Nutrition & Food Science
International Journal
ISSN 2474-767X

Juniper Publishers
key to the Researchers

Mini review
Volume 9 Issue 4 - October 2019
DOI: 10.19080/NFSIJ.2019.09.555766

Nutri Food Sci Int J

Pomegranate's Ellagitannins: Metabolism and Mechanisms of Health Promoting Properties

Paolo Silacci* and Marco Tretola

Agroscope, 1725 Posieux, Switzerland

Submission: October 21, 2019; **Published:** October 30, 2019

***Corresponding author:** Paolo Silacci, Animal Biology Research group, Agroscope, 1725 Posieux, Switzerland

Abstract

The health benefits of pomegranate consumption have been known since the antiquity. More recently, the biological activities of pomegranate, including its anti-bacterial, antioxidant, anti-inflammatory and anticarcinogenic properties, have raised great interests in the scientific community and they have been the subject of a large number of scientific studies. Several phenolic compounds are present in various parts of the fruit, of which ellagitannins, hydrolysable tannins considered responsible for most health benefits of pomegranate. Despite all these efforts, the actual effectors remain unidentified and the mechanisms of action only partially elucidated. This is probably due to the complex catabolic pathway of these molecules that takes place in the gastrointestinal tract, involving both microbial and cellular modifications before entering the bloodstream. The diversity of the intestinal microbiota in the human population leads to the production of different metabolites in individuals, contributing to the heterogeneity of the response to dietary pomegranate assumption. This complex situation determines the need for special attention in designing studies on the action of ellagitannins on both cell cultures and animal models.

Keywords: Pomegranate; Ellagitannins; Ellagic acid; Punicalagin; Urolithins; Human health; Inflammation

Abbreviations: EA: Ellagic Acid; HO-1: Heme Oxygenase 1; Keap1: Kelch-like ECH-Associated Protein 1; MDF: Mucin-Depleted Foci; Nrf2: Nuclear Factor Erythroid 2-related Factor 2; Uros: Urolithins

Introduction

Pomegranate *(Punica granatum L.)* is a fruit native to Asian countries that grows mainly in the Mediterranean region. Since ancient times, pomegranate has been known for its medicinal properties [1]. Most of those beneficial effects have been attributed to the pomegranate metabolites such as polyphenols, with a particular focus on hydrolysable tannins [2]. They can be classified as gallotannins and ellagitannins, which are present in the pericarp of the fruit [3]. The difference in these two categories lies in the different phenolic groups that are esterified to hydroxyl groups of glucose molecules, namely gallic acid in gallotannins and hexahydroxydiphenic acid in ellagitannins [4]. Commercial pomegranate juice is enriched with ellagitannins because it is obtained from the whole fruit. More than 60 hydrolysable tannins have been found in the fruit peel, arils and membranous walls [5]. In particular, punicalagin is the predominant form of the hydrolysable tannins present in pomegranate [2] and is responsible for more than 90% of antioxidant bioactivity contained in pomegranate juice [6]. The amount of punicalagin present in the juice contributes from 5 to 30% of the total phenolic compounds content. Its concentration strongly depends on the geographical origin of the fruit, the type of cultivar (organic or conventional) and the treatment process used for preparation. Normally present at concentrations varying between 100 to 700mg/l, the concentration in industrial processed extracts can reach 1900mg/L [6]. Other hydrolysable tannins present in pomegranate juice include ellagic acid (EA), gallic acid, punicalin and punicafolin, the latter two being particularly present in leaves [7]. The antioxidant properties of pomegranate are of particular interest for a potential therapeutic application in several pathologies characterized by oxidative stress. Partly thanks to their antioxidant activity, pomegranate juice and extracts can attenuate inflammatory processes, inhibit and prevent carcinogenesis, improve diabetes, promote wound healing and be active in many other situations [7-9].

The metabolism of ellagitannins in the gastrointestinal tract is rather complex, with a first hydrolysis of ellagitannins forming EA in the stomach and other decarboxylation reactions carried by the intestinal microbiota in the colon [10]. The diversity in microbiota composition is reflected in the production of different sets of metabolites in individuals. These metabolites are absorbed and chemically modified by enterocytes, before entering the bloodstream and reaching the internal organs. The analysis of the activity of the different metabolites revealed a loss of bioactivity after modification

in the enterocytes, raising several questions about the physiological relevance of studies addressing direct effects of ellagitannins.

The aim of this mini review is to summarize the knowledge on catabolism, tissue distribution of ellagitannins metabolites and in signaling pathways triggered by these molecules to stimulate a cellular response, integrating recent knowledge.

