumor-promoting effects of PFE in a similar animal model of skin cancer development [46] was also studied and found that topical application of PFE on mouse skin produced significant inhibition in a time-dependent manner, against TPA-mediated increase in skin edema and hyperplasia, epidermal ODC activity, and protein expression of ODC and COX-2 [46]. PFE treatment also resulted in inhibition of TPA-induced phosphorylation of ERK1/2, p38, and JNK1/2, as well as activation of NF-κB [46]. These observations provided clear evidence that PFE possesses anti-skin-tumor-promoting effects in CD-1 mouse model by inhibiting conventional as well as novel biomarkers of TPA-induced tumor promotion.

Excessive exposure of solar ultraviolet (UV) radiation, particularly its UV-B component, to humans causes many adverse effects that include erythema, hyperplasia, hyper-pigmentation, immunosuppression, photoaging, and skin cancer. To investigate the effect of PFE on human epidermal keratinocytes (NHEK) exposed to UV-B, PFE dose dependently inhibited UV-B-mediated phosphorylation of ERK1/2, JNK1/2, and p38 protein after 24 hours [39]. PFE treatment of NHEK also resulted in a dose and time-dependent inhibition of UV-B-activation of NF-κB [39,40]. These data demonstrated protective effects of PFE against UV-B radiation and provided a molecular basis for the observed effects. Protective effects of PFE against UVA and UVB-induced damage were also studied in SKU-1064 human skin fibroblast cells [41] and found to be effective in protecting human skin fibroblasts from cell death following UV exposure, which were attributed to a reduced activation of the proinflammatory transcription factor NF-κB, downregulation of proapoptotic caspase-3, and an increased G0/G1 phase associated with DNA repair [41]. However, higher polyphenolic concentrations were needed to achieve a significant reduction in UV-induced reactive oxygen species levels and increased intracellular antioxidant capacity.

UV-A is the major portion of solar radiation reaching the earth's surface and has been shown to lead to formation of benign and malignant tumors. UVA exposure to NHEK led to an increase in phosphorylation of STAT3, AKT, and ERK1/2, which were inhibited when cells were pretreated with PFE (60 - 100 μg/ml) for 24 h [38]. These observations suggest that PFE is an effective agent for ameliorating UVA-mediated damages by modulating cellular pathways. Overall results suggest protective effects of pomegranate against UVA- and UVB-induced cell damage and the potential use of pomegranate polyphenolics in topical applications.

Leukemia

Pomegranate bioactive actions against leukemia were shown by the fact that the polysaccharide PSP001 isolated from the rind of pomegranate fruit exhibited anti-oxidant activity in addition to growth inhibitory effect on leukemic cell lines [64]. Pomegranate juice induced significant apoptosis in lymphoid and myeloid leukemia cell lines, associated with cell cycle arrest [65] and thus pomegranate potential therapy for leukemia may be considered in the future as pomegranate fermented juice, pericarp extracts and 'fatty acids' from seed oil.

Pomegranate juice extract exhibited antiproliferative effects on all tested cell lines, including nontumor cells. Treatment with pomegranate at the highest tested dose (12.5%, v/v) caused a significant S phase arrest in all leukemia cell lines, with the exception of HL-60 and KG-1a, where a small percentage of cells were blocked at the G0/G1 phase [65]. The modulation of cell-cycle arrest in HL-60 and KG-1 cells confirms a different effect of pomegranate juice extract in myeloblastic leukemia cells as compared to lymphoblastic leukemia cells, possibly due to a different modulation of c-myc expression being over expressed in HL-60 [80]. At the lowest pomegranate juice extract tested concentration (6.25% v/v), all cells showed a cell-cycle arrest in G0/G1 phase and the induction of senescence, despite being non-significant for all cell lines [65,66].

