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The Use of Pomegranate (*Punica granatum* L.) Phenolic Compounds as Potential Natural Prevention Against IBDs

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1. Introduction

Phenolic compounds (PCs) are plant secondary metabolites that are integral part of the "normal" human diet. The daily intake of PCs depends on the diet but is commonly evaluated at *ca.* 1g/day for people who eat several fruits and vegetables per day (Scalbert & Williamson, 2000). PCs may be interesting to prevent the development of inflammatory diseases, more particularly in the gastrointestinal tract, where their concentration may reach levels of up to several hundred µM (Scalbert & Williamson, 2000). Many studies have indeed reported on anti-inflammatory properties of different PCs (see (Calixto *et al.*, 2004; Rahman *et al.*, 2006; Romier *et al.*, 2009; Shapiro *et al.*, 2009), for reviews).

Pomegranate *(Punica granatum L.)* belongs to the *Punicaceae* family, which includes only two species. More than 500 cultivars of *Punica granatum* exist with specific characteristics such as fruit size, exocarp and aril color, etc. Originating from the Middle East, pomegranate is now widely cultivated throughout the world, and also widely consumed. Pomegranate has been used for centuries in the folk medicine of many cultures. As described in the review of Lansky *et al.* (2007), the bark and the roots are believed to have anthelmintic and vermifuge properties, the fruit peel has been used as a cure for diarrhea, oral aphthae, and as a powerful astringent, the juice as a blood tonic, and the flowers as a cure for *diabetes mellitus*.

Numerous investigations have highlighted the anti-inflammatory potential of the PCs found in this fruit, and more especially of hydrolysable tannins called ellagitannins (ETs), which are mainly located in pomegranate peels. These ETs are extracted into the juice upon commercial processing of the whole fruit (Gil *et al.*, 2000).

This chapter first describes the PCs found in pomegranate fruit, then focuses on ETs in relation to their metabolic fate after ingestion as well as to their anti-inflammatory properties on the intestine, and finally discusses gut microflora modifications following pomegranate ingestion and their impact on intestinal inflammation.

2. Phenolic compounds identified in the pomegranate fruit

The pomegranate fruit is a berry of 5 to 12 cm diameter with a leathery, deep red peel (husk, rind, and pericarp are synonyms). The fruit's interior is separated by membranous walls

into compartments containing arils filled with pulp. Each aril contains one angular seed. PCs are present in different parts of pomegranate plants; they are found in seeds, arils, fruit peels, leaves, flowers, tree bark, and roots (Lansky & Newman, 2007). Here, we will be focusing on the PCs of the pomegranate fruit since the commercial processing of the whole fruit is largely used in the juice industry. Different classes of PCs are found in the pomegranate fruit. The amounts of each PC are largely affected by the raw material, e.g. pomegranate cultivars and climatic conditions during fruit maturation and ripening (Borochov-Neori *et al.*, 2009), but also by the post-harvest storage, and the technological treatments leading to juice production and distribution (Tomás-Barberan *et al.*, 2000).
One important component of pomegranate PCs is ellagic acid (EA) (**Figure 1**). EA can be found in its free form (aglycone form), in a conjugated form with a glycoside moiety or more commonly complexed in the form of ETs. The occurrence of the free form in the nature is however quite uncommon (Clifford & Scalbert, 2000).

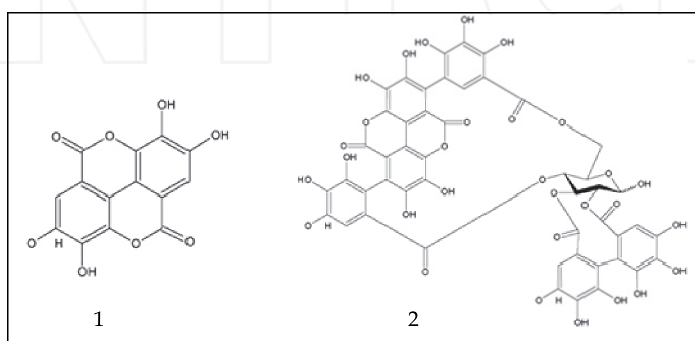


Fig. 1. Chemical structures of EA (1) and punicalagin (2) (Lansky & Newman, 2007).

Regarding pomegranate juice production, the phenolic profile has been reported to be very different if made from isolated arils only or from the whole fruit (Gil *et al.*, 2000). In the same study, commercial pomegranate juices, made by pressing the whole fruit, were reported to show an antioxidant activity three times higher than red wine and green tea infusion, while hand-pressed pomegranate juice showed a lower antioxidant activity. This difference was attributed to the elevated amount of pomegranate peel ETs that are transferred into the juice during whole fruit pressing. Numerous studies have highlighted that commercial pomegranate juices present a very high antioxidant activity, mainly attributed to their high content in ETs and more particularly in punicalagin (**Figure 1**), which offers more than 50% of the total antioxidant activity (Gil *et al.*, 2000; Li *et al.*, 2006; Schubert *et al.*, 1999; Seeram *et al.*, 2005a; Tzulker *et al.*, 2007).
Given the wide difference in antioxidant activity observed with the two methods of juicing, it is useful to distinguish PCs found in juice made from the arils only, from those identified in the peel. Hydroxybenzoic acids were mainly found in the arils, except for gallic acid that was present at a higher level in the peel (Amakura *et al.*, 2000; Fischer *et al.*, 2011). Hydroxycinnamic acids are identified only in the arils, except for free caffeic acid and chlorogenic acid that were reported to be present only in the fruit peels (Amakura *et al.*, 2000; Fischer *et al.*, 2011; Lansky & Newman, 2007). Among the flavonoids, luteolin and luteolin 7-O-glucoside (flavones), as well as naringenin 7-O-rutinoside (flavanones), catechin, epicatechin and epigallocatechin 3-gallate (flavanols), and quercetin, kaempferol,

rutin, kaempferol 3-O-glucoside and kaempferol 3-O-rhamnoglycoside (flavonols), were also exclusively identified in the fruit peel (Lansky & Newman, 2007; de Pascual-Teresa *et al.*, 2000), whereas the flavonol dihydrokaempferol-hexoside was identified in the arils, but not in the peel (Fischer *et al.*, 2011). Still in the class of flavonoids, the pomegranate fruit contains a collection of different anthocyanins (delphinidin 3,5-diglucoside, delphinidin 3-glucoside, cyanidin 3-glucoside, cyanidin-pentoside-hexoside, cyaniding 3-rutinoside, cyaniding 3-pentoside, cyanidin 3-hexoside, cyanidin 3,5-diglucoside, pelargonidin 3-glucoside, pelargonidin 3,5-diglucoside), which are present in both arils and peels in similar concentrations (Fischer *et al.*, 2011). Pomegranate fruits are very rich in hydrolysable tannins, including gallotannins and ETs, essentially present in the peel with about 44g/kg of dry matter of that material (Fischer *et al.*, 2011). Gallotannins represent less than 0.01% and less than 2% of total hydrolysable tannins, respectively in the peel and in the juice made from arils only. ETs are predominantly found in the peel, with about 20 components identified (Fischer *et al.*, 2011). As indicated before, the most abundant ET is punicalagin with a concentration of about 10.5 g/kg of dry matter of pomegranate peel, and its levels can be superior to 2g/L in industrial juice (Amakura *et al.*, 2000; Borges *et al.*, 2010; Fischer *et al.*, 2011; Lansky & Newman, 2007; Martin *et al.*, 2009, Seeram *et al.*, 2005b).

