Pomegranate Fruit for Health Promotion: Myths and Realities

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ABSTRACT

The role of pomegranate on folk medicine has been largely established and in recent years a notable increase of scientific support has occurred. However, what is real? Evidence suggests that phenolic phytochemicals of pomegranate fruit, mainly anthocyanins and ellagitannins, could exert multiple therapeutic properties on health management as playing an essential role in oxidative stress balance, preventing important cardiovascular diseases, and fighting as chemoprotective agent against several kinds of cancer. In addition, pomegranate antioxidant bioactives also could possess a role as neuroprotectors in some neurological disorders just as broad antimicrobial activities among other beneficial implications. Regarding promising prospects of pomegranate phenolics, this review summarizes the available scientific information related to health promotion features of pomegranate-derived products and underlines the influence of multiple constituents on the observed biological actions, pointing out pomegranate juice as an interesting source to obtain health benefits.

Keywords: antioxidants, cancer, cardiovascular diseases, neurological disorders, phytochemicals, Punica granatum L.

INTRODUCTION

In recent years, much about potential health benefits of pomegranate has been published, leading to a substantive increase of the popularity as well as consumption of such fruit. This fact has contributed greatly to the development of both pomegranate juice extraction industry and dietary supplements containing pomegranate extracts. But, are pomegranate beneficial properties really supported? Does pomegranate consumption guarantee health promotion?

The pomegranate, Punica granatum L., is a fruit tree already well-known by ancient civilizations lauded because of its medicinal properties for a wide-range of ailments. Pomegranates fruits are usually earmarked for arils fresh consumption, juice, jam, dried arils, and also for developing nutraceutical ingredients as seed oil and polyphenols extracts.

Nowadays consumers are becoming more aware of the diet contribution to their health and, therefore, willing to buy food rich in bioactive compounds (Niwa 2007). In this framework, pomegranate has shown considerable evidence of therapeutic effects (Cravotto et al. 2010) and actually, consumption of pomegranate derivatives with a high content in bioactive phytochemicals is increasing. These potential health benefits of pomegranate products cover a wide array of maladies and include both prevention and treatment.

The aim of this review was to evaluate and summarize the available scientific information related to health promotion properties of pomegranate fruit derivatives focused on its phytochemical composition, underlining those attributed to antioxidant phenolic bioactives.

POMEGRANATE BIOACTIVE COMPOSITION

From a nutritional point of view, edible part of pomegranate, either arils or their juice, contains mainly high sugar content, dietary fiber (including pectins), and variable quantity of organic acids. In addition, pomegranate has been reported as an interesting source of potassium besides that is rich in some essential vitamins as folate and vitamin K (USDA 2010). Nonetheless, the most important added value of pomegranate is its large content in phenolic compounds, which are present in the edible part as well as in the rest of the fruit and, in fact, is precisely in the husk where they are found in higher concentration (Gil et al. 2000). Moreover, polyphenolic bioactives have been reported to be the major antioxidants of pomegranate and, hence, they have been established as main responsible of pomegranate healthy applications (Gil et al. 2000; Sharma and Maity 2010). However, it is a point worth mentioning the increasing role of bioactive seed oil in establishing the pharmacological mechanisms of pomegranate.

Received: 4 January, 2011. Accepted: 18 April, 2011.
Phenolic compounds are plant secondary metabolites commonly found in vegetables and fruits with attributed pharmacological properties (Parr and Bolwell 2000). Among different kind of phenolic compounds, pomegranate contains anthocyanins, ellagic acid and ellagitannins, gallic acid and gallotannins, flavonols, and proanthocyanidins. The class (and specific chemical structure) of each phenolic has shown to contribute significantly to its unique biological (Seeram 2006). Amounts of these compounds vary depending on the fruit part and thereby varying in pomegranate derivatives as industrialization process does it (Gil et al. 2000; Alighourchi et al. 2008).

Anthocyanins are a group of natural pigments responsible for the red-blue colour of many fruits, including pomegranate, that may play a role in the defence mechanisms of plants (De Pascual-Teresa and Sanchez-Ballesta 2008). Likewise, anthocyanins therapeutic properties are wide-ranging and have been attributed to potential human health benefits of berries (Seeram 2008a).

Pomegranate presents an anthocyanins profile characterized by six anthocyanins: cyanidin 3,5-di- and 3-O-glucoside, delphinidin 3,5-di- and 3-O-glucoside, pelargonidin 3,5-di- and 3-O-glucoside (Gil et al. 1995). Nevertheless, it is important to note that both total anthocyanins amount and predominant individual anthocyanins are largely affected by the cultivar group; for instance, ‘Wonderful’ variety is related to cyanidin 3,5-diglucoside instead of cyanidin 3-glucoside, the main anthocyanin for Spanish ‘Mollar de Elche’ cultivars (Gil et al. 2000; Pérez-Vicente et al. 2004).