Some different fractions of pomegranate extract have also been tested on different leukemia cells (CCRF-CEM, MOLT-3, HL-60, and THP-1) to elucidate which constituents are responsible for the pomegranate anti-leukemic activity [81]. The acetonitrile fraction has been identified to be responsible for apoptosis induction, cell-cycle arrest, and inhibition of cell proliferation as it was the only one able to decrease ATP levels in leukemia cells. The induction of apoptosis by the acetonitrile fraction, tested at 6.25, 12.5, and 25% (v/v), was in line with the results previously obtained with pomegranate juice extract in terms of cell line sensitivity. Dose-dependent induction of caspase-3 and nuclear morphology characteristics confirmed these findings. The acetonitrile fraction was the richest in polyphenols and the HPLC analysis indicated the presence of ellagitannins and ellagic acid and lack of anthocyanins. Punicalagin was the most active among ellagitannins [81]. Moreover, ellagic acid (25 μM) was found to induce caspase-3-dependent apoptosis and S phase cell-cycle block on the promyelocytic cell line (HL60) [82]. Taken together, these findings confirm that the phenolic components were responsible for the main effects induced by pomegranate on leukemia cells including apoptosis, cell-cycle arrest, and inhibition of cell proliferation.

One of the main characteristics of leukemia is the block of cell differentiation at an early stage [83]. Thus, the induction of differentiation is an anti-leukemic strategy with favorable outcomes for substances such as all-trans retinoic acid. Because of the similar structure between plant flavonoids and differentiation-promoting drugs such as retinoids, it was hypothesized that flavonoid-rich pomegranate extracts might have a similar effect on differentiation. Flavonoid-rich fresh and fermented pomegranate juice and aqueous extracts of pomegranate pericarps were evaluated as potential cyto-differentiating agents. Fermented pomegranate juice and pericarp strongly promoted the differentiation of human HL-60 promyelocytic leukemia cells, while fresh juice showed a milder effect [83].

Interestingly, ellagic acid (25 μM) enhanced retinoic acid-induced differentiation of promyelocytic leukemic cells towards granulocytic phenotype [82]. Thus, the association of retinoic acid with ellagic acid might be a promising strategy to reduce the therapeutic dosage of retinoic acid and its cardio-respiratory toxicity [84]. Anti-leukemic activity of pomegranate nanoparticles obtained by partially purifying pomegranate ellagitannins and gelatin were produced to potentially increase bioavailability and bioactivities. Comparing the proapoptotic ability of pomegranate purified ellagitannins with that of pomegranate purified ellagitannin gelatin nanoparticles suspension in promyelocytic leukemia cells, ellagitannins encapsuled in nanoparticles were less effective than pomegranate purified ellagitannins in the induction of the early stage of apoptosis, while having similar effects at the late stage of apoptosis [85].

Once embedded in nanoparticles, the effect of an active component might be altered [86], but the difference in activity might be principally due to nanoparticles toxicity or cell uptake that depends on particle size, zeta-potential, and morphology. Further studies are necessary to optimize the formulation of ellagitannin nanoparticles.

Bladder Cancer

Urinary bladder urothelial carcinoma represents the most frequent cancer affecting the urinary system [64]. Pomegranate extract was shown to inhibit the proliferation of T24 and J82 bladder carcinoma cell lines. The inhibition of cell proliferation induced by pomegranate extract was related to the induction of S phase block, supported by the inhibition of cyclin A protein level and cdk1 expression. The pro-apoptotic effect induced by the extract in T24 cells was probably due to the activation of procaspase-3, procaspase-8, and procaspase-9 and the increase in Bax/Bcl-2 ratio, thus proving that apoptosis in T24 cells was induced by pomegranate through the modulation of both intrinsic and extrinsic pathways. Moreover, pomegranate ethanol extract stimulated procaspase-12 and increased the expression of endoplasmic reticulum stress markers, including CHOP and Bip. This evidence suggests that the endoplasmic reticulum stress may play a crucial role in the proapoptotic effect of pomegranate ethanol extract [87].

