Chapter

Vasculoprotective and Neuroprotective Effects of Various Parts of Pomegranate: In Vitro, In Vivo, and Preclinical Studies

Maria Trapali and Vasiliki Lagouri

Abstract

Pomegranate (*Punica granatum* L.) is one of the oldest edible fruits in the Mediterranean area and has been used extensively in the folk medicine. Popularity of pomegranate has increased especially in the last decade because of the health effects of the fruit. Polyphenols, represent the predominant class of phytochemicals of pomegranate, mainly consisting of hydrolysable tannins and ellagic acid. Pomegranate is a rich source of the ellagitannin punicalagin, which has aroused considerable interest in pomegranate fruit as a new therapeutic agent in recent years. Most studies on the effects of pomegranate juice have focused on its ability to cure diabetes and atherosclerosis. The present review summarizes some recent studies on the vasculoprotective and neuroprotective effect of various parts of pomegranate and its main compounds especially hydrolysable tannins ellagitannins, ellagic acid and their metabolites. The in vitro and in vivo studies, showed that the whole parts of pomegranate as well as its main components had a positive influence on blood glucose, lipid levels, oxidation stress and neuro/inflammatory biomarkers. They could be used as a future therapeutic agent towards several vascular and neurodegenerative disorders such as hypertension, coronary heart disease and Alzheimer.

Keywords: pomegranate, ellagic acid, punicalagin, urolithins, cardiovascular disease, CNS, in vitro, in vivo, pre-clinical trials

1. Introduction

Free radical reactions occur naturally in the human body. An over-production of these reactive species due to oxidative stress can cause oxidative damage to biomolecules and the development of chronic diseases such as aging, coronary heart disease and cancer [1]. The harmful action of free radicals can be inhibited by antioxidant substances which scavenge them and detoxify the organism. Current research has confirmed that dietary antioxidants play an important role in the prevention of cardiovascular diseases and cancers, neurodegenerative diseases and inflammation [2]. Pomegranate (*Punica granatum* L.) is one of the oldest edible fruits in the Mediterranean area and has been used extensively in the folk medicine. Popularity of pomegranate has increased in the last years because of anti-microbial,

anti-viral, anti-cancer, anti-oxidant and anti-mutagenic effects of the fruit [3–5]. Polyphenols, are the main phytochemicals of pomegranate fruits, mainly consisting of hydrolysable tannins, gallotannins, ellagitannins and ellagic acid (EA). It has been found to exhibit antimutagenic, antiviral, whitening of the skin and antioxidative properties [6, 7]. Pomegranate fruit is composed of three different parts: the seeds, the arils and the peels. The therapeutic properties have been reported mostly for pomegranate juice [8–11] however, increasing literature was found lately reporting the inhibition of lipid peroxidation of pomegranate peels and seeds [4, 12, 13].

Even a small number of clinical trials in humans have been reported until now, the results showed positive effects of pomegranate extracts on various vascular diseases.

2. Phytochemical components related to activity

Ellagitannins (ETs) are esters of hexahydroxydiphenic acid (HHDP) and a polyol, usually glucose or quinic acid that when they are hydrolyzed transform through lactonization to the component ellagic acid [14] (**Figure 1**).

The variability in the chemical structures among ETs is associated with different physico-chemical properties, hydrolytic reactions, and biological activity in vivo [15]. The important structural diversity of ET structure is due to the different possible extent of galloylation and formation of aromatic C-glycosides, the number of intramolecular C-C coupling of galloyl groups and hydrolytic cleavage of galloyl-derived aromatic rings, the level of dehydrogenation, and oligomerization [16].

Ellagitannins and ellagic acid with anti-inflammatory and vasculoprotective effects are transformed by the gut microbiota to produce urolithins, bioavailable metabolites [17, 18] (**Figure 2**). There is, however, a large variability in health effects and can be associated with the different polyphenol glucuronide metabolic profiles. Differences in urolithin production, both quantity and chemical type, could explain, at least partly, the large variability in the health effects observed in vivo.

The effects of components of the pomegranate e.g. ellagic acid (EA) are also focusing on its potential protective action towards several neurodegenerative disorders. EA has been investigated as multi-target pharmacological drug on CNS in a review analysis [19]. Pomegranate metabolites such as urolithins prevented β-amyloid fibrillation in vitro and especially methyl-urolithin B (3-methoxy-6H-dibenzo [b, d] pyran-6-one), had a protective effect in *Caenorhabditis elegans* post induction of amyloid β(1–42) induced neurotoxicity and paralysis [20].

