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A pharmacological update of ellagic acid

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Departament de Farmacologia
Prof. José Luis Ríos Cañavate

27 April 2018

Dear Prof Butterweck

Editor-in chief of Planta,

I am sending you the revised version of the manuscript PLAMED-2018-03-0216-REV1, titled now as "A pharmacological update of ellagic acid" revised according to the Editor and referees' suggestions. All the points were answered. Changes in text are in blue color.

Sincerely,

Prof. Jose-Luis Rios
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For Peer Review

A pharmacological update of ellagic acid

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Abbreviations and symbols

ABCA1	ATP-binding cassette transporter-1
AGE	advanced glycation end-products
AGFBP7	insulin-like growth factor-binding protein 7
Akt	protein kinase B
AP-1	activator protein-1
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
cdk	cyclin-dependent kinase
C/EBP	cytosine-cystosine-adenine-adenine-thymine enhancer-binding protein
CHOP	C/EBP homologous protein
COX	cyclooxygenase
CYP	cytochrome P450
DR	death receptor
ERK1/2	extracellular signal-regulated kinase 1/2
HbA1c	glycosylated hemoglobin
HDL	high-density lipoprotein
HO	heme oxygenase
ICAM	intercellular adhesion molecule
IκB	inhibitor of kappa B
IKK	IκB kinase
IL	interleukin
IGFBP7	insulin-like growth factor-binding protein 7
i.p.	intraperitoneal
JNK	c-Jun N-terminal kinase
LDL	low-density lipoprotein
L-NAME	Nω-Nitro-L-arginine methyl ester hydrochloride
LXR	liver X receptor
MAO-B	monoamine oxidase B
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemoattractant protein-1
MIF	migration inhibitory factor
MMP	matrix metalloproteinase
mPGEs-1	microsomal prostaglandin E synthase-1
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor-kappa B
NO	nitric oxide
Nrf2	nuclear factor erythroid 2–related factor 2
p-38	p38 mitogen-activated protein kinase
p-p38	phosphorylated-p38 mitogen-activated protein kinase
PDGFR-β	platelet-derived growth factor receptor-β
PI3K	phosphoinositide 3-kinase
p.o.	pero os, orally
PPAR	peroxisome proliferator-activated receptor
ROS	reactive oxygen species
s.c.	subcutaneous
SRB1	scavenger receptor class B1
STAT3	signal transducer and activator of transcription 3
TGF-β	transforming growth factor-β

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TLR	toll-like receptor
VCAM	vascular cell adhesion molecule
VEGF	vascular endothelial growth factor

Abstract

Ellagic acid is a common metabolite present in many medicinal plants and vegetables. It is present either in free form or as part of more complex molecules (ellagitannins), which can be metabolized to liberate ellagic acid and several of its metabolites, including urolithins. While ellagic acid's antioxidant properties are doubtless responsible for many of its pharmacological activities, other mechanisms have also been implicated in its various effects, including its ability to reduce the lipidemic profile and lipid metabolism, alter pro-inflammatory mediators (TNF-α, IL-1β, IL-6), and decrease the activity of NF-κB while increasing Nrf2 expression. These events play an important role in ellagic acid's anti-atherogenic, anti-inflammatory, and neuroprotective effects. Several of these activities, together with the effect of ellagic acid on insulin, glycogen, phosphatases, aldose reductase, sorbitol accumulation, advanced glycation end-product formation, and resistin secretion, may explain its effects on metabolic syndrome and diabetes. In addition, results from recent research have increased the interest in ellagic acid, both as a potential protective agent of the liver and skin and as a potential anticancer agent, due to the its specific mechanisms affecting cell proliferation, apoptosis, DNA damage, and angiogenesis, and its aforementioned anti-inflammatory properties. Taken together, these effects make ellagic acid a highly interesting compound that may contribute to different aspects of health; however, more studies are needed, especially on the compound's pharmacokinetic profile. In this review we selected papers published from 2005 up to date.