Ellagitannins Metabolism

Dietary absorbed ellagitannins are first hydrolyzed to EA in the small intestine at physiologic alkaline pH [11]. EA is then transformed in the intestine into catabolic intermediates by a series of decarboxylation reactions carried out by bacteria of the intestinal microbiota [10], producing derivatives of dibenzopyran-6-one, also called urolithins (Uro). The complexity of this microbiota, which actively participate in the metabolism of ellagitannins, makes it more difficult to find metabolic pathways of ellagitannins. A two-way interaction exists between ellagitannins and intestinal bacteria. Indeed, polyphenols can modulate the intestinal microbial population [12], itself involved in the transformation of EA into catabolic intermediates [10]. The composition of the bacterial population in the intestine is not homogeneous between individuals and must constantly adapt to changes in the environment caused, for example, by the type of assimilated diet [13,14]. Some pathologies can also have influence or are triggered by imbalances in the intestinal bacterial population (dysbiosis). This diversity is the cause of inter-individual differences in the set of ellagitannins metabolites produced, which may explain the differences in the response observed after consumption of ellagitannins, in terms of health benefits. A study on the ability of individuals to synthetize different Uros after ingestion of walnuts or pomegranate extract capsules revealed the existence of at least three different phenotypes (metabotypes) among the population [15]. This classification is based on the type of Uros present in the urine 24 hours after ingestion. The Uros-content of urine in the three groups is defined as follows: metaboytpe 0 includes those unable to produce Uros; metabotype A includes those able to synthesize only Uro A (Uro-A) and, finally, metabotype B includes those able to synthesize Uros A, B (Uro-B) and isourolithin A (Iso-Uro_A). 60-70% of the cohorts belonged to the metabotype A, 20-30% to metabotype B and the rest to metabotype 0 [15,16]. The identification of intestinal microbiota is far from being complete, but the abundance of two Clostridium strains, C. leptum and C. coccoides has been associated to the ability to produce some Uros [17]. Moreover, intestinal bacterial species from Eggerthellaceae family have been shown to be able to produce some Uros intermediates [18]. As mentioned above, several factors can influence the metabotype. In people with intestinal dysbiosis, the distribution of these three metabotypes was changed [15]. Aging has also been described as one of the major factors influencing Uros-producing microbiota. Until the age of 20, the A metabotype is largely predominant (70-80%) and then declines to values between 50 to 65%. The B and 0 metabotypes are less represented in young people (about 10% each). After the age of 20, the occurrence of B metabotype increases to stabilize around 35 to 45%, while the occurrence of 0 metabotype is less likely to change over life course and remains limited to 10% [19].

The descending colon has recently been identified as the primary site of ellagitannins catabolism by intestinal microbiota, using a gastrointestinal simulation model (TWIN-SHAME) coupled with absorption studies with colon adenocarcinoma-derived cell line Caco-2 [20]. Once formed, Uros are absorbed by the enterocytes and undergo a complex process of phase II-modification including methylation, sulfation and glucuronidation, which make Uros molecules more soluble and suitable to enter bloodstream and to reach internal organs where they can exert different effects [10]. The distribution of ellagitannin-metabolites in tissues was first analyzed in Iberian pigs fed with food containing ellagitannins. Thirty-one different metabolites were detected in plasma and tissues, 26 of which were conjugated. Uro-A was the only metabolite found in feces [11]. In human patients affected by colorectal cancer, the presence of Uros after dietary pomegranate juice absorption was detected in normal intestinal tissue sections of patients with colorectal cancer. Lower concentrations of Uros were also detected in malignant tissue sections [21]. Finally, concentrations of Uro-A glucuronide, Uro-B glucuronide and dimethyl EAs were also measured in human prostate gland of patients with benign prostate hyperplasia and patients with prostate cancer [22]. More recently, in normal and malignant tissues from patients with breast cancer ingesting capsules containing pomegranate extract, only phase II-conjugates Uros have been detected [23]. This complex metabolic pathway suggests that the action of pomegranate on systemic tissues is mediated by conjugated metabolites. However, recent studies showed that conjugation process weakens the bioactivity of these molecules [23,24]; an observation, which raises questions about the mediator of the effects of ellagitannins action and which also raises some concerns on the physiological relevance of in vitro studies directly applying ellagitannins or its unconjugated metabolites on cells. A possible explanation of this paradox may be based on the observation that an increase in glucuronidase activity occurs in systemic tissues under inflammatory conditions [25]. This process of reversal of conjugation has been described for Uro-A, suggesting that an increase in plasma levels of glucuronide-conjugated Uro-A may nevertheless result in a local release of free Uro-A as a consequence of glucuronidase action [25], thus allowing metabolites to exert their bioactivity. A long-term action of conjugated metabolites on cancer cells metabolism is also not excluded.

Given the rapid transformation of ellagitannins into EA in

How to cite this article: Paolo Silacci, Marco Tretola. Pomegranate's Ellagitannins: Metabolism and Mechanisms of Health Promoting Properties. Nutri Food Sci Int J. 2019. 9(4): 555766. DOI: 10.19080/NFSIJ.2019.09.555766

the gastrointestinal tract, the relevance of direct application of ellagitannins, such as punicalagin, in in vitro enterocyte cultures may also be questioned. Nevertheless, in rats receiving punicalagin as dietary supplement, punicalagin was detected in plasma and feces, suggesting that residual punicalagin may be present in feces and be absorbed by enterocytes or exert its action on enterocytes [26]. The observation that direct ellagitannins treatment can induce apoptosis, a reduction of oxidative stress and an increase in glutamate uptake following upregulation of EAAT3 glutamate-transporter gene expression [27-30], support this hypothesis. Interestingly, cell cycle arrest has also been described for proliferating Caco-2 exposed to EA, Uro-A or Uro-B [24], suggesting that ellagitannins and their metabolites may stimulate similar signaling pathways in enterocytes.

