AMERICAN BOTANICAL COUNCIL

PROPRIETARY BOTANICAL FOOD PRODUCT

SCIENTIFIC AND CLINICAL MONOGRAPH

FOR

POM WONDERFUL® POMEGRANATE JUICE

By Allison McCutcheon, PhD; Jay Udani, MD;
Donald J. Brown, ND

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OVERVIEW

This monograph covers the peer-reviewed basic science and clinical research on POM Wonderful® pomegranate juice (PJ) and supporting evidence on generic PJ and PJ components. Research on other parts of the tree (bark, leaf, and root) have not been included in this review. POM Wonderful PJ is a proprietary product made exclusively from the whole fruits of 'Wonderful' pomegranates (*Punica granatum* 'Wonderful', Punicaceae) grown in California by Paramount Farming Company (Bakersfield, CA). The whole fruit POM Wonderful PJ is richer in antioxidant compounds than juice made solely from the arils. POM Wonderful PJ is also distinct from most commercial whole fruit juices in that it has been chemically characterized and normalized to contain specified levels of bioactive constituents to the extent that such characterization is possible for a natural food product.

Pomegranate fruit has a fascinating history of traditional use as food, medicine, and cultural icon, dating back thousands of years.[1] Originating in the Middle East, pomegranates were one of the earliest fruits to be domesticated, and their range now includes the Far East, India, the Mediterranean, and the Americas. A symbol of fertility and immortality, pomegranates' healing properties were discussed in one of the oldest medical texts, the *Ebers papyrus* from Egypt (circa 1500 BCE).[2] The fruit is mentioned in both Greek and Persian mythology representing life, regeneration, and marriage.[3] In Judaism, pomegranate seeds are said to number 613—one for each of the Torah's 613 commandments. The fruit is also one of the 3 blessed fruits in Buddhism. In various forms of traditional Asian medicine, pomegranate fruits were recommended as a health tonic and as a treatment for numerous ailments including diarrhea, dysentery, and diabetes.

Modern scientific research employing *in vitro*, animal, and human models has found that POM Wonderful PJ is a potent antioxidant that promotes cardiovascular health and inhibits the proliferation of many cancers. To date, most pomegranate researchers have focused on the juice's polyphenolic constituents, especially the ellagitannins and gallotannins such as punicalagins, as these compounds exert strong antioxidant, antimicrobial, and

anti-cancer activity. POM Wonderful PJ clinical trials are critically reviewed in the Clinical Review section on page 11.

DESCRIPTION

Pomegranate: The pomegranate (*Punica granatum*, Punicaceae) is an attractive tree that grows up to 5 m in height. It has glossy, leathery leaves and bears showy red flowers at the branch tips. The distinctive shape of the spherical fruit (2.5 to 5 inches in diameter) is imparted by the prominent fleshy red calyx which persists after flowering. The fruit has a tough leathery skin that is variously referred to as the rind, husk, or pericarp. The interior of the fruit is compartmentalized by membranous walls (carpels) and white spongy pith. The resulting locules are packed with 600 to 800 sacs or arils, each of which contains one seed and juicy pulp. Conventional pomegranate juices normally constitute 45 to 65% of the whole fruit. The 50% edible portion (the arils) consists of approximately 80% juice pulp and 20% seeds; traditionally the entire aril is crushed to make the juice so the expressed juice contains liquid from the seeds and the surrounding aril juice.[4,5] The POM Wonderful proprietary process does not crush the seed and hence POM Wonderful PJ does not contain any seed constituents.

POM Wonderful Pomegranate Juice: POM Wonderful PJ is made from whole California-grown Wonderful variety pomegranates. The Wonderful cultivar bears large purplish-red fruit with a medium thick, tough rind and a very juicy, deep crimson flesh with a tangy flavor. The subsequent cloudy juice is filtered and/or enzyme treated before concentrating to 65 Brix for future 16 Brix juice productions, which are flash pasteurized (Brix is a measurement of the mass ratio of dissolved solids to water in a liquid [e.g. fruit juice, wine]). Based on cumulative data from 2002 through 2007, POM Wonderful PJ contains between 2,400 to 4,000 mg/liter (ppm) GAE (gallic acid equivalent) polyphenols.[6] The 2005 through 2007 harvests were consistently at the higher end of this range. The polyphenolic component consisted of approximately 80 to 90% ellagitannins and gallotannins, 8 to 15% anthocyanins, and 2 to 5% ellagic acid.[6] POM Wonderful markets a line of 100% natural pomegranate-based juices launched in 2002, which are sold under the same brand name and are available on a year-round basis in the United States, Canada, and the United Kingdom.

PRIMARY USE

Cardiovascular Health. As noted in the United States patents section on page 11,[7,8] the primary health benefits of POM Wonderful PJ have focused on the antioxidant actions of the juice and its potential to prevent atherosclerosis as well as slow progression of atherosclerotic plaques. Five small human clinical trials testing cardiovascular activities have evaluated POM Wonderful PJ for effects on cholesterol, atherosclerosis, myocardial perfusion, hypertension, and erectile dysfunction.[9–13]

Prostate Cancer. A well-designed phase II clinical study suggests that POM Wonderful PJ may be potentially effective in slowing progression of prostate cancer and reducing recurrence rates of the disease.[14]

DOSAGE AND DURATION OF ADMINISTRATION

Daily dose in clinical trials: All of the clinical studies published to date have used 8 ounces (240 ml) per day (or its equivalent: 50 ml POM Wonderful PJ concentrate) of POM Wonderful PJ.[9–14] Studies have ranged from 2 weeks to 54 months in duration with adults ranging in age from 21 to 80 years.

Manufacturer serving recommendations and nutritional information: The serving for POM Wonderful PJ is 8 fluid ounces per day.

Table 1 on page 14 lists the Nutrition Facts for POM Wonderful PJ based on 4 lots analyzed from 2006 to 2007.[6] With regard to the claim of 0 g of total fat, it is important to note that in the 4 lots analyzed, the total fat measured was < 0.1 g/100 g. According to Food and Drug Administration (FDA) guidelines, "If the serving contains less than 0.5 gram, the content shall be expressed as zero."[15]

A single serving of POM Wonderful PJ (8 ounces or 240 ml) provides 6% of the Recommended Dietary Allowance (RDA) for vitamin B_6 and riboflavin, 4% RDA for biotin, niacin, and pantothenic acid, and 2% RDA for thiamin. Potassium is the most abundant mineral in POM Wonderful PJ, with a single serving providing 520 mg or 15% of the RDA.[6] It also provides 8% RDA manganese, 4% magnesium, and 2% calcium, iron, zinc, and phosphate. According to FDA guidelines, POM Wonderful PJ is a good source of potassium and is low in sodium. The guidelines state, "Diets containing foods that are a good source of potassium and that are low in sodium may reduce the risk of high blood pressure and stroke."[16]

CHEMISTRY

In addition to the conventional nutritional compounds noted just above, PJ was reported to be comprised of 85.4% water, 10.6% total sugars, 1.4% pectin, 0.2–1.0% polyphenols, and organic acids.[5,17,18] Other reported minor compounds include fatty acids, sterols, triterpenoids, and α-tocopherol.[19] The quantitative profile of PJ constituents has been found to vary considerably though, depending upon the cultivar, geoclimatic factors, harvesting, processing, and storage conditions.[20,21]

LIST OF ACRONYMS

ABC	American Botanical Council
ACE	angiotensin converting enzyme
AUC	area under the concentration
COX-2	cyclooxygenase 2
ED	erectile dysfunction
EDV	end diastolic velocity
eNOS	endothelial nitric oxide synthase
GAQ	Global Assessment Questionnaire
GRAS	generally recognized as safe
GSH	glutathione
GSSG	oxidized glutathione
HT	hydrolyzable tannins
IIEF	International Index of Erectile Function
LDL	low density lipoprotein
IMT	intima-media thickness
NIDDM	non-insulin dependent diabetic males
NF-kB	nuclear factor kappa-B
PFE	pomegranate fruit extract
PJ	pomegranate juice
PON1	serum paraoxonase 1
PON2	serum paraoxonase 2
PSA	prostate specific antigen
PSADT	prostate specific antigen doubling time
PSV	peak systolic velocity
RDA	Recommended Dietary Allowance
SDS	summed difference score
TBARS	thiobarbituric acid reactive substances
TPT	total pomegranate tannins

The polyphenols are responsible for the astringent quality of the juice. All parts of the fruit contain polyphenols, including the peel, the inner membranes, and the arils; although the pericarp (peel and membrane) is reported to contain the highest concentrations.[21]

PJ contains 2 major classes of polyphenolic compounds: hydrolyzable tannins (HT) and flavonoids, with very small levels of condensed tannins. Figure 1 shows the chemical structures of the most abundant of these constituents.

The HT are the predominant polyphenols in PJ and include ellagitannins, gallotannins, and gallagoyl esters.[21] The most abundant HT are the gallagyl tannins punicalagins (anomers A and B) and related tannins, which comprise roughly 63% of the constituent polyphenols.[21] Other reported HT include pedunculagin, punicalin, gallagic and ellagic acid esters of glucose (16.8%), and ellagic acid derivatives (4.9%).[19,21] Figure 2 shows a breakdown of the phenolic compounds in PJ.