Ellagitannins are extensively found in pomegranate husk, mainly ellagitannin punicalagin among others (Gil et al. 2000). Punicalagin isomers are ellagitannins in which gallic acid and ellagic acid are linked to a glucose molecule [2,3-(S)-hexahydroxydiphenoyl-4,6-(S,S)-gallagyl-D-glucose] and are extracted into juice during processing. Thus, the extraction process determines the amounts achievable in juice, displaying important differences between whole-fruit juices and arils-made ones (Gil et al. 2000).

These compounds, when exposed to pH variations, are hydrolyzed and the hexahydroxydiphenic acid spontaneously rearranges into the water-insoluble ellagic acid. Likewise, this hydrolysis also renders punicalin, a gallagyl residue bounded to glucose (Clifford and Scalbert 2000). Punicalin is other bioactive compound of pomegranate that has generated interest with regard to human health (Kasimsetty et al. 2010).

Pomegranate ellagitannins have been reported to possess several biological properties. In fact, they are considered as the major antioxidant in vitro of pomegranate juice and their put microbiota metabolites, urolithins, have displayed a broad array of chemopreventive properties (Clifford and Scalbert 2000; González-Sarrías et al. 2009b; Larrosa et al. 2010a). On the other hand, pomegranate also contains other several hydrolysable tannins found in lesser quantities that also possess potential therapeutic applications, like gallic acid, galloyldigalactone, gallic acid, and pedunculagin (Kasimsetty et al. 2010).

In pomegranate fruit other phenolic phytochemicals have been described. Catequin and galloocatechin have been identified as the major flavan-3-ol among others (De Pascual-Teresa et al. 2000). Likewise, prodelphinidins, a kind of condensed tannins derived from the polymerization of galloocatechin, have also been found in pomegranate peel (Plumb et al. 2002). In addition, recently 35 flavanols-anthocyanin adducts have been detected in pressure extracted pomegranate juice (Sentandreu et al. 2010).

Regarding potent antioxidant quercetin, it has also been recorded next to other flavonols, although quantities were not significant (Artik 1998).

POMEGRANATE AND HEALTH IMPLICATIONS

Pomegranate-derived products have displayed extensive pharmacological actions on the treatment and prevention of several ailments due to the potential therapeutic properties of their bioactive constituents.

Although different parts of pomegranate tree as bark, flowers, leaves, and roots have exhibited medicinal properties (Aviram et al. 2008), the present review focuses on the therapeutic benefits of pomegranate juice and fruit extract since they are the most widespread pomegranate products along with its consume as fresh fruit.

Pomegranate and oxidative stress

An unregulated production of reactive oxygen species (ROS), highly oxidant molecules intrinsic from normal metabolism, causes oxidative stress, which is characterized by oxidation of lipids, proteins and nucleic acids. Damage to biomolecules impairs their functions and leads to a dysregulation of cellular mechanisms (Valko et al. 2007). Thus, it is important to keep the oxidative balance to avoid health alterations and it is in this point where pomegranate phytochemicals may act enhancing the effectiveness of the anti-oxidant defence system.

Pomegranate polyphenols have been suggested like antioxidant compounds and it has been confirmed on biological system. Research in animals and humans has demonstrated that pomegranate antioxidant capacity largely depends on the fruit part and thereby varies depending on the processing extraction method (Faria et al. 2007; Guo et al. 2008). The consumption, both fresh fruit and ellagitannins-rich extract, have displayed to raise the antioxidant capacity in plasma of healthy volunteers after a ten days-prolonged intake and an acute dose, respectively (Mertens-Talcott et al. 2006; Hajimahmoodi et al. 2009). Even if these results were assured by two different methods (FRAP and ORAC) and carried out with a thorough experimental design, we should take care of them since they are showing only an unspecified oxidative stress biomarker. Indeed, in order to determine oxidative stress, studying various specific biomarkers seems more logical as they will provide more solid details about the real status. In this way, there are some trials where pomegranate juice consumption increased serum antioxidant status as well as it modified other specific antioxidant biomarkers (Aviram et al. 2004; Rosenblat et al. 2010a); therefore, supporting the former works with direct measures of antioxidant biomarker. Regardless of being an indirect approach, antioxidant capacity measurements are useful as potential bioactivity indicators of pomegranate and other polyphenolic-rich products. Moreover, in vitro antioxidant capacity measurements are correlated with antioxidant effects in vivo (Selvendiran et al. 2010a), at the same time that is interesting to compare pomegranate with other fruits and vegetables.

On the other hand, an improved antioxidant function was shown in a research conducted in elderly population that consumed pomegranate juice daily for a month. Regarding oxidative status, aging process is characterized by an increase in oxidative damage levels; nonetheless, oxidative damage linked to proteins and lipids after the continuous intake of pomegranate juice by elderly subjects was reduced, whereas plasma glutathione peroxidase and catalase antioxidant enzymes were significantly increased (Guo et al. 2008). These anti-aging effects were also appreciated in aged rats, where a protection against blood mononuclear cell DNA damage was observed too (Xu et al. 2005).