Signaling Pathways involved in Bioactivity of Ellagitannins and their Metabolites

The anti-inflammatory and anticarcinogenic effects of pomegranate ellagitannins are directly related to the antioxidant properties of this fruit [31], but the mechanisms of pomegranate interference with these processes are yet not fully understood.

Activation of NF-kB, a redox-regulated transcription factors, is a key target for ellagitannins anti-inflammatory activities. Under normal non-inflammatory conditions, NF-kB is complexed by IkB protein and retained in the cytoplasm. In response to inflammatory stimuli, such as an increase in reactive oxygen species, mitogen-activated protein kinases (MAPK) are activated. MAPKs phosphorylates another kinase, IKKα/β which in turn phosphorylates IkB. IkB phosphorylation releases the two subunits of NF-kB transcription factor, p50 and p65 which can be activated by phosphorylation and can translocate to the nucleus where they exert their action inducing the gene expression of several inflammatory genes [32]. Pomegranate peel extracts has been shown to inhibit the MAPKs-NF-kB pathway in a rat model of diabetic nephropathy, an end-stage renal disorder [33]. A direct effect on NF-kB activation has also been shown in a rheumatoid arthritis rat model in which dietary supplementation with pomegranate rind extract inhibited synovial NF-kB activation, reducing expression of inflammatory cytokines and infiltration of inflammatory cells [34].

Another target for the antioxidative, anti-inflammatory action of pomegranate extracts is the Nuclear factor erythroid 2-related factor 2 (Nrf2) transcription factor [35,36]. As for NF-kB, Nrf2 is normally retained in the cytoplasm by its negative regulator: Kelch-like ECH-associated protein 1 (Keap1). Inactivation of Keap1 results in nuclear translocation of Nrf2, which can then bind to the ARE: Antioxidant Responsive Element, present in the regulatory region of several antioxidant genes including that of heme oxygenase 1 (HO-1) [37]. Punicalagin has been shown to induce HO-1 gene expression via activation of PI3K/Akt/Nrf2 pathway in LPS-stimulated macrophages [38]. In vivo, it has been reported that pomegranate decreased lipid peroxidation in rats with carbon tetrachloride-induced liver-damage by an activation of AMP-activated protein kinase (AMPK)-Nrf2 signaling pathway [39-41]. In spontaneously hypertensive rats, it reduced mitochondrial superoxide anion levels improving mitochondrial function and it increased HO-1 expression by the same mechanisms [35]. AMPK-Nrf2 pathway was also involved in pomegranate extract-mediated reduction of the oxidative stress in the paraventricular nucleus relieving hypertension [35]. Interestingly, a cross talk between NF-kB and Nrf2 has been previously described. Specifically, the cytoplasmic p65 subunit of NF-kB protein can physically associated and retain Keap1, liberating Nrf2 [42]. This provides a "tool" for amplifying protective action of pomegranate polyphenols by the activation of the anti-inflammatory transcription factor Nrf2 and simultaneous inhibition of the pro-inflammatory transcription factor NF-kB.

Potential therapeutic evidence for the use of pomegranate juice or extracts to interfere with cancer pathology has been described. These findings are based on the ability of ellagitannins contained in the pomegranate to interfere with cellular proliferation and apoptotic processes [7]. Breast cancer is the second leading cause for cancer related death in women and the potential therapeutic role of phytochemicals has been intensively investigated [43]. In MCF-7cells, a human breast cancer cell line, pomegranate extract induced the arrest of cell proliferation and promoted pro-apoptotic effects [44, 45]. EA has been shown to arrest proliferation and induce a cell cycle arrest in the G0/G1 phase in MCF-7 cells. This inhibition was mediated by mechanisms involving the activation of TGFβ/Smad3 pathway that down-regulates cyclin A2 and E2, together with an up-regulation of several cyclin-dependent kinase inhibitors, including CDKN1A/p21 (cyclin-dependent kinase inhibitor 1A/p21) protein [46,47]. These mechanisms result in a stop of the cell cycle [48]. The involvement of p21 protein as a target for anticarcinogenic activity of ellagitannins has been demonstrated also using colon carcinoma-derived cell lines. Ellagitannins and/or metabolites present in pomegranate extracts such as punicalagin (used at a concentration of 100µmol/l) inhibit cell proliferation in undifferentiated Caco-2 and HT-29 cells, blocking cell cycle in G0/G1 and G2/S stages [27,28, 49]. Ellagitannins metabolites (EA, Uro-A and Uro-B) have also been shown to affect MAPK signaling pathways leading to a blockade of Caco-2 cell proliferation [24]. The corresponding glucuronide-conjugates failed at once to affect cell proliferation, confirming the negative effect of conjugation on ellagitannins metabolites bioactivities [50]. Cell cycle arrest in Caco-2 cells by ellagitannins metabolites is also mediated by a downregulation of the mIR-224, a microRNA targeting CDKN1A/p21 mRNA and by an activation of the transcription factor TP53 [50]. MicroRNAs are endogenous RNA molecules of

