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Review

Chemical composition of the plant *Punica granatum* L. (Pomegranate) and its effect on heart and cancer

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In this report, the chemical composition and pharmacological properties of *Punica granatum* L. (Punicaceae) have been reviewed. In the past decade, numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis and obesity.

Key words: *Punica granatum* L., pomegranate, chemical composition, pharmacological activities.

INTRODUCTION

The pomegranate, *Punica granatum* L., an ancient, mystical, and highly distinctive fruit, is the predominant member of two species comprising the Punicaceae family. It was lauded in ancient times in the Old Testament of the Bible, the Jewish Torah, and the Babylonian Talmud as a sacred fruit conferring powers of fertility, abundance, and good luck. It also features promi-nently in the ceremonies, art, and mythology of the Egyptians and Greeks and was the personal emblem of the Holy Roman Emperor, Maximilian. Pomegranate is the symbol and heraldic device of the ancient city of Granada in Spain – from which the city gets its name.

The genus name, *Punica*, was the Roman name for Carthage, where the best pomegranates were known to grow. Pomegranate is known by the French as grenade, the Spanish as granada, and literally translates to seeded ("granatus") apple ("pomum") (Abdurazakova et al., 1968).

The pomegranate tree typically grows 12 to16 feet, has many spiny branches, and can be extremely long lived, as evidenced by trees at Versailles, France, known to be over 200 years old. The leaves are glossy and lance-shaped, and the bark of the tree turns gray as the tree ages. The flowers are large, red, white, or variegated and have a tubular calyx that eventually becomes the fruit. The ripe pomegranate fruit can be up to five inches wide with a deep red, leathery skin, is grenade-shaped, and crowned by the pointed calyx. The fruit contains many seeds (arils) separated by white, membranous pericarp, and each is surrounded by small amounts of tart, red juice.

The pomegranate is native from the Himalayas in northern India to Iran but has been cultivated and naturalized since ancient times over the entire Mediterranean region. It is also found in India and more arid regions of Southeast Asia, the East Indies, and tropical Africa. The tree is also cultivated for its fruit in the drier regions of California and Arizona (Albrecht et al., 2004).

In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. In Ayurvedic medicine the pomegranate is considered "a pharmacy unto itself " and is used as an antiparasitic agent, (Aviram and Dornfeld, 2001) a "blood tonic", (Batra et al., 1968) and to heal aphthae, diarrhea, and ulcers (Batta and Rangaswami, 1973).

Pomegranate also serves as a remedy for diabetes in the Unani system of medicine practiced in the Middle

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East and India (Baytop, 1963). The current explosion of interest in pomegranate as a medicinal and nutritional product is evidenced by a MedLine search from 2000 to present, revealing over 130 new scientific papers pertaining to its health effects. Between 1950 and 1999 only 25 such publications appear on MedLine (Borir, 1980). The potential therapeutic properties of pomegranate are wide-ranging and include treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, and protection from ultraviolet (UV) radiation. Other potential applications include infant brain ischemia, Alzheimer's disease, male infertility, arthritis, and obesity. The following abbreviations for various pomegranate extracts will be used throughout the article:

1. Pomegranate juice – PJ
2. Pomegranate by-product – PBP
3. Fermented pomegranate juice – FPJ
4. Cold-pressed seed oil – CPSO
5. Pomegranate peel extract – PPE
6. Pomegranate pulp juice – PPJ
7. Pomegranate fruit extract – PFE
8. Pomegranate flower extract – PFLE
9. Hydroalcoholic extract of pomegranate – HAEP
10. Gel-based pomegranate extract – GPBE

Alkaloid

It was indicated that alkaloid was present at the rate of 0.35 to 0.60% in the body rinds, and over 3% in the roots; but none was found in the fruit rinds (Brieskorn and Keskin, 1954; Caceres et al., 1987). It was also indicated that pseudopelletierine, pelletierine, isopelletierine, methylpelletierine 1-pelletierine, dl-pelletierine and methyl isopelletierines were found in composition of the root, body and branch rinds of *P. granatum*. (Chidambara et al., 2002; Dean et al., 1971).