Key words

Ellagic acid, metabolic syndrome, neuroprotection, hepatoprotection, cardiovascular effects, cancer

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Introduction

Ellagic acid (Fig. 1) is a chromene-dione derivative (2,3,7,8-tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione; $C_{14}H_6O_8$; mw: 302.194 g/mol) [1] possessing a hydrophilic moiety with 4 hydroxyl groups and 2 lactones, along with a lipophilic moiety with two hydrocarbon rings. This endows ellagic acid with the capacity both to accept electrons from different substrates as well as to participate in antioxidant redox reactions [2, 3]. Ellagic acid can present as cream-colored needles or as a yellow powder with a water solubility of less than 1 mg/mL at 21 °C [4]; it is an odorless [4], weak acid that is incompatible with strong reducing agents. It produces an exothermic reaction through the acid-base reaction and is easily sulfonated and nitrated by the corresponding acids [5].

Fig. 1 here

Ellagic acid is present in many fruits (pomegranates, persimmons, raspberries, black raspberries, wild strawberries, peaches, plums), seeds (walnuts, almonds), and vegetables [6]. It can be present in free form or as derivatives, principally as complex polymers called ellagitannins, which can be hydrolyzed by the action of physiological pH and gut microbiota, thus increasing plasma levels of the acid after the ingestion of fruits and vegetables [7]. The actual content of ellagic acid varies from plant to plant, with different concentrations being described depending on the source. Raspberries (fruit of *Rubus idaeus* L., Rosaceae) probably contain the highest concentration, with values (expressed with respect to fresh weight) ranging from 1900 mg/100 g (yellow raspberries) to 270 mg/100 g (wild raspberries), depending on the sample analyzed. Other species from the same genus also have high ellagic acid content; these include cloudberries (*Rubus chamaemorus* L.), blackberries (*Rubus* sp.), and strawberries (*Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozier, Rosaceae). Seeds, such as pecans (*Carya illinoinensis* (Wangenh.) K.Koch, Juglandaceae) and walnuts (*Juglans regia* L., Juglandaceae), and beverages such as cognac and oak-aged red wine obtained from grapes (*Vitis vinifera* L., Vitaceae) also present relevant levels [7]. Other fruits with high levels of ellagic acid are pomegranates (*Punica granatum* L., Lythraceae), persimmons (*Diospyros kaki* L.f., Ebenaceae), peaches (*Prunus persica*

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(L.) Batsch, Rosaceae), and plums (*Prunus domestica* L., and other species and subspecies from the genus *Prunus*, Rosaceae) [8].

In the case of medicinal plants, there are many species described with ellagic acid in their chemical composition. Previously, García-Niño and Zazueta [50] reviewed the presence of ellagic acid in 32 medicinal plants. In the present review we compiled some recent studies in which ellagic acid was isolated or identified (Table 1). For this purpose, we selected a limited number of papers with a total of 43 species, and in some cases the botanical name are not same that the original paper because we use the last taxonomical review.

Table 1 here

Various pharmacological properties of ellagic acid have been reviewed and described by Derosa et al. [6] and Larrosa et al. [8]. Knowledge of several of these properties comes from the use of medicinal plants in folk medicine; many have been studied in animals, while other properties have been evaluated in humans. This review will focus only on the most recent studies and discoveries of ellagic acid's relevant properties, such as its antioxidant activity, implicated in most of its pharmacological activities. These include its anti-inflammatory, neuroprotective, and hepatoprotective effects, as well as the protection it provides against diabetes, cardiovascular disease, and cancer [50,51]. However, ellagic acid's antioxidant effects are not the only factor involved, but act alongside other mechanisms of interest. For this reason, we will also review several relevant aspects of its pharmacokinetic properties and its efficacy in humans.

For this review, we selected the most relevant articles published from 2005 to the present. Papers published before 2005 were used only if they added special insights to be included in the introduction and discussion sections. Our search was conducted in Pubmed, Scopus, the Web of Science, and the Cochrane Library. The keywords selected were 'ellagic acid', either alone or combined with: 'antioxidant', 'anti-inflammatory', 'hepatoprotection', 'liver', 'neuroprotection', 'cardiovascular', 'heart', 'blood pressure', 'hypertension', 'metabolic syndrome', 'cholesterol', 'hypercholesterolemia', 'lipid', 'hyperlipidemia', 'hypertriglyceridemia', 'diabetes', 'hyperglycemia', 'insulin', 'cytotoxic', 'antitumor', 'anti-infectious', 'antiviral', 'antibacterial', 'parasiticidal', 'clinical trials', and 'pharmacokinetic'.

