Review Article

Therapeutic Applications of Pomegranate (*Punica granatum* L.): A Review

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Abstract

The pomegranate, *Punica granatum* L., is an ancient, mystical, unique fruit borne on a small, long-living tree cultivated throughout the Mediterranean region, as far north as the Himalayas, in Southeast Asia, and in California and Arizona in the United States. In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. The synergistic action of the pomegranate constituents appears to be superior to that of single constituents. In the past decade, numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity. (*Altern Med Rev* 2008;13(2):128-144)

Introduction

The pomegranate, *Punica granatum* L., an ancient, mystical, and highly distinctive fruit, is the predominant member of two species comprising the Punicaceae family. It was lauded in ancient times in the Old Testament of the Bible, the Jewish Torah, and the Babylonian Talmud as a sacred fruit conferring powers of fertility, abundance, and good luck. It also features prominently in the ceremonies, art, and mythology of the Egyptians and Greeks and was the personal emblem of the Holy Roman Emperor, Maximilian. Pomegranate is the symbol and heraldic device of the ancient city of Granada in Spain – from which the city gets its name. The genus name, Punica, was the Roman name for Carthage, where the best pomegranates were known to grow. Pomegranate is known by the French as grenade, the Spanish as granada, and literally translates to seeded ("granatus") apple ("pomum").[1]

The pomegranate tree typically grows 12-16 feet, has many spiny branches, and can be extremely long lived, as evidenced by trees at Versailles, France, known to be over 200 years old. The leaves are glossy and lance-shaped, and the bark of the tree turns gray as the tree ages. The flowers are large, red, white, or variegated and have a tubular calyx that eventually becomes the fruit. The ripe pomegranate fruit can be up to five inches wide with a deep red, leathery skin, is grenade-shaped, and crowned by the pointed calyx. The fruit contains many seeds (arils) separated by white, membranous pericarp, and each is surrounded by small amounts of tart, red juice. The pomegranate is native from the Himalayas in northern India to Iran but has been cultivated and naturalized since ancient times over the entire Mediterranean region. It is also found in India and more arid regions of Southeast Asia, the East Indies, and tropical Africa. The tree is also cultivated for its fruit in the drier regions of California and Arizona.[2]

In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. In Ayurvedic medicine the pomegranate is considered "a pharmacy unto itself" and is used as an antiparasitic agent,[3] a "blood tonic,"[4] and to heal aphthae, diarrhea, and ulcers.[5] Pomegranate also serves as

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a remedy for diabetes in the Unani system of medicine practiced in the Middle East and India.[6] The current explosion of interest in pomegranate as a medicinal and nutritional product is evidenced by a MedLine search from 2000 to present, revealing over 130 new scientific papers pertaining to its health effects. Between 1950 and 1999 only 25 such publications appear on MedLine.[7] The potential therapeutic properties of pomegranate are wide-ranging and include treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, and protection from ultraviolet (UV) radiation. Other potential applications include infant brain ischemia, Alzheimer's disease, male infertility, arthritis, and obesity.

The following abbreviations for various pomegranate extracts will be used throughout the article:

- Pomegranate juice – PJ
- Pomegranate by-product – PBP
- Fermented pomegranate juice – FPJ
- Cold-pressed seed oil – CPSO
- Pomegranate peel extract – PPE
- Pomegranate pulp juice – PPJ
- Pomegranate fruit extract – PFE
- Pomegranate flower extract – PFLE
- Hydroalcoholic extract of pomegranate – HAEP
- Gel-based pomegranate extract – GPBE

Biochemical Constituents

Over the past decade, significant progress has been made in establishing the pharmacological mechanisms of pomegranate and the individual constituents responsible for them. Extracts of all parts of the fruit appear to have therapeutic properties,[7] and some studies report the bark, roots, and leaves of the tree have medicinal benefit as well.[3] Current research seems to indicate the most therapeutically beneficial pomegranate constituents are ellagic acid ellagitannins (including punicalagins), punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonols and flavones. Table 1 lists the principal constituents of the *Punica granatum* tree and fruit. Figure 1 depicts the structure of ellagic acid.

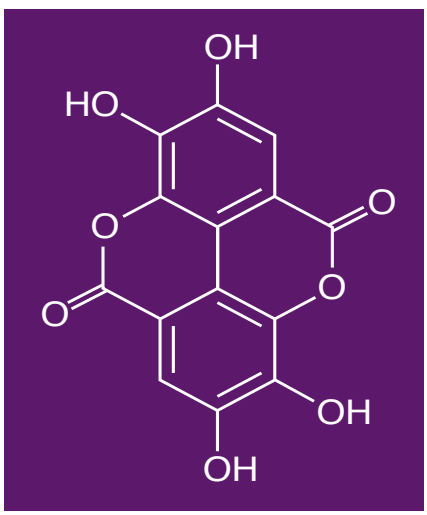
Table 1. Pomegranate Fruit Parts and Constituents[8-21]

PLANT COMPONENT	CONSTITUENTS
Pomegranate juice	anthocyanins;[8] glucose, ascorbic acid;[9] ellagic acid, gallic acid, caffeic acid;[10] catechin, EGCG;[11] quercetin, rutin;[12] numerous minerals, particularly iron;[13] amino acids[7]
Pomegranate seed oil	95-percent punicic acid;[14] other constituents, including ellagic acid;[10] other fatty acids;[14] sterols[15]
Pomegranate pericarp (peel, rind)	phenolic punicalagins; gallic acid and other fatty acids;[10] catechin, EGCG;[11] quercetin, rutin, and other flavonols;[12] flavones, flavonones;[16] anthocyanidins[17]
Pomegranate leaves	tannins (punicalin and punicafolin); and flavone glycosides, including luteolin and apigenin[16]
Pomegranate flower	gallic acid, ursolic acid;[18] triterpenoids, including maslinic and asiatic acid;[19] other unidentified constituents
Pomegranate roots and bark	ellagitannins, including punicalin and punicalagin;[20] numerous piperidine alkaloids[21]