3. Metabolic fate of pomegranate ellagitannins

In order to evaluate the health effects of ETs, it is critical to understand their oral bioaccessibility from the pomegranate matrix as well as their bioavailability.

3.1 Bioaccessibility and intestinal absorption

After ingestion, PCs may undergo changes until they reach the site where they could have an impact. In the gastro-intestinal tract, PCs are submitted, on one hand, to abiotic physico-chemical changes and enzymatic attacks, accompanying the digestion in the upper part of the tract, and, on the other hand, to biotic changes with the participation of the gut microflora found in the lower part of the tract, and especially in the colon.

Concerning the abiotic changes, an *in vitro* simulated gastric digestion showed that ETs are quite stable under acidic conditions and are not hydrolyzed by stomach enzymes, while an important increase of free EA was observed in the duodenal digestion conditions (Gil-Izquierdo *et al.*, 2002). Similarly, in the duodenal conditions, a significant release of EA from the standard punicalagin was also reported by Larrosa *et al.* (2006b) and it was assumed to be due to a spontaneous hydrolysis of punicalagin in the neutral pH conditions. When pomegranate juice was submitted to both *in vitro* gastric and duodenal digestions, no significant differences in total soluble phenolic contents (determined by the Folin-Ciocalteu) were noticed before and after gastric digestion, while, after duodenal digestion, 29% of the total initial phenolic content in a soluble form were available for absorption (Perez-Vicente *et al.*, 2002). These results confirm that pomegranate PCs undergo modifications under duodenal conditions. To our knowledge, no other data are available on the fate of ETs or EA with reference to abiotic changes, related to enzymatic attacks and pH variations, encountered in the gastro-intestinal tract. In the lower part of the gastro-intestinal tract, ETs seem to be transformed by the gut microflora (*i.e.* biotic changes). An *in vivo* study conducted on pigs showed that EA, resulting from ET hydrolysis, is metabolized by the gut microflora that is already active in the jejunum and much more concentrated in the colon. This progressive metabolic processing is illustrated in **Figure 2**. Urolithins D, C, A and B are

sequentially produced, by successive losses of hydroxyl groups, resulting in an increased lipophilicity as well as in an increased intestinal absorption rate (Espín *et al.*, 2007). When rats fed with punicalagin at a daily rate ranging from 0.6 to 1.2 g for 37 days, their feces showed an increased presence of punicalagin and its hydrolysis products (punicalin, gallagic acid, and EA) up to day 18. Afterwards, a decrease of punicalagin and punicalin amounts was observed concomitantly with an increase in urolithin acompounds (Cerdá *et al.*, 2003b). Human fecal samples from 6 healthy volunteers were incubated with EA, punicalagin and an extract of walnut (rich in ETs) to search for the occurrence of urolithin A. Samples were analyzed at 5, 24, 48, and 72h of incubation. Urolithin A in its aglycone form was identified in all the samples collected, but not urolithin A conjugates, suggesting that the aglycones should first be absorbed before being metabolized in the conjugated forms by the intestinal cells and/or liver (Cerdá *et al.*, 2005). In another study, the occurrence of the different urolithins (**Figure 2**) in their aglycone form was analyzed after incubation of pomegranate by-products with human fecal samples obtained from three healthy volunteers. The four urolithins D, C, A and B were identified (Bialonska *et al.*, 2010).

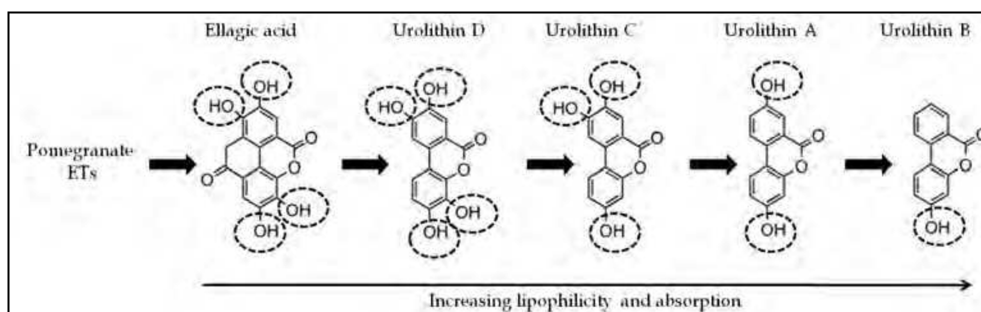


Fig. 2. Metabolism of pomegranate ETs by the human intestinal microflora (adapted from Espín *et al.* (2007)).

Concerning the intestinal absorption, EA was shown to enter into the intestinal cells and to be further metabolized in dimethyl EA conjugates (e.g. dimethyl EA glucuronides and dimethyl EA sulphates) in an *in vitro* study using proliferating Caco-2 cells, suggesting the involvement of phase II enzymes (methylation, glucuronidation and sulphation) (Larrosa *et al.*, 2006b). In another *in vitro* study (Whitley *et al.*, 2003), with differentiated Caco-2 cells in a bicameral system, labeled standard EA was incubated in the apical compartment to evaluate its trans- or para-cellular transport. Cellular uptake seemed very extensive and probably governed by passive diffusion at the apical side. However, the passage across the epithelial cell monolayer appeared very limited since the quantity of EA found in the basolateral compartment was very low in this model. In accordance with this observation, a preferential apical efflux of EA was observed even though the multidrug resistance-associated protein 2 (MRP2) and the P-glycoprotein, two apical efflux transporters expressed in Caco-2 cells, were not involved. In addition, intracellular binding processes were shown to decrease EA passage. Once in the intestinal cells, EA appeared indeed to bind irreversibly to DNA and proteins (Whitley *et al.*, 2003), as it was already demonstrated for the flavonoid quercetin (Walle *et al.*, 2003). The high irreversible binding ability of EA to DNA did not require prior oxidation and may be due to its great ability to intercalate DNA (Dixit & Gold, 1986; Teel *et al.*, 1987; Thulstrup *et al.*, 1999). In contrast, the covalent binding ability of EA to proteins