The flavonoid component consists mainly of anthocycanidins, flavanols, and flavanol glucosides. The anthocyanidins are the pigments which imbue the juice with its ruby red color and include the compounds cyanidin; cyanidin-3-glucoside; cyanidin 3,5-diglucoside; cyanidin-3-rutinoside; delphinidin; delphinidin-3-glucoside; delphinidin 3,5-glucoside; pelargonidin-3-glucoside; and pelargonidin-3,5-glucoside (see Figure 3).[20,22,23,24] Of these, cyanidin derivatives appear to be the most abundant, followed by delphinidin glucosides.[21,25] Other flavonoid constituents include kaempferol, myricetin, rutin, narigenin, luteolin and luteolin glycosides, quercetin, and quercetin glucosides.[26-29]

The main condensed tannins found in the juice are catechin, procyanidin B1, and procyanidin B2.[26] Gallocatechin and its derivatives have also been reported.[30] Other minor phenolics include gallic acid, protocatechuic acid, catechin, and phloridzin.[31]

Citric acid is the most abundant organic acid in fresh PJ, followed by malic acid.[31,32] Other reported organic acids include caffeic, chlorogenic, cinnamic, coumaric, ferulic, gallic, malic, oxalic, protocatechuic, quinic, succinic, and tartaric acid.[26,31,32]

Although the steroid hormone estrone (and to a lesser extent estriol, 17-β-estradiol, and testosterone) have been reported in pomegranate seed,[28,33-37] these findings were not confirmed with definitive mass spectrometry and/or nuclear magnetic resonance analyses. More accurate evaluations utilizing high power liquid chromatography and gas chromatograph mass spectrometer did not detect any of these compounds in pomegranate seed or PJ[38] or a pomegranate fruit extract (PFE).[29]

Similarly, the sterols β-sitosterol, camesterol, cholesterol, daucosterol, and stigmasterol, and the coumestan coumestrol have been reported in pomegranate seed,[35,37,39] but these compounds have not been found in PJ.

PHARMACOLOGICAL ACTIONS/MECHANISM OF ACTION

Antioxidant Activity

In vitro

The potent antioxidant capacity of pomegranate and its components has been reported by numerous scientists using multiple *in vitro* assay systems.[21,24,30,39-55] This activity is largely due to the polyphenolic constituents.[21,24,49,53,56] PJ has both a higher total polyphenolic content and greater antioxidant activity than other commonly consumed fruit juices, including grape, cranberry, orange, and apple juice among others.[49]

POM Wonderful PJ was found to be a much more potent antioxidant in protecting nitric oxide than Concord grape juice, blueberry juice, red wine, vitamin C, and vitamin E.[57] As an antioxidant, POM Wonderful PJ was found to be 100 times more powerful than blueberry juice and 300 times more powerful than grape juice.

PJ made from the Wonderful cultivar exhibited antioxidant activity up to 3 times greater than that of phenolic-rich green tea and red wine.[21] The polyphenolic content and correspondingly antioxidant activity of commercial whole fruit PJ was significantly greater than that of aril juice. Gil et al calculated that the punica-

Figure 1. Chemical Structure of Phenolic Compounds in Pomegranate Juice

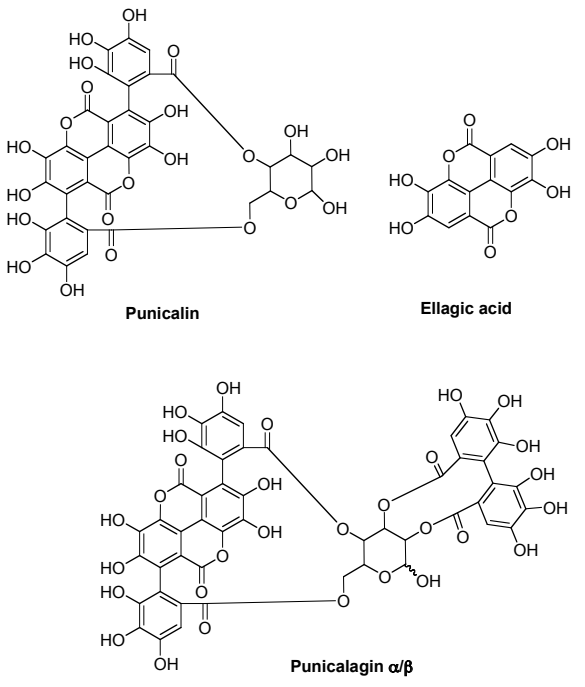
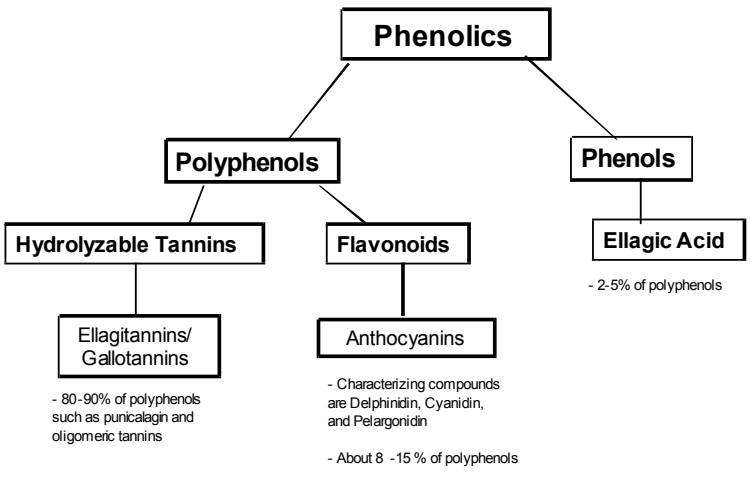


Figure 2. Phenolics in 100% Pomegranate Juice



lagins constituted 62.8% and other HT 16.8% of the total phenolic content, and together they accounted for 78.5% of the antioxidant activity.[21] These results subsequently led many researchers to focus on the HT as the primary antioxidant constituents of PJ. In various *in vitro* models, the punicalagins have been reported to protect lipids, proteins, and DNA against oxidative damage by several mechanisms: scavenging free radicals, transferring electrons to repair oxidatively damaged components, and chelating metal ions.[43,44]

However, as Gil et al point out, PJ anthocyanidins, ellagic acid, and other phenolics also contributed to the overall antioxidant effect of the polyphenols even though they were present in much lower concentrations than the HT.[21] An independent study of the 3 major PJ anthocyanidins found that delphinidin, cyanidin, and pelargonidin scavenged oxygen radicals in a dose-dependent manner with the median infective dose (i.e., the dose that will infect 50% of the experimental group) of 2.4, 22, and 456 µM, respectively.[24] PJ constituent prodelphinidins, gallocatechin, ellagic acid and gallic acid derivatives, fatty acids, and polysaccharides have also been found to exert significant antioxidant effects.[21,22,40,48,50-52,54]

A recent study on POM Wonderful PJ clearly demonstrated the superiority of the whole juice over PJ fractions and isolates. Seeram et al found that the antioxidant activity of the POM Wonderful PJ was not only greater than that of isolated punicalagins or ellagic acid, but also more potent than an experimental PJ total tannin extract.[53]

In vivo—Animals*

In aged rats, animals supplemented with PJ for 4 weeks exhibited significantly higher antioxidant capacity compared to the control group.[58] Similar results were observed in a mouse model.[59] After 4 weeks of PJ ingestion, oxidative stress was decreased according to 3 different indicators: (1) protein and DNA damage was decreased, (2) reduced glutathione (GSH) and oxidized glutathione (GSSG) levels were lowered without changing the GSH/GSSG ratio, and (3) the concentration of antioxidant liver enzymes were reduced. Two investigations of pomegranate extracts have also provided supporting evidence of *in vivo* antioxidant activity.[60,61] A pomegranate fruit extract (PFE) orally administered to rats at 10 mg kg^{-1} day^{-1} significantly reduced liver concentrations of malondialdehyde, hydroperoxides, and conjugated dienes while the activities of the enzymes catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase showed significant elevation. Concentrations of glutathione in the tissues were also increased.[60] Rats fed a PFE and carbon tetrachloride maintained catalase, peroxidase, and superoxide dismutase levels comparable to control animals given carbon tetrachloride, whereas lipid peroxidation was brought back by 54%. Histopathological studies of the liver also indicated hepatoprotective effects.[61]

Human

The effect of POM Wonderful PJ on oxidative stress in patients with non-insulin dependent diabetes mellitus (type 2 diabetes) compared to a control group of 10 healthy subjects is summarized in the Clinical Review section on page 11.[10] A phase II clinical trial of men with recurrent prostate cancer taking POM Wonderful PJ is also summarized in the Clinical Review section.[14] The patient serum antioxidant effect of POM Wonderful PJ consumption was determined by evaluating the basal serum oxidative state and the sensitivity to the antioxidative activity of phycocyanobilin induced oxidation of the patients' serum at baseline and after 9 months of POM Wonderful PJ consumption using the lipid peroxides method. Compared with baseline, patients' serum showed a 40% reduction in the basal oxidative state and a 15% reduction in the resistance of their serum samples to antioxidative activity of phycocyanobilin induced lipid peroxidation after PJ consumption ($p < 0.02$).[14] As noted in the Cardioprotective Actions section below, POM Wonderful PJ increased total antioxidant status by 9% in 13 healthy male volunteers.[62]

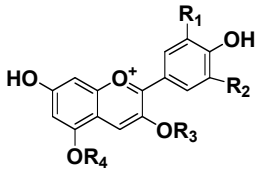
Shorter duration investigations of generic PJ have produced conflicting results. In a randomized, double-blind, placebo-controlled trial with 30 chronic obstructive pulmonary disease patients, no antioxidant benefits were observed after 5 weeks of PJ consumption.[63] In a 5-day pharmacokinetic study involving 6 healthy humans, neither punicalagins nor ellagic acid were detectable in their body fluids, and the metabolites of these compounds did not exert antioxidant activity.[64] However, a study with 11 healthy volunteers found a 32% increase in antioxidant capacity ½ hour after the consumption of a single acute dose of a PFE.[65]

Cardioprotective Actions

In vitro

There is evidence that oxidative stress is an important factor in atherogenesis (the development of lipid deposits in the arteries).[66–69] Oxidatively damaged macrophages have an increased capacity to oxidize low density lipoprotein (LDL), increase peroxide contents, and decrease glutathione levels.[70,71] These factors promote macrophage cholesterol accumulation and foam cell formation,[72,73] early indicators of atherogenesis.[69] Therefore the inhibition of LDL oxidation may play a key role in the prevention of atherosclerosis.