Constituent Standardization versus Synergy

The goal of many pomegranate studies has been to identify the therapeutic constituents. Commonly found in many plants, ellagic acid exhibits powerful anticarcinogenic[22] and antioxidant[23] properties, propelling it to the forefront of pomegranate research. Many commercially available pomegranate extracts are being standardized to contain 40-percent (or more) ellagic acid; however, Lansky, a prominent researcher on the medicinal properties of pomegranate, cautions against focusing on ellagic acid standardization to the exclusion of other therapeutically important pomegranate constituents.[24] Research on ellagic acid with other flavonoids such as quercetin supports his contention.[25,26] Lansky's research confirms the synergistic action of several pomegranate constituents is superior to ellagic acid in suppressing prostate cancer.[27,28] To quote Lansky, "The recent profusion onto the nutraceuticals marketplace of products standardized to 40 percent (or even higher) ellagic acid represents a cynical, lucre-driven attempt to replace the power of the pomegranate with the power of ellagic acid. The pomegranate needs no such tricks or enhancements. It is rather an extraordinary, albeit mysterious (and messy), fruit with a complete medicinal power contained within its juice, peel, and seeds."[24]

Figure 1. Structure of Ellagic Acid



Biochemistry/Pharmacokinetics

Although little is known about the metabolism and bioavailability of ellagitannins from food sources, three small human trials and one case study have investigated the bioavailability, absorption, metabolism, and *in vivo* antioxidant effects of pomegranate. In the case study, consumption of 180 mL pomegranate juice (PJ) by a single subject yielded 31.9 ng/mL plasma ellagic acid at one hour, with rapid plasma clearance by four hours post-ingestion. This was the first direct evidence that ellagic acid consumed from food was absorbed in humans.[29] A study of 18 healthy volunteers by the same researchers confirmed the rapid absorption and plasma clearance of ellagitannins and also confirmed urolithin metabolites excreted in the urine can persist for 48 hours after pomegranate juice ingestion, thereby suggesting an explanation of the benefits of long-term pomegranate administration.[30]

In a 13-day clinical trial involving six healthy subjects (4 men and 2 women), one liter of PJ containing 4.37 g/L punicalagins and 0.49 g/L anthocyanins was consumed by all six subjects for five days. Three pomegranate juice metabolites were detected in the plasma – urolithin A, urolithin B, and a third unidentified minor metabolite; urinalysis at 24 hours revealed six metabolites – the three found in the plasma as well as an aglycone metabolite corresponding to each of three plasma metabolites. Maximum excretion rates occurred 3-4 days after juice ingestion. Significant variability of urinary metabolite concentrations was observed among subjects and may be attributable to differences in colonic microflora, where the ellagitannins are believed to be metabolized.[31] The persistence of urolithin A and B in the urine may be responsible for pomegranate's long-term antioxidant effects, rather than the polyphenols found in the juice.

In another study, 11 healthy men and women were placed on a polyphenol- and antioxidant-free diet for three days prior to consuming pomegranate extract (plant parts used were not specified). Subjects were given 800 mg capsuled pomegranate extract daily containing 330.4 mg punicalagins and 21.6 mg ellagic acid (EA). C_{max} and T_{max} for plasma EA was 33.8±12.7 ng/mL at one hour post-ingestion, similar to values observed in the case study when similar amounts of punicalagins and EA were administered. This study also demonstrated a significant increase (31.8%) in plasma

antioxidant capacity 30 minutes after extract administration; one and two hours post ingestion, values were increased 1.62- and 1.43-fold, respectively.[32]

Mechanisms of Action

Although pomegranate's wide-ranging therapeutic benefits may be attributable to several mechanisms, most research has focused on its antioxidant, anticarcinogenic, and anti-inflammatory properties.

Antioxidant Mechanisms

An *in vitro* assay using four separate testing methods demonstrated pomegranate juice and seed extracts have 2-3 times the antioxidant capacity of either red wine or green tea.[33] Pomegranate extracts have been shown to scavenge free radicals and decrease macrophage oxidative stress and lipid peroxidation in animals[34] and increase plasma antioxidant capacity in elderly humans.[35]

Studies in rats and mice confirm the antioxidant properties of a pomegranate by-product (PBP) extract made from whole fruit minus the juice, showing a 19-percent reduction in oxidative stress in mouse peritoneal macrophages (MPM), a 42-percent decrease in cellular lipid peroxide content, and a 53-percent increase in reduced glutathione levels.[34] *In vitro* assay of a fermented pomegranate juice (FPJ) extract and a cold-pressed seed oil (CPSO) extract found the antioxidant capacity of both are superior to red wine and similar to green tea extract.[14] A separate study in rats with CCl_4-induced liver damage demonstrated pretreatment with a pomegranate peel extract (PPE) enhanced or maintained the free-radical scavenging activity of the hepatic enzymes catalase, super oxide dismutase, and peroxidase, and resulted in 54-percent reduction of lipid peroxidation values compared to controls.[36]

Research in humans has shown a juice made from pomegranate pulp (PPJ) has superior antioxidant capacity to apple juice. Using the FRAP assay (ferric reducing/antioxidant power), Guo et al found 250 mL PPJ daily for four weeks given to healthy elderly subjects increased plasma antioxidant capacity from 1.33 mmol to 1.46 mmol, while subjects consuming apple juice experienced no significant increase in antioxidant capacity. In addition, subjects consuming the PPJ exhibited significantly decreased plasma carbonyl content (a biomarker for oxidant/antioxidant barrier impairment in various inflammatory diseases) compared to subjects taking apple juice. Plasma vitamin E, ascorbic acid, and reduced glutathione values did not differ significantly between groups, leading researchers to conclude pomegranate phenolics may be responsible for the observed results.[35]

Anticarcinogenic Mechanisms

In vitro assays utilizing three prostate cancer cell lines (DU-145, LNCaP, and PC-3) demonstrated various pomegranate extracts (juice, seed oil, peel) potently inhibit prostate cancer cell invasiveness and proliferation, cause cell cycle disruption, induce apoptosis, and inhibit tumor growth. These studies also demonstrated combinations of pomegranate extracts from different parts of the fruit were more effective than any single extract.[27,37]

Several animal studies have elucidated pomegranate's potential anticancer mechanisms. Two studies in mice implanted with the prostate cancer PC-3 cell line demonstrated pomegranate fruit extract (PFE; edible parts of the fruit, excluding the peel) inhibits cell growth and induces apoptosis via modulation of proteins regulating apoptosis.[38,39]

In an open-label, phase II clinical trial in 46 men with recurrent prostate cancer, 16 patients (35%) showed a significant decrease in serum prostate specific antigen (PSA) levels (average=27%) during treatment with eight ounces of pomegranate juice. Corresponding *in vitro* assays using patient plasma and serum demonstrated significant decreases in prostate cancer cell line proliferation and increased apoptosis. Nitric oxide preservation via ingestion of pomegranate polyphenols significantly correlated with lower PSA values. These results indicate pomegranate may affect prostate cancer because of antiproliferative, apoptotic, antioxidant, and possibly anti-inflammatory effects.[40]