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Relevant *in vitro* and *in vivo* studies were analyzed, as well as clinical trials. Only articles written in English and published in peer-reviewed scientific journals were selected. About 1480 papers on ellagic acid were published between 2005 and the present. Of these, we retrieved and analyzed approximately 250 and included 150 in the final review. As an initial reference we used the last review published in each subject, excluding articles if they included data from previous studies or with similar results. Finally, we focused the review on what we considered to be the most relevant topics: antioxidant and anti-inflammatory properties; metabolic syndrome, hepatoprotection, cardiovascular, anti-cancer, and skin disease effects, and pharmacokinetic and clinical trials.

Ellagic acid as an antioxidant agent

Ellagic acid is one of the major antioxidants, along with the well-known vitamins ascorbic acid and α-tocopherol [52]. Its intrinsic antioxidant properties have been attributed to its free radical scavenging activity, which has been proposed to be similar to that of essential vitamins. As commented above, the presence of four hydroxyl and two lactone functional groups enables ellagic acid to scavenge a wide variety of reactive oxygen species (ROS) and reactive nitrogen species [53]. Indeed, studies show that at physiological pH, ellagic acid in aqueous solution can deactivate not only hydroxyl radical ($HO^{\bullet}$), but also peroxyl radicals ($ROO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), and peroxynitrite ($ONOO^{-}$) [54,55]. The scavenging efficacy of an antioxidant can be determined with the scavenging rate constant, which is the rate of the compound's reaction with free radicals in a given system. Tiwari and Mishra [56] demonstrated that ellagic acid is a good radical scavenger, particularly against $OH^{\bullet}$, methoxyl ($OCH_3^{\bullet}$), and nitrogen dioxide ($NO_2^{\bullet}$), in descending order of scavenging rates ($OH^{\bullet} >> OCH_3^{\bullet} > NO_2^{\bullet}$). These authors thus suggested that ellagic acid should, in general, be more a efficient scavenger of ROS than of reactive nitrogen species. This antiradical property is not reduced upon metabolism, as the metabolites of ellagic acid are also capable of efficiently scavenging a wide range of free radicals, often even faster than ellagic acid itself. Moreover, under specific environmental conditions, ellagic acid is predicted to be continuously regenerated after scavenging two free radicals, one peroxyl ($ROO^{\bullet}$) and one superoxide ($O_2^{\bullet -}$) per cycle, until some of the intermediates are consumed in different reactions. This increases protective effects of ellagic acid at low concentrations, which is both a desirable and unusual behavior for an antioxidant [55].

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The mechanism behind ellagic acid's scavenging activity is related to its ability to transfer the phenolic H-atom to a free radical. The formal H-atom abstraction from ellagic acid reaction has been shown to involve complex processes that proceed via at least three different mechanisms: hydrogen atom transfer, single electron transfer followed by proton transfer, and sequential proton loss electron transfer [57]. In aqueous solutions, the predicted mechanism is the loss of a proton from ellagic acid followed by electron transfer to free radicals, whereas in the gas phase or in nonpolar solvents, the reaction probably entails a hydrogen atom transfer to free radicals [58]. Specifically, the hydroperoxyl radical ($HOO^{\bullet}$) scavenging activity of ellagic acid has been found to take place exclusively through the hydrogen atom transfer mechanism, regardless of the polarity of the environment. In contrast, the relative importance of the various reaction paths is influenced significantly by the polarity of the environment. Compared to other antioxidants, the peroxyl radical scavenging activity of ellagic acid in lipid media was found to be lower than that of carotenes, dopamine, canolol, hydroxytyrosol, sesamol, sinapinic acid, protocatechuic acid, capsaicin and α-mangostin; similar to that of tyrosol and melatonin; and higher than that of caffeine. Surprisingly, while ellagic acid is predicted to react about 7.9 times more slowly than Trolox, in aqueous solution it is predicted to react with hydroperoxyl radical 1.8 times faster than Trolox. With regard to other antioxidants, it is predicted to have higher peroxyl radical scavenging activity than melatonin, caffeine, allicin, and thioacrolein, similar activity to that of dopamine, and lower activity than that of canolol, α-mangostin, protocatechuic acid, 2-propenesulfenic acid, glutathione and sesamol [55].