How to cite this article: Paolo Silacci, Marco Tretola. Pomegranate's Ellagitannins: Metabolism and Mechanisms of Health Promoting Properties. Nutri Food Sci Int J. 2019. 9(4): 555766. DOI: 10.19080/NFSIJ.2019.09.555766

20-25 nucleotides in length, which bind to the 3'-untranslated region of target mRNAs causing their degradation [51]. The same study reported a strong heterogeneity in mechanisms triggered by ellagitannins metabolites as a function of the cell line considered. Indeed, levels of TP53 expression induction were not the same in the three cell lines used (Caco-2, SW480 and HT-29) and mIR-224 was only downregulated in Caco-2 cells in response to Uros treatment [50]. Moreover, in SW480 and HT-29, but not in Caco-2, another micro RNA, mIR-215, was upregulated, inducing p53 and p21 accumulation [50] with a subsequent cell cycle arrest [52]. In Caco-2 and SW480 treated with single and mixed metabolites, an increase of c-MYC expression was also observed [50]. The concomitant induction of TP53 and c-MYC favors chemotherapy-induced apoptosis in colon carcinoma cells [53], thus providing evidence for a possible use of ellagitannins metabolites to sensitize tumor cells to anticancer treatment. Finally, the expression of p53 also increased in Uro-A-treated HCT116 human colon cancer cells, determining a stop in G2/M cell cycle phase transition and reducing cellular glycolytic potential [54].

The use of ellagitannins metabolites was also evaluated in prostate cancer. EA have been shown to inhibit proliferation and to induce apoptosis in androgen-dependent, but not in androgen-independent, human prostate cancer cell lines. The anti-proliferative action was mediated by the regulation of the expression of several cell-cycle-related proteins, including CDKN1A/p21, and by the upregulation of the expression of several markers of apoptosis, including caspase-3 [55]. Pro -apoptotic activity has also been described for pomegranate extracts in metastatic prostate cancer cell lines [56]. In these cells, ellagitannins induced an increase in cleavage of poly (ADP-ribose) polymerase and caspase-3, and downregulation of the level of expression of survivin protein [56], an anti-apoptotic protein associated with bone metastasis from prostate cancer [57]. This downregulation was attributed to a reduced activation of Stat3 transcription factor [56], known to bind the survivin gene promoter [58].

Evidence of EA anti-cancer properties have also been obtained in vivo using different animal models. E2-induced mammary carcinogenesis ACI rats, a model of breast cancer, exhibit an aberrant miRNAs expression in breast cancer, resulting in changes in the expression of several target genes participating in the process of carcinogenesis [59]. EA dietary supplementation of E2-induced mammary carcinogenesis in ACI rats reversed aberrant miRNAs expression, reestablishing a normal expression of target genes [60]. Using Pirc rats, a genetic model of colorectal cancer spontaneously developing microscopic pre-neoplastic lesions called mucin-depleted foci (MDF) [61,62], Tortora et al. observed a 30% inhibition of the number and size of MDF after dietary supplementation with pomegranate mesocarp decoction [63]. This inhibitory effect has been attributed to the ability of Uro-A to inhibit the expression of proliferative and inflammatory markers (PCNA, COX-2 and iNOS) and to increase the expression of apoptotic markers (caspase-3) in HT-29 cells. A similar inhibitory effect was observed in Sprague-Dawley rats injected with azoxymethane, a different animal model of colorectal cancer. In this model, the effect was exerted by an increase in the expression of the micro RNA mIR-126 targeting the expression of VCAM1 and PI3Kp85βgenes [64]. Activation of PI3K/Akt – NF-kB pathway was inhibited by pomegranate juice resulting in a decreased VCAM1, COX2, iNOS and VEGF gene expression. Interestingly, these results are consistent with those of a previous study showing a significant decrease in the expression of mIR-126 in colon cancer tissues and metastatic cell lines [65]. Anticarcinogenic properties of pomegranate have also been proven in animal models for prostate cancer. A transgenic rat model for adenocarcinoma of prostate also confirmed caspase-3 involvement in pro-apoptotic action of EA on prostate cancer [55]. In transgenic adenocarcinoma of the mouse prostate mice, another animal model of prostate cancer [66], pomegranate extract significantly reduced the spontaneous onset of palpable abdominal tumors and the number of metastases, in particular in lungs and liver. This inhibitory effect on prostate carcinogenesis determined an increased survival rate of the mice [67]. In the prostate tissue of these mice, clear inhibition of PI3K/Akt/mTor signaling pathway was also observed subsequently to pomegranate extract diet supplementation [67].

Finally, several clinical trials have been conducted to evaluate the therapeutic potential of pomegranate juice or extracts on human prostate cancer. These studies showed that pomegranate derivatives were safe, but their efficacy was limited to a marked increase in prostate-specific antigen concentration doubling time [8].

