Pomegranate fruit extract

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Introduction

Pomegranate *(Punica granatum* L) is a native plant of Northern Africa and the Caucasian Mountains and is widely distributed throughout the southern United States. The name pomegranate comes from the Latin *"pomu*m," meaning apple, and *"granatu*s," meaning full of seeds. The botanical name is derived from old French: *pume grenate* – pomegranate apple.

Pomegranate: Lore and Traditional Uses

The pomegranate tree was said to have flourished in the Garden of Eden and has been used extensively in the folk medicine of many cultures. It was mentioned in the papyrus Ebers of Egypt written about 1550 B.C., and was a favorite motif in the temple of Solomon. The juicy pomegranate fruit with its multitudinous seeds was a popular *symbol of fertility* and fecundity in ancient times and it is counted among the seven kinds of produce with which the land is blessed. Doctors in Greece prescribed pomegranate juice as a remedy for inflammation, intestinal worms, persistent coughs, diarrhea, and dysentery. The Babylonians regarded pomegranate seeds as an agent of resurrection. Persians believed that the seeds conferred strength and invincibility on the battlefield, and in ancient China, the seeds were revered for their powers to promote longevity and immortality. People of the Georgian Republic in Russia used pomegranate for arresting chronic mucous discharges, passive hemorrhages, night sweats and diarrhea. It has also been prescribed to strengthen the human capillary system, and prevent atherosclerosis, asthma, tonsillitis and bronchitis.

Table 1. Phytochemistry of pomegranate

Anthocyanidins
Pelargonidin
Ellagotannins
Gallic acid
Ellagic acid
Pseudopelletierine
Isopelletierine
Methylisopelletierine
Genistein
Diadzein
Genistin
Diadzin
Estrone

Phytochemistry:

Pomegranate is composed of a rich variety of flavonoids, which comprise approximately 0.2% to 1.0% of the fruit (ref h). Table 1 summarize the phytochemistry of pomegranate fruit. Approximately 30% of all anthocyanidins found in pomegranate are contained within the peel. The isoflavones genistein, diadzein, genistin, and diadzin as well as estrone, the metabolic derivative of estradiol, have been isolated from the seeds (ref A and (Moneam et al. 1988). The stems and roots of pomegranate contain alkaloids including isopelletierine, pseudopelletierine, and N-methylisopelletierine (PDR).

Atherosclerosis:

Atherosclerosis is the progressive and irregular distribution of lipid deposits within large and medium-sized arteries. It leads the cause of death in develop countries, manifesting as a stroke or myocardial infarction (heart attack). Atherosclerosis can develop in any part of the circulatory system: in the coronary arteries, leading to angina pectoris and myocardial infarctions; in the carotid arteries that supply the brain and can lead to stroke. Intermittent claudication, a condition characterized by narrowing the arteries in the leg, resulting in pain brought on by walking, is another manifestation of atherosclerosis. Atherosclerosis can attack the kidneys as well, leading to hypertension and atheroembolic disease (blockage of a

blood vessel due to a blood clot). Unfortunately, the majority of us are unaware that we have advanced atherosclerosis until symptoms of chest or leg pain or transient or full-blown stroke occur. Most people in developed countries have some form of atherosclerosis that may develop as early as childhood; not surprising considering the copious amounts of fat and sugar in the typical western diet. To understand how pomegranate attenuates atherosclerosis, it's important to review the process of atherosclerosis itself.

Atherosclerosis

The blood vessels that carry our vital fluids are composed of intricate layers of tissue. The inner layer, called the intima, is the primary sight for atherogenesis. The initial stage of atherosclerosis begins with the "fatty streak," the accumulation of lipids in the intimal layer. This lipid accumulation results from LDL cholesterol (low density lipoproteins) binding, or "sticking," to constituents in the intimal layer. This has the effect of trapping the LDL particle in the intimal layer – a process known as retention. Once trapped, the LDL is susceptible to chemical modifications in its lipid structures that ultimately promote atherosclerosis. These modifications result from oxidative reactions.