In summary, ellagic acid exhibits antioxidant-sparing activity through the scavenging of free radicals, which may account for its protective effect against free radical-induced damage. In their study, Iino et al. [59,60] suggested that the protective effect of ellagic acid (3 to 30 mg/kg, p.o.) on gastric lesions induced by NH_4OH in the ischemic stomach may arise through the scavenging of NH_2Cl, a causative factor in this lesion model, in addition to that of superoxide and hydroxyl anions [59,60]. Treatment with ellagic acid (50 mg/kg b.w., p.o.) also resulted in a significant decrease in the activity of serum liver enzymes as well as a decrease in total bilirubin and direct bilirubin serum levels, which may be responsible for inducing excessive free radical production leading to severe hepatic injury [50]. In addition, through its scavenging action on free radicals, ellagic acid indirectly reduced serum level of triglycerides, total cholesterol, and the fraction of

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low- and very low-density lipoproteins (LDL and VLDL). These results are in agreement with previous reports showing the hypolipidemic activity ellagic acid [53].

Particularly, hydroxyl and peroxyl radicals are involved in the initiation and propagation of lipid peroxidation, respectively. Ellagic acid has been reported to be effective in inhibiting lipid peroxidation, even at μM concentrations [54]. Its protective effect has been attributed mainly to its role as free radical scavenger; indeed, it has been proposed to be a better free radical scavenger than vitamin E succinate [61] and equal to a two- to threefold Trolox [62]. This is in agreement with Böyük et al. [63], who reported for the first time that ellagic acid (85 mg/kg, p.o) was effective in protecting lung tissue against ischemia reperfusion oxidative stress through a reduction in the increase of lipid peroxidation as well as a reduction in oxidative stress parameters. Uzar et al. [64] found that ellagic acid (50 mg/kg/d) caused a decrease in streptozotocin-elicited lipid peroxidation and counteracted streptozotocin-induced impairment of total oxidant status and the oxidative stress index. More recently, Kilic et al. [65] demonstrated that the protective effect of ellagic acid (59%) on the lipid peroxidation of linoleic acid emulsion was similar to that of ascorbic acid (60%), but lower than that of *p*-coumaric acid (72%), while Roche et al. [66] asserted that the reduction in lipid peroxidation provides clear evidence of the antioxidant effects of ellagic acid.

It has been established that ROS are generated during the reaction of cytochrome P450 (CYP) with its substrate; however, the microsomal electron-transfer chain continues to oxidize NADPH-oxidase and produce ROS even in the absence of any substrate. In addition, enzymes such as lipoxygenase, cyclooxygenase (COX), and xanthine oxidase can also contribute to ROS production. Hassoun et al. [67] suggested that ellagic acid (1 mg/kg/d, 13 wk) prevented the up-regulation of CYP expression through an indirect antioxidant effect. However, in their study, they did not elaborate on how ellagic acid decreases superoxide production through the mitochondrial function, an important detail, as this organelle is the main site of superoxide production in mammalian cells. In other research, ellagic acid (5 to 20 μM, *in vitro*) was shown to exert its beneficial effect on oxidized LDL-induced endothelial dysfunction by suppressing the membrane assembly of the NADPH oxidase complex, thereby decreasing the overproduction of superoxide radicals [68]. In this context, Berkban et al. [69] suggested that ellagic acid reduces oxidative stress by decreasing NADPH oxidase subunit $p47^{phox}$ expression. However, to date, no study has produced conclusive evidence for this hypothesis.