The protective effect of pomegranate against hepatic oxidative stress after prolonged pomegranate derivatives ingestion has also been pointed out in two different animal models (Chidambaram Murthy et al. 2002; Faria et al. 2007). Hepatoprotection was assured in a normal oxidative stress models (Chidambaram Murthy et al. 2002; Mertens-Talcott et al. 2006; Hajimahmoodi et al. 2009). Even if these results were assured by two different methods (FRAP and ORAC) and carried out with a thorough experimental design, we should take care of them since they are showing only an unspecified oxidative stress biomarker. Indeed, in order to determine oxidative stress, studying various specific biomarkers seems more logical as they will provide more solid details about the real status. In this way, there are some trials where pomegranate juice consumption increased serum antioxidant status as well as it modified other specific antioxidant biomarkers (Aviram et al. 2004; Rosenblat et al. 2010a); therefore, supporting the former works with direct measures of antioxidant biomarker. Regardless of being an indirect approach, antioxidant capacity measurements are useful as potential bioactivity indicators of pomegranate and other polyphenolic-rich products. Moreover, in vitro antioxidant capacity measurements are correlated with antioxidant effects in vivo (Selvendiran et al. 2010a), at the same time that is interesting to compare pomegranate with other fruits and vegetables.

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levels of antioxidant enzymes (Chidambara Murthy et al. 2002). On the other hand, Faria et al. (2007), feeding high-fat diet pomegranate extract to rats for a prolonged time, associated a decrease of carbonyl groups and 8-hydroxy-2’-deoxyguanosine (8-OHdG), cellular damage biomarkers of proteins and DNA, respectively, with a protective effect against oxidative stress. At the same time, a reduction of antioxidant enzymes as SOD, catalase, and glutathione peroxidase and of glutathione-S-transferase and glutathione synthetase transcription was observed. Initially, these findings could be controversial as several works have indicated how dietary antioxidants intake may act by raising the levels of antioxidant enzymes (Chidambara Murthy et al. 2002). However, these authors considered diminishing endogenous defences is a way of metabolic saving, since antioxidant enzymes are no longer required when a general decline in oxidative stress happens. Thus, it seems that both studies assessed the hepatoprotective effects of pomegranate against oxidative injury, even though the opposite behaviour of oxidative biomarkers as they are different metabolic situations.

A point worth mentioning is how pomegranate antioxidant compounds really act against oxidative stress. The protective effects of pomegranate bioactive compounds on oxidative stress have been traditionally attributed to their ability as free radicals quenchers, diminishing the levels of ROS and, hence, lipid peroxidation and protein damage (Chidambara Murthy et al. 2002). Antioxidant properties of phenolic compounds are usually attributed to the capacity of electron or hydrogen donation from the hydroxyl moieties to free radicals such as superoxide anion, hydrogen peroxide, etc. (Rice-Evans et al. 1996). Nevertheless, pomegranate health features should not be related to an antioxidant activity of polyphenols per se, as free radicals scavengers, rather than the role of polyphenols metabolites in vivo, as signalling molecules able to exert modulatory actions in cell pathways. First of all, original pomegranate phytochemicals are usually absorbed and metabolized in other bioavailable compounds that can vary their biological properties, even losing their free-radical scavenging activity, as it occurs to the main ellagitannins metabolites registered in plasma, urolithins (Cérda et al. 2004). However, this lack of direct antioxidant activity does not prevent these metabolites from exerting beneficial effects. Concerning the main ellagitannins metabolites, and not those phenolics present originally in fruits, seem to be the real responsible compounds for the protective effects linked to pomegranate phytochemicals consumption, and not necessarily due to a radical scavenging ability. As it has been observed, pomegranate may act by up-regulating the expression of genes encoding antioxidant enzymes and, therefore, raising the levels of proteins related to the endogenous defences (Rosenblat et al. 2006b; De Nigris et al. 2007a; Guo et al. 2008; Rosenblat et al. 2010b). Indeed, it is possible that the modulation of the transcription and expression of defensive enzymes occurs because of the interaction between dietary antioxidant and antioxidant response elements in gene promoter regions of genes encoding these enzymes, as it has been reported for other polyphenols (Myhrstad et al. 2002). Despite of this, underlying mechanisms of how pomegranate polyphenols act against oxidative injury are still unclear.

### Pomegranate and Cardiovascular Diseases

Cardiovascular diseases (CVD) continue leading the ranking among causes of morbidity and mortality in developed countries (WHO 2008). Oxidative stress is the major contributor to CVD, and inflammation its main manifestation (Levonen et al. 2008). Reduction on the prevalence of CVD by fruit and vegetable consumption has been well established in several epidemiological studies (Banerjee and Maulik 2002; Bazzano 2006). In the same way, pomegranate intake has shown a high potential in the management of CVD.