Conclusion

There is an important public and scientific interest in the health benefits of pomegranate fruit and its derived metabolites. However, several aspects require further investigation. The link between pomegranate consumption and the related effects is represented by ellagitannins and their metabolites. In particular EA, a phenolic lactone produced by hydrolysis of ellagitannins in the gastrointestinal tract and widespread in many fruits and vegetables. Among the many beneficial effects attributed to EA, there are the anti-inflammatory and anticarcinogenic effects, but its bioactivity is not yet well understood. It is known that different Uros can be produced by the gut microbiota starting from EA. However, the isolation and identification of bacteria strains involved in the metabolism of EA in the large intestine is far from being complete. In enterocytes, Uros undergoes an additional modification leading to a loss of bioactivity, which raises several questions on the real benefits of pomegranate consumption. Nevertheless, MAPK, PI3K/Akt, AMPK have been identified as major targets for ellagitannins and metabolites action on NF-

How to cite this article: Paolo Silacci, Marco Tretola. Pomegranate's Ellagitannins: Metabolism and Mechanisms of Health Promoting Properties. Nutri Food Sci Int J. 2019. 9(4): 555766. DOI: 10.19080/NFSIJ.2019.09.555766

kB and Nrf2 transcription, two transcription factors involved, with different roles, in inflammatory processes. Control of cell proliferation and apoptosis appears as the main targets for anticarcinogenic action and it takes place mainly through the regulation of CDKN1A/p21, PCNA and caspases expressions and activations. Cell cycle control by ellagitannins metabolites also involves the expression modulation of specific micro RNAs, but this mechanism appears to be dependent on origin and specific properties of tumor cells. Despite the existing evidence on potential therapeutic action of ellagitannins and their metabolites, given the ability to elicit different cellular responses in different pathological situations, further investigations are required to confirm the real physiological relevance of these observations.

Acknowledgments

We would like to acknowledge Bill Crews for his valuable help in the drafting of the manuscript.

