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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.

CONTENTS

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 (Niva 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, phenolic 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.

Invited Review

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, flavanols, and proanthocyanidins. The class (and specific chemical structure) of each phenolic has shown to contribute significantly to its unique biological properties (Seeram 2008a). 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 gallagic and ellagic acids 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 gut 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, gallagyldilactone, gallagic acid, and pedunculagin (Kasimsetty *et al.* 2010).

In pomegranate fruit other phenolic phytochemicals have been described. Catequin and gallocatechin 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 gallocatechin, have also been found in pomegranate peel (Plumb *et al.* 2002). In addition, recently 35 flavanol-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 antioxidant 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 pomegranate derivatives increase plasma antioxidant capacity and decrease oxidative stress, as lipid peroxidation as formation of protein carbonyl (Chidambara Murthy *et al.* 2002; Mertens-Talcott *et al.* 2006; 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 to antioxidant effects *in vivo* (Rosenblat *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 conduced 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 (Chidambara Murthy *et al.* 2002; Faria *et al.* 2007). Hepatoprotection was assured in a normal oxidative stress status as well as in a CCl4-induced oxidative stress acute episode. One of these experiences was performed inducing severe oxidative injury in liver by a single-dose of CCl4 after feeding rats with a pomegranate peel methanolic extract for two weeks. Catalase, peroxidase, and superoxide dismutase (SOD) values were comparable with control

values in contrast to those rats without pomegranate extract pretreatment, where ROS-combating enzymes were not preserved, being significantly decreased. Similarly, lipid peroxidation was lower in pretreated with pomegranate extract rats than in non-pretreated ones. In addition, the hepatoprotective effects of pomegranate extract pretreatment were verified by histopathological methods (Chidambara Murthy *et al.* 2002). On the other hand, Faria *et al.* (2007), feeding healthy mice with pomegranate juice, for a prolonged time, associated a decrease of carbonyl groups and 8-hydroxy-2´-deoxiguanosine (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 antioxidants 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 (Cerdá *et al.* 2004). However, this lack of direct antioxidant activity does not prevent these metabolites from exerting health benefits. Concerning this, *in vivo* 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; Rosenblat *et al.* 2006b; De Nigris *et al.* 2007a; De Nigris *et al.* 2007b; Aviram *et al.* 2008). 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 (Basu and Penugonda 2009). Thus, it is hardly surprising that most of the trials are about elucidating the role of pomegranate in atherosclerotic injury, focused on the endothelial function and the NO biochemistry as these are implicated in this pathology.

Pomegranate phenolics have demonstrated their ability to protect blood vessels of inflammation. Both pomegranate juice and pomegranate fruit extract have demonstrated to be able to decrease vascular inflammation markers, thrombospondin-1 (TSP-1) and cytokine transforming growth factor-β1 (TGFβ1). Moreover, endothelial NO synthase (eNOS) expression as well as NO levels were increased in both supplements regimens (De Nigris *et al.* 2007a). The up-regulation of eNOS and NO augment can stop the pernicious influence of oxidative damage on endothelial tissue, thereby decreasing endothelial dysfunction (Vaskonen *et al.* 2002; Napoli *et al.* 2006). These results were achieved in obese Zucker rats fed with an atherogenic diet, a model of metabolic syndrome, thus, showing the anti-atherosclerotic benefits of pomegranate consumption in so severe metabolic condition and suggesting potential clinical applications.

Likewise, pomegranate juice was tested for its role in the protection of NO and whether it could modify some of the biological activities of NO (Ignarro *et al.* 2006). *In vitro* assays indicated that pomegranate juice can protect against superoxide anion-mediated NO degradation, even much more than potent antioxidant juices like grape or blueberry ones. On the other hand, the well-established anti-proliferative action of NO (Ignarro *et al.* 2001) was enhanced in cell culture by pomegranate juice. Actually, the 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.* 2006).

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 by increasing the levels of eNOS and NO, with along a drop in the content of isoprostanes and redox-sensitive gene expression. These findings were consistent with those already reported for obese Zucker rats (De Nigris *et al.* 2007a). In fact, a reduction in atherosclerotic lesions was also registered, suggesting that atherogenic damage could also be reversed by chronic administration of pomegranate extract (De Nigris *et al.* 2007b).