The effects of pomegranate-derived products on prevention and attenuation of atherosclerosis have been largely tested, showing multiple anti-atherogenic effects (Aviram et al. 2004; Bazzano 2006). De Nigris et al. (2007b) and Aviram et al. (2008) showed that pomegranate has a certain role in atherosclerotic injury. Atherosclerosis is one of the CVD with a major incidence, and involves inflammatory and oxidative processes that entail to endothelial dysfunction by affecting nitric oxide (NO) bioavailability among others (Lavi et al. 2008). Consequently, both oxidative stress and inflammation have been suggested like the main targets of atherosclerosis treatment by dietary phytochemicals, as they are potent antioxidant juices like grape or blueberry ones. On the other hand, the well-established anti-proliferative effect of pomegranate juice on vascular smooth muscle cell growth was ascertained by NO-dependent mechanisms both protecting NO against oxidative destruction and enhancing its biological actions (Ignarro et al. 2002). A study carried out with hypercholesterolemic mice, fed with high-fat diet, also exhibited the protective effects on atherosclerosis of a prolonged (6-months) pomegranate derivatives supplementation (De Nigris et al. 2007b). Both a regular pomegranate juice and an ellagitannins-rich pomegranate fruit extract were tested, without notable differences between their performances. They both elicited therapeutic beneficial effects both protecting NO against oxidative destruction and enhancing its biological actions, in vivo and in vitro.

The role of oxidative stress in atherosclerosis is also mediated by macrophage and LDL oxidation since an impaired oxidative metabolism entails lipid peroxidation of macrophages and LDL, increasing the levels of oxidized low-density lipoproteins (Ox-LDL) as well as the capacity of macrophages for engulfing this Ox-LDL. The Ox-LDL uptake raising leads to the formation of lipid-laden foam cells, with the subsequent development of plaques and atherosclerotic lesions (Fuhrman et al. 1997; Aviram and Rosenblat 2004). Significant reductions in atherosclerotic
lesion size were noted upon consumption of pomegranate by-products for 3 months by apolipoprotein E-deficient mice (which develop atherosclerotic injury similar to that displayed in humans). The cardioprotective effects of pomegranate by-products on atherosclerotic lesion were attributed to macrophage oxidative stress attenuation with a decrease in the extent of Ox-LDL uptake by macrophages at their lipids were oxidized in a lesser degree (Rosenblat et al. 2010b). On the contrary, these healthy products (Rouanet et al. 2010). In the same way, anti-atherosclerotic benefits of pomegranate should be due to the concerted action of a combination of phytochemicals and other pomegranate nutrients, rather than the sole effects of a unique compound. Moreover, both anthocyanins and ellagitannins, main pomegranate phenolics, have demonstrated possessing large cardiovascular health-promoting effects as it has been previously reviewed (de Pascual-Teresa et al. 2010; Larrosa et al. 2010a). The superior anti-proliferative and anti-atherogenic activity of pomegranate juice above pomegranate extracts and purified phenolics have already been reported (Seeram et al. 2005; Aviram et al. 2008). Likewise, phenolics-complexed sugars of pomegranate also ameliorated the atherosclerotic injury, just as a dietary fiber-rich extract of pomegranate flowers, that even led the highest fall in atherosclerotic lesion area when it was compared to polyphenols-rich pomegranate extracts (Aviram et al. 2008). Therefore, these data seems to indicate a synergistic or additive effect of pomegranate compounds, not only polyphenols but also complexed sugars and dietary fiber, on anti-atherogenic activity of pomegranate.

On the other hand, pomegranate phenolics do not seem to modify serum cholesterol (both LDL- and HDL-cholesterol) levels, as well as other serum biochemical parameters (glucose, triacylglycerol, sodium, and potassium), in in vivo trials (Aviram et al. 2004; De Nigris et al. 2005; Sumner et al. 2005; De Nigris et al. 2007a; Aviram et al. 2008; Rosenblat et al. 2010a), in accordance with other polyphenolic-rich beverages (Rosenblat et al. 2010a; Rouanet et al. 2010), except for studies performed either in hyperlipidemic or hypercholesterolemic patients or animals, where LDL-cholesterol values were lowered (Esmailzadeh et al. 2004; Huang et al. 2005; Bagri et al. 2009).

Cardiovascular health benefits of pomegranate have not been limited to atherosclerosis. Pomegranate juice has also shown cardioprotective effects on coronary diseases (Sumner et al. 2005; Jadedia et al. 2010). Daily consumption of pomegranate juice for 3 months by ischemic coronary heart disease (CHD) patients may decrease myocardial ischemia (Sumner et al. 2005). Likewise, rats with ischemic-reperfusion-induced cardiac necrosis supplemented with pomegranate juice for 1 month showed a lesser infarct size and lipid peroxidation as well as a protective effect on endogenous antioxidant defense, when compared to non-supplemented control; these effects were attributed to the potential of pomegranate juice phytochemicals, for overcoming isoproterenol-induced oxidative stress and related biochemical and structural distortions (Jadedia et al. 2010).

Concerning this vast number of research, there is enough in vivo evidence about the protective effects on CVD of pomegranate derivatives, especially pomegranate juices, and this should be taken into account for clinical purposes.