Urolithin A (UA) allayed hypoxia/reoxygenation abuse in myocardial cells, decreased myocardial cell death in mice after ischemia/reperfusion. UA enhanced antioxidant quantity in cardiomyocytes following hypoxia/reoxygenation reducing

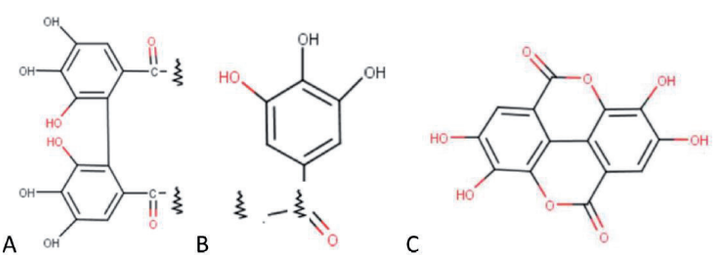

Figure 1.
Basic structures of ellagitannins: (A) HHDP acid (R radical); (B) galloyl unit (G radical); (C) ellagic acid.

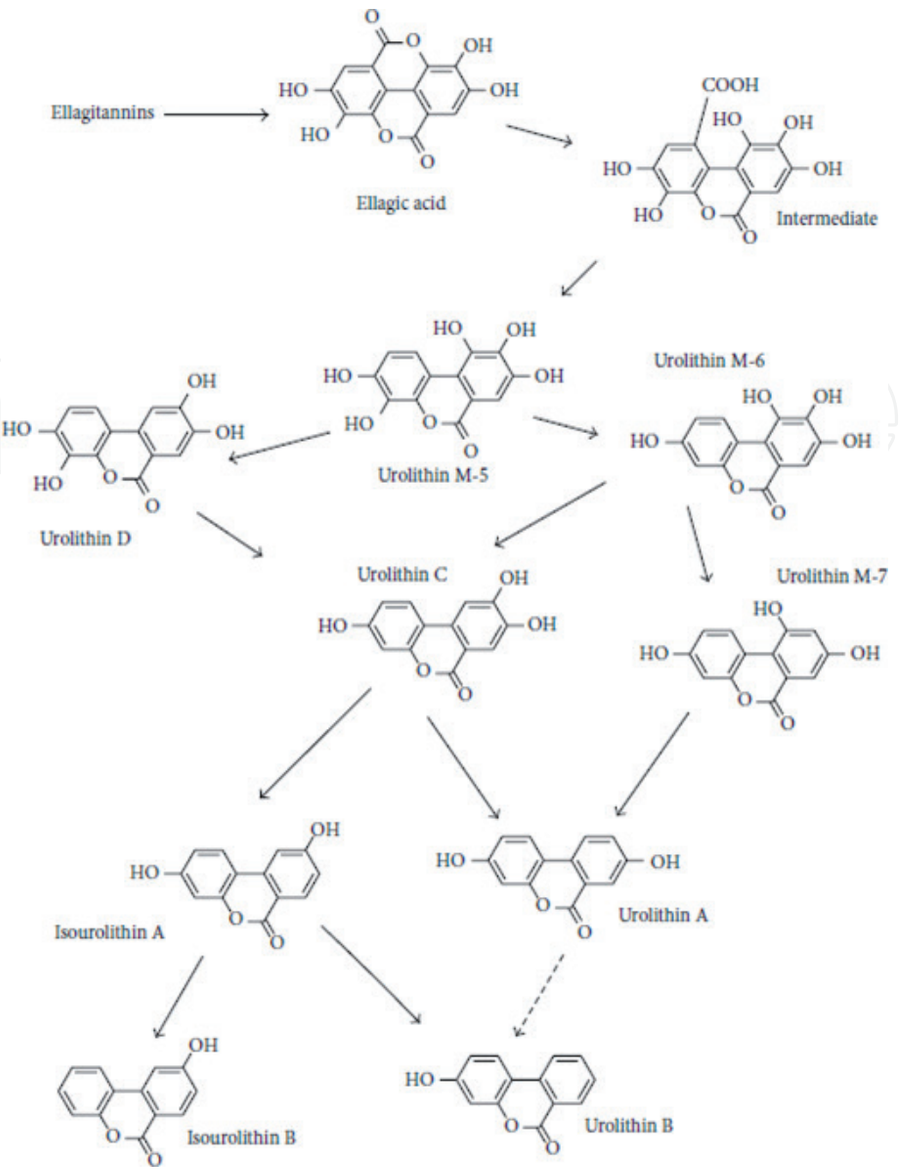

Figure 2.
Gut microbiota metabolism of ellagitannins and ellagic acid.