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 displays in humans). The cardioprotective effects of pomegranate by-products on atherosclerotic lesion were attributed to macrophage oxidative stress attenuation with along a decrease in the extent of Ox-LDL uptake by macrophages at their lipids were oxidized in a lesser degree (Rosenblat *et al.* 2006b). Indeed, in the case of pomegranate juice, the antioxidant effects on macrophages have been pointed out recently to be mediated via macrophage paraoxonase 2 (PON2) up-regulation, but not by increasing serum paraoxonase 1 (PON1) (Rosenblat *et al.* 2010b), despite both enzymes were shown to protect against lipid-laden foam cell formation and atherosclerosis onset (Rosenblat and Aviram 2009). Nevertheless, the inhibitory effects on macrophage cholesterol and triglyceride metabolism have not been related to paraoxonases activity, if not to the direct effects of pomegranate juice unique constituents on these cells (Rosenblat *et al.* 2010b). Likewise, pomegranate juice effects on atherosclerosis have been related not only to macrophages protection but also to a decrement in the basal levels of serum lipid peroxides (Aviram *et al.* 2004; Aviram *et al.* 2008), whereas this effect has not been occurred after pomegranate peel extract consumption (Aviram *et al.* 2008).

In addition, these same positive results have been observed in diabetic patients, who did not aggravate their condition despite the high sugars content of pomegranate juice (~ 15%), but they even improved serum diabetic parameters as well as decreased both serum and macrophages oxidative stress after juice supplementation for 3 months (Rosenblat *et al.* 2006a).

Otherwise, an increase in carotid intima-media thickness (CIMT) results in hypertension, as it used to happen in atherosclerotic patients. One of the most common hypertensive effects is an augment in blood pressure which leads to many related complications; hence, avoiding developing this pathology is crucial. Pomegranate has been effective on reducing significantly systolic blood pressure and CIMT in carotid artery stenosis patients who consumed pomegranate juice for one year (Aviram *et al.* 2004), thus exhibiting antihypertensive properties. However, in subjects with moderate coronary hearth disease risk these effects were not observed after 18 months of consumption (Davidson *et al.* 2009). Concerning this data, we may point out that pomegranate seem to be useful in hypertension treatment but not in prevention.

In another trial carried out in hypertensive patients a decrement in systolic blood pressure was also noted with along a reduction in serum angiotensin converting enzyme (ACE) activity, which indicates pomegranate juice effects on attenuating the progression of atherosclerosis (Aviram and Dornfeld 2001).

So far, the anti-atherogenic benefits of pomegranate intake had been associated with a long-term consumption; however, these beneficial properties have also been linked recently to a short-term consumption. After a period of time of one week having pomegranate juice, it has been possible to enhance the oxidative status and decrease the lipid peroxidation, rather than an acute dosage (Rosenblat *et al.* 2010a). Indeed, beneficial anti-atherogenic activity of pomegranate derivatives has resulted to be dose-dependent (Aviram and Dornfeld 2001; Rosenblat *et al.* 2006b).

An approach on elucidating the responsible compound(s) of this anti-atherosclerotic activity by testing different pomegranate fruit parts has been reported. Aviram *et al.* (2008) pointed out that pomegranate juice and pomegranate arils extract, they both rich in anthocyanins, show a major influence in the drop of serum oxidative stress in contrast to pomegranate peel extracts, rich in hydrolysable tannins, which display a higher beneficial effect on the extent of Ox-LDL uptake by macrophages and on their oxidative status. On the whole, pomegranate juice showed better anti-atherogenic properties than pomegranate peel extracts. Likewise, pomegranate juice was more potent than pomegranate peel purified phenolics like anti-atherogenic product.

Concerning the preceding data, more than one compound should be implicated in the anti-atherosclerotic effects of pomegranate. A recently reported study on supplementation of berry juices and teas in hamsters has proposed that a diversity of phenolic compounds, rather than a few specific components, could induce the anti-atherogenic activity of 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 (Esmaillzadeh *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; Jadeja *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 isoproterenol-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 defence, 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 (Jadeja *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 counteracting 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 bioactives come in direct contact with cancerous cells. Hence, antioxidant pomegranate juice and its constituents have been broadly studied for their antiproliferative and 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 procaspases 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).

Therefore, investigations suggest 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 proapoptotic activities of pomegranate derivatives 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 22R*v*1) 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 (Lansky *et al.* 2005a, 2005b).

Likewise, the effects of pomegranate extract on prostate adenocarcinoma have also been assessed in severe combined immunodeficient mice injected subcutaneously with human cancerous cells 22R*v*1 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 trial 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; Sartippour *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 variability 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-

loped 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 phytochemical compounds 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 leukemic cell lines (Khan and Mukhtar 2007; Ampasavate *et al.* 2010; Glauert *et al.* 2010). Action mechanisms to inhibit proliferation and to induce apoptosis are based in those former exposed for colon and prostate cancers.

In general, the anti-oncogenic potential of pomegranate has been related to ellagitannins and its *in vivo* metabolites and even seed oil almost exclusively. In contrast, anthocyanins have hitherto not received adequate attention albeit they have shown to delay carcinogenesis 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.