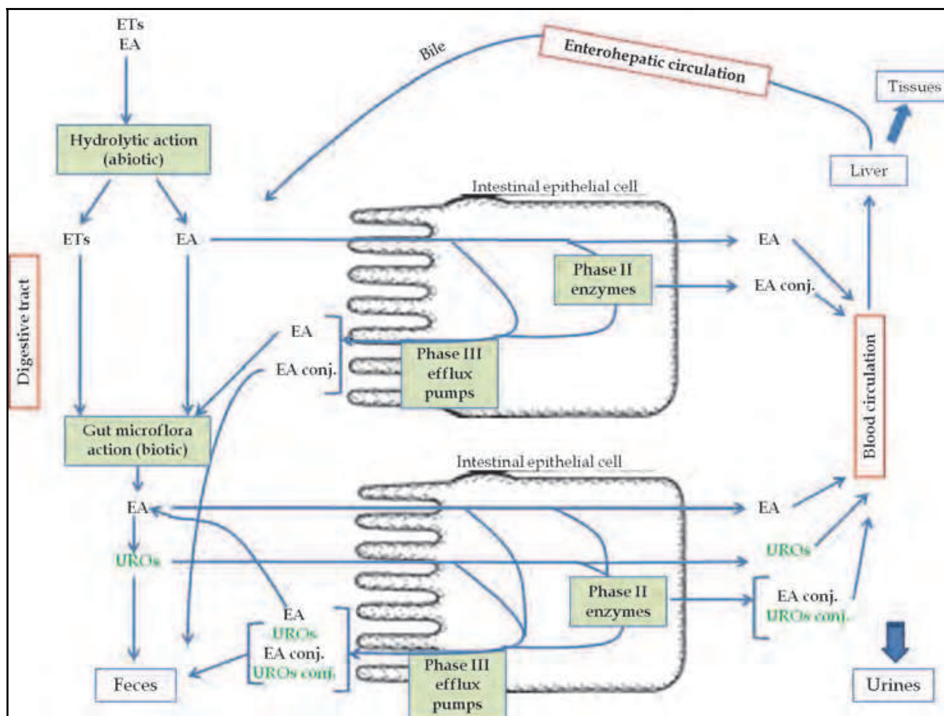
may require its prior oxidation by reactive oxygen species that could be abolished by glutathione (Whitley *et al.*, 2003). In the *in vivo* study on pigs mentioned above (Espín *et al.*, 2007), ET metabolites were shown to be absorbed in the intestinal tissues. The metabolites detected in the pig jejunum tissues were, in increasing amounts, urolithin D, urolithin C, urolithin A, urolithin A glucuronide, and urolithin C methyl ether, following the increase degree of lipophilicity. By contrast, EA conjugates were not detected in the intestinal tissues but were present in bile and in urines, suggesting EA absorption in the stomach. The colon tissues were also analyzed and only small amounts of urolithins A and B were detected. The results of these *in vitro* and *in vivo* studies indicate that the resulting products of microbial transformation, namely the urolithins, are absorbed along with EA and further metabolized in the enterocytes by phase II (methylation, glucuronidation and sulphation) enzymes.

3.2 Blood circulation, distribution and excretion

Except for the studies of Cerdá *et al.* (2003a & 2003b) reporting trace amounts of punicalagin in rat plasma after a high daily consumption of 0.9 g punicalagin for 37 days, many *in vivo* animal studies indicate that ETs are not found as such in the blood circulation (Borges *et al.*, 2007; Cerdá *et al.*, 2004a & 2004b; Espín *et al.*, 2007; González-Barrio *et al.*, 2010; Mertens-Talcott *et al.*, 2006; Seeram *et al.*, 2006). In their pig study, Espín *et al.* (2007) reported that animals fed with acorns rich in ETs and EA (single feeding) showed the occurrence of urolithin A and B as aglycone and conjugates in their plasma 24h, but not 3h, after ingestion. The same ET metabolites were also detected in trace amounts in the 24h urines. These observations indicate that pigs are able to produce rapidly the microflora metabolites (24h after a single ingestion), in contrast with rats that needed several days (Cerdá *et al.*, 2003b). In pigs regularly fed with acorns and after a 24h fasting period, urolithins A and B as aglycone and as conjugates and EA conjugates were detected in both plasma and urines as well. The bile content of these animals, after gall bladder removal, showed a wide range of urolithins (urolithin A, C and D) conjugates, mainly the glucuronides, as well as EA conjugates, while no urolithin B aglycone and/or conjugates were found. The metabolites found in the bile coincide with those found in the lumen, except for the aglycone EA that was not detected in the bile. The presence of a wide range of ET metabolites in the bile and the very low clearance of these compounds in urines suggested an important enterohepatic circulation (Espín *et al.*, 2007). Interestingly enough, the study of Cerdá *et al.* (2003b) on rats fed with punicalagin for 37 days did not only report on fecal analyses (see section 3.1), but also on plasma and urine levels of ET metabolites. They showed that, during a first period of about 20 days, the main metabolites detected in plasma and urines were derived only from punicalagin hydrolysis through conjugation in methyl ether or glucuronide forms, whereas, after 20 days, urolithins and their glucuronides were detected, which may be due, as for the fecal observations, to a modification of the gut microflora composition. After mice ingested a single dose (0.8 mg per animal) of an ET-enriched pomegranate peel extract, standardized to 37% ETs and 3.5% EA, plasma samples were collected over 24h. EA was detected in the plasma 30 min after pomegranate extract ingestion and was cleared after 2h, while neither urolithin A nor urolithin A conjugates were detected during the 24h (Seeram *et al.*, 2007). The pharmacokinetics of EA was evaluated after oral administration of a pomegranate leaf extract to rats. A rapid increase of EA was observed in the plasma with a maximum concentration reached 0.54h after ingestion and a plasma half-life of 5h (Lei *et al.*, 2003).

Human studies on healthy volunteers seem to confirm the results obtained in animal studies. A first trial was conducted on one subject who ingested 180 mL of pomegranate juice containing 25 mg of EA and 318 mg of ETs. In order to evaluate EA bioavailability, blood samples were collected until 6h after consumption. A maximum concentration was detected 1h post-ingestion and EA was cleared after 4h (Seeram *et al.*, 2004). On the basis of that preliminary study, another study was performed on 18 healthy human volunteers who consumed 180 mL of pomegranate juice containing 1561 mg/L of punicalagin, 121 mg/L of EA, and 417 mg/L of other ETs. Blood samples were analyzed during the 6h following ingestion and urines were collected in 12h batches the day before, the day of, and the day after ingestion. Again, no ETs were detected as intact form in plasma. By contrast, EA could be detected. It peaked at 0.98±0.06 h post-ingestion (T_{max}), with a maximum concentration (C_{max}) of 0.06±0.01 µmol/L (18.64 ng/mL), and an elimination half-life ($T_{1/2}$) of 0.71±0.08 h. It was cleared within 5h. The area under the curve (AUC) was 0.17±0.02 (µmol*h)*L^{-1} (50.07 ng*h/mL). Interestingly, urolithin A and B as aglycone and as conjugates began already to appear in blood collected 0.5h after ingestion and higher concentrations were found in 6h plasma samples. EA and dimethyl EA glucuronide were detected in urines of the day of juice consumption, respectively for 5 and 15 of the 18 subjects, but not of the following day. Urolithin A and B glucuronides appeared in the urines collected the second 12h of the day of the study, and in the urines collected the day after (Seeram *et al.*, 2006). After 6 healthy volunteers ingested 1 L of pomegranate juice per day for 5 days, the occurrence of metabolites in plasma and urines was examined (Cerdá *et al.*, 2004a). Neither punicalagin nor EA, in free or in conjugated form, was found in plasma samples, whereas urolithins conjugates were detected (urolithin A glucuronide, an unidentified aglycone metabolite and urolithin B glucuronide). The urines revealed the presence of 3 additional microflora metabolites (urolithin A, urolithin B, and an unidentified aglycone metabolite). The metabolites found in plasma and urines presented high inter-individual variability (Cerdá *et al.*, 2004a). Another study conducted on 40 healthy volunteers investigated the metabolic fate of ETs from 4 different sources of ET-rich foodstuffs, *i.e.* strawberries, red raspberries, walnuts, and oak-aged red wine. Each group of 10 volunteers received a single dose of ET-containing foodstuff and the urines were collected in 5 fractions, at 8, 16, 32, 40, and 56h after food intake (Cerdá *et al.*, 2004b). As previously observed with pomegranate juice consumption (Cerdá *et al.*, 2004a), neither ETs nor EA were detected in none of the urine fractions collected. Whatever the foodstuff ingested, urolithin B glucuronide was detected in all the urines from 32h to 56h following ingestion, and urolithin B in some of them (Cerdá *et al.*, 2004b). In another human study, 2 capsules corresponding to 800 mg of pomegranate extract (330.4 mg of punicalagin and 21.6 mg of EA), were administered to 11 healthy volunteers. No punicalagin was found in human plasma, while EA appeared in plasma with similar pharmacokinetic parameters as previously observed: T_{max}=1h, C_{max}=33.8±12.7 ng/mL, $T_{1/2}$=0.94h and AUC=118.01 ng*h/mL. The microflora metabolites were detected as well (e.g. urolithin A, hydroxyl urolithin A, urolithin A glucuronide, urolithin B and dimethyl urolithin B glucuronide). Again, these different metabolites were not present in all the subjects tested, which could be explained by the difference in microflora composition responsible for ET degradation (Mertens-Talcott *et al.*, 2006). A single intake of raspberry containing ETs was given to 10 healthy humans and 4 humans with ileostomy. Blood was collected during 24h and urines were collected at 4, 7, 24 and 48h post-ingestion. Urolithins were detected in the plasma of the healthy volunteers, but not in the plasma of the patients with ileostomy, confirming that urolithins are formed in the large intestine. No ETs in an