Brain Tumors

Gliomas are the most frequent brain tumors, with still poor prognosis because of their resistance to surgical and medical treatments. Interestingly, punicalagins have been found to induce cell death in U87MG human glioma cells [88]. The decrease in cell viability was associated with an increased expression of cyclin E and decreased expression of cyclins A and B. Punicalagin induced apoptosis in U87MG as shown by the increase in caspase-9 and caspase-3 activity and PARP cleavage. Apoptosis was not the only mechanism of cell death induced by punicalagins as the pretreatment with the caspase inhibitor z-DEVD-fmk did not completely prevent cell death. Accordingly, punicalagins caused autophagic cell death, as confirmed by the increased LC3-II cleavage. Although the role of AMPK in determining autophagy remains to be verified, ectopic expression of p27(Kip1) or phosphorylation on Thr 198 increase autophagy. Also, punicalagin raised the level of phosphorylated AMPK and phosphorylated p27 at Thr198. The dose-dependent decrease in punicalagin-induced cell death after chloroquine treatment, a suppressor of autophagy, further confirmed the ability of punicalagins to induce autophagic cell death [88]. These preliminary data are encouraging and justify further investigations on the antitumor activity of punicalagin in gliomas.

Liver cancer

Pomegranate emulsion has been found to exert chemoprevention of hepatic cancer through antioxidant, antiproliferative, and pro-apoptotic mechanisms. The emulsion seems to reduce the number and area of γ-glutamyl transpeptidase-positive hepatic foci induced in rat by diethylnitrosamine treatment. The effect was associated with the upregulation of several housekeeping genes under the control of Nrf2 such as glutathione S-transferase, NAD(P)H: quinone oxidoreductase 1, and uridine diphosphate-glucuronosyltransferase [89]. Nrf2 acts as a key mediator of NF-κB-regulated inflammatory pathway. The pomegranate emulsion suppressed several inflammatory markers including NO synthase, 3-nitrotyrosine, heat shock protein 70 and 90, COX-2, and NF-κB induced in rats following exposure to diethylnitrosamine [90]. Since pomegranate juice consumption has been reported to decrease total hepatic cytochrome P450 content as well as cytochrome P4501A2 expression in rodents [34,35], the chemopreventive effect of pomegranate emulsion might be attributable to the attenuation of diethylnitrosamine activation through pomegranate-induced cytochrome P450 inhibition [89]. However, *in vivo* studies have not demonstrated unequivocally that the consumption of pomegranate juice may interfere with drug metabolism and clearance [91]. Pomegranate emulsion was also shown to reverse hepatic proliferation induced in rat by diethylnitrosamine treatment and apoptosis through the upregulation of the proapoptotic protein Bax and the downregulation of the antiapoptotic protein Bcl-2. Canonical NF-κB and Wnt/β-catenin pathways; two interconnected molecular circuits implicated in liver physiology and pathology through regulation of proliferation, differentiation, survival, inflammation, and regeneration [92], have been shown to be involved in the above reported effects and thus in the hepato-carcinogenesis prevention by pomegranate [93].

The potent antioxidant, anti-atherosclerotic and anti-cancer activities of pomegranate seem to be largely attributed to its polyphenols contents. Ellagitannins (ETs) have also been identified as active antiatherogenic compounds in PJ. It has been shown that pomegranate fruit extracts and its purified ETs inhibit the proliferation of human cancer cells and modulate inflammatory subcellular signalling pathways and apoptosis. The crude aqueous extract derived from the peel of *Punica granatum* was evaluated as a cytotoxic agent. The results

indicated that the extract is potentially cytotoxic to the Hep2 cell line with an inhibition value of 73.9%. The grind extract from pomegranate decreased the viable cell number of Hep-2 cell line [15,21,51-56]. In study to investigate the activity of punicalagin, ellagic acid and pomegranate tannin as anti-tumor agents, it was found that these compound from pomegranate have the ability to decrease the viable cell number of human oral, prostate and colon tumor cells; however superior activity was obtained with pure pomegranate juice.

Conclusions

Cancer is the second leading cause of death and is becoming the leading one in the old age. Vegetable and fruit consumption are inversely associated with reduced cancer incidence and mortality. The fruits which are high in polyphenols are reported to have antioxidant and chemo preventive and/or chemotherapeutic potential. There is a major need for less toxic but yet effective therapies to treat cancer. Intake of Pomegranate products has been shown to exert anti-atherosclerotic, anti-inflammatory, anti-hypertensive and antioxidant activities in human subjects. Pomegranate has been shown to exert anticancer and antioxidant activity, which is generally attributed to its high content of polyphenols due to their effects of neutralizing free radicals. It was shown that pomegranate juice and/or pomegranate extracts or seed oil can inhibit the growth of prostate cancer cells in culture and also inhibit cell proliferation and induce apoptosis in human breast cancer cells, human pancreatic cancer cells, colon cancer and hepato-cellular carcinoma cell lines. It seems that the old statement that ancient tradition of keeping diseases away would be through consuming the right food, from the green world that worked both at preventive as well as potential curative levels.