Recent research also indicates pomegranate constituents inhibit angiogenesis via downregulation of vascular endothelial growth factor in MCF-7 breast cancer and human umbilical vein endothelial cell lines.[41]

Anti-inflammatory Mechanisms

Cold pressed pomegranate seed oil has been shown to inhibit both cyclooxygenase and lipoxygenase enzymes *in vitro*. Cyclooxygenase, a key enzyme in the conversion of arachidonic acid to prostaglandins (important inflammatory mediators), was inhibited by 37 percent by a CPSO extract. Lipoxygenase, which catalyzes the conversion of arachidonic acid to leukotrienes, also key mediators of inflammation, was inhibited by 75 percent by a CPSO extract. By comparison, an FPJ extract resulted in a 23.8-percent inhibition of lipoxygenase *in vitro*.[14]

Another *in vitro* study that may have far-reaching implications for those suffering from osteoarthritis (OA) demonstrated PFE has a significant and broad inhibitory effect on matrix metalloproteinases (MMPs), a subgroup of collagenase enzymes expressed in high levels in arthritic joints and involved in the turnover, degradation, and catabolism of extracellular joint matrix. In pretreated human femoral OA chondrocytes, PFE inhibited IL-1beta-induced destruction of proteoglycan, expression of MMPs at the cellular level, and phosphorylation and activation of mitogen-activated protein kinases (signal transduction molecules involved in MMP expression). The suppression of MMP expression in OA chondrocyte cultures by PFE suggests pomegranate constituents prevent collagen degradation and may inhibit joint destruction in OA patients.[42]

Other Mechanisms

A pilot study in type 2 diabetic patients with hyperlipidemia found concentrated PJ decreased cholesterol absorption, increased fecal excretion of cholesterol, had a beneficial effect on enzymes involved in cholesterol metabolism, significantly reduced total and LDL cholesterol, and improved total/HDL and LDL/HDL cholesterol ratios.[43]

PJ consumption by hypertensive patients inhibits serum angiotensin converting enzyme (ACE; a catalyst for the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor) activity, thereby reducing systolic blood pressure[44] and potentially protecting against cardiovascular disease.

Animal studies have revealed three possible hypoglycemic mechanisms for *Punica granatum* extracts. Pomegranate flower extract (PFLE) improved insulin sensitivity and lowered glucose levels in rats as early as 30 minutes post-glucose loading. PFLE also inhibited alpha-glucosidase *in vitro*, thereby decreasing the conversion of sucrose to glucose.[45] PPE demonstrates significant hypoglycemic activity in diabetic rats, via enhanced insulin levels and regeneration of pancreatic beta cells.[46]

Numerous *in vitro* studies[3,47,48] and two human trials[49,50] demonstrate the antimicrobial activity of pomegranate extracts. The growth of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Diplococcus pneumoniae*, *Escherichia coli* O157:H7, and *Candida albicans* was inhibited via direct bacteriocidal or fungicidal activity.

Clinical Applications

Prostate Cancer

Among males in the United States and other Western countries, prostate cancer is the second-leading cause of cancer-related death. *In vitro* studies show several PFEs inhibit prostate cancer cell growth, induce apoptosis of several prostate cancer cell lines (including highly aggressive PC-3 prostate carcinoma cells), suppress invasive potential of PC-3 cells, and decrease proliferation of DU-145 prostate cancer cells.[27,37,38] Lansky et al found combining equal amounts of FPJ, PPE, and CPSO extracts resulted in a 99-percent suppression of DU-145 prostate cancer cell invasion across a Matrigel matrix. CPSO extract or FPJ extract alone resulted in 60-percent suppression of invasion, and combining any two extracts induced 90-percent suppression. Studies in mice have also demonstrated PFE inhibits prostate tumor growth and decreases PSA levels.[38,39]

These promising results led some of the same researchers to conduct a two-stage phase II clinical trial in men with recurrent prostate cancer and rising PSA levels. All eligible patients had previous surgery or radiation therapy for prostate cancer, Gleason scores (a grading system for predicting the behavior of prostate cancer) ≤7, rising PSA value of 0.2-5.0 ng/mL, no prior hormonal therapy, and no evidence of metastases. Baseline PSA doubling times were established for 22 participants who were then started on eight ounces PJ (570 mg total polyphenol gallic acid equivalents) daily until meeting disease progression endpoints. Endpoints measured were: effect on PSA levels, serum lipid peroxidation and nitric oxide levels, *in vitro* induction of proliferation and apoptosis of LNCaP cells in patient serum containing pomegranate constituents, and overall safety of extract administration.[40]

Based on preliminary results achieved in phase I, 24 additional patients were enrolled and 46 patients were evaluated over 13 months in both stages of the trial. Of these, 35 percent (n=16) demonstrated decreased PSA levels, the primary trial endpoint – average decrease=27%; median decrease=18%; range 5-85%. Four of 46 patients (8.7%) met objective response criteria and exhibited >50-percent reduction in PSA values, meeting criteria for a phase III trial. In addition, an average 40-percent reduction in serum oxidative state was observed in patients accompanied by a significant reduction in serum lipid peroxidation compared to baseline. Nitric oxide serum metabolites measured at nine months after study initiation revealed an average 23-percent increase, which significantly correlated with baseline PSA levels.[40]

An *in vitro* arm of the trial using patient serum investigated whether PJ consumption had any effect on growth rates or apoptosis of LNCaP prostate cancer cells in culture. Serum collected at nine months after study initiation and incubated with LNCaP decreased cell growth by an average of 12 percent in 84 percent of patients compared to baseline. An average 17.5-percent increase in apoptosis in 75 percent of patients was also noted. This study indicates PJ or PJ constituents may have promise as a therapy for prostate cancer, particularly recurrent type with rising PSA levels; phase III studies are currently underway.[40]