Figure 3. Constituent Anthocyanins and Anthocyanidins in Pomegranate Juice



	R1	R2	R3	R4
1. Cyanidin	OH	H	H	H
2. Cyanidin-3-glucoside	OH	H	Glucose	H
3. Cyanidin-3,5-diglucoside	OH	H	Glucose	Glucose
4. Cyanidin-3-rutinoside	OH	H	Rutinose	H
5. Delphinidin	OH	OH	H	H
6. Delphinidin-3-glucoside	OH	Glucose	H	
7. Delphinidin-3,5-glucoside	OH	OH	Glucose	Glucose
8. Pelargonidin-3-glucoside	H	H	Glucose	H
9. Pelargonidin-3,5-glucoside	H	H	Glucose	Glucose

POM Wonderful PJ showed the highest capacity to decrease LDL oxidation and inhibit oxidative stress in macrophages as compared to red wine, green tea, blueberry, or orange juice.[56] In macrophages treated with PJ, degradation of oxidized LDL was reduced by 40% and cholesterol synthesis was inhibited by 50%.[73] In J774A1 macrophages, PJ treatment decreased peroxide content by 23% more than the PJ phenolic fraction.[51] The polysaccharide fraction dose-dependently decreased peroxide concentrations up to 72%, while in marked contrast a white grape juice polysaccharide fraction dose-dependently increased peroxide by up to 72%. In a mouse diabetes model, PJ polysaccharides reduced cellular glutathione by 18% in peritoneal macrophages.[51]

Nitric oxide is arguably the body's most important native defense against cardiovascular disease. Oxidized LDL inhibits the production of nitric-oxide synthase, reducing nitric oxide activity in a dose-dependent manner.[74] The subsequent application of POM Wonderful PJ significantly decreased oxidized LDL induced down-regulation of nitric-oxide synthase in human endothelial coronary cells.[74] POM Wonderful PJ significantly protected nitric oxide against oxygen radical mediated damage and enhanced the anti-proliferative action of nitric oxide on rat aorta smooth muscle cells.[57] However, in bovine pulmonary artery endothelial cells it did not affect endothelial nitric oxide synthase (eNOS) expression or eNOS activity and did not stimulate eNOS promoter activity.[57]

Atherosclerosis is promoted in arterial regions subjected to disturbed blood flow. This shear stress increases the expression of oxidation sensitive genes such as ELK-1, p-JUN, and p-CREB, leading to increased production of free radicals and suppression of eNOS activity. POM Wonderful PJ increased eNOS activity in a dose-dependent manner and significantly reduced ELK-1, p-CREB, and p-JUN in human endothelial coronary cells.[75,76]

In addition to direct antioxidant activity, there are several other mechanisms by which PJ may convey protective effects against cardiovascular disease. POM Wonderful PJ was found to exert a dose-dependent inhibitory effect (31%) on angiotensin converting enzyme (ACE) activity *in vitro*.[12]

Serum paraoxonase 1 (PON1) and PON2 decrease macrophage oxidative stress.[25,55,77] PJ and the polysaccharide fraction were reported to dose-dependently increase expression and activity of PON2 and reduce macrophage oxidative stress.[25,55]

An increase in prostacyclin synthesis may also provide a protective effect against cardiovascular disease. An acute dose of PJ produced an increase in media prostacyclin while chronic exposure resulted in a 61% rise in prostacyclin synthesis in human aortic endothelial cells.[78]

In vivo—Animals*

The prophylactic effect of POM Wonderful PJ was assessed in a rabbit model of arteriogenic erectile dysfunction (ED).[56] Eight weeks of daily supplementation with 3.87 ml of PJ concentrate (equivalent to 112 uM polyphenols daily) increased intracavernous blood flow, improved smooth muscle relaxation and erectile response, but it did not significantly affect nitric-oxide synthase expression. The PJ intake also prevented erectile tissue fibrosis in the ED group. The authors concluded that supplementation may help prevent smooth muscle dysfunction and fibrosis in ED.

Two studies have found that POM Wonderful PJ supplementation exerted cardio-protective effects in hypercholesterolemic mice.[75,76] In both an atherosclerosis prevention and a treatment protocol, 24 weeks of POM Wonderful PJ consumption resulted in a suppression of ELK-1, increased eNOS expression, and the progression of atherosclerosis in animals at various stages of disease development was delayed.[75] Both the atherosclerotic lesion area and macrophage foam cell formation were reduced approximately 20% in both POM Wonderful PJ protocols. As measured by plasma isoprostanes, plasma lipid peroxidation was significantly reduced while cholesterol levels were not affected.

These results were reproduced and expanded upon in a subsequent evaluation by de Nigris et al.[76] POM Wonderful PJ was again found to reduce the activation of ELK-1 (and p-CREB) and increase eNOS expression in atherosclerosis-prone areas. Plasma lipid peroxidation was reduced by 25% and plasma nitrates increased over 44%. Atherosclerotic lesion area and foam cell formation were both decreased by approximately 25% and atherosclerotic disease progression was significantly inhibited. Maximum arterial relaxation was also significantly increased, and other tests indicated an improvement in endothelium-dependent and endothelium-independent vasomotor reactivity. The authors concluded that chronic POM Wonderful PJ consumption reversed the proatherogenic effects induced by perturbed shear stress.

A study employing obese Zucker rats as a model of metabolic syndrome provided evidence that the protective effects of POM Wonderful PJ are attributable to its polar components.[79] Expression of vascular inflammation markers, thrombospondin, and cytokine TGFbeta1 was significantly decreased by POM Wonderful PJ intake. Plasma nitrate and nitrite levels were significantly increased ($p < 0.05$) and eNOS expression rose in the POM Wonderful PJ group. In contrast to the above mouse experiments, only endothelium-dependent (acetylcholine induced) arterial relaxation was increased in this rat model.

In atherosclerotic apolipoprotein E–deficient (E^0) mice, 8 weeks of PJ supplementation reduced macrophage lipid peroxides by 37%.[80] In contrast, Aviram et al reported that in E^0 mice, 11 weeks of supplementation with PJ reduced macrophage LDL oxidation by 90% with an associated reduction in cellular lipid peroxidation and superoxide release.[62] The size of atherosclerotic lesions was reduced by 44% and the number of foam cells declined compared to the controls. Uptake of oxidized LDL and native LDL was decreased by 20%.

The latter finding is in general agreement with the findings of Kaplan et al.[81] In E^0 mice, 8 weeks of POM Wonderful PJ supplementation reduced macrophage oxidized LDL uptake by 31%, decreased cholesterol esterification, and increased cholesterol efflux by 39%. Lipid peroxide content was 42% lower in the 6-month old PJ-treated mice compared to 6-month old placebo-treated control mice and 20% lower than that of 4-month old control mice. The rate of cholesterol esterification in macrophages from the PJ mice was 80% lower than that of age-matched, placebo-treated mice and 57% lower than control mice. At the end of the experiment, the oxidized LDL and cholesterol esterification levels in the 6-month old PJ group were lower than those of untreated 4-month old control group. Serum paraoxonase activity was 43% higher than that of the placebo-control mice and 26% higher than 4-month old control mice. In mice with advanced disease, atherosclerotic lesions were reduced by 17%.

Macrophage oxidative stress levels were reduced in both streptozotocin-induced diabetic Balb/C mice and healthy control mice that were fed PJ polysaccharides for 10 days.[25] A decrease in total peroxide content and PON2 activity, and an increase in glutathione levels, were observed in peritoneal macrophages from animals supplemented with PJ sugars. The opposite effect was seen in animals fed white grape juice: a 22% rise in peroxide and 45% decline in glutathione.[51] In an 8-week study with E^0 mice, PJ consumption increased PON3 activity by 23%.[80]

PJ may also provide protection against stroke. In a mouse model, maternal consumption of POM Wonderful PJ protected the pups against hypoxic-ischemic brain injury.[82] Brain tissue loss was reduced by 60%. Caspase-3 activation, a marker of apotopic death, was reduced by 84% in the hippocampus and 64% in the neonatal brain cortex. A recent study suggested that it may also convey some protective effects against Alzheimer's disease. Activity guided fractionation of PFE identified ellagic acid and punicalagin as non-competitive beta-secretase inhibitors.[83]

Human

The cardioprotective effects of POM Wonderful PJ have been investigated in 4 clinical trials.[9–12] The Clinical Review section contains a critical overview of these studies.

In 13 healthy male volunteers, 2 weeks of daily POM Wonderful PJ consumption (50 ml PJ concentrate; the concentrated PJ was diluted 1:5 [v:v] with water to obtain a single strength juice equivalent to 8 ounces per day) resulted in an 11% decrease in collagen-induced platelet aggregation compared to baseline values ($p < 0.02$).[62] LDL oxidative susceptibility was decreased by 43%, as measured by the prolongation of the lag time to oxidation initiation. Plasma susceptibility to oxidation was reduced by 6% and total antioxidant status was increased by 9%. A trend towards a reduction in LDL susceptibility to aggregation and retention was observed in 7 of the 13 subjects. The activity of PON1 was increased by 18%.

In another study, PJ (6-9 mL/kg) was provided to 28 fasted, healthy adult subjects (8 men and 20 women).[78] After juice consumption, epinephrine/collagen-induced clotting time ($p < 0.05$) was significantly prolonged, indicating an inhibition of platelet aggregation. However, it did not significantly affect plasma prostacyclin concentrations.