Pomegranate and cancer

Treatment options for advanced stage cancers have several limitations in countering the pathology and remain inadequate; thus, formulating effective strategies for the prevention of cancer is part from the increasing efforts to reduce cancer burden. One of these preventive strategies is through the diet, increasing the consumption of foods rich in chemoprotective compounds. Pomegranate, due to its phytochemical composition, has demonstrated to possess potential effects on multiple cancer such as colon, prostate, and breast, using cell lines and animal models assays.
Colon cancer represents one of the most frequent cancers in high-income countries and also constitutes a leading cause of cancer death (WHO 2008). Epidemiological evidence indicates that a diet rich in phytochemicals from fruit and vegetables sources reduces the risk of colon cancer (Riboli and Norat 2003), that allows to food-related bioactivities come in direct contact with cancerous cells. Hence, antioxidant pomegranate juice and its constituents have been widely studied for their antiproliferative and pro-apoptotic activities in human cell cultures of colon cancer since they could exert their chemoprotective properties on the colon epithelium through a direct contact. Ellagitannin-derived compounds (punicalagins and ellagic acid) showed antiproliferative activity against all colon tumour cell lines tested (HT-29, HCT116, SW480, SW620), but in a lesser degree than pomegranate juice, which displayed the most prominent effect (Seeram et al. 2005). The superior bioactivity of pomegranate juice compared to its purified polyphenols was attributed to synergies in the way of action of multiple compounds presented in pomegranate juice. Nevertheless, nowadays the additive effects of other ellagitannins-related constituents contained in juice should be taken into account as they have also displayed antiproliferative effects even superior to those shown by ellagic acid and punicalagins (Kasimsetty et al. 2010). Likewise, pomegranate juice and its purified ellagic acid-related polyphenols induced apoptosis in colon cancer HT-29 cells, whereas ellagic acid and punicalagin, but not pomegranate juice, also induced apoptosis in HCT116 (Seeram et al. 2005). Similarly, ellagic acid and punicalagin (as ellagic acid precursor), induced apoptosis in human colon adenocarcinoma Caco-2 cells but interestingly not in normal colon CCD-112CoN cells (Larrosa et al. 2006b). Caco-2 cells underwent apoptosis via mitochondrial pathway as consequence of cytochrome c release into cytosol, down-regulation of the bcl-XL protein expression (antiapoptotic protein) and caspases 9 and 3 activation. Cell cycle analysis revealed both punicalagin and ellagic acid provoked down-regulation of cyclins A and B1 and up-regulation of cyclin E, which led to cell-cycle arrest in S phase. Recently, ellagic acid and its in vivo colonic metabolites, urolithin-A and -B, have been studied in Caco-2 cells by this same research group as an attempt to link gene expression and functional analysis results with the antiproliferative response of the cells exposed to dietary polyphenols and their colonic metabolites (González-Sarrías et al. 2009b). Novel gene expression profiles and deregulation of cellular functions related to cell cycle and proliferation have been identified, suggesting that both ellagic acid and urolithins-A and -B may exert a modulating role in the progression of colorectal cancer at achievable concentrations in the intestinal lumen. At the same time, it has been shown that both, ellagic acid and urolithins-A and -B, modulate phase I and phase II detoxifying enzymes in Caco-2 cells, which may play an important role in the chemoprotective action of pomegranate phenolics against colon cancer (González-Sarrías et al. 2009a). However, critical effects of the matrix in which these compounds are dissolved have been noted: food matrix interferences result even in neutralizing the likely anti-carcinogenic effects of phase I and phase II detoxifying enzymes.

On the other hand, ellagitannins-derived colonic metabolites have also shown to inhibit Wnt signalling pathways (Sharma et al. 2010), which play a pivotal role in 90% of colon cancer (Klaus and Birchmeier 2008). Some studies have observed that pomegranate ellagitannins reduce the proliferation of colon cancer in vivo by arresting the cell cycle and inducing apoptosis, as well as modulating xenobiotic metabolism and Wnt signalling, which is in accordance with the multitargeted role of ellagitannins in carcinogenesis (Heber 2008).

Prostate cancer is currently one of the most common malignancies and a leading cause of cancer-related mortality among men (WHO 2008). The implications of dietary patterns in prostate cancer development have been well-defined and, hence, chemoprevention through nutritional agents has been recognized as a plausible approach directed to prevent or delay the initiation of the disease (Syed et al. 2007). Thus, the former considerations, in accordance with the occurrence of urolithins in the human prostate gland upon consumption of pomegranate juice (González-Sarrías et al. 2010), have positioned pomegranate as an ideal chemopreventive agent against prostate carcinoma in humans.

Antiproliferative and pro-apoptotic activities of pomegranate-derived compounds have been evaluated in vitro in various cell lines of human prostate cancer. Treatment of highly aggressive cancer PC3 cells with pomegranate fruit extract caused inhibition of cell growth and induction of apoptosis (Malik et al. 2005). Indeed, antiproliferative properties of pomegranate ellagitannins metabolites ellagic acid and urolithins on androgen-dependent (LNCaP) and -independent prostate carcinoma cell lines (LNCaP-AR, DU145, and 22Rv1) have also been confirmed (Seeram et al. 2007). In addition, the antiproliferative effect resulted in a dose-dependent manner in all cell lines tested. It is also a point worth mentioning the fact that a combination of both different compounds and discrete fractions of pomegranate fruit have reflected possible synergies against cell proliferation (Mansky et al. 2005).