It was detected that saturated alkaloids present in the root and body rinds are not present in the leaves, whereas 2-(2-propenyl)-piperidine of unsaturated alkaloids was present in the leaf extract (Du et al., 1975).

Tannin and similar compounds

It was stated that punicacortein A, B, C, D in the structure of hydrolysable C-glycoside, which is a new ellagitannin, as well as punigluconin which contains one gluconic acid; and also casuariline and casuarine were present in the fresh body roots of *P. granatum* (Drillien and Viel, 1963; Fayez et al., 1963). Punicafolin as well well four ellagitannins and two gallotannins were isolated from the leaves. These were indicated to be granatin A and B, strictinin, corilagin, 1,2,4,6-tetra-O-galloyl D-glucose with 1,2,3,4,6-penta-O-galloyl D-glucose (Feldman and Markh, 1970). *Pericarpium Granati* on the other hand contains granatin A and B with punicalin and punicalagin (Gabbasova and Abdurazokova, 1968; Gil et al., 2000).

Anthocyanosides

Anthocyanosides are present in the fruit and flower sections of the plant. In comparison of anthocyanoside content of partly purified fruit rind extract and pomegranate arils; it is stated that pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside found in high amounts in the rinds are present in less amounts in the arils. Cyanidin-3-glucoside and cyanidin-3,5-diglucoside were detected in both arils and fruit rinds. On the other hand, it was not possible to detect in the fruit rinds delfinidin-3,5-diglucoside and delfinidin-3-glucoside, the major anthocyan in pomegranate juice (Guo et al., 2008; Hartwell, 1971). Flowers contain pelargonidin-3,5-diglucoside (Hartwell, 1971). It is furthermore stated that the amount of anthocyan varies by altitude of the location where the plant grows; and diminishes and disintegrates by keeping it waiting (Guo et al., 2008, Haddock et al., 1982).

Flavonoids

Flavonoids which display vitamin P activity are present in *P.granatum*. It is indicated that the fruits contain compounds in structure of flavonoid, quercetol in particular (Heftman et al., 1966, http://www.crfg.org).

Triterpenic acids

Presence of ursolic acid, one of the compounds in triterpenic structure, was determined in different sections of the pomegranate plant. Amount of ursolic acid is at the rate of 0.45% in the leaves and flowers as it reaches to 0.6% in the fruit rinds (Isamuhamedov and Akramov, 1982).

Polyholosides

Free oses (SUGERS) (fructose, glucose, and raffinose in low amounts), pectic substances, hemicellulose A and B, and water-soluble polyholosides are found in *P. granatum*. It was determined that the fruit rinds contained polyholoside at the rate of 2.58% (Jurkovic et al., 1976; Keogh and Donovan, 1970).

In result of pectin-related studies conducted on the fruit rinds, it was revealed that mannose, galactose, rhamnose, arabinose, glucose and galacturonic acid were present in its composition. They were found to be present in the form of calcium pectate in the lamella (Khodzhaeva and Yuldasheva, 1985).

Other compunds

Sitosterol, maslinic acid, asiatic acid and alkanes are present in the composition of pomegranate flower. It was expressed that D-mannitol, ellagic acid and gallic acid were present in its alcoholic extract (Hartwell, 1971). It is stated that in the pomegranate juice almost all the amino acids are present; while valine and methionine are in a very high concentration (Koleva et al., 1981; Konowalchuk and Speirs, 1976). It was found that pomegranate juice also contained invert sugar, thiamin, vitamin C, riboflavin and protein (Heftman et al., 1966, Lad and Frawley, 1986, Malik et al., 2005). Moreover, organic acids such as citric acid, malic acid and oxalic acid are present in the pomegranate juice, with 14.31% carotenoid and carotene being present in the edible part of the fruit (Nakov et al., 1982; Naqvi et al., 1991; Okuda et al., 1980). Composition of phenolic acids in cultivated and wild pomegranate fruits was determined, and it was reported to contain vanillic acid, neochlorogenic acid, chlorogenic acid, sinapic acid, kumic acid, ferulic acid and caffeic acid (Pantuck et al., 2006).