ANTI-CANCER ACTIONS

In vitro

The anti-cancer effects of pomegranate and its components have been observed in a wide variety of *in vitro* models, including breast, prostate, colon, leukemia, and skin cancer cell lines, among others.[23,28,53,84-94]

The *in vitro* antiproliferative effects of POM Wonderful PJ concentrate (1.74 mg/ml punicalagin and 0.14 mg/ml ellagic acid) was evaluated in human colon and oral cancer cell lines using a luminescence assay.[53] POM Wonderful PJ exhibited 100% inhibition of the 2 oral cancer cell lines: (1) at a concentration 12.5 ug/ml in the CAL 27 and (2) at 25 ug/ml in KB cells. At a concentration of 25 ug/ml POM Wonderful PJ, a 100% reduction in growth was also observed in 4 colon cancer lines (the non-metastatic SW 460, metastatic SW 620, HT29, and HCT 116). The ability of POM Wonderful PJ to induce apoptosis was assessed in the latter 2 colon cancer lines using a photometric ELISA assay. In HCT 116 cells, apoptosis was only induced 0.7-fold. However, in HT29 cells apoptosis was induced 2.66-fold over the controls.

The anticancer activity of pomegranate tannin fractions and the isolated pure constituents ellagic acid and punicalagins have been reported by a number of investigators.[44,89,95,96] Seeram et al also tested the antiproliferative effects of 3 POM Wonderful PJ components: ellagic acid, punicalagins, and a total pomegranate tannins (TPT) extract.[53] While all 3 components exhibited some inhibitory effects in the cancer cell lines, in every case their activity was significantly less than that of the POM Wonderful PJ—even though the stock solutions of juice, TPT, and punicalagins had been normalized to provide the equivalent amount of punicalagins w/w. At a concentration of 12.5 ug/ml, ellagic acid inhibition was less than 55% in all lines while POM Wonderful PJ exhibited ≥ 80% inhibition. In

comparison, punicalagins and TPT activity did not even reach 20% inhibition. These results suggest that other POM Wonderful PJ components significantly contribute to the juice's antiproliferative effects, in addition to the tannin constituents. In this regard, it is noteworthy that pomegranate anthocyanidins, flavonoids, and oils have also been reported to exert anticancer effects against leukemia, colon, breast, prostate, skin, and lung tumors.[28,88,89,97-102] These 3 components induced apoptosis only at concentrations equivalent to 100 ug/ml in the 2 colon cancer lines. In HT29 cells, ellagic acid, punicalagins, and TPT induced apoptosis 2.44-, 2.65-, and 2.59-fold, respectively, as compared to the controls (cv 2.66 for POM Wonderful PJ). For HCT 116, the values were 2.85-, 1.52-, and 2.87-fold, respectively.[53] The fact that POM Wonderful PJ did not significantly induce apoptosis in the HCT 116 line even though it decreased proliferation 100%, suggests that POM Wonderful PJ anti-cancer effects may occur via at least 2 different mechanisms. In this study, the antioxidant activity of POM Wonderful PJ was also significantly greater than that of the 3 components (POM Wonderful PJ > TPT > punicalagins > ellagic acid), implying that direct inhibition of oxidation may be one of these mechanisms.

Ellagic acid and punicalagins were also evaluated in Caco-2 colon cancer and normal colon cells.[95] They dose-dependently inhibited Caco-2 proliferation but their effect was additive, not synergistic. Both compounds significantly increased Caco-2 apoptosis but neither induced apoptosis in the normal colon cells. It appeared that ellagic acid was the actual apoptosis inducer though, as punicalagins treatment did not induce apoptosis until its hydrolysis product, ellagic acid, accumulated in the media.

The protein kinase/nuclear factor kappa-B (NF-kB) signaling pathway promotes the production of cyclooxygenase 2 (COX-2) and there is evidence that over expression COX-2 plays an important role in carcinogenesis.[102,103] NF-kB inhibition in particular has been identified as a major therapeutic target, as this transcription factor regulates the expression of over 200 genes involved in cancer progression.[102,104]

The effect of POM Wonderful PJ and its components on COX-2 and protein kinase/NF-kB activity was evaluated in HT29 colon cancer cells.[102] At concentrations normalized to provide the equivalent of 50 ug/ml punicalagins, POM Wonderful PJ, TPT, and punicalagins all significantly inhibited COX-2 expression in a dose-dependent manner. POM Wonderful PJ exhibited the greatest effect, inhibiting COX-2 by 79% compared to 55% TPT and 48% punicalagins. POM Wonderful PJ abolished protein kinase B activity completely and also significantly affected NF-kB activity at a concentration of 50 ug/ml. Pretreatment with POM Wonderful PJ resulted in a 6.4-fold reduction in NF-kB (p65) DNA binding and a 92% reduction in phosphorylation of the p65 subunit. Considering POM Wonderful PJ's superior potency to TPT and punicalagins, the authors concluded that POM Wonderful PJ's activity is most likely due to significant interactions with other bioactive constituents of the juice.

Pretreatment with the anthocyanidin constituent delphinidin was found to provide protective effects against UV-B induced decreases in cell viability and apotosis induction in immortalized HaCaT keratinocytes. It inhibited numerous markers of UV-B oxidative stress including increased lipid peroxidation, increased the pro-apoptosis Bax, decreased Bcl-2, and down-regulated the anti-apoptosis Bcl- X_L.[97]

In other studies, PJ was also shown to inhibit the proliferation of leukemia and breast cancer cells.[28,90] PJ and PJ polyphenol fractions were found to inhibit proliferation and induce differentiation in HL-60 human promyelocytic leukemia cells, as assessed in 4 different assays.[90] PJ and 3 fractions all inhibited growth of estrogen sensitive (MCF-7) and estrogen resistant (MDA-MB-231) breast cancer cells.[28] The maximum inhibition (80%) of MCF-7 was induced by fermented PJ at a concentration of 50 ug/ml; the same concentration was 3 to 4 times less effective in the MDA-MB-231 cells and produced minimal toxicity in immortalized normal human breast epithelial cells (MCF-10A).

The anti-angiogenic effects of pomegranate polyphenol and oil fractions were evaluated in breast cancer and normal breast cells by measuring vascular endothelial growth factor and migration inhibition factor.[94] The angiogenic promoter vascular endothelial growth factor was significantly down-regulated in normal human breast epithelial (MCF-10A) and estrogen sensitive (MCF-7) breast cancer cells but not estrogen resistant (MDA-MB-231) breast cancer cells. Conversely, expression of the angiogenesis suppressor migration inhibition factor was not affected in the normal and estrogen sensitive cells while it increased in the estrogen resistant cells. All extracts inhibited the proliferation of myometrial and amnionotic fluid fibroblasts, while only fermented PJ polyphenols inhibited tubule growth in human umbilical vein endothelial cells. These results suggest that the antiangiogenic effects of pomegranate polyphenols arise via multiple mechanisms.

*In vivo—Animals**

A PFE (70% acetone-water extract) was found to significantly improve survival time in athymic male nude mice xenografted with human A549 non-small cell lung cancer.[91] Human clinical relevance of the 2 doses assessed (0.1% and 0.2% PFE) was based on the assumption that a typical healthy person (70 kg) would reasonably consume 250 to 500 ml of PJ per day. The latency period before the appearance of solid tumors was extended to 19 days compared to 15 days in the controls, an increase of 27%. An average tumor volume of 1200 mm^3 was reached after 55 days in the controls. At this time point, the average tumor volume in the PFE groups was 621 and 540 mm^3, respectively. In the verum groups, 1200 mm^3 average tumor volume was reached at 67 days (0.1%) and 79 days (0.2%), prolonging survival time 22% and 44%, respectively.

In female A/J mice, the PFE was also found to inhibit the growth and progression of lung tumors induced by 2 chemical carcinogens: NTCU and B(a)P.[92] The mice treated with PFE had significantly lower lung tumor multiplicities in both models, with a tumor reduction of 61.6% in the B(a)P group and a decrease of 65.9% in the NTCU mice. The extract inhibited a number of cell survival pathways, including NF-kB and IkBα kinase activation, and phosphorylation of IkBα, MAPK, protein kinase B, and c-met. Markers of cell proliferation and angiogenesis were also significantly inhibited.

Oral feeding of PFE also inhibited markers of UV-B induced carcinogenesis in SKH-1 hairless mice.[105] In SKH-1 hairless mice, the PJ constituent delphinidin also inhibited UV-B induced apoptosis and markers of DNA damage.[97]

PJ and PFEs have also been reported to be radioprotective and chemo-protective against liver and gastric damage.[40,60,106–108]

Prostate Cancer

In vitro

The antiproliferative effects of POM Wonderful PJ (1.74 mg/ml punicalagin and 0.14 mg/ml ellagic acid) was evaluated in the metastatic 22RV-1 and the immortal epithelial RWPE-1 prostate cancer cell lines.[53] At the lowest concentration evaluated in this study (12.5 ug/ml), POM Wonderful PJ significantly inhibited the proliferation of both prostate cancer lines, reducing the growth of RWPE-1 by 90% and that of 22RV-1 by > 95%.

The PJ constituent ellagic acid and its urolithin A metabolites were assessed in 4 prostate cancer cell lines: (1) androgen-dependent LNCaP, (2) androgen-independent LNCaP-AR, (3) DU 145, and (4) 22RV1.[109] The compounds exhibited dose-dependent inhibition in all of the cell lines, with urolithin A inducing a lower IC_{50} than ellagic acid in all cases. The lowest IC_{50} values were observed with the derivative murolithin A in LNCaP and 22RV1 cells, at 15.9 and 6.2 umol/L, respectively.