Bibliography

1. Stavridi F., *et al*. "Targeted therapeutic approaches for hormone-refractory prostate cancer". *Cancer Treat Reviews* 36.2 (2010): 122-130.

2. Chuu CP., *et al.* "Androgens as therapy for androgen receptor-positive castration-resistant prostate cancer". *Journal of Biomedical Science* 18 (2011): 63.

3. Hoffman-Censits J and Fu M. "Chemotherapy and targeted therapies: Are we making progress in castrate-resistant prostate cancer". *Seminars in Oncology* 40.3 (2013): 361-374.

4. Al-Dujaili EAS., *et al*. "Natural Polyphenols: Potential for Disease Prevention". *EC Nutrition* 2.2 (2015): 337-345.

5. Swathi Sudhakar., *et al*. "Anticancer Activity of the Pomegranate and Their Role in Cancer Prevention and Therapy". *International Journal of Life Sciences Research* 3.3 (2015): 77-84.

6. Longtin R. "The pomegranate: nature's power fruit?" *Journal of the National Cancer Institute* 95.5 (2003): 346-348.

7. Gil MI., *et al*. "Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing". *Journal of Agricultural and Food Chemistry* 48.10 (2000): 4581-4589.

8. Noda Y., *et al*. "Antioxidant activities of pomegranate fruit extract and its anthocyanidins: delphinidin, cyanidin, and pelargonidin". *Journal of Agricultural and Food Chemistry* 50.1 (2002): 166-171.

9. Schubert SY., *et al*. "Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids". *Journal of Ethnopharmacology* 66.1 (1999): 11-17.

10. Al-Dujaili E A S., *et al*. "Intake of polyphenol-rich pomegranate pure juice influences urinary glucocorticoids, blood pressure and homeostasis model assessment of insulin resistance in human volunteers". *Journal of Nutritional Science* 1 (2012): e9.

11. Kim ND., *et al*. "Chemoprevetive and adjuvant therapeutic potential of pomegranate (Punica granatum) for human breast cancer". *Breast Cancer Research and Treatment* 71.3 (2002): 203-217.

12. Mehta R and Lansky EP. "Breast cancer chemopreventive properties of pomegranate (Punica granatum) fruit extracts in a mouse mammary organ culture". *European Journal of Cancer Prevention* 13.4 (2004): 345-348.

13. Toi M., *et al*. "Preliminary studies on the anti-angiogenic potential of pomegranate fractions *in vitro* and *in vivo*". *Angiogenesis* 6.2 (2003): 121-128.

14. Jeune MA., *et al*. "Anticancer activities of pomegranate extracts and genistein in human breast cancer cells". *Journal of Medicinal Food* 8.4 (2005): 469-475.

15. Stockton A and Al-Dujaili EAS. "Effect of Pomegranate Extract Consumption on Cardiovascular Disease Risk Factors, Stress Hor¬mones, and Quality of Life in Human Volunteers: An Exploratory Randomised, Double-Blind, Placebo-Controlled Trial". *EC Nutrition* 2.4 (2015): 396-411.

16. Emad A S Al-Dujaili., *et al*. "Consumption of Pomegranate Juice Attenuates Exercise - Induced Oxidative Stress, Blood Pressure and Urinary Cortisol/Cortisone Ratio in Human Adults". *EC Nutrition* 4.6 (2016): 982-995.

17. Aviram M., *et al*. "Dietary antioxidants and paraoxonases against LDL oxidation and atherosclerosis development". *Handbook of Experimental Pharmacology* 170 (2005): 263-300.

18. Adhami V M., *et al*. "Cancer Chemoprevention by Pomegranate: Laboratory and Clinical Evidence". *Nutrition and Cancer* 61.6 (2009): 811-815.