myocardial apoptosis [21]. The flavonoids naringin and narirutin have a significant beneficial effect in reducing diastolic blood pressure, in patients with hypertension [22]. Human umbilical vein endothelial cells (HUVECs) were pretreated with ellagic acid and then incubated with oxidized low-density lipoprotein (oxLDL). The results indicated inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, enhancing cellular antioxidant defenses, and attenuating oxLDL-induced Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) up-regulation and endothelial nitric oxide synthase (eNOS) down-regulation. Lectin-like oxidized LDL (oxLDL) receptor-1 (LOX-1, also known as OLR-1, is a class E scavenger receptor that mediates the uptake of oxLDL by vascular cells. LOX-1 seems to represent an attractive therapeutic target for the treatment of human atherosclerotic diseases [23]. Adipocyte cells were pretreated with punicalagin and ellagic acid and that caused inhibition of lipolysis reducing MAO activity [24].

Urolithin C, a combination of urolithins A and B metabolites of pomegranate and ellagic acid also reduced cholesterol accumulation in the human monocytic cell line THF-1-derived macrophages, but were unable to promote cholesterol outflow. Atherosclerotic processes can be attenuated by urolithins, but future human

intervention tests are needed to see if it translates in vivo [23]. The ability of punicic acid (PUA) to modulate peroxisome proliferator-activated receptor PPAR activity was determined in 3 T3-L1 pre-adipocytes. PUA activates PPAR, increases PPAR - responsive gene expression and ameliorates diabetes and inflammation [25].

2.1 In vitro studies

PJ concentrate reduced the activation of redox-sensitive genes (ELK-1 and p-JUN) and increased eNOS expression in cultured human coronary artery endothelial cells (EC) exposed to high shear stress in vitro [26]. In vitro study showed that pomegranate leaf, seed and juice repressed cholinesterase activity in a dose dependent manner. Pomegranate juice had also protective effects against hydrogen peroxide induced toxicity in the *Artemia salina* (a species of brine shrimp) and HepG2 models (in vitro model system for the study of polarized human hepatocytes), antiproliferative activities in HeLa and PC-3 cancer cells inhibiting COX-2 and MAO enzymes [27].

Microglial cells are the resident macrophages of the CNS. The immortalized murine microglial cell line BV-2 has been used frequently as a substitute for primary microglia. Urolithin B inhibited the production of NO and pro-inflammatory cytokines, inhibited NF-κB activity by reducing the phosphorylation and degradation of a nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, IκBα. In addition, urolithin B suppressed the phosphorylation of c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinases (ERK), and Protein kinase B Akt, and enhanced the phosphorylation of AMPK, which is associated with anti-inflammatory and antioxidant processes [28, 29]. In another study, lipopolysaccharide LPS-treated cultured astrocytes and microglial BV-2 cells were investigated for anti-neuroinflammatory effects of punicalagin (PUN). It was found that PUN inhibits LPS-induced memory impairment via anti-inflammatory and anti-amylogenic mechanisms through inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells NF-κB activation [30]. The above results may be a solution to Alzheimer Disease [31].

2.2 In vivo studies

Clinical studies in hypertensive and/or obese patients receiving pomegranate juice have shown a reduction in systolic and diastolic blood pressure [32–36] and a concomitant increase in high density lipoprotein (HDL) cholesterol. Juice intake also led to a significant reduction in the by-products of fat peroxidation and protein and inflammatory biomarkers. Patients taking pomegranate-containing nutrient supplements had lowered systolic and diastolic blood pressure levels but the cardiovascular risk did not recover [37].

A number of clinical trials in humans proved the positive effects of pomegranate juice in the protection of central nervous system (CNS). Maternal pomegranate juice absorption in pregnancies with intrauterine growth restriction (IUGR) showed differences in the infant brain and structure [38].

2.3 Preclinical studies

When PJ was given in diabetic rats it was observed decreased blood glucose, lipid levels, and inflammatory biomarkers [39]. In another study using obese Zucker rats, intake of pomegranate juice (PJ) or fruit extract PFE caused a decrease of inflammation factors and increase of plasma nitrate and nitrite (NOx) [40]. In a study involving diabetic rats, they were given pomegranate seed powder (PS).