Recruitment of leukocytes, or white blood cells, in the formation of a fatty streak is the second step in the process of atherosclerosis. Modifications in the structure of LDL spark the activity of monocytes, a class of leukocytes. Once activated, monocytes migrate to the intima where oxidized LDL has been detected and differentiate into macrophages. The primary role of the macrophage is to seek out foreign material and, essentially, eat it (hence, the name macrophage, which means "large eater"). Macrophages consume oxidized LDL at a rampant pace, eventually becoming overloaded with cholesterol from the LDL particle and ultimately become what is known as a "foam cell." Foam cells are characteristic of atherosclerotic lesions. Why macrophages consumed modified LDL is still speculative, but is thought to be a sort of cleaning process to remove oxidized LDL, which may be seen as a foreign body. During the normal course of LDL consumption, some macrophages leave the intimal layer of the blood vessel, taking the cholesterol with it. Therefore, atherosclerosis occurs when more lipids enters the artery wall than leaves via macrophage transport or other pathways.

Factors that Affect Atherosclerosis

High cholesterol, hypertension, diabetes, smoking, and obesity are primary risk factors for atherosclerosis. Table two lists other risk factors for atherosclerosis. Platelet aggregation at the lesion sight can lead to further progression of the fatty streak. Platelets help repair slightly damaged blood vessels by promoting blood clotting. Any damage, such as an atherosclerotic lesion, will instigate platelet aggregation and blood clotting. Thus, a lesion development and growth is the result of the accumulation of foam cells platelets. Conversely, high levels of high density lipoproteins (HDL) are associated with a decrease risk because they provide an independent pathway for the removal of lipids from fatty streaks. By a process known as

Table 2. Risk Factors of Atherosclerosis

- High cholesterol levels
- Low HDL level
- Hypertension
- Male gender
- Diabetes mellitus
- Cigarette smoking
- Post-menopausal state
- Physical inactivity
- Obesity
- Angiotensin-converting enzyme
- Family history

"reverse cholesterol transport," HDL can mediate a net removal of cholesterol from lipid-laden macrophages thus, preventing or decreasing the size of an atherosclerotic lesion. With this cursory overview of atherosclerosis as a frame of reference, we can delve into pomegranate's ability to treat both the early and late stages of atherosclerosis.

Western approach: Cholesterol Drugs

Contrary to popular opinion, cholesterol is essential to life. The human body contains about 100 g of cholesterol. Most of this is incorporated in the membranes from which cells are constructed and is an indispensable component of them. It is used to synthesize several steroid hormones, including the sex hormones estrogen, progesterone, and testosterone as well as the corticosteroids, and it is also the precursor from which the body synthesizes vitamin D. Human liver synthesizes 1500-2000 mg of new cholesterol each day. The amount synthesized and metabolized daily by the body itself is far greater than the amount usually consumed in the diet.

In response to soaring rates of atherosclerosis, pharmaceutical companies have concocted a variety of drugs collectively known as "statin drugs," the most popular being lovastatin, pravastatin, simvastatin, and fluvastatin and fibrates. Their effect is one-dimensional; lower cholesterol levels by decreasing the body's ability to synthesize it. Even worse, their use has been linked to undesirable side effects such as stomach, liver, and lung tumors in mice (reference). Lovastatin reduces levels of coenzyme Q10 and L-carnitine, two very important molecules involved in energy production and fat metabolism (Mortensen et al. 1997). Forty-five hyper-cholesterolemic patients were randomized in a double-blind trial were taken either lovastatin (20-80 mg/day) or pravastatin (10-40 mg/day) over a period of 18 weeks (Mortensen et al. 1997). Serum levels of coenzyme Q10 were measured parallel to the levels of cholesterol at baseline on placebo and diet and during active treatment. A dose-related significant decline of the total serum level of coenzyme Q10 was found in the pravastatin group In another study forty-seven patients were treated with 10 or 20 mg of Simvastatin per day for 14 weeks (Human et al. 1997). As expected, total cholesterol and LDL cholesterol concentrations decreased considerably, but unfortunately, the decline in circulating vitamin E and coenzyme Q10 concentrations were also documented. The five hospitalized patients, 43-72 years old, revealed increased cardiac disease from lovastatin, which was life threatening for patients having class IV cardiomyopathy before lovastatin or after taking lovastatin (Folkers et al. (1990). Lovastatin was shown in some patients develop a secondary deficiency in muscle carnitine. Clinically, this may manifest as a myalgia/myositis. Myalgia refers to generalized muscle pain, may accompany myositis, which is inflammation of the muscles, either in response to an immune system disorder refers to inflammation of the muscles. Treatment with lovastatin significantly alters the carnitine. The use of statins or statins containing products such as *Meniscus purpureus* (known as "red rice yeast") in treatment of high cholesterol could lead to a secondary deficiency in carnitine, which may manifest clinically as a myalgia/myositis-a side effect that is occasionally seen with this class of drugs