19. Syed DN., *et al*. "Pomegranate derived products for cancer chemoprevention". *Seminars in Cancer Biology* 17.5 (2007): 377-385.

20. Kaufman M and Wiesman Z. "Pomegranate oil analysis with emphasis on MALDI-TOF/MS triacylglycerol fingerprinting". *Journal of Agricultural and Food Chemistry* 55.25 (2007): 10405-10413.

21. Adams LS., *et al*. "Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells". Journal of Agricultural and Food Chemistry 54.3 (2006): 980-985.

22. Turrini E., *et al*. "Potential effects of pomegranate polyphenols in cancer prevention and therapy". *Oxidative Medicine and Cellular Longevity* (2015).

23. Seeram NP., et al. "*In vitro* antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice". *Journal of Nutritional Biochemistry* 16.6 (2005): 360-367.

24. Seeram NP., *et al*. "Pomegranate ellagitannin-derived metabolites inhibit prostate cancer growth and localize to the mouse prostate gland". *Journal of Agricultural and Food Chemistry* 55.19 (2007): 7732-7737.

25. Espin JC., *et al*. "Iberian pig as a model to clarify obscure points in the bioavailability and metabolism of ellagitannins in humans". *Journal of Agricultural and Food Chemistry* 55.25 (2007): 10476-10485.

26. Albrecht M., *et al*. "Pomegranate extracts potently suppress proliferation, xenograft growth, and invasion of human prostate cancer cells". *Journal of Medicinal Food* 7.3 (2004): 274-283.

27. Lansky EP., *et al*. "Possible synergistic prostate cancer suppression by anatomically discrete pomegranate fractions". *Investigational New Drugs* 23.1 (2005): 11-20.

28. Hong MY., *et al*. "Pomegranate polyphenols down-regulate expression of androgen-synthesizing genes in human prostate cancer cells overexpressing the androgen receptor". *Journal of Nutritional Biochemistry* 19.12 (2008): 848-855.

29. Malik A., *et al*. "Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer". *Proceedings of the National Academy of Sciences of the United States of America* 102.41 (2005): 14813-14818.

30. Koyama S., *et al*. "Pomegranate extract induces apoptosis in human prostate cancer cells by modulation of the IGF-IGFBP axis". *Growth Hormone & IGF Research* 20.1 (2010): 55-62.

31. Rettig MB., *et al*. "Pomegranate extract inhibits androgen-independent prostate cancer growth through a nuclear factor-kappaB-dependent mechanism". *Molecular Cancer Therapeutics* 7.9 (2008): 2662-2671.

32. Pantuck AJ., *et al*. "Phase II study of pomegranate juice for men with rising prostate-specific antigen following surgery or radiation for prostate cancer". *Clinical Cancer Research* 12.13 (2006): 4018-4026.

33. Kasimsetty SG., *et al*. "Effects of pomegranate chemical constituents/intestinal microbial metabolites on CYP1B1 in 22Rv1 prostate cancer cells". *Journal of Agricultural and Food Chemistry* 57.22 (2009): 10636-10644.

34. Faria A., *et al*. "Pomegranate juice effects on cytochrome P450S expression: in vivo studies". *Journal of Medicinal Food* 10.4 (2007): 643-649.

35. Wang L., *et al*. "Cellular and molecular mechanisms of pomegranate juice-induced anti-metastatic effect on prostate cancer cells". *Integrative Biology* 3.7 (2011): 742-754.

36. Adhami VM., *et al*. "Oral infusion of pomegranate fruit extract inhibits prostate carcinogenesis in the TRAMP model". *Carcinogenesis* 33.3 (2012): 644-651.

37. Paller CJ., *et al*. "A randomized phase II study of pomegranate extract for men with rising PSA following initial therapy for localized prostate cancer". *Prostate Cancer and Prostatic Diseases* 16.1 (2013): 50-55.

38. Syed DN., *et al*. "Photochemopreventive effect of pomegranate fruit extract on UVA-mediated activation of cellular pathways in normal human epidermal keratinocytes". *Photochemistry and Photobiology* 82.2 (2006): 398-405.