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 deposition in the hippocampus, a close related to disease progression process. Moreover, formation and deposition of amyloid-β has been associated with oxidative stress (Barnham *et al.* 2004) so antioxidative-related properties of pomegranate chemopreventive agents could be involved.

Concerning neonatal hypoxic-ischemic brain injury, a significant cause of infant illness and death linked to a ROS increment (Gulcan *et al.* 2005; Huang and Castillo 2008), pomegranate juice has also shown interesting results as neuroprotective agent in mice (Loren *et al.* 2005). Neonatal brain protection against a hypoxic-ischemic insult was observed after maternal dietary supplementation with pomegranate juice. Data displayed a significant decreased brain tissue loss in neonates among different regions assessed, with a slight dose-dependent effect, as well as a decrement in caspase-3 activation. Thus, this work demonstrated how maternal pomegranate juice intake can provide neuroprotection to neonates and how this neuroprotection is effective even 7 days after birth. Later, this lasting neuroprotection transferred from mother to neonates have been attributed to whole-fruit pomegranate polyphenols (West *et al.* 2007).

The role of an ethanolic extract of pomegranate seeds on central nervous system (CNS) in mice after a single dose has also been studied employing different behaviour animal models (Kumar *et al.* 2008). Pomegranate extract exhibited anxiolytic, antidepressant, and antinociceptive activity just as a dose-dependent response, while no significant differences between aged and young mice were showed. It is important to note the wide effects of pomegranate on CNS and how pomegranate phytochemicals might modulate the neurotransmitter activity depending on the stimulus received: anxiolytic activity of pomegranate extracts was related by authors to an action on γ-aminobutyric acid (GABA)-mediated transmission via a possible binding of phenolic bioactives to $GABA_A$-benzodiazepines complex; on the contrary, antidepressive-like effects were similar to CNS stimulant drugs which are related to a glutamate increment. Thus, pomegranate constituents may modify the levels of two of the most important neurotransmitter at CNS: GABA and glutamate.

Another pomegranate ethanolic extract, in this case from bark, caused inhibition in acetylcholinesterase in the nervous tissue of the snail *Lymnaea acuminata* (Tripathi *et al.* 2004). Acetylcholinesterase is the enzyme responsible of the degradation of acetylcholine, a neurotransmitter linked mainly to peripheral nervous system (PNS) and muscular motor functions, at the same time that also possesses a role in the CNS (Huh and Fuhrer 2002). Thus, pomegranate bioactives may affect not only to CNS neurotransmitters if not also to PNS neurotransmitters.

Pomegranate benefits in neurological disorders might be severely conditioned by phytochemicals bioavailability in nervous system. CNS is protected by blood-brain barrier (BBB), an endothelium formed by brain microvessel endothelial cells with complex tight junctions and specific transporters which selectively restrict traffic of molecules from the blood to the brain regions, exerting strict control over passage of metabolites and contributing to brain composition regulation (Abbott and Romero 1996). However, although xenobiotic transport across BBB is limited, many kind of flavonoids have shown to be able to cross this barrier and, thus, to be present into brain tissues where they may exert their biological properties (Youdim *et al.* 2004; Spencer 2010). Consequently, taking into account the phenolic profile of pomegranate, it seems reasonable to attribute the neuroprotective effects of pomegranate, at least in part, to polyphenols.

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 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 properties 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 neuropreventive prospects of pomegranate.

On the other hand, ellagitannins or their *in vivo* metabolites have not been detected in brain regions (Espín *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 flavanols 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; El-Sherbini *et al.* 2010; Endo *et al.* 2010). 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 *in vivo* achievable concentrations 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 antiparasitic activity and the inhibition of pro-inflammatory mechanisms involved in the onset of malaria (Dell'Agli *et al.* 2010).

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 extract 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 (Larrosa *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 cosmeceutical 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 (Bae *et al.* 2010). Thus, photoprotective effects of pomegranate on UVB-mediated skin damage could delay (or even prevent) photoaging.

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 urolithins 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 promising chemoprevention of pomegranate. In addition, future pomegranate interventions should be conduced 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.* 2011).

With respect to other pomegranate health implications, a cream containing pomegranate extract has recently shown to improve skin hydration, providing photoprotective effects (Kaur and Saraf 2012). Furthermore, prospects of pomegranate juice consumption on muscle strength and soreness after eccentric exercise has been displayed (Trombold *et al.* 2011).

ACKNOWLEDGEMENTS

The authors wish to thank Fundación Agroalimed for the financial support of this work (Project "Producción de Zumo de Caqui e Industrialización de Granada"). PM was funded by a grant of the FPU Fellowship Programme from the Spanish Ministry of Education. AGV wishes to thank the National Research Council (CSIC) for a JAE Fellowship Grant.

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