Pomegranate seeds contain 4 g/kg of estrone, with its surface parts containing 8.7 g/kg and flowers containing 2.5 g/kg of that (Rosenblat et al., 2006; Saxena and Vikram, 2004). When fatty acid composition of the seeds were examined; punicic acid, 4-methyl lauric acid, 1,3-dimethyl stearic acid, sterols (stigmasterol, sitosterol), phospholipids (phosphatidyletanolamine, phosphatidylcholine, phosphatidylinositol) along with mono, di- and triglycerides and free fatty acids were detected (Santagati et al., 1984; Sergeeva, 1973). Preparations made up of different sections of *P. granatum* have been applied to cancer therapy (Sharaf, 1966). The fruit extract shows antiviral activity (Schubert et al., 1999), and also antimicrobial effect due to its anthocyans (Tanaka et al., 1986).

Biochemical constituents

Over the past decade, significant progress has been made in establishing the pharmacological mechanisms of pomegranate and the individual constituents responsible for them. Extracts of all parts of the fruit appear to have therapeutic properties (Borir, 1980) and some studies reported that the bark, roots, and leaves of the tree have medicinal benefit as well. Three current research seems to indicate the most therapeutically beneficial pomegranate constituents are ellagic acid ellagitannins (including punicalagins), punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonols and flavones.

ANTİOXİDANT MECHANİSMS

An *in vitro* assay using four separate testing methods demonstrated pomegranate juice and seed extracts have 2 to 3 times the antioxidant capacity of either red wine or green tea. (Tanaka et al., 1986b). Pomegranate extracts have been shown to scavenge free radicals and decrease macrophage oxidative stress and lipid peroxidation in animals (Tanaka et al., 1985) and increase plasma antioxidant capacity in elderly humans (Torres and Fresno, 1970).

Studies in rats and mice confirm the antioxidant properties of a pomegranate by-product (PBP) extract made from whole fruit minus the juice, showing a 19-percent reduction in oxidative stress in Mouse peritoneal macrophages (MPM), a 42% decrease in cellular lipid peroxide content, and a 53% increase in reduced glutathione levels (Tanaka et al., 1985). *In vitro* assay of a fermented pomegranate juice (FPJ) extract and a coldpressed seed oil (CPSO) extract found the antioxidant capacity of both are superior to red wine and similar to green tea extract (Zelepukha et al., 1975). A separate study in rats with CCl4- induced liver damage demonstrated pretreatment with a pomegranate peel extract (PPE) enhanced or maintained the free-radical scavenging activity of the hepatic enzymes catalase, super oxide dismutase, and peroxidase, and resulted in 54% reduction of lipid peroxidation values compared to controls (Tsuyuki et al., 1981). Research in humans has shown a juice made from pomegranate pulp (PPJ) has superior antioxidant capacity to apple juice. Using the FRAP assay (ferric reducing/antioxidant power), Guo et al. (2008) found 250 ml PPJ daily for four weeks given to healthy elderly subjects increased plasma antioxidant capacity from 1 to 1.46 mmol (Tanaka et al., 1986a), while subjects consuming apple juice experienced no signi-ficant increase in antioxidant capacity.

In addition, subjects consuming the PPJ exhibited significantly decreased plasma carbonyl content (a biomarker for oxidant/antioxidant barrier impairment in various inflammatory diseases) compared to subjects taking apple juice. Plasma vitamin E, ascorbic acid, and reduced glutathione values did not differ significantly between groups, leading researchers to conclude pomegranate phenolics may be responsible for the observed results (Torres and Fresno, 1970).

CLINICAL APPLICATIONS

Prostate cancer

Among males in the United States and other Western countries, prostate cancer is the second-leading cause of cancer-related death. *In vitro* studies show several PFEs inhibit prostate cancer cell growth, induce apoptosis of several prostate cancer cell lines (including highly aggressive PC-3 prostate carcinoma cells), suppress invasive potential of PC-3 cells, and decrease proliferation of DU-145 prostate cancer cells (Khodzhaeva et al., 1985; Ulja, 1972; Veres, 1977). Lansky et al. (2007) found that combining equal amounts of FPJ, PPE, and CPSO

extracts resulted in a 99% suppression of DU-145 prostate cancer cell invasion across a Matrigel matrix. CPSO extract or FPJ extract alone resulted in 60% suppression of invasion, and com-bining any two extracts induced 90% suppression. Studies in mice have also demonstrated PFE inhibits prostate tumor growth and decreases prostate specific antigen (PSA) levels (Veres 1977, Wills et al., 1986).