39. Afaq F., *et al*. "Pomegranate fruit extract modulates UV-B-mediated phosphorylation of mitogen-activated protein kinases and activation of nuclear factor kappa B in normal human epidermal keratinocytes paragraph sign". *Photochemistry and Photobiology* 81.1 (2005): 38-45.

40. Zaid MA., *et al*. "Inhibition of UVB-mediated oxidative stress and markers of photoaging in immortalized HaCaT keratinocytes by pomegranate polyphenol extract POMx". *Photochemistry and Photobiology* 83.4 (2007): 882-888.

41. Pacheco-Palencia LA., *et al*. "Protective effects of standardized pomegranate (Punica granatum L.) polyphenolic extract in ultraviolet-irradiated human skin fibroblasts". *Journal of Agricultural and Food Chemistry* 56.18 (2008): 8434-8441.

42. Aslam MN., *et al*. "Pomegranate as a cosmeceutical source: pomegranate fractions promote proliferation and procollagen synthesis and inhibit matrix metalloproteinase-1 production in human skin cells". *Journal of Ethnopharmacology* 103.3 (2006): 311-318.

43. Afaq F., *et al*. "Oral feeding of pomegranate fruit extract inhibits early biomarkers of UVB radiation-induced carcinogenesis in SKH-1 hairless mouse epidermis". *Photochemistry and Photobiology* 86.6 (2010): 1318-1326.

44. Khan N., *et al*. "Pomegranate Fruit Extract Inhibits UVB-induced Inflammation and Proliferation by Modulating NF-kappaB and MAPK Signaling Pathways in Mouse Skin(dagger)". *Photochemistry and Photobiology* (2011).

45. Hora JJ., *et al*. "Chemopreventive effects of pomegranate seed oil on skin tumor development in CD1 mice". *Journal of Medicinal Food* 6.3 (2003): 157-161.

46. Afaq F., *et al*. "Anthocyanin- and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-kappaB pathways and inhibits skin tumorigenesis in CD-1 mice". *International Journal of Cancer* 113.3 (2005): 423-433.

47. George J., *et al*. "Synergistic growth inhibition of mouse skin tumors by pomegranate fruit extract and diallyl sulfide: evidence for inhibition of activated MAPKs/NF-kappaB and reduced cell proliferation". *Food and Chemical Toxicology* 49.7 (2011): 1511-1520.

48. Murthy KN., *et al*. "Study on wound healing activity of Punica granatum peel". *Journal of Medicinal Food* 7.2 (2004): 256-259.

49. Hayouni EA., *et al*. "Hydroalcoholic extract based-ointment from Punica granatum L. peels with enhanced in vivo healing potential on dermal wounds". *Phytomedicine* 18.11 (2011): 976-984.

50. Kasimsetty SG., *et al*. "Colon cancer chemopreventive activities of pomegranate ellagitannins and urolithins". *Journal of Agricultural and Food Chemistry* 58.4 (2010): 2180-2187.

51. Sharma M., *et al*. "Effects of fruit ellagitannin extracts, ellagic acid, and their colonic metabolite, urolithin A, on Wnt signaling". *Journal of Agricultural and Food Chemistry* 58.7 (2010): 3965-3969.

52. Gonzalez-Sarrias A., *et al*. "NF-kappaB-dependent anti-inflammatory activity of urolithins, gut microbiota ellagic acid-derived metabolites, in human colonic fibroblasts". *British Journal of Nutrition* 104.4 (2010): 503-512.

53. Kohno H., *et al*. "Pomegranate seed oil rich in conjugated linolenic acid suppresses chemically induced colon carcinogenesis in rats". *Cancer Science* 95.6 (2004): 481-486.

54. Rosillo MA., *et al*. "Protective effect of ellagic acid, a natural polyphenolic compound, in a murine model of Crohn's disease". *Biochemical Pharmacology* 82.7 (2011): 737-745.

55. Boussetta T., *et al*. "Punicic acid a conjugated linolenic acid inhibits TNFalpha-induced neutrophil hyper-activation and protects from experimental colon inflammation in rats". *PloS one* 4.7 (2009): e6458.

56. Larrosa M., *et al*. "Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on phenolic metabolism". *Journal of Nutritional Biochemistry* 21.8 (2010): 717-725.