intact or conjugated form were detected in the plasma of any subjects during 24h after raspberry intake. Small amounts of EA and EA glucuronide were detected in the urines from both groups. However, urolithin A and B glucuronides were only identified in the urines of healthy subjects collected at 7 to 48h post-ingestion (González-Barrio *et al.*, 2010). In sum, these human studies indicate that there is no absorption of the ETs in an intact form. Concerning the distribution in non-intestinal tissues, an *in vitro* study reported that urolithins A and B entered into the human breast cancer MCF-7 cells and were metabolized in urolithin sulphate and glucuronide conjugates (Larrosa *et al.*, 2006a). In the mice study of Seeram *et al.* (2007), prostate, liver, kidney, lung and brain tissues were analyzed 24h after pomegranate extract ingestion. Neither EA, nor ETs, nor urolithin A in free or conjugated form was detected in any tissues when pomegranate was orally administrated. In the pig study performed by Espín *et al.* (2007), liver, kidney, heart, lung, brain, and muscle tissues were analyzed after a 117 day-ET rich diet, and again no ET metabolites were detected. Nevertheless, in the rat study performed by Cerdá *et al.* (2003a), 5 punicalagin metabolites (two EA conjugates, gallagic acid, urolithin A glucuronide, and urolithin C glucuronide) were detected in the two organs investigated, namely liver and kidney.



Abbreviations: conj., conjugates ; EA, ellagic acid; ETs, ellagitannins; UROs, urolithins.
The compounds in green result from a microbial action on EA.

Fig. 3. Model of the metabolic fate of ellagitannins and ellagic acid in the human intestine.

As summarized in **Figure 3**, upon ingestion of pomegranate, ETs seem to be partially hydrolyzed in the upper part of the gastrointestinal tract to release EA that is further

metabolized by the colon microflora to form the bioavailable urolithins. Free EA found in pomegranate could already be absorbed in the small intestine epithelial cells, while intact ETs need to reach the large intestine where they are shown to be extensively transformed in urolithins by the intestinal microflora before being absorbed. Once urolithins and EA are absorbed, they are conjugated to give methyl ether, glucuronide or sulphate conjugates by phases II enzymes. These conjugates can be found in the plasma, as well as in the intestinal lumen, directly after conjugation in the intestinal cell, or via the enterohepatic circulation. Part of them is not absorbed and is excreted in the feces. The urolithins and EA, either conjugated or in their free form, can temporarily accumulate in the intestinal tissues before being liberated in the plasma.

4. Effects of pomegranate phenolic compounds on the intestinal inflammatory response in IBD-related models

Since the last years, anti-inflammatory effects related to pomegranate consumption were reported in intestinal *in vitro* and in *vivo* studies, suggesting a role for pomegranate-derived products in IBD prevention. Causes of IBDs are not well known but two main hypotheses are put forward. The first hypothesis suggests that a deregulation of the mucosal immune system provokes excessive immunologic responses against the normal gut microflora, while the second one proposes that changes in the composition of the gut microflora associated with a disrupted epithelial barrier lead to an abnormal inflammatory response from the intestinal mucosa (Stecher & Hardt, 2008). In this section, *in vitro* and *in vivo* studies highlighting the anti-inflammatory properties of pomegranate PCs will be reviewed.

4.1 *In vitro* anti-inflammatory effects of pomegranate phenolic compounds

Anti-cancer effects of pomegranate have been described in *in vitro* studies on cell proliferation and apoptosis using human colon cancer cells (Larrosa *et al.*, 2006b; Seeram *et al.*, 2005b). Even if IBDs are associated with increased risk for colorectal cancer and if there are similarities in the biology of IBD-associated colon cancer and sporadic cancer (Rhodes & Campbell, 2002), these highlighted effects are not, strictly speaking, related to the intestinal inflammation response developed by IBD patients, and therefore, will not be detailed in this section. Among *in vitro* studies on intestinal epithelial cells, only three studies investigated the intestinal anti-inflammatory potential of pomegranate extracts, pomegranate juice or pomegranate PCs. They were carried out on human colon epithelial cells: Caco-2 cells (Romier-Crouzet *et al.*, 2009; Sergent *et al.*, 2010) and HT-29 cells (Adams *et al.*, 2006). In these studies, intestinal inflammation was induced either by IL-1β (Romier-Crouzet *et al.*, 2009), or TNF-α (Adams *et al.*, 2006) or by a mixture of pro-inflammatory molecules, *i.e.* IL-1β, IFN-γ, TNF-α and LPS (Romier-Crouzet *et al.*, 2009; Sergent *et al.*, 2010). Additional *in vitro* studies have evaluated the potential role of pomegranate for preventing IBDs by using other types of cells involved in the intestinal immune response, such as murine splenic lymphocytes CD4+ T cells (S.I. Lee *et al.*, 2008), RAW 264.7 murine macrophages (C.J. Lee *et al.*, 2010; Panichayupakaranant *et al.*, 2010), or the human basophilic cell line KU812 (Rasheed *et al.*, 2009). In these studies, inflammation was induced either by LPS (C.J. Lee *et al.*, 2010; Panichayupakaranant *et al.*, 2010) or by phorbol-12-myristate 13-acetate plus calcium ionophore A23187 (PMACI) (Rasheed *et al.*, 2009).

4.1.1 *In vitro* effects on inflamed intestinal cells

Ellagic acid. Anti-inflammatory effects of EA were evaluated by measuring inflammatory–related cytokine (the pro-inflammatory cytokines IL-6, IL-8, MCP-1 and the anti-inflammatory IL-10) secretion and mRNA expression in differentiated Caco-2 cells stimulated with the pro-inflammatory cocktail (IL-1β, IFN-γ, TNF-α and LPS) (Sergent *et al.*, 2010). For the experiments, cells were seeded on a microporous membrane in bicameral inserts. Two-day treatments were started at day-19 by adding EA (50 µM final concentration) to the apical side of the cells, while the cocktail of pro-inflammatory stimuli was concomitantly introduced to the basolateral side. The amounts of pro-inflammatory cytokines were quantified with ELISA assays after pooling the media from both compartments. EA decreased MCP-1 and IL-8 secretion but not significantly, and had no effect on IL-6 and IL-10 secretions, while mRNA expression of the four cytokines was not affected. Nevertheless, in that study, EA down-regulated the transcription of three genes involved in the intestinal inflammation: CD14 (Cluster of differentiation 14 gene) encoding a protein acting as LPS receptor, IL1R1 (Interleukin 1 receptor type 1 gene), and PLA2G2A (phospholipase A2 gene). EA also significantly down-regulated the transcription factor STAT3 (signal transducer and activator of transcription 3) involved in the persistent activation of NF-κB (the nuclear factor-kappa B), an inducible nuclear transcriptional factor associated with the intestinal inflammatory response (Yu *et al.*, 2009) and the gene CYP1A1 (Cytochrome P450, family 1, subfamily A, polypeptide 1) (Sergent *et al.*, 2010).