Likewise, the effects of pomegranate extract on prostate adenocarcinoma have also been assessed in severe combined immunodeficient mice injected subcutaneously with human cancerous cells 22Rv1 and it was observed how pomegranate extract inhibited xenograft growth with concomitant reduction in secretion of prostate-specific antigen (PSA) in the serum (Malik et al. 2005). In the same way, a phase II clinical triad in men with recurrent prostate cancer and rising PSA levels was conducted (Pantuck et al. 2006). Patients were supplemented with 8 ounces (~240 mL) of pomegranate juice daily until disease progression and results showed a significant prolongation of PSA doubling time. Consequently, prospects regarding chemopreventive properties of pomegranate juice and its constituents on prostate adenocarcinoma were substantially raised.

On the other hand, underlying mechanisms of pomegranate chemoprotection on prostate carcinoma have not been clarified yet. So far, some attempts have been carried out and both angiogenesis inhibition and drug metabolism modulation via cytochrome P450 1 just as the involvement of cell cycle regulation-mediated apoptosis as mechanism cell growth inhibition have been proposed (Malik et al. 2005; Saritpouri et al. 2008; Kasimsetty et al. 2009). In fact, some of these approaches as cell cycle arrest may be behind chemopreventive properties of pomegranate juice in colon cancer. Nevertheless, these in vitro results pointing out the role of pomegranate in the suppression of cell growth by modulating proliferation markers have not been confirmed in a recent report in humans (González-Sarrías et al. 2010), probably due to that both concentrations and time of exposure in cell assays were unrepresentative of the human normal physiological situation. Moreover, this trial has exposed the very high inter-individual existing variablity with regard to the occurrence of ellagitannins-derived metabolites in prostate gland. Thus, despite preliminary promising cancer chemoprevention through pomegranate juice and/or its derived compounds consumption, more human clinical trials focused on ability of subjects as urolithins-producers or -non producers should be performed to achieve a deep body of evidence in order to confirm the chemopreventive potential of pomegranate bioactives on prostate cancer.

As in the aforementioned cancer types, mostly of studies focused on anti-carcinogenic properties of pomegranate and its constituents on breast cancer have been performed in vitro, with the subsequent lacks in their extrapolation to humans. In spite of this, chemoprevention through pomegranate could be considered in the management of breast cancer, a cancer with a highest prevalence in deve-
lated countries. Several constituents of pomegranate as juice, fermented juice, and seed oil have shown antiproliferative activity against both estrogen-dependent (MCF-7) and -independent (MB-MDA-231) cancerous cells (Kim et al. 2002). In fact, special interest has been focused on pomegranate seed oil as antiproliferative agent owing to its high concentration in punicic acid. However, regardless single purified compounds efficacy, combination of phytochemicals has shown significant higher growth inhibition (Jeune et al. 2005).

Proposal mechanisms for suggested breast carcinoma chemoprotection of pomegranate may happen by arresting cell cycle progression, regulating gene expression of proliferation markers, and provoking apoptosis (Jeune et al. 2005; Khan et al. 2009; Dai et al. 2010). In addition, action on estrogen-responsive breast cancers seems more feasible since punicic acid and urolithins have exhibited estrogenic and antiestrogenic activities and have been termed selective estrogen receptor modulators (SERMs) in vitro (Larrosa et al. 2006a; Tran et al. 2010), which are under intense research for their potential to treat estrogen-related conditions such as osteoporosis and menopause symptoms.

Pomegranate-derived products have also displayed anticarcinogenic properties against lung and skin cancers just as osteoporosis and menopause symptoms. Several constituents of pomegranate as single purified compounds efficacy, combination of phytochemicals has shown significant higher growth inhibition (Jeune et al. 2005; Khan et al. 2009; Dai et al. 2010). In addition, action on estrogen-responsive breast cancers seems more feasible since punicic acid and urolithins have exhibited estrogenic and antiestrogenic activities and have been termed selective estrogen receptor modulators (SERMs) in vitro (Larrosa et al. 2006a; Tran et al. 2010), which are under intense research for their potential to treat estrogen-related conditions such as osteoporosis and menopause symptoms.

Pomegranate and neurological disorders

Unlike many other pathologies, protective effects of pomegranate on neurological disorders have been scarcely studied. There are only a few works reporting the incidence of pomegranate derived products on nervous system and they have not been carried out in humans, with the subsequent lack of information. Nonetheless, a few studies have been performed on animal models suggesting the promising potential of pomegranate bioactives in the prevention and treatment of some neurological disorders.

Like that, neuroprotective effects of pomegranate phytochemicals on Alzheimer’s disease have been tested (Hartman et al. 2006). Transgenic mice with an Alzheimer’s disease-like pathology were supplied with pomegranate juice during their old age delaying the onset of cognitive impairment and enhancing the learning, as well as reducing significantly the accumulation of soluble amyloid-β and amyloid deposits in the hippocampus in several animal models and to contribute greatly to berry fruits attributed chemoprevention (Seeram 2008b). Therefore, anthocyanins as well as other pomegranate polyphenols should be taken into consideration in an attempt to identify the real impact of pomegranate on cancer prevention.