Pomegranate and atherosclerosis

Out of its many traditional uses, scientific evidence suggests that pomegranate fruit is a potent inhibitor of atherosclerosis. Several studies, using juice from the whole fruit, demonstrate that pomegranate reduces the development and progression of

atherosclerosis by several mechanisms. One mechanism relates to the concentration of antioxidant nutrients found in pomegranate. Pomegranate juice pressed from the whole fruit possesses antioxidant activity three times greater than the antioxidant activity of red wine or of green tea (ref 84 of h). Mice fed pomegranate juice showed dose-dependent increase in serum levels of antioxidants (ref g).

Perhaps pomegranate's greatest mechanism for combating atherosclerosis is its association with an enzyme known as paraoxonase. Paraoxonase (PON) is an HDL-associated esterase enzyme whose activity is related to atherosclerosis: decreased activity of PON is associated with increased risk for atherosclerosis. The antiatherogenic properties of PON may be related to its ability to protect against LDL oxidation. Paraoxanase has been shown to protect LDL against lipid peroxidation. Paraoxonase has also been shown to destroy oxidized lipids found in LDL, essentially "undoing" the damage oxidative processes have done (ref 31, 32 of E). Therefore, by restoring LDL to its native form, their incorporation into macrophages will halt and thus, decrease the formation of foam cells. The protective interaction between PON and oxidized LDL and free radicals comes at a cost; the paraoxonase enzyme gradually becomes inactivated and looses effectiveness. Decreased PON activity is also seen in hypercholesterolaemia (high blood cholesterol levels) and diabetes. However, consumption of pomegranate juice by human volunteers demonstrated a significant elevation of paraoxonase activity as compared to values before consumption. Therefore, the ability of pomegranate to preserve, and even enhance PON activity affords greater protection against LDL oxidation and subsequent atherosclerosis.

A third mechanism that may explain pomegranate's effect is its ability to lower LDL's susceptibility to binding and accumulation in the intimal layer of blood vessels. Platelet aggregation is also reduced via pomegranate consumption. Finally, pomegranate may accelerate cholesterol transport out of an atherosclerotic lesion by inhibiting cholesterol metabolism in macrophages, thus slowing their progression to foam cells, and promoting reverse cholesterol transport via HDL. Both in vitro and in vivo studies support pomegranate's various roles in attenuating atherosclerosis.

The Studies

A team of researchers found that the juice of pomegranate prevented the buildup of plaque in the arteries of both young mice and mice with advanced atherosclerotic lesions (although the effect was greater in the younger mice). In young mice, macrophage-mediated oxidation of LDL was 54% lower in the group consuming pomegranate juice. Macrophage uptake of LDL particles was also inhibited. Platelet aggregation, a contributor to the progression of atherosclerosis was inhibited in a dose-dependent manner by up to 90% with pomegranate juice. After the treatment period, the size of the atherosclerotic plague was ascertained. In the group consuming pomegranate juice, plague sizes were significantly lower, by 44%, compared to the plagues in the aortas from control mice.