57. Singh K., *et al*. "Exploring the ameliorative potential of Punica granatum in dextran sulfate sodium induced ulcerative colitis in mice". *Phytotherapy Research* 23.11 (2009): 1565-1574.

58. Bachoual R., *et al*. "An aqueous pomegranate peel extract inhibits neutrophil myeloperoxidase *in vitro* and attenuates lung inflammation in mice". *Food and Chemical Toxicology* 49.6 (2011): 1224-1228.

59. Khan N., *et al*. "Pomegranate fruit extract inhibits prosurvival pathways in human A549 lung carcinoma cells and tumor growth in athymic nude mice". *Carcinogenesis* 28.1 (2007): 163-173.

60. Khan N., *et al*. "Oral consumption of pomegranate fruit extract inhibits growth and progression of primary lung tumors in mice". *Cancer Research* 67.7 (2007): 3475-3482.

61. Adams LS., *et al*. "Pomegranate ellagitannin-derived compounds exhibit antiproliferative and antiaromatase activity in breast cancer cells *in vitro*". *Cancer Prevention Research* 3.1 (2010): 108-113.

62. Grossmann ME., *et al*. "Punicic acid is an omega-5 fatty acid capable of inhibiting breast cancer proliferation". *International Journal of Oncology* 36.2 (2010): 421-426.

63. Khan GN., *et al*. "Pomegranate fruit extract impairs invasion and motility in human breast cancer. Integrat". *Cancer Therapies* 8.3 (2009): 242-253.

64. Joseph MM., *et al*. "Evaluation of antioxidant, antitumor and immunomodulatory properties of polysaccharide isolated from fruit rind of Punica granatum". *Molecular Medicine Reports* 5.2 (2012): 489-496.

65. Dahlawi H., *et al*. "Bioactive actions of pomegranate fruit extracts on leukemia cell lines in vitro hold promise for new therapeutic agents for leukemia". *Nutrition and Cancer* 64.1 (2012): 100-110.

66. Li Z., *et al*. "Fabrication of nanoparticles using partially purified pomegranate ellagitannins and gelatin and their apoptotic effects". *Molecular Nutrition and Food Research* 55.7 (2011): 1096-1103.

67. R. Siegel., *et al*. "Cancer statistics, 2014". *CA: A Cancer Journal for Clinicians* 64.1 (2014): 9-29.

68. Lansky EP., *et al.* "Pomegranate (Punica granatum) pure chemicals show possible synergistic inhibition of human PC-3 prostate cancer cell invasion across Matrigel". *Invest New Drugs* 23.3 (2005): 121-122.

69. Hui C., *et al*. "Flavonoids, Flavonoid Subclasses and Breast Cancer Risk: A Meta-Analysis of Epidemiologic Studies". *PLoS ONE* 8.1 (2013): e54318.

70. Tran HN., *et al.* "Pomegranate (Punica granatum) seed linolenic acid isomers: concentration-dependent modulation of estrogen receptor activity". *Endocrine Research* 35.1 (2010): 1-16.

71. T Sakamoto., *et al*. "Effects of diverse dietary phytoestrogens on cell growth, cell cycle and apoptosis in estrogen-receptor-positive breast cancer cells". *Journal of Nutritional Biochemistry* 21.9 (2010): 856-864.

72. S Banerjee., *et al*. "Pomegranate sensitizes Tamoxifen action in ER-α positive breast cancer cells". *Journal of Cell Communication and Signaling* 5.4 (2011): 317-324.

73. Z Dai, *et al*. "Pomegranate extract inhibits the proliferation and viability of MMTV-Wnt-1 mouse mammary cancer stem cells *in vitro*". *Oncology Reports* 24.4 (2010): 1087-1091.

74. Waly A Ali., *et al*. "Pomegranate (Punica granatum) peel extract efficacy as a dietary antioxidant against azoxymethane-induced colon cancer in rat". *Asian Pacific Journal of Cancer Prevention* 13.8 (2012): 4051-4055.

75. Waly A S., *et al*. "Amelioration of azoxymethane induced-carcinogenesis by reducing oxidative stress in rat colon by natural extracts". *BMC Complementary and Alternative Medicine* 14 (2014): 60.