These promising results led some of the same researchers to conduct a two-stage phase II clinical trial in men with recurrent prostate cancer and rising PSA levels. All eligible patients had previous surgery or radiation therapy for prostate cancer, Gleason scores (a grading system for predicting the behavior of prostate cancer) ≤7, rising PSA value of 0.2 to 5.0 ng/ml, no prior hormonal therapy, and no evidence of metastases. Baseline PSA doubling times were established for 22 participants who were then started on eight ounces PJ (570 mg total polyphenol gallic acid equivalents) daily until meeting disease progression endpoints. Endpoints measured were: effect on PSA levels, serum lipid peroxidation and nitric oxide levels, *In vitro* induction of proliferation and apoptosis of LNCaP cells in patient serum containing pomegranate constituents, and overall safety of extract administration (40 based on preliminary results achieved in phase I), 24 additional patients were enrolled and 46 patients were evaluated over 13 months in both stages of the trial. Of these, 35% (n=16) demonstrated decreased PSA levels, the primary trial endpoint–average decrease=27%; median decrease =18%; range 5 to 85%. Four of 46 patients (8.7%) met objective response criteria and exhibited >50% reduction in PSA values, meeting criteria for a phase III trial.

In addition, an average 40% reduction in serum oxidative state was observed in patients accompanied by a significant reduction in serum lipid peroxidation compared to baseline. Nitric oxide serum metabolites measured at nine months after study initiation revealed an average 23% increase, which significantly correlated with baseline PSA levels. An *in vitro* arm of the trial using patient serum investigated whether PJ consumption had any effect on growth rates or apoptosis of LNCaP prostate cancer cells in culture. Serum collected at nine months after study initiation and incubated with LNCaP decreased cell growth by an average of 12% in 84% of patients compared to baseline. An average of 17.5% increase in apoptosis in 75% of patients was also noted. This study indicated that PJ or PJ constituents may have promise as a therapy for prostate cancer, particularly recurrent type with rising PSA levels; phase III studies are currently underway (Yurtaev, 1959).

Hypertension

A small clinical trial demonstrated PJ inhibits serum angiotensin converting enzyme (ACE) and reduces systolic blood pressure in hypertensive patients. Ten hypertensive subjects (ages 62 to 77; seven men and three women) were given 50 ml/ day PJ containing 1.5 mmol total polyphenols for two weeks. Two of seven patients were also diabetic and two were hyperlipidemic. Seven of 10 subjects (70%) experienced a 36% average decrease in serum ACE activity and a small, but significant, five percent decrease in systolic blood pressure (Yurdasheva et al., 1978).

Alzheimer's disease

The neuroprotective properties of pomegranate polyphenols were evaluated in an animal model of Alzheimer's disease. Transgenic mice with Alzheimer's like pathology treated with PJ had 50% less accumulation of soluble amyloid-beta and less hippocampal amyloid deposition than mice consuming sugar water, suggesting PJ may be neuroprotective. Animals also exhibited improved learning of water maze tasks and swam faster than control animals (Zelepukha et al., 1975).

CONCLUSION

An explosion of interest in the numerous therapeutic properties of *P. granatum* over the last decade has led to numerous *in vitro*, animal, and clinical trials. Pomegranate is a potent antioxidant, superior to red wine and equal to or better than green tea. In addition, anticarcinogenic and anti-inflammatory properties suggest its possible use as a therapy or adjunct for prevention and treatment of several types of cancer and cardiovascular disease.

The possibility that pomegranate extracts may also have an effect on several other disease processes, such as Alzheimer's disease, osteoarthritis, neonatal brain injury, male infertility, and obesity, underscores the need for more clinical research.

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