References

1. Longtin R (2003) The pomegranate: nature's power fruit? J Natl Cancer Inst 95(5): 346-348.
2. Dey D, Debnath S, Hazra S, Ghosh S, Ray R, et al. (2012) Pomegranate pericarp extract enhances the antibacterial activity of ciprofloxacin against extended-spectrum beta-lactamase (ESBL) and metallo-beta-lactamase (MBL) producing Gram-negative bacilli. Food Chem Toxicol 50(12): 4302-4309.
3. Ben Nasr C, Ayed N, Metche M (1996) Quantitative determination of the polyphenolic content of pomegranate peel. Z Lebensm Unters Forsch 203(4): 374-378.
4. Liu Y, Seeram NP (2018) Liquid chromatography coupled with time-of-flight tandem mass spectrometry for comprehensive phenolic characterization of pomegranate fruit and flower extracts used as ingredients in botanical dietary supplements. J Sep Sci 41(15): 3022-3033.
5. Howell AB, D Souza DH (2013) The pomegranate: effects on bacteria and viruses that influence human health. Evid Based Complement Alternat Med 2013: 606212.
6. Gil MI, Tomas-Barberan FA, Hess-Pierce B, Holcroft DM, Kader AA (2000) Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem 48(10): 4581-4589.
7. Syed DN, Chamcheu JC, Adhami VM, Mukhtar H (2013) Pomegranate extracts and cancer prevention: molecular and cellular activities. Anticancer Agents Med Chem 13(8): 1149-1161.
8. Paller CJ, Pantuck A, Carducci MA (2017) A review of pomegranate in prostate cancer. Prostate Cancer Prostatic Dis 20(3): 265-270.
9. Singh B, Singh JP, Kaur A, Singh N (2018) Phenolic compounds as beneficial phytochemicals in pomegranate (Punica granatum L.) peel: A review. Food Chem 261: 75-86.
10. Espin JC, Larrosa M, Garcia-Conesa MT, Tomas-Barberan F (2013) Biological significance of urolithins, the gut microbial ellagic Acid-derived metabolites: the evidence so far. Evid Based Complement Alternat Med 2013: 270418.
11. Espin JC, Gonzalez-Barrio R, Cerda B, Lopez-Bote C, Rey AI, et al. (2007) Iberian pig as a model to clarify obscure points in the bioavailability and metabolism of ellagitannins in humans. J Agric Food Chem 55(25): 10476-10485.
12. Bialonska D, Ramnani P, Kasimsetty SG, Muntha KR, Gibson GR, et al. (2010) The influence of pomegranate by-product and punicalagins on selected groups of human intestinal microbiota. Int J Food Microbiol 140(2-3): 175-182.
13. Fan Y, Zhang J (2019) Dietary Modulation of Intestinal Microbiota: Future Opportunities in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis. Front Microbiol 10: 740.
14. Tang C, Ding R, Sun J, Liu J, Kan J, et al. (2019) The impacts of natural polysaccharides on intestinal microbiota and immune responses- a review. Food Funct 10(5): 2290-2312.
15. Tomas-Barberan FA, Garcia-Villalba R, Gonzalez-Sarrias A, Selma MV, Espin JC (2014) Ellagic acid metabolism by human gut microbiota: consistent observation of three urolithin phenotypes in intervention trials, independent of food source, age, and health status. J Agric Food Chem 62(28): 6535-6538.
16. Tulipani S, Urpi-Sarda M, Garcia-Villalba R, Rabassa M, Lopez-Uriarte P, et al. (2012) Urolithins are the main urinary microbial-derived phenolic metabolites discriminating a moderate consumption of nuts in free-living subjects with diagnosed metabolic syndrome. J Agric Food Chem 60(36): 8930-8940.
17. Garcia-Villalba R, Beltran D, Espin JC, Selma MV, Tomas-Barberan FA (2013) Time course production of urolithins from ellagic acid by human gut microbiota. J Agric Food Chem 61(37): 8797-8806.
18. Selma MV, Beltran D, Luna MC, Romo-Vaquero M, Garcia-Villalba R, et al. (2017) Isolation of Human Intestinal Bacteria Capable of Producing the Bioactive Metabolite Isourolithin A from Ellagic Acid. Front Microbiol 8: 1521.
19. Cortes-Martin A, Garcia-Villalba R, Gonzalez-Sarrias A, Romo-Vaquero M, Loria-Kohen V, et al. (2018) The gut microbiota urolithin metabotypes revisited: the human metabolism of ellagic acid is mainly determined by aging. Food Funct 9(8): 4100-4106.
20. Garcia-Villalba R, Vissenaekens H, Pitart J, Romo-Vaquero M, Espin JC, et al. (2017) Gastrointestinal Simulation Model TWIN-SHIME Shows Differences between Human Urolithin-Metabotypes in Gut Microbiota Composition, Pomegranate Polyphenol Metabolism, and Transport along the Intestinal Tract. J Agric Food Chem 65(27): 5480-5493.
21. Nunez-Sanchez MA, Karmokar A, Gonzalez-Sarrias A, Garcia-Villalba R, Tomas-Barberan FA, et al. (2016) *In vivo* relevant mixed urolithins and ellagic acid inhibit phenotypic and molecular colon cancer stem cell features: A new potentiality for ellagitannin metabolites against cancer. Food Chem Toxicol 92: 8-16.
22. Gonzalez-Sarrias A, Gimenez-Bastida JA, Garcia-Conesa MT, Gomez-Sanchez MB, Garcia-Talavera NV, et al. (2010) Occurrence of urolithins, gut microbiota ellagic acid metabolites and proliferation markers expression response in the human prostate gland upon consumption of walnuts and pomegranate juice. Mol Nutr Food Res 54(3): 311-322.
23. Avila-Galvez MA, Garcia-Villalba R, Martinez-Diaz F, Ocana-Castillo B, Monedero-Saiz T, et al. (2019) Metabolic Profiling of Dietary Polyphenols and Methylxanthines in Normal and Malignant Mammary Tissues from Breast Cancer Patients. Mol Nutr Food Res 63(9): e1801239.
24. Gonzalez-Sarrias A, Espin JC, Tomas-Barberan FA, Garcia-Conesa MT (2009) Gene expression, cell cycle arrest and MAPK signalling regulation in Caco-2 cells exposed to ellagic acid and its metabolites, urolithins. Mol Nutr Food Res 53(6): 686-698.
25. Avila-Galvez MA, Gimenez-Bastida JA, Gonzalez-Sarrias A, Espin JC (2019) Tissue deconjugation of urolithin A glucuronide to free urolithin A in systemic inflammation. Food Funct 10(6): 3135-3141.
26. Cerda B, Llorach R, Ceron JJ, Espin JC, Tomas-Barberan FA, et al. (2003) Evaluation of the bioavailability and metabolism in the rat of punicalagin, an antioxidant polyphenol from pomegranate juice. Eur J Nutr

How to cite this article: Paolo Silacci, Marco Tretola. Pomegranate's Ellagitannins: Metabolism and Mechanisms of Health Promoting Properties. Nutri Food Sci Int J. 2019. 9(4): 555766. DOI: 10.19080/NFSIJ.2019.09.555766

42(1): 18-28.

27. Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, et al. (2005) *In vitro* antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. J Nutr Biochem 16(6): 360-367.

28. Larrosa M, Tomas-Barberan FA, Espin JC (2006) The dietary hydrolysable tannin punicalagin releases ellagic acid that induces apoptosis in human colon adenocarcinoma Caco-2 cells by using the mitochondrial pathway. J Nutr Biochem 17(9): 611-625.

29. Kojadinovic MI, Arsic AC, Debeljak-Martacic JD, Konic-Ristic AI, Kardum ND, et al. (2017) Consumption of pomegranate juice decreases blood lipid peroxidation and levels of arachidonic acid in women with metabolic syndrome. J Sci Food Agric 97(6): 1798-1804.