Pomegranate peel extract. A pomegranate peel extract, used at the concentration of 50 µM gallic acid equivalent (GAE), was reported to inhibit NF-κB activity in confluent Caco-2 cells temporarily transfected with a NF-κB-luciferase construct and under IL-1β stimulation. The same extract tested on Il-1β-stimulated and non-transfected confluent Caco-2 cells was also found to inhibit slightly Erk1/2 phosphorylation, but had no effect on JNK phosphorylation (2 major members of the mitogen-activated-protein kinase (MAPK) cascades involved in the intestinal inflammatory response). In addition, it significantly decreased IL-8 secretion as well as cyclooxygenase-2 (COX-2) activity, as measured by the synthesis of prostaglandin-E2 (PGE2) upon incubation of the cells with arachidonic acid (Romier-Crouzet *et al.*, 2009).

Pomegranate juice, tannins and punicalagin. Effects of pomegranate juice, total pomegranate tannins and punicalagin on inflammatory proteins involved in cell signaling cascades were evaluated using HT-29 colon cancer cells stimulated with TNFα. The juice was commercially available (POM Wonderful LLC, Los Angeles, CA) and was used in a concentrated form containing 1.74 g/L punicalagin, while purified punicalagin as well as total pomegranate tannins were isolated from the fruit peel and normalized at equivalent concentrations of those found in the juice. The induced COX-2 protein expression was decreased with all the three preparations, in a dose dependent manner. In addition, the pomegranate juice was shown to significantly inhibit the protein kinase B (AKT) activity, which is known for increasing COX-2 expression (Adams *et al.*, 2006).

4.1.2 *In vitro* effects on immune cells

Other pomegranate anti-inflammatory properties have been highlighted not directly on the intestinal epithelial cells but also on immune cells since both cell types are interacting during the intestinal inflammatory response.

Macrophage cells. In inflammatory events, macrophage cells, stimulated by the pro-inflammatory cytokines secreted by the activated T cells, produce in turn a large amount of

pro-inflammatory mediators (e.g. TNFα, interleukins, and reactive oxygen species (ROS)). RAW 264.7 murine macrophages were used to study the anti-inflammatory properties of 4 ETs isolated from pomegranate peels, namely punicalagin, punicalin, strictinin A, and granatin B (C.J. Lee *et al.*, 2010). Inflammation was induced by 24h pre-incubation with LPS (1 µg/mL), leading to a high secretion of NO and PGE2 as well as to an up-regulation of iNOS and COX-2 protein expression. Inhibitory effects on NO production were observed for the 4 isolated ETs (at 100 µM) with the following decreasing order of intensity: granatin B > strictinin A > punicalagin > punicalin. That NO inhibition was neither due to NO-scavenging activity, nor to iNOS activity inhibition, but was the consequence of a decrease in iNOS protein expression with the strongest effect attributed to granatin B. Only granatin B significantly reduced PGE2 production and COX-2 expression in a dose dependent manner after 8h of treatment, while granatin B showed no effect on COX-2 expression after 18h exposure (C.J. Lee *et al.*, 2010). An inhibition of NO production was also found with a standardized pomegranate peel extract containing 13% w/w EA, in LPS-induced (100 µg/mL) RAW 264.7 cells. This peel extract revealed marked anti-NO effects, equivalent to that of L-nitroarginine, a NO synthase inhibitor, with an IC_{50} (concentration at which 50% of the inhibitory effect is observed) of 10.7 µg/mL. Standard EA was also tested and revealed even higher anti-NO effects with an IC_{50} value of 1.9 µg/mL (Panichayupakaranant *et al.*, 2010).

Mast cells. Mast cells are other key players in the inflammation that release, upon activation, numerous mediators by discharging their granules and/or by synthesizing them. These mediators play a role by recruiting leucocytes to the inflammation site, and by activating many of them to produce their own mediators of inflammation. The anti-inflammatory potential of a standardized pomegranate fruit extract (POMx, POM Wonderful brand) has been evaluated on KU812 cells, a model of human mast cells, which were stimulated with 40 nM of phorbol 12-myristate 13-acetate (PMA) plus 1 µM of calcium ionophore (CI) (Rasheed *et al.,* 2009). The cells were pre-treated with POMx (20-100 µg/mL) for 1h prior to stimulation with PMA-CI for 4h. POMx significantly inhibited IL-6 and IL-8 secretions and decreased the corresponding gene expressions in a dose-dependent manner. Furthermore, POMx attenuated JNKp54/p46 and ERKp44/p42 phosphorylation, but had no effect on p38-MAPK phosphorylation in PMA-CI-induced mast cells. POMx inhibited the PMA-CI-induced degradation of IκBα and nuclear translocation of p65 NF-κB. Finally, POMx significantly inhibited the NF-κB DNA binding activity in KU812 cells transfected with a NF-κB-luciferase construct and exposed to PMA-CI (Rasheed *et al.,* 2009).

T cells. Naïve T cells (Th0) activation initiates an adaptive immune response, and then, plays a central role in the development of autoimmune diseases. In IBDs, effector T cells (Th1, Th2 and Th17) predominate over regulatory T cells (Th3, Tr). These activated T cells secrete pro-inflammatory cytokines (IL-4, IL-5, IL-12, IL-13, IL-17, INFγ, etc.) that stimulate macrophages. Punicalagin was identified as a potent immune suppressant in activated murine splenic CD4+ T cells. Indeed, a 24h exposure of these cells to punicalagin (5 µM) decreased the secretion of IL-2, a protein stimulating growth and differentiation of T cells. A reduction of IL-2 mRNA levels was also observed with 5 µM punicalagin (S.I. Lee *et al.*, 2008). Furthermore, after 6h incubation, punicalagin (2.5-40 µM) significantly inhibited the activation of the nuclear factor activated T cells (NFAT), a transcription factor for IL-2 expression after T cell activation, in a dose-dependent way, in NFAT-Jurkat cells stimulated with PMA (10 ng/mL) and CI (1 µM) (S.I. Lee *et al.*, 2008).

4.2 *Ex vivo* intestinal anti-inflammatory effects of pomegranate PCs

A single dose (34 mg/kg body weight (bw)) of pomegranate fruit extract (POMx, POM Wonderful brand) was orally administrated to experimental rabbits. After 2h, blood samples of the experimental and the control groups were collected in order to test the inhibitory effects of plasma samples on the activities of COXs *ex vivo* by using purified enzyme preparations. Both COX-1 and COX-2 activities were reduced *ex vivo* in presence of the experimental plasma (2h post-supplementation with POMx) *versus* the control plasma (prior supplementation), with a higher effect on COX-2 activity. These results suggested that pomegranate fruit extract component and/or metabolites may inhibit the activity of eicosanoid generating enzymes, and then exert anti-inflammatory effects *ex vivo* (Shukla *et al., 2008).*

4.3 *In vivo* intestinal anti-inflammatory effects of pomegranate phenolic compounds

Although all the *in vitro* studies seem to show anti-inflammatory effects of the pomegranate fruit in intestinal inflammation, its biological activity should be proven *in vivo*.
Among the *in vivo* studies on the effects of pomegranate PCs on intestinal inflammation related to IBDs, four studies conducted on murine models were reported in the literature: three with rats (Larrosa *et al.*, 2010; Ogawa *et al.*, 2002; Rosillo *et al.*, 2011) and one with mice (Singh *et al.*, 2009). Experimental models of IBD were induced by a daily oral administration of dextran sulphate sodium (DSS) at 2-5% (v/v) in drinking water or by intra-colonic administration of trinitrobenzene sulfonic acid (TNBS).