In the aforementioned report, focused on hypoxic-ischemic brain injury (Loren et al. 2005), pomegranate neuroprotective effects were observed in neonates whereas supplementation was effectuated in mothers, thereby suggesting that phenolic responsible compounds (West et al. 2007), or their metabolic effects, are effective at least one week after the last intake and they are able to cross tight cell barriers, as BBB and placental one. Anthocyanins and ellagitannins derivatives, mainly pomegranate phenolic bioactives, have displayed differences with regard to their brain bioavailability: while a growing number of studies has detected anthocyanins in several brain regions (Andres-Lacueva et al. 2005; Kalt et al. 2008). No ellagic acid or its in vivo metabolites have been found, at least to our knowledge, in these areas.

Recently, anthocyanins as well as their in vivo colonic metabolites have also shown to reach the brain and to be accumulated in these tissues beyond the BBB, where they confer protection (Kalt et al. 2008; Del Bò et al. 2010). Dietary interventions have demonstrated that consumption of anthocyanins-rich both foods or extracts may exert beneficial effects in neurological functions by retarding age-related declines (Joseph et al. 1999), preventing behavioural deficits (enhancing memory) in Alzheimer’s disease (Joseph et al. 2003), and improving spatial learning (Andres-Lacueva et al. 2005), at the same time that these novel neuro-
protective agents may ameliorate ethanol-induced damage to CNS (Chen and Luo 2010). Likewise, anthocyanins supplementation has shown diminishing oxidative modifications of proteins and lipids in the brain, as well as dopamine neurotransmitter abnormalities caused by emotional stress (Rahman et al. 2008). Moreover, equal to pomegranate juice, dietary supplementation with foods rich in anthocyanins provides neuroprotection in animal models of ischemia (Shin et al. 2006). Thus, anthocyanins may exert protective effects against oxidative damage implicated in some neurodegenerative disorders and it is possible to be the main responsible of neuroprotective prospects of pomegranate.

On the other hand, ellagitannins or their in vivo metabolites have not been detected in brain regions (Espin et al. 2007), which may indicate that these compounds are not able to cross the BBB. Therefore, their effects on CNS and neurological diseases would be restricted. Nevertheless, evidence indicates that ellagic acid may modulate cerebral activity in rodent models (Carlsen et al. 2003; Hassoun et al. 2004). These results prompt us to consider that either an unsuitable determination of ellagitannins metabolites had been performed (owing to assay protocols or detection levels) or systemic effects of these compounds affecting SNC take place.

Furthermore, pomegranate also contains flavonols that have shown to protect against brain lipid peroxidation and to exert many protective functions in brain tissues (Spencer 2010).

But, despite of the vast quantity of polyphenols presents in pomegranate and their neuroprotective effects, neurological benefits of pomegranate intake could also be associated to other minor compounds such as saponins, sugars, and even seed oil (Tripathi et al. 2004; Kumar et al. 2008).

According to the WHO, neurological disorders are a leading cause of morbidity and mortality as well as due to the extension of life expectancy and the prolonged ageing of populations globally is estimated to increase the prevalence of these disorders; for example, it is forecast that the number of people affected by dementia (already counted in tens of millions) will double every 20 years. Consequently, the WHO is trying to establish mental health promotion, focused on disease prevention as the first step (WHO 2006). Because of this, considering the protective role of many rich-polyphenols fruits on brain health (Spencer 2010), and the phenolic profile of pomegranate, it seems logical to think in pomegranate and its bioactives, mainly anthocyanins, as promising agents in the fight against neurological disorders.

**Pomegranate and antimicrobial effects**

Pomegranate has been employed in folk medicine for the treatment of various microbial infections and, in fact, the potential antimicrobial properties of pomegranate are recently being studied with promising results.

Pomegranate extracts have displayed antagonist effects against all type of microorganisms causing urinary tract infections (Gopalakrishnan and Benny 2009). Methanolic extract has shown broad-spectrum activity against 159 multi-drugs resistant bacterial strains isolated from urine of patients belonging to different age and sex who had urinary infection (Gopalakrishnan and Benny 2009). In vitro antifungal activity of punicalagins against Candida albicans and Candida parapsilosis has also been reported. Moreover, assays were performed with Punica granatum rind to achieve the concentration of punicalagins, showing a powerful synergistic interaction with commonly used as antifungal fluconazole (Endo et al. 2010). In addition, effectiveness of pomegranate juice against Trichomonas vaginalis has also been guarantee in vivo in a cohort of 20 women as well as in vitro using metronidazole refractory strains (El-Sherbini et al. 2010). Consequently, pomegranate-derived products represent an attractive prospect for the development of new management therapies for treatment of multi-drug resistant urinary tract infections.