A PFE exerted a significant dose-dependent inhibition of proliferation and induced apotosis in androgen-independent PC-3.[93] Mechanistic studies showed that it induced the pro-apoptopic Bax and Bak genes; down-regulated the anti-apoptopic BcL-X_L and Bcl-2; decreased cyclins D1, D2, and E; and decreased cyclin-dependent kinase (cdk) 2, 4, and 6 expression. In CWR22Rv1 cells, the PFE significantly decreased androgen receptor expression (90%) at a concentration of 100 ug/ml and significantly decreased prostate specific antigen (PSA) protein levels (67% at 100 ug/ml).

In vivo—Animals*

The inhibitory effect of a PFE was evaluated in severe combined immunodeficient mice xenografted with human prostate cancer cells (LAPC-4).[109] Oral administration of the PFE significantly inhibited LAPC-4 proliferation, producing a 1.8 cm^3 reduction in tumor volume 6 weeks after inoculation.

Clinically relevant doses of a PFE (0.1% and 0.2% wt/vol) significantly inhibited the growth of androgen-dependent CWR22Rv1 tumors in athymic nude mice. Serum prostate specific antigen (PSA) levels also declined significantly, with a 70% and 85% reduction respectively for the 2 doses after 30 days.[93]

Pomegranate pericarp polyphenol and oil extracts were tested in a PC-3 xenograft model employing athymic nude mice. At a dose of 2ug/g body weight, both fractions reduced tumor volume by 72% compared to controls at 35 days post-inoculation.[85]

Human

The results of a phase II prostate cancer clinical trial are reviewed in the Clinical Review section on page 11.[14]

Estrogenic Activity

In vitro

The purported estrogenicity of pomegranate is a potential concern for prostate and breast cancer patients in particular. There are conflicting results regarding the effects of PJ on estrogen-sensitive MCF-7 breast cancer cells. Maru et al reported that PJ stimulated the proliferation of MCF-7 cells,[110] while Kim et al asserted that it inhibited proliferation of this line.[28] Jeune et al also reported that undescribed PFEs inhibited MCF-7 proliferation via apotopic induction.[87] Mixed results have also been reported regarding androgen-sensitive and androgen-resistant prostate cancer cell lines (see Prostate Cancer section above).

In vitro binding assays have also produced conflicting results. Maru et al reported that PJ exhibited estrogen-like activity, competing with 17β-estradiol for estrogen receptor binding.[110] Kim et al asserted that fermented PJ, fresh PJ, pericarp polyphenols, and oil significantly inhibited estrogen synthase (aromatase) and 17-β-hydroxysteroid dehydrogenase (17-β-HSD) type 1.[28] At a concentration of 1 mg/100ul, the fresh juice by itself displayed only minimal estrogenic action, while the lyophilized juice effected a 55% inhibition of the estrogenic activity of 17-β-estradiol. The fermented PJ induced 51% and the pericarp polyphenol 24% inhibition of aromatase at a concentration of 0.02 ug/ml. All extracts inhibited 17- β-HSD at 1000 ug/ml, but only the oil was active at 100 ug/ml.

However, POM Wonderful PJ did not exhibit estrogenic activity and there was no significant additive effect of POM Wonderful PJ with 17-β-estradiol in an estrogen agonist assay utilizing transfected MCF-7 cells.[111] A small antagonistic effect was noted with the highest concentrations of POM Wonderful PJ concentrate tested at estrogen receptor β.

As previously discussed in the Chemistry section, early reports of steroid hormone constituents in pomegranate seed have not been substantiated by mass spectrometry/nuclear magnetic resonance or by more accurate high power liquid chromatography and gas chromatograph mass spectrometer analysis of seed oil or PJ.[29,38] PJ does contain non-steroidal "phytoestrogenic" compounds though, which may account for the weak estrogenic effects some researchers have reported.[26,29] Using liquid chromatograph-mass spectrometry analysis teamed with an on-line β-estrogen receptor assay, the flavonoids luteolin, kaempferol, and quercetin were identified as the major PJ estrogenic constituents.[29]

In vivo—Animals*

In 1964, Sharaf and Nigm reported that pomegranate seed oil exhibited estrogenic activity in mouse and rabbit models.[112] Heftmann et al also reported estrogenic activity in ovariectomized mice by a seed constituent, which they identified as estrone based upon TLC and chemical tests only.[33] However, the identity of this compound as estrone was never further substantiated.

PJ was also reported to have increased uterine weight in ovariectomized rats[110] and in an ovariectomized mouse model of menopausal syndrome; a PFE improved bone properties and depression.[113] The clinical relevance of the concentrations used in these studies and the preceding *in vitro* investigations has not been established.

Human

In a small open label study with 11 post-menopausal women, 7 days of POM Wonderful PJ consumption (8 ounces per day) significantly increased serum estrone levels.[114] No significant changes in estradiol, follicle stimulating hormone, luteinizing hormone, or pituitary gonadotropins were observed, and estriol remained undetectable. No significant biological estrogenic effects (as measured by vaginal cornification) were noted and the authors pointed out that while the increase in estrone levels was statistically significant, they would be expected to exert physiological effects only at much higher concentrations.

Pharmacokinetics

In vivo—Animals*

The pharmacokinetics of the PJ constituents ellagic acid and punicalagins has been evaluated in rats. Following oral administration of 0.8 g/kg extract, the maximum ellagic acid plasma concentration (213 ng/ml) was observed at 0.55 h.[115] In another oral administration study with rats, approximately 10% was excreted in the feces and urine as 3,8-dihydroxy-6H-dibenzo[b,d]pyran-6-one (urolithin A) and a second metabolite was detected but not identified.[116] Both compounds were apparently of microfloral origin, as their presence was not detected in germ-free rats but was observed when ellagic acid was incubated *in vitro* with normal rat gastro-intestinal microorganisms. Ellagic acid itself was not detected in the feces or urine of any normal rat, but small amounts were detected in the feces of germ-free animals.

In another study, oral administration of PFE led to increased ellagic acid plasma levels, but this compound was not detectable in the prostate.[109] Intraperitoneal administration produced roughly 10-

fold higher ellagic acid plasma levels and detectable amounts in the prostate. Both oral and intraperitoneal administration of urolithin A resulted in significant levels in the prostate, colon, and intestinal tissues.

In animals fed standard rat diet plus 0.6 to 1.2 g punicalagins per day, approximately 3-6% of the punicalagins was excreted as identified metabolites in feces and urine.[117] Presumably, the majority of this compound was converted to undetectable metabolites or accumulated in non-analyzed tissues. Only trace amounts of 5 punicalagins metabolites were detected in the liver or kidney.[118]

Human

A human subject consumed 180 ml POM Wonderful PJ containing 25 mg ellagic acid and 318 mg punicalagins.[119] Ellagic acid was detected in the plasma at a maximum concentration (31.9 ng/ml) after 1 hour but was rapidly eliminated by 4 hours.

Eighteen healthy volunteers were given 180 mL of POM Wonderful PJ concentrate, and blood and urine samples were collected.[120] Ellagic acid was detected in the plasma of all subjects with a maximum concentration of 0.06 mmol/L, area under the concentration-time curve (AUC) of 0.17 (mmol/h)/L^{-1}, t_{max} of 0.98 h, and elimination half-life of 0.71 h. Ellagic acid metabolites, including dimethylellagic acid glucuronide and hydroxy-6H-benzopyran-6-one derivatives (urolithins), were also detected in plasma and urine. On day 0, dimethylellagic acid glucuronide was found in the urine obtained from 15 of 18 subjects, but it was not detected on the day prior or the day after consumption. Urolithin A was found in the urine from 11 subjects on day 0 and in the urine from 16 subjects on the following day. Urolithin B was found in the urine of 3 subjects on day 0 and in the urine of 5 subjects on the next day.

CONTRAINDICATIONS AND PRECAUTIONS

There are no known contraindications for POM Wonderful PJ. Pomegranates and PJ have a long history of use as foods in many cultures. Although consumption of pomegranate fruit and juice has grown rapidly in the United States in the past decade, pomegranate has been widely grown and consumed in other countries for centuries. Clinical studies with POM Wonderful PJ have included sensitive subpopulations such as patients with atherosclerotic disease, carotid artery stenosis, diabetes, hypertension, and prostate cancer.[9–12,14] Despite the sugar content of PJ, one small clinical trial found no negative effect on glycemic control in type 2 diabetics consuming 8 ounces of POM Wonderful PJ daily for 3 months.[10]

PREGNANCY AND LACTATION

No known restrictions.

ADVERSE EFFECTS/SAFETY DATA

There is limited published safety data specific to POM Wonderful PJ. The 6 published human clinical studies summarized in the Clinical Review section on page 11 included a total of 201 subjects who were exposed to POM Wonderful PJ for time periods ranging from 2 weeks to 54 months.[9-14] Adverse event reporting included both clinical events and laboratory events. No clinical or laboratory adverse events were reported in any of these studies.

PJ is regulated as a food and has GRAS (generally recognized as safe) status with the FDA in the United States. Since it is not a drug or even a dietary supplement, adverse event reporting is not required. POM Wonderful has not formally implemented any Adverse Event Reporting system and therefore post-marketing surveillance data is not available on this product.

Although there are no reports on adverse effects with POM Wonderful PJ, there have been a total of 5 published case reports of allergic reactions to the ingestion or handling of pomegranate fruit/seeds.[121,122,123] In summary, it appears that ingestion and/or handling of pomegranate seeds is capable of eliciting a type I hypersensitivity reaction. Due to a proprietary juice processing, POM Wonderful PJ does not contain pomegranate seeds and this reaction has not been noted in subjects consuming POM Wonderful PJ in clinical studies. It is possible that other commercial PJ may contain seed extracts and it may be prudent to advise patients with documented fruit and/or nut allergies to watch for potential signs and symptoms of allergic reaction when consuming these juices.