In older mice with advance atherosclerosis, pomegranate juice increased PON activity by 43% compared to placebo group. Consumption of pomegranate juice also decreased lipid peroxidation in macrophages by 42% and decreased LDL uptake into macrophages by 31%. In the placebo group, LDL uptake *increased* by 34%. Cholesterol esterification in macrophages (a process that leads to the development of a foam cell), was 110% greater in the control group and 80% *lower* in the group consuming pomegranate juice. Reverse cholesterol transport, the process by which

cholesterol is remove from fatty lesions, was 39% greater in the group consuming pomegranate juice and 46% lower in the placebo group. Finally, the ultimate marker of atherosclerosis, the size of the lesion, was examined. In the placebo group, lesion size increased 214%! In the group of mice consuming pomegranate juice, although the size of the lesion was bigger than in younger mice, it was 17% smaller when compared to the placebo group.

An *ex vivo* study using a group of healthy males who drank pomegranate juice daily for 2 weeks showed similar result from animal studies. Decreased susceptibility of LDL to lipid peroxidation was observed. Additionally, a significant 9% increase in plasma antioxidant status was observed after two weeks of pomegranate juice consumption. Increased consumption of juice to 20 ml/day and then to 50 ml/day decreased lipid peroxidation by 11% and 21%, respectively. Greater amounts of juice did not have a greater effect. Two weeks of consuming pomegranate juice also resulted in a significant 18% increase in paraoxonase activity, which correlated to increased resistance of HDL to peroxidation. Oxidative damage to LDL gradually decreased by 43%. Both retention and aggregation of LDL decreased as well. In the latter case, aggregation decreased by as much as 75%. In another study using ten hypertensive patients, pomegranate juice caused a small but significant decrease in blood pressure. In seven out of the ten participants, serum angiotensin-converting enzyme activity decreased by 36%. Angiotensin-converting enzyme (ACE) plays a critical role in the regulation of blood pressure. Drugs that specifically target this enzyme (called ACE inhibitors) are used for the treatment of hypertension.

An *In vitro* study corroborates previous studies mentioned. Pomegranate juice inhibited lipid peroxidation in a dose-dependent manner and increased paraoxonase activity by 33%. This study also confirmed that the aqueous extracts of the inner and outer peel contain a higher concentration of antioxidants than the juice alone. This suggests that any supplements incorporate the whole fruit.

Table 3. Antiatherogenic Effect of Pomegranate

- Inhibits LDL oxidation and modifications
- Attenuates LDL aggregation
- Attenuates platelet aggregation
- Increases the antioxidant status of the body
- Enhances paraoxonase activity
- Facilitates reverse cholesterol transport
- Decreases the formation of foam cells
- Reduces blood pressure by inhibiting ACE

Although the body of evidence is relatively small, the results are impressive. Consumption of pomegranates can reduce the risk of atherosclerosis and, unlike pharmaceutical drugs, accomplishes this task by a several mechanisms as outlined in table three. The exact constituents responsible for pomegranate's effect is still speculative but is most likely attributed to the polyphenols found in the whole fruit (peel and edible parts). In all studies, no adverse changes in lipid, carnitine, or CoQ10 levels were observed. Pomegranate's unique ability to influence paraoxonase activity is very interesting and important considering the enzyme's role in protecting HDL and LDL particles from oxidation. In fact, pomegranate has a greater effect on paraoxonase activity than other antioxidant compounds such as flavonoids from red wine!

Additional Health Benefits of Pomegranate: Ellagic acid

Pomegranate fruit extract

Pomegranate is also a rich source of ellagic acid, a phenolic normally found as a polymer with gallic acid, known as ellagitannin. There is a large body of evidance suggesting that ellagic acid is an inhibitor of chemically-induced cancers. Initial studies in mice showed that Ellagic acid reduces lung and skin tumors. Treatment of mice with ellagic acid shortly before injecting them with the carcinogen benzo[a]pyrene caused a 44 to 75% inhibition in the formation of lung tumors. Boukharta et al. observed that at doses of 0.06-4.0g/kg diet, Ellagic acid inhibited the multiplicity of lung tumors. Mukhtar showed that topical application of ellagic acid to the skin of mice exerted strong protective effects against skin carcinogens. Ellagic acid significantly reduces the incidence of polycyclic aromatic hydrocarbon-induced carcinomas. Dietary EA has been shown to reduce the incidence of N-2-fluorenylacetamide-induced hepatocarcinogenesis in rats and N-nitrosomethylbenzylamine (NMBA)-induced rat esophageal tumors.