On the other hand, pomegranate sun-dried rind is employed in some regions of India as an anti-malarial herbal preparation. The role of tannins-rich pomegranate rind methanolic extract on the treatment of cerebral malaria, a complication of the infection by Plasmodium falciparum, has been studied. Positive results were attributed to the antimalarial activity and the inhibition of pro-inflammatory mechanisms involved in the onset of malaria (Dell’Agli et al. 2010b).

Concerning responsible compound(s) of pomegranate antimicrobial properties, authors seem to be agree with associating them to ellagitannins content of pomegranate derivatives, mainly to punicalagin (Dell’Agli et al. 2010; Endo et al. 2010).

**Pomegranate and other diseases**

The most significant or, at least, more researched pomegranate fruit therapeutic properties have been aforementioned, nevertheless, there are some other applications which have offered satisfactory results.

Consumption of pomegranate derivatives has been related to possess anti-inflammatory activity and has been tested in various animal models. Pomegranate polyphenols were strongly delayed the initiation, reduced the morbidity, and lowered the severity of collagen-induced arthritis in mice (Shukla et al. 2008). Likewise, another ellagitannins-rich pomegranate extract has recently shown to decrease oxidative stress in an inflammatory bowel model of rat, although it cannot avoid colonic damage instead of urolithins-A, which reduced significantly colonic lesions. These differences regarding ellagitannins and their gut microbiota metabolites actions could be due to the inability for urolithins formation of colon-damaged rats (Larroso et al. 2010b). All these properties have been linked to the anti-inflammatory activity of pomegranate phenolic and their gut microbiota metabolites, specifically urolithins-A, since they decreased inflammation markers as well as caused the down-regulation of inflammatory response pathway (Shukla et al. 2008; Larrosa et al. 2010b).

Finally, pomegranate derivatives also present considerable prospects as cosmeceuticals because of their protective effects on UVB-induced damage. Two different trials have been carried out with similar results, pointing out the inhibition of increase and activity of matrix metalloproteinases in both human reconstituted skin and human skin fibroblasts (Afaq et al. 2009; Park et al. 2010). Pomegranate photoprotection could be related to ellagic acid among others, as it has been displayed to alleviate UVB-induced skin wrinkles and inflammation (Bai et al. 2010). Thus, photoprotective effects of pomegranate on UVB-mediated skin damage could delay (or even prevent) phototoaging.

**CONCLUSIONS**

A plethora of pharmacological and therapeutic features have been associated to pomegranate fruit. Pomegranate fruit derivatives contain a very different range of phenolics among other bioactive phytochemicals that may be implicated in protective effects, being possible that these health promotion properties are generated from the influence of multiple constituents working in a concerted action more than the observed biological actions of a single compound. Hence, it seem logical to take into consideration the consumption of a complete and balanced pomegranate-derived products instead of a occlusive supplement rich in a specific group of phytochemicals. Therefore, pomegranate juice is proposed as an interesting beverage, in order to achieve the chemopreventive effects attributed to pomegranate fruit.

On the other hand, research has mainly been focused on the role of ellagitannins as responsible of potential applications of pomegranate without almost regarding the prospects of anthocyanins, a kind of phenolics that have displayed a wide array of therapeutic benefits when contained in many other fruits. Likewise, evidence indicates that des-
pite significant ellagitannins-derived metabolites health promotion, the existence of both ulothrins producers and non-producers could limit severely the contribution of pomegranate fruit or its extracts to those ellagitannins in vivo metabolites non-producers subjects.

Consequently, more trials with clinical perspectives should be performed to assess the real potential of pomegranate fruit and its antioxidant agents. For that, gut microbiota influence on phenolic metabolites formation should be remarked when studying the action mechanisms of the promoting chemoprevention of pomegranate. In addition, future pomegranate interventions should be conducted taking into account a nutrigenomic approach to establish a more integral evaluation of pharmacological actions of pomegranate fruit.

LATEST DEVELOPMENTS

Research on pomegranate fruit health implications is continuously growing and focused on the elucidation of underlying mechanisms of possible pomegranate bioactivity features. In fact, one of the most promising areas in pomegranate disease prevention, their antiatherogenic effects, is still being widely assessed (Haber et al. 2011). Likewise, studies to determine the impact of pomegranate bioactives on different kinds of cancer have been also performed. In this aspect, a pomegranate extract was tested in human pancreatic cancer cells and those mechanisms involved in colon and prostate cancer prevention, inhibition of cell proliferation and cell cycle arrest, were showed to account for the anticarcinogenic potential of pomegranate on pancreatic cancer (Nair et al. 2011). Moreover, significant results have been provided on chemoprevention of hepatocarcinogenesis by attenuating oxidation (Bishayee et al. 2011). In addition, a reduction of serum oxidative status in patients with active rheumatoid arthritis reduced clinical symptoms of these patients (Balbir-Gurman et al. 2011). On the other hand, pomegranate juice consumption in patients with obesity did not alter insulin secretion although it halted weight increase (González-Ortiz et al. 2012). Furthermore, prospects of pomegranate consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. A pilot study. Israel Medical Association Journal 13, 474-479


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