DRUG INTERACTIONS

There are no known specific drug interactions for POM Wonderful PJ. As noted in the Clinical Overview on page 11, the juice has been given to type 2 diabetics taking oral hypoglycemic drugs for 3 months with no apparent negative effects on glycemic control.[10]

Due to reports of grapefruit juice's inhibiting cytochrome P450 isoenzymes and subsequent hepatic and enteric (within the intestine) drug metabolism,[124] there has been concern about the potential for drug interactions with other commercial fruit juices including PJ. A case report of a 48 year-old man developing rhabdomyolysis while taking rosuvastatin (5 mg every other day) and PJ (200 ml twice weekly) concomitantly, has also drawn focus to this concern for PJ.[125] It should be noted that the link between PJ and development of rhabdomyolysis is quite weak in this case because the subject had increased creatine kinase levels while not taking medication and because rosuvastatin is metabolized by P450 isoenzymes (e.g., CYP 2C9, CYP 2C19), which is not known to be affected by fruit juices such as grapefruit juice or PJ.

One study examined the effect of PJ on CYP3A-mediated drug metabolism both *in vitro* and *in vivo*.[126] Among the members of the P450 family, CYP3A is thought to be the most important enzyme and is involved in the majority of P450-catalyzed metabolism. Using liver microsomes, it was found that the addition of PJ inhibited the metabolism of carbamazepine, a drug known to be metabolized by CYP3A. The *in vivo* interaction between PJ and carbamazepine was then studied in male Wistar rats. Orally consumed PJ was found to increase the AUC when given 1 hour prior to oral administration of carbamazepine. However, when PJ was injected into the bloodstream, there was no effect on the AUC for the drug. The investigators suggest that PJ may impair the function of enteric but not hepatic CYP3A. Similar results on enteric but not hepatic activity were also found in another study with rats examining the effect of PJ on CYP2C9 and tobultamide pharmacokinetics.[127]

The effect of POM Wonderful PJ and grapefruit juice on CYP3A activity was studied *in vitro* and in human volunteers.[128] Using human liver microsomes and triazolam (a drug metabolized by CYP3A), the investigators found that pre-incubation with grapefruit juice and POM Wonderful PJ led to inhibition of CYP3A. However, a study with healthy human volunteers found that pretreatment with POM Wonderful PJ (8 ounces) did not alter the elimination half-life, volume of distribution, or clearance of intravenous midazolam (another drug metabolized by CYP3A). POM Wonderful PJ also did not affect C_{max}, AUC, or clearance of oral midazolam (6 mg). Alternatively, the same amount of oral grapefruit juice was found to impair clearance and elevate plasma levels of oral midazolam. The results of this study suggest that oral consumption of 8 ounces of POM Wonderful PJ does not alter the activity of hepatic or intestinal CYP3A in humans.

PATENTS

United States Patent (6,361,807 B1, issued September 21, 1999): Michael Aviram, Leslie Dornfeld. Pomegranate extracts and methods of using thereof.[7] The patent provides antioxidative compositions comprising an extract from pomegranate fruit. Antioxidative compositions for treating disorders associated with lipoprotein oxidation, lipoprotein aggregation, macrophage atherogenicity, platelet activation, or atherosclerosis are also provided. (*Note:* US Patents 6,375,993 B1 [August 23, 2002] and 6,387,418 B1 [May 14, 2002] are continuations of US Patent 6,361,807 B1).

United States Patent (6,641,850 B1, issued November 4, 2003): Michael Aviram, Leslie Dornfeld. Methods of using pomegranate extracts for causing regression in lesions due to atherosclerosis in humans.[8] Patent provides methods of administering pomegranate extracts for treating patients with atherosclerosis or intima-media thickness of an artery. The methods comprise the step of administering to the patient a composition comprising a therapeutically effective amount of an extract from pomegranate. The invention also provides methods of decreasing the incidence of stroke or heart attack in a patient by administering to the patient a composition comprising a therapeutically effective amount of an extract from pomegranate. (*Note:* US Patent 6,977,089 B1 [December 20, 2005] is a continuation of US Patent 6,641,850 B1).

CLINICAL REVIEW

There are a total of 6 published efficacy human clinical trials on POM Wonderful PJ[9-14] on a total of 201 subjects. (Table 2 on pages 16 and 17 summarizes these 6 clinical trials.) All except one[14] are pilot studies. The 4 cardiovascular studies evaluated POM Wonderful PJ for atherosclerosis, antioxidant activity, myocardial perfusion, and hypertension.[9-12] One study evaluated POM Wonderful PJ for erectile dysfunction,[13] and the only phase II study evaluated POM Wonderful PJ in slowing the progression of prostate cancer.[14]

CARDIOVASCULAR DISEASE

Atherosclerosis/Antioxidant Status

Aviram et al, 2004. A small open label, parallel group clinical trial was conducted with 19 patients (5 women and 14 men, aged 65-75 years) with severe carotid artery stenosis (CAS, 70-90% occlusion of the internal carotid arteries as confirmed by Doppler ultrasound) who were recruited from the Vascular Surgery Clinic at the Rambam Medical Center in Haifa, Israel.[9] Patients were randomized to receive 50 ml POM Wonderful PJ concentrate per day (treatment group; n = 10) or no POM Wonderful PJ (control group; n = 9). A subgroup of the POM Wonderful PJ subjects (n = 2) along with 7 additional matched control subjects underwent carotid endarterectomy. Subjects received 50 ml of POM Wonderful PJ per day. The concentrated POM Wonderful PJ was diluted 1:5 (v:v) with water to obtain a single strength juice equivalent to 8 ounces per day. The study period lasted for one year and 5 patients consuming POM Wonderful PJ continued for another 2 years.

The primary outcome was the change in intima-media thickness (IMT) over time measured at the distal common carotid artery by Doppler ultrasound. Additional outcomes included peak systolic velocity (PSV); end diastolic velocity (EDV); total cholesterol; high density lipoprotein cholesterol; triglycerides; apolipoproteins A-1 and B-100; serum paraoxonase 1 (PON 1) arylesterase activity (a high density lipoprotein associated enzyme that can reduce lipid peroxides, thereby decreasing oxidative stress); total antioxidant status; serum antioxidized LDL antibodies; LDL oxidation; and chemical analyses of atherosclerotic plaques obtained by endarterectomy for cholesterol, lipid peroxides, and reduced glutathione concentrations.

Compared to pretreatment values, mean IMT decreased significantly in the treatment group after 3, 6, 9 and 12 months (–13%, –22%, –26% and –35%, respectively; $p < 0.01$). After 12 months of treatment the mean IMT had decreased from 1.5 ± 0.2 mm at baseline to 1.1 ± 0.1 mm ($p < 0.01$) and remained at that approximate mean thickness for the duration of the study. In contrast, the mean IMT in the control group significantly increased from baseline to 12 months from 1.52 ± 0.03 to 1.65 ± 0.04 mm, ($p < 0.01$). Comparisons between groups were not provided. Significant decreases after 1 year of treatment were noted for mean PSV (cm/s), which decreased from 135 ± 6 to 103 ± 10 ($p < 0.01$), and for mean EDV, which decreased from 38 ±1 to 30 ± 12 ($p < 0.01$), with no additional significant reductions for the remainder of the trial. The large standard deviation calculated for EDV after one year of supplementation (30 ± 12) narrowed during the rest of the trial. The mean EDV at 22 months was 27 ± 5, 29 ± 2 at 28 months and 29 ± 2 at 36 months.

Systolic, but not diastolic, blood pressure (mmHg) was significantly reduced after 1 month of treatment from 174 ± 8 to 162 ± 9 ($p < 0.05$), and, compared to baseline, was further significantly reduced after 12 months to 153 ± 7 ($p < 0.01$). Blood pressure was

not significantly changed in the control group at any time period compared to baseline. Compared to baseline, antioxidized LDL antibodies (EU/ml) decreased by 24% after 1 month of treatment, from 2070 ± 61 to 1563 ± 69 and by 19% after 3 months, to 1670 ± 52 ($p < 0.01$). Mean total antioxidant status (nmol/L) increased after 12 months of PJ consumption from 0.95 ± 0.12 at baseline to 2.20 ± 0.25; however, 1 month after stopping treatment, mean total antioxidant status decreased to 1.4 ± 0.1. Mean serum lipid oxidation (nmol lipid peroxides/ml) significantly decreased after 12 months of treatment from 1670 ± 66 to 691 ± 43 ($p < 0.01$). Mean serum lipid oxidation was further significantly decreased to 690 ± 40 after 28 months and 660 ± 30 after 36 months compared to baseline ($p < 0.01$). PON 1 (U/ml) significantly increased in the treatment group after 1 year from baseline to 56 ± 5 to 97 ±10 ($p < 0.01$) and continued to significantly increase throughout the duration of the study to 107 ± 10 ($p < 0.01$) at 3 years; however, one month after stopping treatment, PON 1 activity decreased to 88 ± 18 U/mL.

Carotid endartherectomy was performed in 2 patients, one after 3 months and one after 12 months of consuming PJ, due to clinical deterioration during the trial. Compared to 7 controls, their carotid lesions had significantly lower mean concentrations of cholesterol (58% and 20% lower, respectively; $p < 0.01$), lipid peroxides (61% and 44%, respectively; $p < 0.01$), and lesion-induced LDL oxidation (43% and 32%, respectively; $p < 0.01$), and they had significantly greater reduction of glutathione (2.5 times greater in both samples; $p < 0.01$). Exact values for samples obtained by endartherectomy were not reported. No adverse events were reported.