Many people's lives have been touched in some way by cancer. Maybe they've lost a relative, a friend or an acquaintance; maybe they had a scare as a result of an annual physical. Regardless of what drives people to ask about cancer prevention, it is a perfect opportunity for them to learn about diet and supplements. Empowering people to preserve their health through intelligent choices puts responsibility in the patient's hands.

Cancer is a prominent killer of Americans – second only to heart disease – and is responsible for more than a half million deaths yearly. We are exposed to oxidizing and cancer producing substances daily. Fruit phenolics help limit the free radical initiation and DNA damage caused by these carcinogens and, therefore, fruit phenolics should lower the incidence of various types of cancer. These protective phytochemicals include the ellagic and hydroxycinnamic acids, which have been shown to degrade carcinogenic substances. Among other things, hydroxycinnamic acid helps degrade carcinogens and helps prevent nitrates in the digestive tract from being converted into the carcinogenic nitrosamines. Ellagic acid, which is particularly plentiful in pomegranates, also prevents carcinogen oxidation of cellular membranes.

Interest in ellagic acid has increased greatly during the last decade due to its extraordinary effectiveness as an antimutagen and anticarcinogen, and its potential as an inhibitor of chemically induced cancer. Dr. Gary Stoner at Ohio State University contributed pioneering research in our understanding of the phytomedicinal properties of ellagic acid. Initial studies on rodents by Dr. Gary Stoner have shown that ellagic acid significantly helps prevent, and reduce, certain cancers. Lesca (1983) investigated the effect of ellagic acid on carcinogens-induced lung tumors in mice. When administered *ip* or as a dietary admixture, ellagic acid decreased the multiplicity of tumors. Chang et al. (1985) showed that treatment of mice with ellagic acid shortly before an injection of different carcinogens caused a 44 to 75% inhibition in the number of lung tumors. Application of ellagic acid by Boukharta et al. (1992) inhibited the multiplicity of lung tumors in mice by 54%.

Other tissues in which ellagic acid has been shown to exhibit anticarcinogenic effects include the esophagus and liver. Mandal and Stoner (1990) reported inhibitory effects of ellagic acid on tumorigenesis in the esophagi of rats. The ellagic acid inhibited the development of both preneoplastic and eoplastic lesions by 21 to 50%. Ellagic acid also was found to be an effective inhibitor of tumorigenesis in the rat esophagus when administered before, during and after the carcinogen (Siglin et al. 1995). Mandal and Stoner (1990) demonstrated inhibition of nitrosobenzylmethylamine esophageal carcinoma in rats by dietary ellagic acid, apparently by inhibiting carcinogen metabolism and DNA damage to the esophagus. Daniel and Stoner (1991) later demonstrated 60% inhibition of nitrosobenzylmethylamine-induced esophageal tumors in rats by dietary ellagic acid.

Tanaka et al. (1988) investigated the effect of ellagic acid on the genesis of liver cancer in male rats. Rats were fed a diet containing 400 ppm of ellagic acid before, during and after administration of carcinogen in the diet. Ellagic acid reduced the number of altered foci and the incidence of hepatocellular neoplasms in the carcinogen-treated rats. Clinical tests conducted at the Hollings Cancer Center of the Medical University of South Carolina under the direction of Dr. Bhagavathi A. Narayanan (1999) reveal that the human body readily absorbs ellagic acid. Moreover, ellagic acid clinically has been shown to cause apoptosis (cell death) in cancer cells. The findings of Dr. Narayanan and her colleagues point to what may be the most potent way to prevent cancer, inhibit the development of cancer cells, and arrest the growth of cancer in persons with a genetic predisposition for the disease, through "normal diet."