Other Cancer Types

Numerous *in vitro* studies have investigated the therapeutic effect of pomegranate extracts against several other cancer cell lines. In HT-29 colon cancer cells, cyclooxygenase-2 (COX-2) expression is increased via activation of nuclear factor kappa-B (NFκB) by tumor necrosis factor-alpha (TNF-α), an inflammatory cell signaling process that may be a cause of cancer initiation and progression. Treatment of HT-29 colon cancer cells with PJ, total pomegranate tannins, or concentrated pomegranate punicalagin induced a significant decrease in COX-2 expression. PJ treatment resulted in the highest level of COX-2 suppression (79%) compared to treatment with single constituents. The effects were attributed to synergistic activity of the bioactive constituents thought to be necessary for pomegranate's anti-inflammatory and anticarcinogenic activity.[51]

Another *in vitro* study investigated the effects of punicalagin, ellagic acid, total pomegranate tannins, and PJ on several cell lines. Although all preparations decreased viable cell numbers in KB and CAL-27 oral cancer cell lines, as well as in HT-29 and HCT-116 colon cancer cell lines, a higher degree of suppression was obtained with pure PJ, an affect attributed to the synergy of its bioactive constituents.[52]

Research utilizing breast cancer cell lines MCF-7 and MB-MDA-231 demonstrates pomegranate constituents effectively inhibit angiogenesis,[41] tumor growth,[53] proliferation, and invasiveness,[54] and induce apoptosis.[55] To examine the effect of FPJ and CPSO extracts, and an HPLC-isolated peak B (from the fruit extract), Mehta and Lansky used the mouse mammary organ culture, an animal model of breast cancer having ≥75-percent accuracy of predicting *in vivo* carcinogenesis. They found cancerous glands treated with each pomegranate compound exhibited decreased lesion incidence – 37 percent for FPJ, and 75-90 percent for both peak B and CPSO. Seed oil is comprised mainly of punicic acid, a trienoic acid with anticarcinogenic properties and effective at very low doses (1 μg/mL in organ culture). Peak B is believed to be a phenolic compound with potent chemopreventative properties.[53]

Research in mice has shown PFE inhibits tumorigenesis in lung cancer and skin cancer models. In the lung cancer study, mice given daily oral dosages of PFE comparable to what humans could reasonably consume (exact dosages were not available) exhibited significantly less lung tumor growth than mice not receiving PFE.[56] In mice treated with skin-cancer-inducing 12-O-tetradecanoylphorbol-13-acetate (TPA), animals treated topically with PFE had significantly reduced incidence of skin tumors. In the PFE-treated group, only 30 percent of mice exhibited tumors compared to 100 percent of mice treated with TPA and no PFE. This result was attributed to suppression of inflammation (COX-2, MAPKs, NFκB) and the tumor proliferation marker ornithine decarboxylase.[57]

Lansky and Kuwaii investigated the effect of flavonoid-rich PJ and FPJ and pomegranate pericarp extracts on HL-60 human leukemia cell differentiation (the ability of cancer cells to revert to normal cells) and proliferation. Because of the structural similarity between plant flavonoids and retinoids (the latter be-

ing established pro-differentiating agents), it was hypothesized that flavonoid-rich pomegranate extracts might have a similar effect on differentiation. *In vitro* assays confirmed both the FPJ and pericarp extracts strongly promoted cellular differentiation and inhibited proliferation in HL-60 cell cultures; the effect of PJ on cellular differentiation was less significant. This study suggests another mechanism by which pomegranate constituents impart an anticarcinogenic effect.[58]

Atherosclerosis

In vitro, animal, and human trials have examined the effects of various pomegranate constituents on prevention and attenuation of atherosclerosis. One of the preeminent researchers in endothelial function and nitric oxide (NO) biochemistry, Louis J. Ignarro, PhD, investigated the effects of pomegranate juice and other fruit juices on endothelial function, comparing propensities to protect NO from destruction by reactive oxygen species *in vitro*. Results of the antioxidant portion of the study demonstrate pomegranate juice possesses significantly greater antioxidant capacity at much lower concentrations (>1000-fold dilutions) than either grape or blueberry juice, which was attributed to the high anthocyanin flavonoid content and higher total flavonoid content in PJ than the other juices.[59]

Because impaired endothelial function is an early indicator of atherosclerosis, this study examined the effect of PJ on proliferation of rat aortic smooth muscle cells in culture. PJ proved superior to other juices, significantly enhancing NO's effect on cardiac endothelium even at 2,000-fold dilutions. PJ did not influence endothelial nitric oxide synthase (eNOS) expression, leading Ignarro et al to conclude the antioxidant properties of PJ protect NO from free radical destruction and augment the antiproliferative action of NO on rat aortic smooth muscle cells.[59]

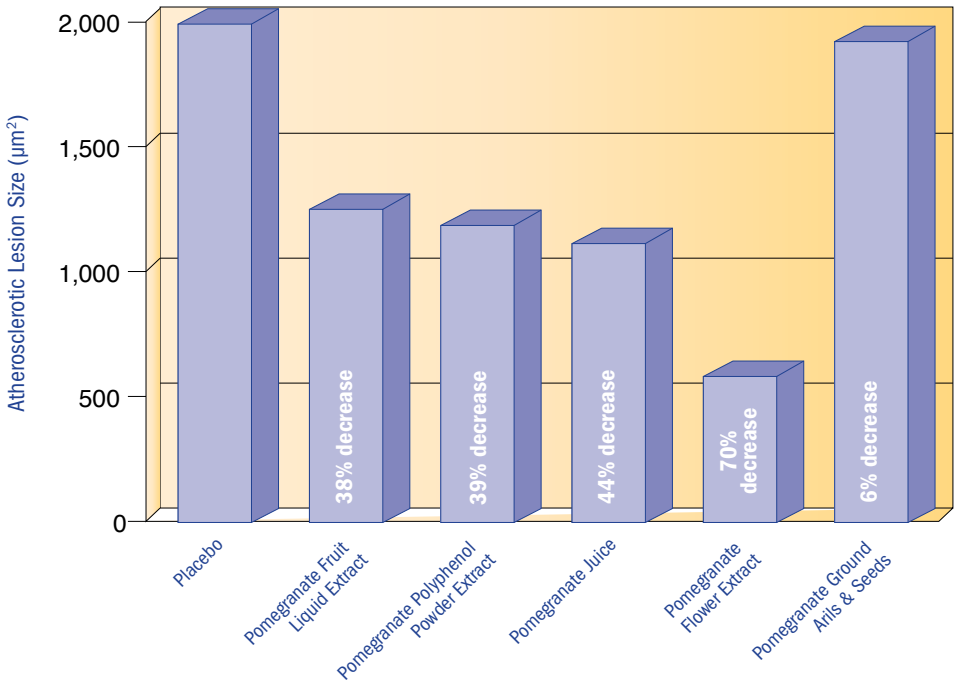
In early-stage atherosclerosis, elevated plasma cholesterol, increased oxidative stress, and increased cholesterol esterification rates are factors contributing to foam cell formation and development of atherosclerotic lesions.[60-62] Research in atherosclerotic apolipoprotein-E deficient (E°) mice by Aviram et al at the Lipid Research Laboratory in Haifa, Israel, has focused on the ability of pomegranate extracts to inhibit atherogenesis.[34,63] Two months of PJ to E° mice with advanced atherosclerosis reduced MPM lipid peroxide content by 42 percent compared with placebo-treated mice; MPM lipid peroxide content in PJ-treated mice was 20-percent lower than in four-month-old control mice. In addition, MPM harvested from PJ-treated mice exhibited 80-percent lower rates of cholesterol esterification than placebo-treated mice. In PJ-treated mice atherosclerotic lesion size in the aorta was 17-percent smaller than in the age-matched placebo group. PJ and an isolated tannin fraction from PJ were also given to young E° mice prior to development of significant atherosclerosis. Researchers found 25- and 17-percent reductions in plasma lipid peroxide concentrations with the isolated tannin fraction and PJ, respectively.[64]