The main limitation of this study is that both groups were not treated equally. While there was a control group, there was no placebo, and the PJ group received many more interventions than the control group, including blood draws and carotid ultrasounds at 1, 3, 6, 9, and 12 months, whereas the control group only received these interventions at 1 year. The carotid lesion analysis introduced a new group of controls who appear not to have been part of the original study. Finally, analysis between groups was not performed for any of the outcomes, only analysis within group.

Rosenblat et al, 2006. An open label, control group comparison was conducted with 10 non-insulin dependent diabetic (NIDDM) males (ages 35–71 years old; mean 50 ± 10 years) and 10 healthy age-matched controls (non-smokers).[10] The NIDDM subjects were non-smokers, did not have ischemic heart disease, had been diabetic for at least 4 years (up to 10 years), had a fasting serum glucose above 160 mg%, and a hemoglobin A1c ranging from 7.5 to 11.3%. Half had hypertriglyceridemia. All NIDDM subjects were treated with oral hypoglycemic drugs and 2 were treated with angiotensin II converting enzyme (ACE) inhibitors for hypertension. All subjects received 50 ml of POM Wonderful PJ concentrate per day for 3 months. The concentrated PJ was diluted 1:5 (v:v) with water to obtain a single strength juice equivalent to 8 ounces per day. The PJ was noted to contain 10% sugar and have a glycemic index "similar to that of other fruit juices."

Pre- and post-PJ blood samples from the NIDDM subjects were analyzed for several parameters including glucose, HbA1c, cholesterol, triglycerides, antioxidant measures including lipid peroxides, cellular peroxides, glutathione levels, thiobarbituric acid reactive substances (TBARS), PON1 activity, and serum total sulfhydryl groups (SH groups in serum). Cellular uptake of oxidized LDL was also measured *ex vivo*.

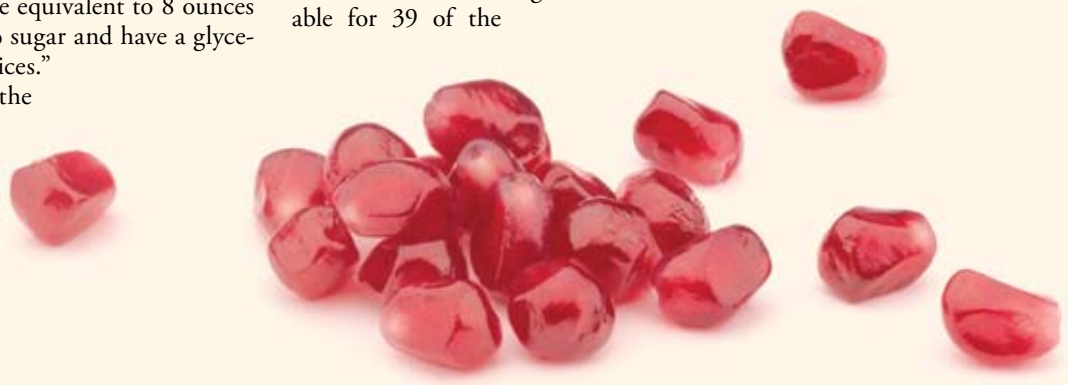
PJ consumption did not affect serum glucose, cholesterol, and triglyceride levels. At baseline, serum lipid peroxides and TBARS levels were increased by 350% and 51%, respectively, in diabetic subjects compared to controls, whereas serum SH groups content and PON1 activity were both decreased by 23% compared to controls. After PJ consumption, lipid peroxides and TBARS levels were decreased by 56% and 28%, respectively, as compared to baseline in diabetic subjects. Total SH groups and PON1 activity were significantly increased by 12% and 24%, respectively. At baseline, patient versus control measures of HMDM found increased levels of cellular peroxides (by 36%) and decreased glutathione content (by 64%). After PJ consumption, there was a 71% decrease in cellular peroxides and a 141% increase in glutathione in the diabetic patients compared to baseline. Compared to controls at baseline, diabetic patients had a cellular uptake of oxidized LDL at an enhanced rate of 37%. After 3 months of PJ consumption, this was decreased by 39%.

Ischemic Coronary Heart Disease

Sumner et al, 2005. A randomized, placebo-controlled, double-blind study was conducted by researchers at the Preventive Medicine Research Institute, Sausalito, CA, and the California Pacific Medical Center and the School of Medicine, University of California, San Francisco.[11] Forty-five patients (mean age 69 years) with stable coronary heart disease were recruited for the trial. Patients were confirmed to have stress-induced ischemia that was documented by ≥ 1 reversible myocardial perfusion defect on single-photon emission computed tomographic technetium-99m tetrofosmin scintigraphy. Patients were randomized to receive either 240 ml (8 ounces) per day of POM Wonderful PJ or a placebo drink ("modified sports beverage of similar caloric content, flavor, and color") for 3 months.

At baseline and at 3 months, patients underwent radionucleotide exercise treadmill or pharmacologic (adenosine or dipyridamole) stress testing by gated, single-isotope myocardial perfusion with single-photon-emission computed tomography using a radiotracer. Beta blockers, ACE-inhibitors, calcium antagonists, and nitrates were withheld for 24 hours before stress testing. A semiquatitiative scoring method analyzed radiotracer uptake in 17 myocardial segments. Using a 5-point scoring system for each segment (ranging from 0 = normal uptake to 4 = absent uptake), a summed stress score and a summed rest score were calculated. A summed difference score (SDS) was calculated using the difference between summed stress and summed rest scores. The SDS is a measurement of inducible myocardial infarction and has been found to be predictive of myocardial infarction.

Three month testing data was available for 39 of the



original 45 patients (2 dropouts in each group and 1 in each group who had unreadable perfusion tests). After 3 months of treatment, the extent of stress-induced ischemia decreased in the PJ group compared to baseline (SDS: – 0.8 ± 2.7) and increased in the placebo group (SDS: 1.2 ± 3.1); the difference between the 2 groups was significant ($p < 0.05$). There were no significant changes in summed stress or summed rest scores in the PJ group, while the placebo group had a significant increase in summed stress score ($p < 0.05$). The change in SDS in the PJ group was not accompanied by any changes in plasma lipids, blood glucose, hemoglobin A1c, body weight, or blood pressure. Angina episodes decreased by 50% in the PJ group (from 0.26 to 0.13) as compared to a 38% increase in the placebo group (0.53 to 0.75); this difference was not statistically significant. There were several reported cardiac adverse events during the study, but the authors did not attribute any of these events to the intervention, nor was an analysis done demonstrating whether the rate of events was different in the 2 groups.

Hypertension

Aviram and Dornfeld, 2001. A small open-label study was conducted with 10 hypertensive (mean blood pressure levels of 155 ± 7/83 ± 7 mm Hg) patients (7 males and 3 females, aged 62–77 years old).[12] All patients were nonsmokers, 2 were diabetic, and 2 were hyperlipidemic. Eight patients were on ACE inhibitors (enalapril or ramipril) and 2 were on calcium channel blockers. It was not made clear whether or not the subjects continued their antihypertensive regiments during the study. Patients received 50 ml of POM Wonderful PJ concentrate per day for 2 weeks. The concentrated PJ was diluted 1:5 (v:v) with water to obtain a single strength juice equivalent to 8 ounces per day. Serum ACE was determined at baseline and after 2 weeks of PJ ingestion. Blood pressure was also measured at the end of 2 weeks.

After 2 weeks of PJ ingestion, 7 out of 10 patients had a mean decrease in serum ACE activity of 36% (significance is claimed but no statistical analysis is reported). Mean blood pressure levels were 147 ± 10/82 ± 5 mmHg—a small (5%) but significant ($p < 0.05$) decrease in systolic blood pressure. Adverse events were not reported.

Two significant factors confound the results of this trial: (1) 3 of the 10 subjects are not included in the analysis nor are they accounted for, and (2) it is unclear whether 8 patients continued their use of ACE inhibitor medication (the change in serum ACE levels was one of the primary endpoints).

Discussion

In summary, these 4 pilot studies show that PJ can demonstrate statistically significant improvements in intima-media thickness (IMT), end-diastolic volume, peak systolic velocity (PSV), systolic blood pressure, TBARS, lipid peroxides, serum ACE activity, summed stress and rest scores, and the number of angina episodes. No improvements were seen in lipids, glucose, or HbA1c. The clinical relevance of these improvements varies, but in general they can be taken to represent positive trends in overall improvement of cardiovascular risk.

ERECTILE DYSFUNCTION

Forest et al, 2007. A randomized, double-blind, placebo controlled crossover study of 61 subjects (21–70 years old) was performed at an erectile dysfunction (ED) clinic in Beverly Hills, CA.[13] The rationale for the study was that the antioxidant properties of PJ would increase nitric oxide production, improve smooth muscle relaxation, and reduce atherosclerotic plaque, all of which can contribute to ED.

Seventy-four subjects with a history of ED for at least 3 months and who were in a monogamous heterosexual relationship were screened. Of those, 61 qualified and were enrolled. Subjects were excluded if they had a documented secondary cause of ED. The primary outcome was Global Assessment Questionnaire (GAQ), which represents the subject self-evaluation of the study beverage effect on erectile activity. The secondary outcome was the International Index of Erectile Function (IIEF) questionnaire, which evaluates intercourse satisfaction, overall satisfaction, orgasm, and desire. The study design incorporated a screening period during which any prescription, nonprescription (over-the-counter), or dietary supplement ED therapies were discontinued. The first arm of the crossover was 28 days, followed by a 2-week washout, and then the second 28-day arm. The group was split into 2 cohorts whose baseline characteristics were similar. Subjects consumed 8 ounces of POM Wonderful PJ with their evening meal.