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The presence of ionic metals such as copper or iron in a system can accelerate the rate of oxidation in that system. Phenolic compounds such as ellagic acid have been shown to inhibit the pro-oxidative action of metals by means of a chelation process in which the phenolics bind with the metal ions to form a complex incapable of promoting oxidation [70]. Through this chelation process, they operate as 'secondary' or 'preventive' antioxidants, thus inhibiting oxidation without directly interacting with oxidative species [71]. In a previous study, Ahmed et al. [72] had demonstrated that ellagic acid (500 µmol/kg b.w.) was a potent chelating compound for suppressing nickel-induced oxidative stress in the liver and kidney of female Wistar rats. In another study, ellagic acid (30 µM for 24 h) effectively counteracted cadmium-induced ROS generation, thus blocking the cadmium-mediated apoptosis of astrocytes, the most abundant glial cells in the central nervous system [73]. Even though the authors did not describe the molecular mechanism by which ellagic acid exerts such protective effects upon primary astrocytes, its ability to chelate metal ions may be involved. Moreover, ellagic acid was shown to have an effective chelating effect on ferrous ion (45 µg/mL, 49%), which was similar to that of other phenolic compounds such as caffeic acid (15 µg/mL, 53%) [65]. More recently, Saha et al. [74] demonstrated that ellagic acid (10 µM) is capable of binding iron in a similar way to epigallocatechin gallate. The presence of a catechol group in the former was identified as being responsible for iron chelation (Fig. **2**) [75]. Finally, Galano et al. [55] concluded that after deprotonation, ellagic acid is also capable of chelating copper in aqueous solution, yielding stable complexes. All these reactions predictably decrease free radical production; thus, metal chelation is another way in which ellagic acid exerts its protective effect against oxidative stress.

Fig. 2 here

Another potential protective mechanism of ellagic acid against oxidative stress involves shielding DNA from attack through a direct association with this macromolecule. The formation of 8-oxo-2'-deoxyguanosine is a benchmark of oxidative DNA damage. Various studies have shown that ellagic acid significantly decreases the amount of 8-oxo-2'-deoxyguanosine produced after oxidative DNA damage [63,76], which was consistent with an earlier report [77]. This protective behavior also correlates with a previous study on the interaction between ellagic acid and DNA, where ellagic acid was shown to bind directly to DNA, likely leading to the protection of binding sites from

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free radicals [78,79]. In their study, Spencer et al. [80] showed that ellagic acid substantially inhibited dopamine/Cu^{2+}-mediated oxidative DNA decomposition at all doses tested (1, 6, 30 and 150 μM); in fact, even at doses as low as 1 μM, the inhibition was nearly 50%. These results, coupled with those of a recent study indicating that dietary ellagic acid (1 mg/kg/d, 13 wk) significantly protected rat brain tissue from tetrachlorodibenzo-*p*-dioxin-induced superoxide anion production, lipid peroxidation, and DNA strand breaks [67], support the potential role of ellagic acid in neuroprotection from oxidatively-generated DNA damage.

Regulation of endogenous antioxidant enzymes by exogenous antioxidants should be an effective way to prove ellagic acid's antioxidant capacity. The nuclear factor erythroid 2–related factor 2 (Nrf2) is a redox-sensitive transcription factor that acts as the master antioxidant response regulator in the cell. Under stressful and toxic conditions, Nrf2 translocates into the nucleus where, after binding to DNA, it induces transcription of genes related to the antioxidant defense system [81]. In this regard, ellagic acid (5, 12 and 30 μM) has been shown to play a defensive role against ultraviolet (UV)-B light-induced oxidative stress through up-regulation of the Nrf2 signaling pathway in human dermal fibroblasts [82] and may even augment the nuclear translocation and transcriptional activation of Nrf2 [83] in human keratinocyte cells. A study by Gu et al. [84] reported that ellagic acid (5, 10, 20 mg/kg b.w., i.p.) may protect against acute hepatic injury in mice by inducing the expression of Nrf2 and heme oxygenase (HO)-1. In addition, consistent ellagic acid consumption within a nutritional range (0.5 g/kg diet; for a dose of about 30 mg/kg/d, 14 wk) has been shown to attenuate endothelial dysfunction and atherosclerosis in mice fed with a high-fat (21%) diet, a finding which has been partly attributed to ellagic acid's induction of the oxidative defense system through its effects on Nrf2. However, although these results confirm that the intake of ellagic acid can have a pharmacological effect, the authors did not evaluate the molecular mechanism through which ellagic acid modulates the nuclear translocation of Nrf2 [85]. Other studies have since revealed that the activation of Nrf2 in human keratinocyte cells by ellagic acid involves an ROS-independent pathway, suggesting that the presence of the α,β-unsaturated ketone moiety chemical backbone in ellagic acid is responsible for its potent activation of Nrf2 [83].