Aviram et al also investigated the anti-atherosclerotic effects of a PBP extract after the juice was removed. Four-month-old E° mice with significant atherosclerosis were given PBP extract (containing 51.5 µg gallic acid equiv/kg/day) with an eight-fold higher polyphenol concentration than PJ for three months. This resulted in a significant reduction in MPM oxidative status as evidenced by a 27-percent decrease in total macrophage peroxide levels, a 42-percent decrease in cellular lipid peroxide levels, and a 19-percent decrease in peritoneal macrophage uptake of oxidized LDL.[34]

To further identify the most potent anti-atherogenic pomegranate components, Aviram et al analyzed several more pomegranate extracts from all parts of the plant. Atherosclerotic E° mice were given six different pomegranate preparations with varying amounts of total polyphenols and gallic acid content for three months. Antioxidant activity, atherosclerotic lesion size, MPM oxidative status, blood sugar, and lipid profiles were examined. Confirming earlier results, this study demonstrated PFLE more significantly affects atherosclerotic lesion size (Figure 2), lipid profiles, and blood sugar levels than other extracts tested; two PPEs demonstrated the most potent antioxidant effects. Mechanisms associated with the anti-atherogenic effects of pomegranate in this study include increased MPM uptake of oxidized LDL, decreased lipid peroxidation, and decreased cholesterol levels.[65]

The effect of PJ consumption on lipid peroxidation in plasma and HDL- and LDL-lipoproteins was examined in a double-armed human trial. In the first study, 13 healthy, nonsmoking men (ages 20-35) were given 50 mL PJ daily (containing 1.5 mmol total polyphenols) for two weeks. In the second study (duration ≤10 weeks), three healthy men (same age range)

Figure 2. Atherosclerotic Lesion Size with Various Pomegranate Extracts



Aviram M, Volkova N, Coleman R, et al. Pomegranate phenolics from the peels, arils, and flowers are antiatherogenic: studies *in vivo* in atherosclerotic apolipoprotein E-deficient (E°) mice and in vitro cultured macrophages and lipoproteins. *J Agric Food Chem* 2008;56:1148-1157.

were given increasing doses of PJ ranging from 20-80 mL daily (0.54-2.16 mmol total polyphenols). Fasting blood samples were drawn from participants pre-study and after one and two weeks of PJ supplementation. No significant effect was observed in either study on plasma lipid profile or lipoprotein patterns. The results did show, however, for the first time in humans, that PJ has an inhibitory effect on lipid peroxidation in plasma and in lipoproteins, with the middle dose (50 mL daily) being the most effective, yielding a 32-percent decrease in plasma lipid peroxidation. PJ (in a dose-dependent manner) also demonstrated up to 90-percent inhibition of collagen-induced platelet aggregation in human platelets *ex vivo*.[63]

Hyperlipidemia

Pomegranate flowers have been used in both the Unani and Ayurvedic systems of medicine as a remedy for diabetes. Based on historical use, a study in diabetic rats explored the effects of PFLE on cardiac lipid metabolism in 13- to 15-week old Zucker diabetic rats. Animals were given 500 mg/kg PFLE or placebo for six weeks, and total cholesterol, triglyceride, and nonesterified free fatty acids (NEFA) were determined prior to treatment (nonfasting), at week 4 (nonfasting), and week 5 (fasting) in both rat plasma and cardiac tissue. PFLE was shown to activate peroxisome proliferator-activated receptor (PPAR-α), a cardiac transcription factor involved in myocardial energy production via fatty acid uptake and oxidation. PPAR-α activation decreased cardiac uptake and circulation of lipids. Decreases were observed in cardiac tissue triglyceride content at the end of the study and in plasma total cholesterol and NEFA after four weeks of treatment.[66]

A pilot study involving 22 type 2 diabetic patients (8 men and 14 women) investigated the cholesterol-lowering effects of 40 g concentrated PJ for eight weeks. Statistically significant decreases were observed in total cholesterol (from 202.4±27.7 mg/dL at baseline to 191.4±21 mg/dL at study conclusion), LDL cholesterol (124.4±31.9 mg/dL at baseline to 112.9±25.9 mg/dL at study conclusion), total/HDL cholesterol ratio (5.5±1.3 at baseline to 5.1±1.1 at study conclusion), and LDL/HDL ratio (3.4±1.2 at baseline to 3.0±0.9 at study conclusion). The authors attributed these effects to decreased absorption and increased fecal excretion of cholesterol, as well as possible affects on HMG-CoA reductase and sterol O-acyltransferase, two enzymes key to cholesterol metabolism.[43]

Table 2. Antioxidant Activity of Pomegranate Juice Extract in Patients with Carotid Artery Stenosis

ANALYSIS	BASELINE	1 MONTH	3 MONTHS	1 YEAR	3 YEARS
Total Antioxidant Status (nmol/liter)	0.95 ± 0.12			2.20 ± 0.23 (⇧130%)	
Serum Antibodies against LDL Oxidation (EU/mL)	2670 ± 61	1563 ± 69	1670 ± 52	⇩19%	
AAPH-induced Serum Lipid Peroxidation (nmol/mL)	1670 ± 66			691 ± 43 (⇩59%)	⇩75%
Serum Paraoxonase 1 (PON1) Arylesterase Activity (Units/mL)	56 ± 5			97 ± 10 (⇧73%)	107 ± 10 (⇧83%)
Lipid Peroxide Content of Carotid Lesions (nmol/mg of lesion protein)			⇩61%	⇩44%	