4.3.1 Rat studies

Ellagic acid. To evaluate the prophylactic effects of EA in the treatment of IBDs (Ogawa *et al.*, 2002), EA was administered to rats, as such or in an encapsulated form. The microsphere capsules allowed EA to reach the terminal ileum and colon where microcapsules dissolved. Encapsulated or not, EA was administrated orally twice daily for the last 6 days during the 7 day treatment with DSS to one group of rats. Another DSS-induced colitis rat group received superoxide dismutase (SOD), a well-known anti-oxidative agent, intra-rectally twice daily for the last 6 days. Colonic mucosa of DSS-treated animals showed typical inflammatory changes such as erosions, ulcerations, and infiltration of immune cells. The administration of encapsulated EA (1-10 mg/kg bw) prevented the development of DSS-induced colitis with an effective dose on 50% of rats (ED_{50}) of 2.3 mg/kg bw. The same effects were observed with non-encapsulated EA, but at higher concentrations (ED_{50} 32.9 mg/kg bw). Treatments with EA also prevented the decrease in colon length due to DSS treatment, and again a higher effect was observed with the encapsulated EA. Lipid peroxidation in the colonic mucosa, determined by the thiobarbituric acid-reactive substance (TBARS) assay, was significantly decreased by the encapsulated EA treatment, but was not affected by free EA. Similar effects were observed after SOD administration, supporting the idea that EA prevents the colitis development by radical scavenging or other anti-oxidative actions (Ogawa *et al.*, 2002). EA was orally administrated to rats at both doses (10 and 20 mg/kg bw) 48, 24 and 1h before TNBS intra-colonic administration and 24h after. Both doses of EA significantly decreased the extent and severity of the colonic damage and the leukocyte infiltration, and increased the mucus production by goblet cells. EA decreased COX-2 and iNOS protein expressions induced by TNBS. EA also inhibited both MAPK (ERK, JNK, and p38) pathways by preventing their phosphorylation and NF-κB pathway by preventing the IκBα degradation and the nuclear translocation of p65 in the intestinal epithelial cells from inflamed rat colon (Rosillo *et al.*, 2011).

Pomegranate polyphenolic extract and urolithin A. A DSS-induced colitis rat model was used to study the anti-inflammatory effects of a commercial pomegranate extract ("Nutragranate from Nutracitrus S.L., Elche, Spain), and of urolithin A (see section 3). Rats received a standard diet for 25 days with 5% DSS for the last 5 days. The test groups received the standard diet supplemented either with pomegranate extract (250 mg/kg bw/day) or with urolithin A (15 mg/kg bw/day) (Larrosa *et al.*, 2010). Typical histological changes of inflammation were observed in the colon of DSS-induced colitis rats, together with increases in the prostaglandin E synthase (PTGES) and COX-2 protein levels and in their catalysis product, namely PGE_2, and with an up-regulation of iNOS mRNA expression associated with higher levels of NO in the colon mucosa. Urolithin A supplementation significantly attenuated the histological changes induced by DSS in colon mucosa, while the pomegranate extract showed non-significant attenuations. Both supplementations decreased the PTGES and COX-2 protein levels, and lowered PGE_2 production in the colon mucosa with more efficient effects in the case of the pomegranate extract. In addition, both supplementations allowed to significantly decrease NO levels in colon mucosa (Larrosa *et al.*, 2010).

4.3.2 Mice studies

Pomegranate flower extract and EA-rich fraction. The potential beneficial properties of a pomegranate flower extract and of its EA-rich fraction were evaluated in mice with ulcerative colitis induced by a daily administration of DSS (2%) in drinking water for 7 days (Singh *et al.*, 2009). Treatments were administrated concomitantly with DSS and during a period of 48h before. Colonic tissues were harvested on the day 8 after DSS treatment for macroscopic and biochemical analyses. As expected, DSS administration induced mucosal injuries and reduced colon length. Administration of the pomegranate extract and its EA-rich fraction significantly attenuated most of the DSS-induced macroscopic changes. Body weight loss, stool consistency, and bleeding, were also improved by both treatments, as well as the histopathological changes induced by DSS in the colon. DSS administration was also associated with an increase in myeloperoxidase (MPO) activity from an intense infiltration of neutrophils in the colon in association with an acute inflammation. A decrease of MPO activity in colonic tissues was also observed with both treatments. In addition, both treatments decreased histamine levels in colonic tissues, suggesting a prevention of histamine release. Finally, they also decreased the oxidative stress, measured by the TBARS assay, and the superoxide anion generation, which were significantly increased in DSS–treated mice (Singh *et al.*, 2009).

4.3.3 Human studies

To our knowledge, no human study has yet been conducted to evaluate anti-inflammatory properties of pomegranate in subjects suffering from IBDs. There is a serious need for this kind of studies to validate the purported beneficial effects of pomegranate-derived products. Nevertheless, a study conducted on healthy human subjects showed an increase by 6% of serum antioxidant status, 2h after consumption of a commercial pomegranate juice (POM Wonderful LLC, Los Angeles, CA), and by 11% after a daily consumption of 250 mL of that juice for 1 week (Rosenblat *et al.*, 2010). The increment of antioxidant status and reduction of oxidative damage after pomegranate juice consumption were also observed in another study conducted on healthy elderly subjects who consumed daily 250 mL of juice for 4 weeks (Guo *et al.*, 2008). In physiological inflammation, ROS are scavenged by

substances naturally found in our organism. However, in IBDs, the chronic pathological inflammatory response is characterized by an overproduction of ROS leading to persistent oxidative stress and to functional alterations in DNA, proteins and lipids (Bartsch & Nair, 2006; Kapoor *et al.*, 2005). It can thus be speculated that oxidative status improvements offered by the consumption of pomegranate products can attenuate IBD severity.