In cases of oxidative stress or lipid peroxidation, the cellular defense system operates mainly via antioxidant enzymes such as catalase, superoxide dismutase, glutathione

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peroxidase, glutathione *S*-transferase, and glutathione reductase. Earlier reports [83,86] showed that ellagic acid increased the levels of the antioxidant glutathione *S*-transferase (as well as the enzyme involved in the synthesis of glutathione, glutamate-cysteine ligase) several-fold in rat livers. Induction of glutathione *S*-transferase and the enhancement of glutathione levels can both protect against the oxidative damage generated by many carcinogens, as well as influence redox-sensitive signaling pathways involved in response to stress [87]. More recently, Mishra and Vinayak [88] demonstrated that ellagic acid (60 and 80 mg/kg b.w., p.o.) improves the antioxidant defense system by increasing the expression and activity of the antioxidant enzymes catalase, superoxide dismutase, glutathione peroxidase-4, and glutathione reductase, both in liver and ascites cells of Dalton's lymphoma-bearing mice. Thus, because of its indirect activity inhibiting ROS formation, ellagic acid has been proposed as potential new drug in the treatment of cirrhosis induced by chemical agents, such as CCl_4 [89].

Anti-inflammatory activity of ellagic acid

Inflammation and oxidative stress are closely related pathophysiological events that are tightly linked with one another [90]. Apart from its well-known antioxidative effects, ellagic acid has also been shown to exert potent anti-inflammatory activities [88]. For this reason, several studies have emphasized the potential of ellagic acid as a candidate for the treatment of many chronic inflammatory diseases and conditions [91-93]. In this review, we will cite the most relevant studies, specifically those focused on the mechanism of action. For example, ellagic acid has been shown to inhibit key cell functions and activation of pancreatic stellate cells, which play a pivotal role in the pathogenesis of pancreatic fibrosis and inflammation. In their study, Masamune et al. [94] demonstrated that ellagic acid (1, 5, 10, 25 μg/mL) inhibits interleukin (IL)-1β-, and tumor necrosis factor (TNF)-α-induced activation of activator protein-1 (AP-1) and mitogen-activated protein kinases (MAPK), such as extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), and p38, but not nuclear factor-κB (NF-κB). These results are concordant with those from a study carried out by González-Sarrías et al. [95], who reported that ellagic acid (10 μM) did not attenuate NF-κB activation, as seen by the lack of anti-inflammatory activity in colon fibroblasts after IL-1β treatment. However, the inhibition of MAPK activation was likewise not observed in this study. These contradictory results regarding MAPK activation were attributed to the different MAPK activation patterns, depending on the cell type. In their

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work, Cornélio Favarin et al. [96] showed that ellagic acid (10 mg/kg b.w., p.o.) reduced the pro-inflammatory cytokine IL-6 and increased the anti-inflammatory cytokine IL-10 in the bronchoalveolar lavage fluid in acid-initiated acute lung injury without down-regulating the nuclear factor-kappa B (NF-κB) and AP-1 signaling pathways. This was in contrast with the effects of dexamethasone (1 mg/kg b.w., s.c.), suggesting that the effect of ellagic acid on acute lung injury-associated inflammation is not NF-κB and AP-1 dependent. In contrast, NF-κB was a potential target for the anti-inflammatory effect of ellagic acid incorporated into the normal diet (0.5%) of mice in an ulcerative colitis experimental model [97]. Similar results for ellagic acid as an anti-inflammatory agent through modulation of the NF-κB activation pathway have been observed in several other studies [98-102].