Pomegranate part/substance	**Vasculoprotective effect (in vitro/in vivo)**	**Ref.**	**Neuroprotective effect (in vitro/in vivo)**	**Ref.**
Pomegranate juice/peel extract/seed	Reduction in systolic and diastolic blood pressure (clinical studies/in vivo) Significant reduction in the by-products of fat peroxidation and protein and inflammatory biomarkers (clinical studies/in vivo) Decreased blood glucose, lipid levels, and inflammatory biomarkers (preclinical studies/ in vivo) Improved insulin sensitivity, increased levels of interleukin-10 and activated PPARγ (preclinical studies/ in vivo) Reduction of systemic oxidative stress (preclinical studies/in vivo) Reduced the activation of redox-sensitive genes (ELK-1 and p-JUN) and increased eNOS expression (in vitro)	Asgary et al. [32], Lynn et al. [35], Haghighian et al. [34], Asgary et al. [33], Moazzen and Alizadeh [36] Wu et al. [37] Taheri et al. [39], De Nigris et al. [40], Dos Santos et al. [42] Harzallah et al. [43], Hontecillas et al. [25] Asgary et al. [33] Nigris et al. [26]	Protection against oxidative destruction and improvement of neuronal durability (preclinical studies/ in vivo) Inhibition of fetal brain apoptosis, neuronal nitric oxide synthase, and nuclear factor-κB activation (preclinical studies/ in vivo) Repressed cholinesterase activity, Inhibition COX-2 and MAO-A enzymes (in vitro)	Kujawska et al. [44] Ginsberg et al. [45] Amri et al. [46], Les et al. [27]
Ellagic acid, punicalagin, urolithin	Inhibition of NADPH oxidase, enhancing cellular antioxidant defenses, attenuating oxLDL-induced LOX-1 up-regulation and eNOS down-regulation (in vitro) inhibition of lipolysis reducing MAO activity (in vitro) Increased PPAR -responsive gene expression and amelioration of diabetes and inflammation (in vitro)	Lee et al. [23] Les et al. [24] Hontecillas et al. [25] Yuan et al. [20]	Protective effect in neurotoxicity and paralysis (in vitro) Inhibition of the production of NO, pro-inflammatory cytokines, NF-κB activity, IκBα and Protein kinase B Akt (in vitro)	Yuan et al. [20] Lee et al. [29], DaSilva et al. [28], Kim et al. [30], AlMatar et al. [31]

Table 1.
Vasculoprotective and neuroprotective effects of pomegranate and their substances/metabolites in in vitro and in vivo pre-clinical studies.

Increased blood cholesterol, LDL and HDL lipoprotein were found [39, 41] while systolic blood pressure, angiotensin-converting enzyme coronary activity decreased [42]. Pomegranate peel (PPE), flower (PFE) and seed (PSO) given in obese mice decreased fasting blood glucose, improved insulin sensitivity, increased levels of the anti-inflammatory cytokine interleukin-10 [43] and activated peroxisome proliferator-activated receptor gamma (PPARγ) [25]. PPARγ, a ligand-activated transcription factor, has a role in various cellular functions as well as glucose homeostasis, lipid metabolism, and avoidance of oxidative stress. Pigs with hypercholesterolemia were given a pomegranate extract which caused reduction of systemic oxidative stress [33]. Pomegranate supplementation also exhibits cardiovascular protection improving cardiac hypertrophy in cigarette smoke in sight animals [11].

Preclinical trials in animal models added research results to the positive effects of pomegranate in CNS. In a rat model of Parkinsonism induced by rotenone, pomegranate juice treatment resulted in protection against oxidative destruction and improvement of neuronal durability [44]. Besides, in a rat model of maternal inflammation, pomegranate juice caused inhibition of fetal brain apoptosis, neuronal nitric oxide synthase, and nuclear factor-κB activation [45] (**Table 1**).

Methods used are extensively described in literature (e.g. [21, 37, 46–50]).

3. Possible therapeutic applications

The in vitro and in vivo studies showed that the whole parts of pomegranate as well as its main components such as hydrolysable tannins, ellagic acid and urolithins had a positive influence on blood glucose, lipid levels, oxidation stress and neuro/inflammatory biomarkers.

4. Future perspective and recommendations

The reviewed studies emphasize the potential benefits and suggest of a wider use of pomegranate and its components as dietary supplements or as adjuncts in the treatment of vascular and neurodegenerative diseases such as hypertension, coronary heart disease, peripheral artery disease and Alzheimer disease.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this chapter.

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