5. Effects of pomegranate phenolic compounds on the gut microflora associated to IBD pathogenesis

The human gut microflora is estimated to contain 10^{18} of microorganisms (Davis & Milner, 2009) with about 10^{14} bacteria classified within 4 bacterial phyla, namely Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria (Seksik, 2010). Gut microflora is composed of beneficial bacteria (like *Bifidobacterium* spp. from the Actinobacteria phylum, and *Lactobacillus* spp. from the Firmicutes phylum), but also comprises deleterious bacteria (like certain members of *Clostridium* spp. or *Staphylococcus* spp. from the Firmicutes phylum). Archae, fungi and protozoa also make up the human gut microflora but little is known about their activities. The gut microflora is a critical component in the development and prevention/treatment of IBDs and its composition differs in IBD patients compared to healthy patients (Swidsinski *et al.*, 2002). Nutrients, such as PCs, may influence that composition by enhancing or depleting the growth of beneficial bacteria, and by increasing or decreasing deleterious bacteria (Laparra & Sanz, 2010). Changes of gut microflora composition after consumption of pomegranate PCs have thus been investigated in different ways. Several studies have highlighted the antimicrobial effects of pomegranate extracts or PCs on isolated bacteria or fungi. A pomegranate peel extract showed antimicrobial effects (evaluated by measuring the zone of inhibition (IZ)) against different multi-drug resistant pathogenic organisms according to the following decreasing order: *Staphylococcus aureus*, *Salmonella paratyphi, Shigella dysenteriae* > *Candida albicans* > *Bacillus subtilis*, *Escherichia coli* (Ahmad & Beg, 2001). EA, gallagic acid, punicalin, and punicalagin, which were isolated from a pomegranate peel extract, were also evaluated for their antimicrobial activities against pathogenic fungi (*C. albicans, Cryptococcus neoformans,* and *Aspergillus fumigatus*), a non-pathogenic strain of *E. coli,* and pathogenic bacteria (*Pseudomonas aeruginosa* and *Mycobacterium intracellulare*). EA and punicalin did not show any antimicrobial activity at the highest concentration tested (20 µg/ml). However, gallagic acid and punicalagin inhibited the growth of *E. coli, P. aeruginosa,* and *C. neoformans* with IC_{50} values lower than 15 µg/mL (Reddy *et al.*, 2007). Another study also highlighted the inhibitory effects of a pomegranate peel extract against antibiotic-resistant pathogenic bacteria and fungi. Significant inhibitory effects were observed against a pathogenic strain of *E. coli,* and the pathogenic *S. aureus*, *B. subtilis, Listeria monocytogenes, P. aeruginosa* and *Yersinia enterocolitica.* An antifungal activity was also observed against *Candida utilis, Saccharomyces cerevisae,* and *Aspergillus niger* (Al-Zoreky, 2009). The effects of the standardized commercial pomegranate peel extract POMx and of pure punicalagin, punicalin, EA, and gallic acid were evaluated on the growth of intestinal bacteria in liquid cultures. POMx and punicalagin inhibited the growth of *S. aureus* and of *Clostridium* spp.. Interestingly enough, the growths of the probiotic *Lactobacillus* spp. and *Bifidobacteria* spp. were relatively unaffected neither by the extract POMx nor by the pure polyphenols, except for the growths of the probiotics *Bifidobacterium breve* and *Bifidobacterium infantis,* which were significantly enhanced by POMx. In this study, POMx application resulted in a decrease of pH media that could

partially explain its inhibition towards pathogenic bacteria that are more susceptible to low pH than probiotic bacteria (Bialonska *et al.*, 2009). Another pomegranate peel extract was evaluated for its antibacterial activity towards *Propionibacterium acnes, S. aureus*, *Staphylococcus epidermidis*, *E. coli*, *Salmonella typhimurium*, *Salmonella typhi* and *Shigella sonnei*. After exposure to pomegranate peel extract (200 mg/mL), inhibitory effects were observed against the deleterious gram-positive bacteria *P. acnes, S. aureus* and *S. epidermidis*, while no inhibitory effect was seen on the non-pathogenic gram-negative bacteria *E. coli*, and on the pathogenic gram-negative *S. typhimurium*, *S. typhi* , and *S. sonnei* (Panichayupakaranant *et al.*, 2010). Normal human gut microflora also contains harmless saprophyte yeasts, like *Candida* spp., in a normal gastrointestinal tract. However, if the immune defenses are compromised, these yeasts can cause infections. In a recent study, *Candida* spp. were incubated with punicalagin or a pomegranate peel extract (Endo *et al.*, 2010).

Punicalagin showed strong antifungal activities against *C. albicans* and *Candida parapsilosis*, while the pomegranate peel extract did not show any antifungal activity. In the same study, the pomegranate peel extract was also tested against *E. coli, P. aeruginosa, B. subtilis,* and *S. aureus* and showed inhibitory effects only against *S. aureus*. The antimicrobial and antifungal activities of pomegranate PCs are summarized in **Table 1.**

The antimicrobial potential of pomegranated-derived products has also been tested in feces, which represent a more complex model than isolated bacteria or fungi. Bialonska *et al.* (2010) investigated the potential antibacterial activities of POMx and punicalagin on bacteria present in fecal samples obtained from 3 healthy human subjects without any gastrointestinal disease history. The samples were used to inoculate batch-culture vessels and both POMx and punicalagin were added under anaerobic conditions. Bacterial enumeration was assessed by a fluorescence *in situ* hybridization technique (FISH) using ribosomal RNA-targeted oligonucleotide probes for *Bifidobacterium* spp., *Lactobacillus-Enterococcus* group, *Clostridium coccoides-Eubacterium rectale* group, *Clostridium histolyticum* group, as well as for the totality of bacteria. POMx significantly increased the number of *Bifidobacterium* spp., as well as the *Lactobacillus-Enterococcus* group and the total number of bacteria. By contrast, it did not change the growth of the commensal *Clostridium coccoides-Eubacterium rectale* group and *C. histolyticum* group. Punicalagin had no significant effect on any bacteria (Bialonska *et al.*, 2010). Another study (Larrosa *et al.*, 2010) investigated the effects of Nutragranate and urolithin A (see section 3) on the gut microflora composition in DSS-induced colitis rats *vs.* control rats. Both supplementations for 10 days resulted in an increase of *Bifidobacterium* spp, *Lactobacillus* spp. and *Clostridium* spp. in the control rats. After DSS administration for 5 days, the increases of *Bifidobacterium* spp, *Lactobacillus* spp. and *Clostridium* spp were maintained in rats fed with urolithin A, but were reduced in rats eating the pomegranate extract. In addition, significant increases of *E. coli*, of the whole *Enterobacteriaceae* family and of total aerobic bacteria were observed in the DSS-induced colitis model (without any supplementation). These increases were significantly lower in the groups supplemented with the pomegranate extract or urolithin A. The inflammatory status induced by DSS generated differences in the metabolism of pomegranate PCs. As such, in the healthy rats eating the pomegranate extract, the expected metabolite urolithin A was significantly recovered in feces (190 µg/g), but not ETs or EA. In the DSS-treated rats eating the pomegranate extract, EA was predominant in feces, while urolithin A was detected in much lower quantities (8 µg/g). These differences can be put in parallel with the microbial effects observed and suggest that urolithin A formation in the gut lumen is an important

Pomegrenate-derived products	Antimicrobial activity of pomegranate-derived products								
	PPE [1]	**PPE [2]**	**PPE [3]**	**PPE [4]**	**POMx [5]**	**Gallic acid [5]**	**EA [5]**	**Punicalin [5]**	**Punicalagin [5]**
Dose tested	8 mg/mL	150 mg/mL	200 mg/mL		0.01% (v/v)	0.05% (v/v)	0.05% (v/v)	0.05% (v/v)	0.05% (v/v)
Microbia (CFU)	10^6	10^4	10^8	10^6	10^8	10^8	10^8	10^8	10^8
BACTERIA									
Firmicutes									
Staphylococcus aureus	IZ: 13 mm MIC: 2 mg/ml	IZ: 31-40 mm	IZ: 16-19 mm MIC: 7.8 µg/ml	MIC: 125 µg/ml	-3%[a] *	97% [a]	75% [a]	86% [a]	-27% [a] *
Staphylococcus epidermidis			IZ: 19 mm MIC: 15.6 µg/ml						
Bacillus subtilis	IZ: 17 mm MIC: 0.5 mg/ml	IZ: 10-20 mm							
Listeria monocytogenes	IZ: 20 mm								
Lactobacillus acidophilus					83% [a] *	126% [a]	66% [a] *	131% [a]	95% [a]
Lactobacillus casei ssp. casei					81% [a]	102% [a]	67% [a] *	101% [a]	70% [a]
Lactobacillus paracasei ssp. paracasei					90% [a]	110% [a]	79% [a] *	107% [a]	81% [a]