EU = Enzyme units
AAPH = 2.2'-azobis, 2-amidinopropane hydrochloride

Hypertension

A small clinical trial demonstrated PJ inhibits serum ACE and reduces systolic blood pressure in hypertensive patients. Ten hypertensive subjects (ages 62-77; seven men and three women) were given 50 mL/day PJ containing 1.5 mmol total polyphenols for two weeks. Two of seven patients were also diabetic and two were hyperlipidemic. Seven of 10 subjects (70%) experienced a 36-percent average decrease in serum ACE activity and a small, but significant, five-percent decrease in systolic blood pressure.[44]

Carotid Artery Stenosis

In a small, long-term study, 19 subjects (ages 65-75) with severe carotid artery stenosis (70-90% stenosis of internal carotid arteries) were randomized to receive either 50 mL PJ daily containing 1.5 mmoles total polyphenols (n=10) or no treatment (n=9) for one year; five subjects continued PJ for an additional two years. Study participants were treated with similar hypocholesterolemic and antihypertensive medications and no dietary or lifestyle changes occurred in either group. Blood samples were collected and echo Doppler analysis was performed at baseline and at 3, 6, 9, 12, 22, 28, and 36 months. Control subjects demonstrated a mean nine-percent increase in intima-media thickness (IMT) of left and right carotid arteries during the first year. Conversely, those consuming PJ had reduced IMT at 3, 6, 9, and 12 months ranging from 13 percent at three months to 35 percent at one year compared to baseline values.[67]

Most serum biochemistry parameters remained unchanged by PJ consumption over the first year, with the exception of triglyceride concentrations, which increased 16 percent but remained in the normal range. Serum lipid peroxidation in subjects consuming PJ was significantly reduced by 59 percent after one year, and levels of LDL-associated lipid peroxides

were also decreased by as much as 90 percent after six months of supplementation. Body mass index did not change in treated subjects but systolic blood pressure was reduced an average of 16 percent during the three-year study.[67] In addition to previous reports of reduced systolic blood pressure[44] and inhibition of lipid peroxidation,[63] this study demonstrated that PJ consumption (via antioxidative mechanisms) significantly reduces various aspects of IMT in patients with severe carotid artery stenosis (Table 2).

Myocardial Perfusion

In a double-blind, randomized, placebo-controlled trial, 39 patients were given either 240 mL PJ (polyphenol content not specified) (n=23) or a sports beverage of similar color, flavor, and caloric content daily for three months (n=16). Although both control and treatment patients demonstrated similar levels of stress-induced ischemia at baseline, at three months stress-induced ischemia increased in the placebo group (from 5.9±4.3 to 7.1±5.5) but decreased in the treatment group (from 4.5±3.1 to 3.7±3.7). In addition, angina episodes increased 38 percent in the placebo group but decreased 50 percent in the treatment group (a net change of 88 percent). These results demonstrate a reduction in myocardial ischemia and improved myocardial perfusion (as measured by stress-induced ischemia) in patients consuming pomegranate juice.[68]

Diabetes

In an animal model of diabetes, Huang et al demonstrated the favorable effect of PFLE on lipid profiles[66] and cardiac fibrosis[18] of Zucker fatty diabetic rats. Rosenblat et al investigated the effect of 50 mL/day PJ for three months on oxidative stress, blood sugar, and lipid profiles in 10 type 2 diabetic patients (history of diabetes for 4-10 years) and 10 healthy controls (ages 35-71).[69] In diabetic patients, triglyceride levels were 2.8 times greater, HDL cholesterol was 28-percent lower, and hemoglobin A1C (HbA1C) values were 59-percent higher than in control patients. Insulin was only slightly lower in patients than controls, and C-peptide (a proinsulin metabolite marker for endogenously secreted insulin) was slightly higher in diabetic patients than in healthy controls at baseline (indicating slight hyperinsulinemia). Consuming PJ for three months did not significantly affect triglyceride, HDL cholesterol, HbA1C, glucose, or insulin values, but did lower serum C-peptide values by 23 percent compared to baseline in diabetic patients – a sign of improved insulin sensitivity.

PJ consumption also significantly reduced oxidative stress in the diabetic patients as evidenced by a 56-percent reduction in lipid peroxides and a 28-percent reduction in TBARS compared to baseline serum levels. In addition, a 39-percent decrease in uptake of oxidized LDL by human monocyte-derived macrophages (an early development in foam cell formation and atherogenesis) was observed in diabetic patients after PJ consumption. Researchers concluded that despite the sugars naturally present in pomegranate juice, consumption did not adversely affect diabetic parameters but had a significant effect on atherogenesis via reduced oxidative stress.[69]

Dental Conditions

Topical applications of pomegranate preparations have been found to be particularly effective for controlling oral inflammation, as well as bacteria and fungal counts in periodontal disease and Candida-associated denture stomatitis.

Dental Plaque

A hydroalcoholic extract of *Punica granatum* fruit (HAEP) was investigated for antibacterial effect on dental plaque microorganisms. Sixty healthy patients (33 females/27 males; ages 9-25) with fixed orthodontic appliances were randomized to three groups of 20: (1) control group who rinsed with 15 mL distilled water; (2) a group who rinsed with 15 mL chlorhexidine, a standard antiplaque mouth rinse; and (3) a group who rinsed with a 15-mL HAEP solution. Rinsing duration was one minute and dental plaque material was collected from each patient prior to and after rinsing. Samples were diluted and plated on Meuller-Hinton agar and incubated at 37° C for 48 hours. HAEP decreased the number of colony forming units (CFU) of dental plaque bacteria 84 percent, comparable to chlorhexidine (79-percent inhibition) but significantly better than the control rinse (11-percent inhibition). Both HAEP and chlorhexidine were effective against Staphylococcus, Streptococcus, Klebsiella, and Proteus species, as well as *E. coli*. The ellagitannin, punicalagin, is thought to be the fraction responsible for pomegranate's antibacterial activity.[49]