Fifty-three of the 61 subjects completed the study. Seven were lost to follow-up and one subject was discontinued "for reasons not related to the study." No serious adverse events were noted and no subjects discontinued due to adverse events. The results of the GAQ showed that a total of 42 subjects attributed an improvement in erectile activity to drinking the beverage, and of those 25 were attributed to the PJ beverage. Subjects were more likely to report an improvement in GAQ score while drinking the PJ beverage, and the difference between beverages approached statistical significance ($p = 0.058$). There was a sizable difference between cohorts in their response rates. There was no difference in the secondary endpoint within any domains on the IIEF.

While generally well designed, the authors admit that a sexual encounter diary would have been helpful since the GAQ and IIEF were administered only at the end of each crossover arm (28 days), which may limit the interpretation of the efficacy (or lack thereof) of the study product. In addition the significant between-cohort differences may point to a sequence effect indicating that the washout period between arms was not long enough.

PROSTATE CANCER

Pantuck et al, 2006. A phase II, open-label, single-arm clinical trial was conducted at the Clark Urologic Center, David Geffen School of Medicine, University of California at Los Angeles.[14] Forty-six men (ages are not given) with recurrent prostate cancer and rising prostate-specific antigen (PSA) after surgery or radiotherapy were recruited for the study. Eligible patients had a detectable PSA > 0.2 and < 5 ng/ml that was documented as rising pretreatment PSA time points enough to calculate a baseline PSA doubling time (PSADT): no hormonal therapy before entering the study, no evidence of metastatic disease, and Gleason score ≤ 7. Of the patients enrolled, 68% were originally treated by radical prostatectomy, 10% by external beam radiotherapy, 10% by brachytherapy, 7% by surgery and radiation, and 5% by cryotherapy. Patients were treated with 8 ounces per day of POM Wonderful PJ until disease progression end points.

Serial PSA measurement before study entry determined a baseline PSADT. Each patient had a minimum of 3 pretreatment PSA values measured over a minimum of 6 months before study entry. Patients were then treated with PJ until meeting disease progression end points. Patients were followed in 3-month intervals for serum PSA, and blood and urine were collected for laboratory studies. Clinical end points included safety, effect on serum PSA, effect on serum hormone levels (testosterone, estradiol, sex-hormone binding globulin, dehydroepiandrosterone, insulin-like growth factor,

and androstenedione), and *in vitro* assays that measure the effect of patients' serum on LNCaP cell (a prostate cancer cell line) growth and apoptosis as well as nitric oxide and lipid peroxidation. The primary end point was effect on PSA variables, such as change in PSADT.

Mean PSADT significantly increased with treatment from a mean of 15 months at baseline to 54 months post-treatment ($p < 0.001$). Seven subjects actually had negative PSADT slopes (their PSA decreased over time) and were not included in the analysis. This exclusion results in an underestimation of the true PSADT. *In vitro* assays comparing pretreatment and post-treatment patient serum on the growth of LNCaP showed a 12% decrease in cell proliferation and a 17% increase in apoptosis ($p = 0.048$ and 0.0004, respectively). Also found was a 23% increase in serum nitric oxide ($p = 0.0085$) as well as significant reductions in oxidative state and sensitivity to oxidation of serum lipids after PJ consumption ($p < 0.02$). There were no significant differences in pretreatment and post-treatment hormone levels. There were no serious adverse events and the PJ was well tolerated.

This robust open label study demonstrates a clinically significant improvement in PSADT. As noted by the investigators of this study, the benefits noted are in assays that are not yet validated. Future clinical trials will be needed to demonstrate whether improvement in biomarkers (e.g., PSDAT) correlate with clinical benefit. A confirmatory phase III placebo-controlled clinical trial using POM Wonderful PJ began recruiting patients in April 2006 and hopes to address some of the limitations of the phase II study summarized above.

MANUFACTURER INFORMATION

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***Manufacturer Statement on Animal Testing:** POM Wonderful LLC completed all animal testing on PJ as of October 2006 and has no plans for future testing.

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CONFLICT OF INTEREST DISCLOSURE

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Table 1. Nutrition Facts For Pom Wonderful® Pomegranate Juice Based On 4 Lots Analyzed 2006-2007

Nutrition Facts

Serving Size 8 fl oz (240 mL)
Servings Per Container _____

Amount Per Serving	
Calories 150	Calories from Fat 0
	% Daily Value *
Total Fat 0g	**0%**
Saturated Fat 0g	**0%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 0mg	**0%**
Potassium 520mg	**15%**
Total Carbohydrate 40g	**13%**
Dietary Fiber 0g	**0%**
Sugars 32g	
Protein < 1g	

Vitamin A 0%	• Vitamin C 0%
Calcium 2%	• Iron 2%
Thiamin 2%	• Riboflavin 6%
Niacin 4%	• Vitamin B6 6%
Biotin 4%	• Pantothenic Acid 4%
Phosphorus 2%	• Magnesium 4%
Zinc 2%	• Manganese 8%

* Percent Daily Values are based on a 2,000 calorie diet. Your calorie needs may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Potassium		3,500mg	3,500mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

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Table 2. Published Clinical Studies on POM Wonderful® Pomegranate Juice

CARDIOVASCULAR DISEASE

ATHEROSCLEROSIS/ANTIOXIDANT STATUS

Author/Year	Subject	Design	Duration	Dosage	Results/Conclusion
Aviram et al, 2004[9]	Atherosclerosis (change in intima-media thickness (IMT) at the distal carotid artery); antioxidant status; blood pressure (BP)	OL, PG n=19 patients with severe carotid artery stenosis (n=14 males, n=5 females; aged 65–75 yrs); 10 patients treated with PJ & remainder received no treatment & served as controls.	1 year (5 patients continued for another 2 yrs)	50 ml of concentrated PJ diluted 1:5 (v:v) with water to obtain single strength juice equivalent to 8 oz. per day	Compared to pretreatment values, IMT decreased significantly in treatment group at 3, 6, 9, and 12 months compared to baseline. ($p<0.001$). Systolic BP, but not diastolic BP, was significantly reduced from 174±8 mmHg at baseline to 153±7 at 12 mos. ($p<0.01$) in treatment group. Measures of oxidative stress were also significantly improved in treatment group at 12 mos.
Rosenblat, et al, 2006[10]	Antioxidant status	OL, PG n=10 male patients with non-insulin dependent diabetes (aged 35–71 years old); n=10 healthy age-matched controls	3 months	50 ml of concentrated PJ diluted 1:5 (v:v) with water to obtain a single strength juice equivalent to 8 oz. per day	Compared to controls, diabetic patients consuming PJ lipid peroxides & thiobarbituirc acid reactive substances were decreased by 56% and 28%, respectively compared to baseline. Total serum sulfhydryl groups & paraoxonase activity were increased by 12% & 24%, respectively. A substantial decrease (71%) cellular peroxides was shown in diabetic group as was an increase (141%) in glutathione compared to baseline.

ISCHEMIC CORONARY DISEASE

Author/Year	Subject	Design	Duration	Dosage	Results/Conclusion
Sumner et al, 2005[11]	Decrease in stress-induced ischemia	R, DB, PC n=45 patients (mean age 69 yrs) with stable coronary heart disease	3 months	240 ml (8 oz) per day	Stress-induced ischemia decreased significantly in PJ group compared to placebo group ($p<0.05$). Angina episodes decreased by 50% in PJ group compared to 38% increase in placebo group (difference did not reach statistical significance).

Author/Year	Subject	Design	Duration	Dosage	Results/Conclusion
Aviram, Dornfeld, 2001[12]	Hypertension; Serum angiotensin II converting enzyme (ACE) activity	OL n=10 hypertensive patients (n=7 males, n=3 females; aged 62–77 yrs)	2 weeks	50 ml concentrated PJ diluted 1:5 (v:v) with water to obtain single strength juice equivalent to 8 oz. per day	7 out of 10 patients had mean decrease in serum ACE activity of 36%. Mean blood pressure showed small (5%) but significant ($p<0.05$) decrease in systolic BP but not diastolic BP.

ERECTILE DYSFUNCTION

Author/Year	Subject	Design	Duration	Dosage	Results/Conclusion
Forest et al, 2007[13]	Erectile Dysfunction	R, DB, PC, CO n=61 subjects (aged 21–70 years); 53 completed study	28 days (2 day washout between arms)	8 oz. per day	Subjects were more likely to report an improvement in Global Assessment Questionnaire (subjective evaluation) while on PJ but this did not reach statistical significance ($p=0.058$). There were no differences between PJ or placebo on International Index of Erectile Function.

PROSTATE CANCER

Author/Year	Subject	Design	Duration	Dosage	Results/Conclusion
Pantuck et al, 2006[14]	Change in prostate specific antigen (PSA) doubling time (PSADT)*. * Note: men with greater PSADT are thought to have a greater chance of survival & decreased chance of recurrence	OL, SA n=46 patients with recurrent prostate cancer & rising PSA after surgery or radiotherapy	Patients were treated with PJ until meeting disease progression endpoints (e.g. safety, effect on serum, PSA, hormone levels, etc.)	8 oz. per day	Mean PSADT significantly increased with treatment from mean of 15 mos to 54 mos post-treatment ($p<0.001$). In vitro assays comparing pre- & post-treatment serum effects on LNCaP cells (a prostate cancer line) showed 12% decrease in cell proliferation ($p=0.048$) & 17% increase in apoptosis ($p=0.0085$).

Key: R = randomized, DB = double-blind, PC = placebo-controlled, OL = open label, PG = parallel groups, SA = single-arm, CO = crossover study, n = number of patients

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