Ellagic acid: Positive effects against cancers of the breasts and cervix

Breast cancer is second only to skin cancer as the most common form of cancer among women in the United States. The incidence of breast cancer has been rising for the past two decades, although researchers feel that much of the increase is associated with increased and improved screenings and detection. It is improvements in both screening and treatments that are believed to have led to the significant decrease in breast cancer mortality witnessed between 1992 and 1996. Nonetheless, about 40,600 American women will die from breast cancer during 2001. During 2001 as well, an estimated 192,000 new cases of invasive breast cancer will be diagnosed (ACS 2001). Epidemiological research by the National Cancer Institute (NCI 2001) estimates that, if current rates stay constant, 1 in 8 women born today will develop breast cancer sometime during their lives. Previously cited studies have described ellagic acid's abilities to prevent, inhibit, reduce and even destroy cancer cells. Research by Dr. Wendy A. Smith and colleagues (2001, 1999, 1998, 1997) at the University of Kentucky's Graduate Center for Toxicology also shows that ellagic acid specifically helps prevent the earliest chemical reactions which can lead to the development of breast cancer.

Ellagic Acid Combats Carcinogens from Cirgarette Smoke

Dibenzo [*a, l*] pyrene (DBP) is a potentially deadly environmental chemical found in cigarette smoke, diesel exhaust and in other products of combustion. Research on rodent models has shown that DBP is one of the most potent mammary carcinogens known and, therefore, it often is used in laboratory research to create cancers to be studied in otherwise healthy cells. DBP, like other carcinogens, metabolizes with enzymes to produce specific carcinogenic chemicals which bind to DNA, resulting in the

corruption of cellular DNA. Unrepaired or misrepaired damage to cellular DNA produces mutant cells which may become cancerous and proliferate. Dr. Smith's team found that ellagic acid inhibited the binding of DBP carcinogenic chemicals to the DNA of human breast cells by 45%, thereby reducing carcinogen bioactivation by 45%. The carcinogen, DBP, itself, though, also can bind directly to DNA without first metabolizing with enzymes to create byproduct carcenogenic chemicals. In a 1998 study of six cancer chemopreventive agents, Dr. Smith's team found that ellagic acid was the only test agent observed that inhibited the binding of the carcinogen DBP directly to DNA in the absence of microsomal enzymes. Intervention with ellagic acid inhibited the direct binding of DBP to DNA by 64%, thereby reducing carcinogen bioactivation by 64%. One of the most potent carcinogens to which tobacco smokers expose themselves is nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanon (NNK). Drs. Teel and Castonguay (1992) at Loma Linda University in California tested the antimutagenic efficacies of polyphenolics, including ellagic acid, on NNK in Salmonella typhimurium TA1535. Ellagic acid was found to inhibit mutagenesis by 67%. For ellagic acid an almost complete inhibition of the mutagenicity of CSC and SNUS in STY was indicated. Along with chlorogenic acid, the ellagic acid reduced the mutagenicity of CSC and also strongly inhibited SNUS mutagenicity (Romert et al. 1994).

The protective effect of Ellagic acid is thought to be it ability to induce cellular detoxification enzymes such as NAD(P)H:quinone reductase (QR). Rats fed *Ellagic* acid demonstrated a 9-fold increase in hepatic and a 2-fold increase in pulmonary QR. Induction of glutathione S-transferase (GST) enzymes can increase detoxification of carcinogens and reduce carcinogen-induced mutagenesis and tumorigenesis. Rats fed ellagic acid demonstrated significant increases in total hepatic GST activity.

Thus, in addition to pomegranate's profound effect on atherosclerosis, it may also protect against chemical toxins frequently found in our environment.

Pomegranate fruit extract appears to be a promising supplement for combating two of the biggest killers in developed countries: cancer and heart disease.