Periodontal Disease

A preliminary and follow-up study by a group of Thai researchers investigated the effect of biodegradable chips impregnated with *Centella asiatica* and *P. granatum* pericarp on periodontal disease in 20 patients with gum pocket depths of 5-8 mm. A baseline exam was performed and followed by root planing and scaling of target teeth. Subgingival placement of the medicated chips (treatment group) and non-medicated chips (placebo/control group) followed, and pocket depth, attachment level, bleeding, and gingival and plaque indexes were measured at baseline and after three and six months. All treatment sites demonstrated a trend toward decreasing plaque and significant improvements were noted in pocket depth and attachment level at three months compared to placebo.[70]

In the follow-up study, 15 patients who had completed standard periodontal therapy but still had pocket depths of 5-8 mm were implanted with the same medicated chips. The same parameters were measured again at baseline and after three and six months, but researchers also measured inflammatory markers interleukin-1ß (IL-1ß) and IL-6. Significant improvement was noted in all re-measured parameters and confirmed by significant decreases in IL-1ß and IL-6 at three and six months compared to baseline.[71]

Denture Stomatitis

The primary etiologic factors for denture stomatitis are poor oral hygiene, inflammation from ill-fitting dentures, and Candida infection,[72,73] which manifest as swelling, pain, burning in the mouth, and aphthous ulcers.[74] In a randomized, double-blind study of 60 subjects (ages 19-62) with candidiasis confirmed via mycologic examination, the effect of a gel-based *P. granatum* bark extract (GPBE) was evaluated for its effect on healing of oral lesions and direct fungicidal effect. Patients were randomized into two groups of 30: one received miconazole oral gel (a standard therapy) and the other used GPBE, both three times daily for 15 days. Gels were applied to oral surfaces, dentures were removed and cleaned nightly, then brushed with the corresponding oral gels. All subjects reported an improvement in symptoms and general oral health. Clinical symptoms of those using miconazole were slightly better (27/30 satisfactory improvement) compared to GPBE (21/30 satisfactory improvement). Clearing of Candida infection was approximately the same in both groups (25/30 in the miconazole group and 23/30 in the GPBE group).[50]

Interestingly, despite randomized subject placement, there were three times more subjects with good oral hygiene scores in the miconazole group compared to the GPBE group, possibly accounting for the superior results observed by miconazole therapy. Also, because the initial step in the development of Candida denture stomatitis is adherence of organisms to dentures and the miconazole gel was stickier than GPBE, contact duration of miconazole was longer. A stickier GPBE might result in improved clinical response.[50]

Bacterial Infections

The only human trials examining the antibacterial properties of pomegranate extracts have focused on oral bacteria.[49,50,70,71] However, several *in vitro* assays demonstrate its bacteriocidal activity against several highly pathogenic and sometimes antibiotic-resistant organisms. Brazilian researchers evaluated the synergistic effect of a *P. granatum* methanolic extract with five antibiotics on 30 clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *S. aureus*.[75] Antibiotics tested were chloramphenicol, gentamicin, ampicillin, tetracycline, and oxacillin. Although synergistic activity between the pomegranate extract and all five antibiotics was noted in the *S. aureus* isolates, synergy with ampicillin was the most pronounced. A combination of the two increased the lag time to bacterial growth by three hours (over that of ampicillin alone) and was also bacteriocidal as evidenced by a 72.5-percent reduction in methicillin-sensitive organisms and a 99.9-percent reduction in MRSA. Based on earlier research[76] and the results of this study, the ellagitannin, punicalagin, is thought to be the primary constituent responsible for the observed antibacterial effects.

Another organism that can cause significant disease in humans is enterohemorrhagic *Escherichia coli* (*E. coli* O157:H7), which can present with diarrhea, hemorrhagic colitis, thrombocytopenic purpura, and hemolytic uremic syndrome. *P. granatum* and seven other Thai medicinal plant extracts were tested for *in vitro* activity against *E. coli* O157:H7. An ethanolic PPE, one of the two most effective extracts against *E. coli* O157:H7, was shown to be both bacteriostatic and bacteriocidal, indicating PPE may be an effective adjunct treatment for *E. coli* O157:H7 infection.[47]

Ultraviolet Radiation

In vitro studies using normal human epidermal keratinocytes and PFE demonstrate PFE incubation with cell cultures ameliorates ultraviolet A and B radiation-induced cell damage in a dose- and time-dependent manner, providing evidence at a cellular level that PFE may be an effective photo-chemopreventive agent.[77,78]

A double-blind, placebo-controlled trial evaluated the protective and ameliorative properties of pomegranate extract and its EA constituent on UV-induced skin pigmentation. An ethanolic PPE was prepared containing 89.5 percent EA, confirmed by HPLC analysis. Thirty-nine healthy women (ages 20-49) were randomly assigned to one of three groups: (1) high-dose (200 mg/day) EA tablets; (2) low-dose (100 mg/day) EA tablets; and (3) placebo (0 mg EA) tablets for four weeks. Prior to the first dose, subjects received a 1.5 minimum erythema dose (MED) of UV radiation on the inside upper right arm. Melanin, luminance, and erythema values were measured at baseline and at the end of each of the next four weeks. A questionnaire was completed by subjects to evaluate PPE's effectiveness on improvement of UV-induced slight sunburn. Rate of change for luminance, melanin, and erythema values was not significantly different for subjects receiving either EA dose compared to placebo or compared to baseline values. However, analysis of the questionnaire results demonstrated a trend toward amelioration of UV-induced damage in both EA groups compared to placebo.[79]

Erectile Dysfunction

A study using a rabbit model of arteriogenic erectile dysfunction (ED) measured the effect of PJ concentrate on intracavernous blood flow and penile erection. Azadzoi et al found eight weeks administration of 3.87 mL PJ concentrate (112 µmol polyphenols) daily significantly increased intracavernous blood flow and smooth muscle relaxation, probably via its antioxidant effect on enhanced NO preservation and bioavailability.[80]

A randomized, double-blind, placebo-controlled, 10-week crossover trial in 53 men (mean age 46) investigated PJ's therapeutic effect on mild-to-moderate ED. Subjects with other medical conditions that might contribute to ED were excluded, and subjects were asked to refrain from taking ED medication for the duration of the study. The trial consisted of two four-week treatment periods separated by a two-week washout. During the first four weeks, subjects were given PJ (1.5 mmol polyphenols daily) or placebo beverage, followed by washout and crossover to the other group. Although assessment via the International Index of Erectile Function and Global Assessment Questionnaires demonstrated a trend toward improvements in ED, statistical significance was not achieved. This may be attributable to small sample size, short study duration, subject compliance with beverage consumption, or may indicate the PJ dosage did not have an appreciable effect on ED.[81]