Lactobacillus pentosus		88% [a] *	107% [a]	77% [a] *	109% [a]	86% [a]
Lactobacillus rhamnosus		79% [a] *	105% [a]	74% [a] *	108% [a]	82% [a]
Bifidobacterium breve		275% [a] *	112% [a]	81% [a]	121% [a]	130% [a] *
Bifidobacterium infantis		241% [a] *	99% [a]	122% [a]	106% [a]	106% [a]
Bifidobacterium longum		99% [a]	96% [a]	93% [a]	121% [a]	68% [a]
Bifidobacterium bifidum		83% [a] *	83% [a] *	114% [a]	96% [a]	86% [a]
Bifidobacterium animalis ssp. lactis		112% [a]	109% [a]	52% [a] *	78% [a] *	78% [a] *
Clostridium perfringens		-13% [a] *	46% [a]	0% [a] *	90% [a]	-26% [a] *
Clostridium clostriidoforme		58% [a]	114% [a]	0% [a] *	103% [a]	0% [a] *
Clostridium ramosum		0% [a] *	70% [a]	26% [a] *	65% [a]	-16% [a] *
Bacteroidetes						
Bacteroides fragilis		73% [a]	107% [a]	24% [a] *	117% [a]	83% [a]
Actinobacteria						
Propionibacterium acnes	IZ: 22 ± 2 mm MIC: 15.6 µg/ml					

Pomegrenate-derived products	PPE [1]	PPE [2]	PPE [3]	PPE [4]	POMx [5]	Gallagic acid [6]	EA [6]	Punicalin [6]	Punicalagin [6]
Dose tested	8 mg/mL	150 mg/mL	200 mg/mL		0.01% (v/v)	20 µg/ml	20 µg/ml	20 µg/ml	20 µg/ml
Microbia (CFU)	10^6	10^4	10^8	10^6	n.d.	n.d.	n.d.	n.d.	n.d.
Actinobacteria									
Mycobacterium intracellulare						/	/	/	/
Proteobacteria									
Salmonella paratyphi		IZ: 31-40 mm							
Salmonella enteritidis	MIC : 4 mg/ml								
Salmonella typhimurium			/						
Salmonella typhi			/						
Shigella dysenteriae		IZ: 31-40 mm							
Shigella sonnei			/						
Escherichia coli	IZ: 16 mm MIC : 1 mg/ml	IZ: 10-20 mm	/			IC_{50}: 15 µg/ml	/	/	IC_{50}: 10 µg/ml
Pseudomonas aeruginosa	IZ: 18 mm					IC_{50}: 6.0 µg/ml	/	/	IC_{50}: 3.5 µg/ml

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Yersinia enterocolitica	IZ: 19 mm MIC : 0.25 mg/ml				
FUNGI					
Candida albicans		IZ: 21-30 mm	MIC: 3.9 µg/ml	/	/
Candida parapsilosis			MIC: 3.9 µg/ml		
Candida utilis	IZ: 18 mm				
Cryptococcus neoformans				IC_{50}: 10 µg/ml MIC: 20 µg/ml	/
Aspergillus fumigatus				/	/
Aspergillus niger	IZ: 12 mm				
Saccharomyces cerevisae	IZ: 14 mm				

Abbreviations: CFU, colony forming unit; IZ, inhibitory zone; MIC, minimum inhibitory concentration; n.d., no da
extract (POM Wonderful brand); PPE, pomegranate peel extract.
[a.] Means of human gut bacteria growth (with PCs) compared to means of control growth (significant differences
0.05))
/ : No inhibitory activity
[1.]Al-Zoreky, 2009; [2.]Ahmad & Beg, 2001; [3.]Panichayupakaranant *et al.*, 2010; [4.]Endo *et al.*, 2010; [5.]Bialonska *et al.*, 2

Table 1. Antimicrobial effects of pomegranate or PCs on isolated microorganisms.

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step for the pomegranate extract to affect the gut microflora composition (Larrosa *et al.*, 2010). The effect of pomegranate extracts or of their derived PCs on the gut microbiota can occur at different levels and could partially explain their beneficial role in IBDs. First, tannins are known to complex enzymes, in particular those secreted by the gut microbiota, leading to changes in their structural conformation and thereby inhibiting their enzymatic activities. Furthermore, tannins might form complexes with proteins of cell walls, and by that way, could result in the decrease of both cell permeability and substrate transport into cells. Tannins may also form stable complexes with metal ions (*e.g.*, Fe and Cu), resulting in the decrease of their availability to bacteria and therefore affecting the activity of their metalloenzymes. Finally, as already mentioned, another effect of pomegranate extracts could be the decrease of pH within the intestinal lumen. Most of the time, a low pH favors probiotic bacteria, while deleterious bacteria would be more affected by acidic conditions (Puupponen-Pimia *et al.*, 2005).

6. Conclusion

Plant-derived secondary metabolites are the basis for many drugs or food supplements currently used to treat or prevent pathologic conditions. Since recent research works have shown that the repeated oral administration of high doses of pomegranate to rats and mice was not toxic (Cerdá *et al.*, 2003a, Patel *et al.*, 2008), it is expected that pomegranate peel polyphenolic extracts do not show any severe toxicity in humans. However, further investigations are needed to confirm its safety in humans. The pomegranate peel is a by-product of the pomegranate juice industry that contains high amounts of ETs. When the pomegranate is ingested, these ETs are metabolized mainly in active urolithins by the gut microflora but a lower metabolization occurred in inflamed conditions. In addition to its high antioxidant activity, pomegranate presents anti-inflammatory properties potentially interesting against IBDs by acting on several mechanisms involved in the intestinal inflammatory response. These mechanisms include the interaction with the NF-κB and MAPK cascade pathways, the reduction of the mRNA expression and protein secretion of various pro-inflammatory cytokines, the decrease of the inducible isoforms of iNOS and COX-2 and their resulting products NO and PGE2, known to participate in an increase of the inflammatory status. It also improves the luminal microbiota composition. These different effects were mainly observed in *in vitro* models of intestinal epithelial and immune cells. Results generated *in vivo* go in the same direction, *i.e.* pomegranate PCs can decrease the inflammatory status of animals with induced-inflammation. However, the number of animal studies remains quite limited and to our knowledge, no one human study has been published yet in a peer-reviewed journal. Therefore, since increasing evidence *in vitro* and *in vivo* converge to indicate beneficial effects of pomegranate PCs on intestinal inflammation, further *in vivo* studies are necessary and trials on human subjects should be designed to investigate the pomegranate potential and especially that of pomegranate peel extracts on IBDs.

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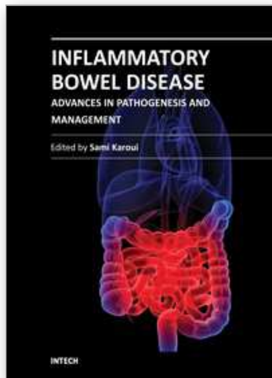

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This book is dedicated to inflammatory bowel disease, and the authors discuss the advances in the pathogenesis of inflammatory bowel disease, as well as several new parameters involved in the etiopathogeny of Crohn's disease and ulcerative colitis, such as intestinal barrier dysfunction and the roles of TH 17 cells and IL 17 in the immune response in inflammatory bowel disease. The book also focuses on several relevant clinical points, such as pregnancy during inflammatory bowel disease and the health-related quality of life as an end point of the different treatments of the diseases. Finally, advances in management of patients with inflammatory bowel disease are discussed, especially in a complete review of the recent literature.

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