30. Biolley C, Tretola M, Bee G, Jud C, Silacci P, et al. (2019) Punicalagin increases glutamate absorption in differentiated Caco-2 cells by a mechanism involving gene expression regulation of an EAAT3 transporter. Food Funct 10(9): 5333-5338.

31. Kaneyuki T, Noda Y, Traber MG, Mori A, Packer L (1999) Superoxide anion and hydroxyl radical scavenging activities of vegetable extracts measured using electron spin resonance. Biochem Mol Biol Int 47(6): 979-989.

32. Liu T, Zhang L, Joo D, Sun SC (2017) NF-kappaB signaling in inflammation. Signal Transduct Target Ther 2.

33. Manna K, Mishra S, Saha M, Mahapatra S, Saha C, et al. (2019) Amelioration of diabetic nephropathy using pomegranate peel extract-stabilized gold nanoparticles: assessment of NF-kappaB and Nrf2 signaling system. Int J Nanomedicine 14: 1753-1777.

34. Karwasra R, Singh S, Sharma D, Sharma S, Sharma N, et al. (2019) Pomegranate supplementation attenuates inflammation, joint dysfunction via inhibition of NF-kappaB signaling pathway in experimental models of rheumatoid arthritis. J Food Biochem 43(8): e12959.

35. Sun W, Yan C, Frost B, Wang X, Hou C, Zeng M, et al. (2016) Pomegranate extract decreases oxidative stress and alleviates mitochondrial impairment by activating AMPK-Nrf2 in hypothalamic paraventricular nucleus of spontaneously hypertensive rats. Sci Rep 6: 34246.

36. Bishayee A, Bhatia D, Thoppil RJ, Darvesh AS, Nevo E, et al. (2011) Pomegranate-mediated chemoprevention of experimental hepatocarcinogenesis involves Nrf2-regulated antioxidant mechanisms. Carcinogenesis 32(6): 888-896.

37. Nguyen T, Nioi P, Pickett CB (2009) The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J Biol Chem 284(20): 13291-13295.

38. Xu X, Li H, Hou X, Li D, He S, et al. (2015) Punicalagin Induces Nrf2/HO-1 Expression via Upregulation of PI3K/AKT Pathway and Inhibits LPS-Induced Oxidative Stress in RAW264.7 Macrophages. Mediators Inflamm 2015: 380218.

39. Wei XL, Fang RT, Yang YH, Bi XY, Ren GX, et al. (2015) Protective effects of extracts from Pomegranate peels and seeds on liver fibrosis induced by carbon tetrachloride in rats. BMC Complement Altern Med 15: 389.

40. Turk G, Ceribasi S, Sonmez M, Ciftci M, Yuce A, et al. (2016) Ameliorating effect of pomegranate juice consumption on carbon tetrachloride-induced sperm damages, lipid peroxidation, and testicular apoptosis. Toxicol Ind Health 32(1): 126-137.

41. Pirinccioglu M, Kizil G, Kizil M, Kanay Z, & Ketani A (2014) The protective role of pomegranate juice against carbon tetrachloride-induced oxidative stress in rats. Toxicol Ind Health 30(10): 910-918.

42. Ganesh Yerra V, Negi G, Sharma SS, Kumar A (2013) Potential therapeutic effects of the simultaneous targeting of the Nrf2 and NF-kappaB pathways in diabetic neuropathy. Redox Biol 1: 394-397.

43. Libson S, Lippman M (2014) A review of clinical aspects of breast cancer. Int Rev Psychiatry 26(1): 4-15.

44. Shirode AB, Bharali DJ, Nallanthighal S, Coon JK, Mousa SA, et al. (2015) Nanoencapsulation of pomegranate bioactive compounds for breast cancer chemoprevention. Int J Nanomedicine 10: 475-484.

45. Jeune MA, Kumi-Diaka J, Brown J (2005) Anticancer activities of pomegranate extracts and genistein in human breast cancer cells. J Med Food 8(4): 469-475.

46. Chen HS, Bai MH, Zhang T, Li GD, Liu M (2015) Ellagic acid induces cell cycle arrest and apoptosis through TGF-beta/Smad3 signaling pathway in human breast cancer MCF-7 cells. Int J Oncol 46(4): 1730-1738.

47. Zhang T, Chen HS, Wang LF, Bai MH, Wang YC, et al. (2014) Ellagic acid exerts anti-proliferation effects via modulation of Tgf-beta/Smad3 signaling in MCF-7 breast cancer cells. Asian Pac J Cancer Prev 15(1): 273-276.

48. Maiti B, Li J, de Bruin A, Gordon F, Timmers C, et al. (2005) Cloning and characterization of mouse E2F8, a novel mammalian E2F family member capable of blocking cellular proliferation. J Biol Chem 280(18): 18211-18220.

49. Kasimsetty SG, Bialonska D, Reddy MK, Ma G, Khan SI, et al. (2010) Colon cancer chemopreventive activities of pomegranate ellagitannins and urolithins. J Agric Food Chem 58(4): 2180-2187.