Male Infertility

Research in rats demonstrates PJ consumption improves epididymal sperm concentration, spermatogenic cell density, diameter of seminiferous tubules, and sperm motility, and decreases the number of abnormal sperm compared to control animals. An improvement in antioxidant enzyme activity in both rat plasma and sperm was also noted.[82]

Neonatal Hypoxic-Ischemic Brain Injury

Neonatal hypoxic-ischemic (HI) brain injury in severely preterm, very low birth-weight infants is a major cause of infant illness and death[83] and has been associated with an increase in reactive oxygen species.[84] Two studies in which pregnant mice were given PJ in drinking water revealed the neonatal offspring, when subjected to experimentally-induced HI brain injury, had significantly less brain tissue loss (64% decrease) and significantly decreased hippocampal caspase-3 activity (84% decrease) compared to neonates with experimentally-induced HI brain injury from dams who consumed a control beverage.[85,86] These results suggest PJ has an antioxidant-driven neuroprotective effect conferred from mother to neonate.

Alzheimer's Disease

The neuroprotective properties of pomegranate polyphenols were evaluated in an animal model of Alzheimer's disease. Transgenic mice with Alzheimer's-like pathology treated with PJ had 50-percent less accumulation of soluble amyloid-beta and less hippocampal amyloid deposition than mice consuming sugar water, suggesting PJ may be neuroprotective. Animals also exhibited improved learning of water maze tasks and swam faster than control animals.[87]

Obesity

PFLE (400 or 800 mg/kg/day) given to obese hyperlipidemic mice for five weeks caused significant decreases in body weight, percentage of adipose pad weights, energy intake, and serum cholesterol, triglyceride, glucose, and total cholesterol/HDL ratios. Decreased appetite and intestinal fat absorption were also observed, improvements mediated in part by inhibition of pancreatic lipase activity.[88]

Potential Drug Interactions

Based on pomegranate's current popularity and research suggesting its therapeutic benefit in cancer, cardiovascular disease, and other diseases treated with prescription medications, it has been of interest to determine whether pomegranate extracts have any effect on cytochrome P450-3A, the hepatic enzyme system responsible for metabolism of many prescription medications. A randomized, single-dose, crossover study in

Table 3. Ongoing Pomegranate Trials

CLINTRIALS.GOV IDENTIFIER	STUDY FOCUS	SPONSOR	ESTIMATED ENROLLMENT	STUDY START DATE	ESTIMATED COMPLETION DATE	STATUS
NCT00413530	Rising PSA levels in men with previous prostate cancer	M.D. Anderson Cancer Center; Houston, TX	300 subjects	December 2006	December 2008	Currently recruiting
NCT00060086	Recurrent prostate cancer	Jonsson Comprehensive Cancer Center; National Cancer Institute	29-40 subjects	March 2003	September 2004 or longer	Ongoing
NCT00433797	Prostate cancer	University of Oslo; Norwegian Cancer Society; The Research Council of Norway	102 subjects	June 2007	March 2009	Currently recruiting
NCT00381108	Benign prostatic hyperplasia	University of California, Irvine; Jarrow Pharmaceuticals	20 subjects	September 2005	March 2009	Currently recruiting
NCT00455416	Follicular lymphoma	University of Oslo, Norway	45 subjects	April 2007	December 2009	Currently recruiting
NCT00336934	Rising PSA levels in men with previous prostate cancer	Jonsson Comprehensive Cancer Center; National Cancer Institute	250 subjects	November 2005	December 2009	Currently recruiting
NCT00428532	Atherosclerosis in diabetics	HaEmek Medical Center, Israel	10 males 10 females	March 2007	August 2007	Completed; publication pending
NCT00655031	Prevention of rhino-virus infection	Pom Wonderful LLC	150 subjects	April 2008	June 2008	Currently recruiting

From: http://www.clinicaltrials.gov

13 healthy human volunteers demonstrated PJ pretreatment did not affect elimination half-life or distribution of intravenous midazolam (a benzodiazepine derivative with anxiolytic, amnestic, hypnotic, anticonvulsant, and muscle relaxant properties), nor did it affect the C_{max} or clearance of oral midazolam.[89] This human study contradicts a rat study showing PJ has an inhibitory effect on carbamazepine pharmacokinetics, an anticonvulsant medication also metabolized by cytochrome P450-3A.[90]

Safety of Pomegranate Extracts

Pomegranate and its constituents have safely been consumed for centuries without adverse effects. Studies of pomegranate constituents in animals at concentrations and levels commonly used in folk and traditional medicine note no toxic effects.[91] Toxicity of the polyphenol antioxidant punicalagin, abundant in pomegranate juice, was evaluated in rats. No toxic effects or significant differences were observed in the treatment group compared to controls, which was confirmed via histopathological analysis of rat organs.[92]

Research in 86 overweight human volunteers demonstrated the safety of a tableted PFE in amounts up to 1,420 mg/day (870 mg gallic acid equivalents) for 28 days, with no adverse events reported or adverse changes in blood or urine laboratory values observed.[93] Another study in 10 patients with carotid artery stenosis demonstrated PJ consumption (121 mg/L EA equivalents) for up to three years had no toxic effect on blood chemistry analysis for kidney, liver, and heart function.[67]

Conclusion

An explosion of interest in the numerous therapeutic properties of *Punica granatum* over the last decade has led to numerous *in vitro*, animal, and clinical trials. Pomegranate is a potent antioxidant, superior to red wine and equal to or better than green tea. In addition, anticarcinogenic and anti-inflammatory properties suggest its possible use as a therapy or adjunct for prevention and treatment of several types of cancer and cardiovascular disease. Because of pomegranate's antimicrobial properties, it may aid in preventing infection by dental pathogens, pathogenic *E. coli* O157:H7, and antibiotic-resistant organisms such as MRSA. Pomegranate's effect on bacterial pathogens has only been tested *in vitro*, however, necessitating human trials to refute or substantiate any clinical effect. The possibility that pomegranate extracts may also have an effect on several other disease processes, such as Alzheimer's disease, osteoarthritis, neonatal brain injury, male infertility, and obesity, underscores the need for more clinical research. Currently, numerous clinical trials are in progress exploring the therapeutic potential of pomegranate extracts (Table 3).[94]

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