76. Larrosa M., *et al*. "The dietary hydrolysable tannin punicalagin releases ellagic acid that induces apoptosis in human colon adenocarcinoma Caco-2 cells by using the mitochondrial pathway". *Journal of Nutritional Biochemistry* 17.9 (2006): 611-625.

77. Nair V., *et al*. "Pomegranate extract induces cell cycle arrest and alters cellular phenotype of human pancreatic cancer cells". *Anticancer Research* 31.9 (2011): 2699-2704.

78. Aggarwal S., *et al*. "[DLys6]-luteinizing hormone releasing hormone–curcumin conjugate inhibits pancreatic cancer cell growth *in vitro* and *in vivo*". *International Journal of Cancer* 129.7 (2011): 1611-1623.

79. J George., *et al*. "Synergistic growth inhibition of mouse skin tumors by pomegranate fruit extract and diallyl sulfide: evidence for inhibition of activated MAPKs/NF-κB and reduced cell proliferation". *Food and Chemical Toxicology* 49.7 (2011): 1511-1520.

80. Renneville A., *et al*. "Cooperating gene mutations in acute myeloid leukemia: a review of the literature". *Leukemia* 22.5 (2008): 915-931.

81. H Dahlawi., *et al*. "Polyphenols are responsible for the proapoptotic properties of pomegranate juice on leukemia cell lines". *Food Science & Nutrition* 1.2 (2013): 196-208.

82. Y Hagiwara., *et al*. "Ellagic acid, a natural polyphenolic compound, induces apoptosis and potentiates retinoic acid-induced differentiation of human leukemia HL-60 cells". *International Journal of Hematology* 92.1 (2010): 136-143.

83. S Kawaii and E P Lansky. "Differentiation-promoting activity of pomegranate (Punica granatum) fruit extracts in HL-60 human promyelocytic leukemia cells". *Journal of Medicinal Food* 7.1 (2004): 13-18.

84. P Fenaux., *et al*. "All-trans retinoic acid and chemotherapy in the treatment of acute promyelocytic leukemia". *Seminars in Hematology* 38.1 (2001): 13-25.

85. Li Z., *et al*. "Fabrication of nanoparticles using partially purified pomegranate ellagitannins and gelatin and their apoptotic effects". *Molecular Nutrition and Food Research* 55.7 (2011): 1096-1103.

86. K W Powers., *et al*. "Research strategies for safety evaluation of nanomaterials. Part VI. characterization of nanoscale particles for toxicological evaluation". *Toxicological Sciences* 90.2 (2006): 296-303.

87. ST Lee., *et al*. "Suppression of urinary bladder urothelial carcinoma cell by the ethanol extract of pomegranate fruit through cell cycle arrest and apoptosis". *BMC Complementary and Alternative Medicine* 13 (2013): 364.

88. S G Wang., *et al*. "Punicalagin induces apoptotic and autophagic cell death in human U87MG glioma cells". *Acta Pharmacologica Sinica* 34.11 (2013): 1411-1419.

89. Bishayee A D., *et al*. "Pomegranate-mediated chemoprevention of experimental hepatocarcinogenesis involves Nrf2-regulated antioxidant mechanisms". *Carcinogenesis* 32.6 (2011): 888-896 .

90. Bishayee R J., *et al*. "Pomegranate phytoconstituents blunt the inflammatory cascade in a chemically induced rodent model of hepatocellular carcinogenesis". *Journal of Nutritional Biochemistry* 24.1 (2013): 178-187.

91. D Farkas., *et al*. "Pomegranate juice does not impair clearance of oral or intravenous midazolam, a probe for cytochrome P450-3A activity: comparison with grapefruit juice". *Journal of Clinical Pharmacology* 47.3 (2007): 286-294.

92. Y Takigawa and A M C Brown. "Wnt signaling in liver cancer". *Current Drug Targets* 9.11 (2008): 1013-1024.

93. D Bhatia., *et al*. "Pomegranate bioactive constituents suppress cell proliferation and induce apoptosis in an experimental model of hepatocellular carcinoma: role of Wnt/β -catenin signaling pathway". *Evidence-based Complementary and Alternative Medicine* (2013): 15.

Volume 6 Issue 1 December 2016