50. Gonzalez-Sarrias A, Nunez-Sanchez MA, Tome-Carneiro J, Tomas-Barberan FA, Garcia-Conesa MT, et al. (2016) Comprehensive characterization of the effects of ellagic acid and urolithins on colorectal cancer and key-associated molecular hallmarks: MicroRNA cell specific induction of CDKN1A (p21) as a common mechanism involved. Mol Nutr Food Res 60(4): 701-716.

51. Garzon R, Croce CM (2011) MicroRNAs and cancer: introduction. Semin Oncol 38(6): 721-723.

52. Braun CJ, Zhang X, Savelyeva I, Wolff S, Moll UM, et al. (2008) p53-Responsive micrornas 192 and 215 are capable of inducing cell cycle arrest. Cancer Res 68(24): 10094-10104.

53. Arango D, Corner GA, Wadler S, Catalano PJ, Augenlicht LH (2001) c-myc/p53 interaction determines sensitivity of human colon carcinoma cells to 5-fluorouracil in vitro and in vivo. Cancer Res 61(12): 4910-4915.

54. Norden E, Heiss EH (2019) Urolithin A gains in antiproliferative capacity by reducing the glycolytic potential via the p53/TIGAR axis in colon cancer cells. Carcinogenesis 40(1): 93-101.

55. Naiki-Ito A, Chewonarin T, Tang M, Pitchakarn P, Kuno T, et al. (2015) Ellagic acid, a component of pomegranate fruit juice, suppresses androgen-dependent prostate carcinogenesis via induction of apoptosis. Prostate 75(2): 151-160.

56. Wang Y, Zhang S, Iqbal S, Chen Z, Wang X, et al. (2013) Pomegranate extract inhibits the bone metastatic growth of human prostate cancer cells and enhances the in vivo efficacy of docetaxel chemotherapy. Prostate .

57. Akfirat C, Zhang X, Ventura A, Berel D, Colangelo ME, et al. (2013) Tumour cell survival mechanisms in lethal metastatic prostate cancer differ between bone and soft tissue metastases. J Pathol 230(3): 291-297.

58. Gritsko T, Williams A, Turkson J, Kaneko S, Bowman T, et al. (2006) Persistent activation of stat3 signaling induces survivin gene expression and confers resistance to apoptosis in human breast cancer cells. Clin Cancer Res 12(1): 11-19.

59. Bahrami A, Aledavood A, Anvari K, Hassanian SM, Maftouh M, et al. (2018) The prognostic and therapeutic application of microRNAs in breast cancer: Tissue and circulating microRNAs. J Cell Physiol 233(2):

How to cite this article: Paolo Silacci, Marco Tretola. Pomegranate's Ellagitannins: Metabolism and Mechanisms of Health Promoting Properties. Nutri Food Sci Int J. 2019. 9(4): 555766. DOI: 10.19080/NFSIJ.2019.09.555766

774-786.

60. Munagala R, Aqil F, Vadhanam MV, Gupta RC (2013) MicroRNA 'signature' during estrogen-mediated mammary carcinogenesis and its reversal by ellagic acid intervention. Cancer Lett 339(2): 175-184.

61. Amos-Landgraf JM, Kwong LN, Kendziorski CM, Reichelderfer M, Torrealba J, et al. (2007) A target-selected Apc-mutant rat kindred enhances the modeling of familial human colon cancer. Proc Natl Acad Sci U S A 104(10): 4036-4041.

62. Femia AP, Dolara P, Caderni G (2004) Mucin-depleted foci (MDF) in the colon of rats treated with azoxymethane (AOM) are useful biomarkers for colon carcinogenesis. Carcinogenesis 25(2): 277-281.

63. Tortora K, Femia AP, Romagnoli A, Sineo I, Khatib M, et al. (2018) Pomegranate By-Products in Colorectal Cancer Chemoprevention: Effects in Apc-Mutated Pirc Rats and Mechanistic Studies *In Vitro* and Ex Vivo. Mol Nutr Food Res 62: 2.

64. Banerjee N, Kim H, Talcott S, Mertens-Talcott S (2013) Pomegranate polyphenolics suppressed azoxymethane-induced colorectal aberrant crypt foci and inflammation: possible role of miR-126/VCAM-1 and miR-126/PI3K/AKT/mTOR. Carcinogenesis 34(12): 2814-2822.

65. Li XM, Wang AM, Zhang J, Yi H (2011) Down-regulation of miR-126 expression in colorectal cancer and its clinical significance. Med Oncol 28(4): 1054-1057.

66. Hurwitz AA, Foster BA, Allison JP, Greenberg NM, Kwon ED (2001) The TRAMP mouse as a model for prostate cancer. Curr Protoc Immunol Chapter 20: 20-25.

67. Adhami VM, Siddiqui IA, Syed DN, Lall RK, Mukhtar H (2012) Oral in-

DOI: 10.19080/NFSIJ.2019.09.555766

How to cite this article: Paolo Silacci, Marco Tretola. Pomegranate's Ellagitannins: Metabolism and Mechanisms of Health Promoting Properties. Nutri Food Sci Int J. 2019. 9(4): 555766. DOI: 10.19080/NFSIJ.2019.09.555766