The transcription factor NF-κB has been shown to be a critical regulator of COX-2 expression [103]. For this reason El-Shitany et al. [104] studied ellagic acid (100 mg/kg b.w., i.p.), demonstrating its ability to modulate the production of COX-2 mRNA mainly through the inhibition of ROS production which, in turn, inhibits NF-κB activation. In their study, COX-2 mRNA expression was also blocked by meloxicam (4 mg/kg b.w., i.p.), but was not affected by indomethacin (10 mg/kg b.w., i.p.). Moreover, it was shown that ellagic acid has an even higher binding affinity than that of diclofenac or meloxicam. The binding pattern of ellagic acid with the COX-2 active site shows that it makes four hydrogen bonds with Arg120, Ser530, Tyr355, and Tyr385, while meloxicam makes three hydrogen bonds and diclofenac makes only two hydrogen bonds. Thus, researchers have suggested that ellagic acid may inhibit carrageenan-induced acute inflammation by blocking the COX-2 receptor, as is the case with both diclofenac and meloxicam [104]. In parallel, prostaglandin E_2 (a metabolite of COX-2) is considered to be one of the strongest mediators in the inflammatory response. Ellagic acid was able to inhibit this compound in human monocytes in a dosage range of 10–30 μM. This effect was mediated by the inhibition of the lipopolysaccharide-induced expression of the cytosolic phospholipase $A_2\alpha$, COX-2, and microsomal prostaglandin E synthase-1 (mPGEs-1) proteins, not through a direct effect on enzyme activity. The mechanism by which ellagic acid inhibited the lipopolysaccharide-induced expression of all three enzymes was thought to involve effects on protein kinases and/or transcription factors. As mentioned above, ellagic acid has been shown to inhibit various protein kinases [105]. Among these, MAPK [94,97,99,106] are of special

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interest since they seem to participate in the regulation of the expression of both COX-2 [107-109] and mPGEs-1 [110]. Both *in vitro* and *in vivo* studies have indicated an existing cross-talk between the release of prostaglandins and nitric oxide (NO) in the modulation of inflammation. It is thus significant that ellagic acid (10 and 30 mg/kg b.w., i.p.) has been shown to inhibit NO production significantly by down-regulating inducible NO synthase [111,112].

The binding and recruitment of circulating monocytes to vascular endothelial cells are early steps in the development of inflammation and atherosclerosis. These processes are mediated through cell adhesion molecules that are expressed on the surface of endothelial cells. In their study, Papoutsi et al. [113] tested ellagic acid at a concentration range of 0.1–10 μM and found that it inhibited the TNF-α-induced endothelial activation and expression of both vascular cell adhesion molecule (VCAM)-1 and intracellular adhesion molecule (ICAM)-1. In contrast, Yu et al. [114] demonstrated that ellagic acid at a concentration of 25–50 μM reduced IL-1β-induced expression of VCAM-1 and E-selectin, but not of ICAM-1. Their finding that ellagic acid exerted its anti-inflammatory effects via modulation of NF-κB activity is noteworthy. In other work, ellagic acid (10 mg/kg b.w., p.o.) has been consistently shown to decrease the expression of P-selectin in the bronchial epithelium of ovoalbumin-immunized and challenged mice [115].

Various secreted pro-inflammatory cytokines, such as macrophage migration inhibitory factor (MIF), play key roles in mediating inflammatory responses. Particularly, it has been shown that MIF induces nuclear translocation of NF-κB and chemotaxis of peripheral blood mononuclear cells to promote inflammation. Sarkar et al. [116] have shown that ellagic acid (50 μM) inhibits the tautomerase activity of MIF and MIF-mediated pro-inflammatory responses in peripheral blood mononuclear cells (IC_{50} 4.77 ± 0.52 μM). Although the exact mechanism was not elucidated, the authors suggest that ellagic acid's ability to block tautomerase activity or the tautomerase-active site of the cytokine could contribute to the inhibition. The cytokines TNF-α and IL-6 are related to humoral and cellular inflammation, respectively. In lipopolysaccharide-stimulated RAW 264.7 cells, ellagic acid significantly inhibited TNF-α and IL-6 production, even at μM concentrations [117]. It also decreased production of IL-13 (at 100 μM) from stimulated human peripheral blood mononuclear cells, whereas no change was observed in IL-4 and TNF-α production [118]. Moreover, dietary administration of ellagic acid (5

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g/100 g standard power diet) has been shown to lower cardiac and renal levels of IL-1β, IL-6, TNF-α, and monocyte chemoattractant protein-1 (MCP-1) while also significantly down-regulating TNF-α and MCP-1 mRNA expression in the kidney. In addition, intake of ellagic acid substantially decreased renal IL-1β, IL-6, and TNF-α levels in diabetic mice [91]. Topical application of ellagic acid (1 to 10 μM) diminished production of pro-inflammatory cytokines IL-1β and IL-6 as well as adhesion molecule ICAM-1 in the dermis, and also mitigated infiltration of inflammatory macrophages in UVB-inflamed hairless mouse skin [119]. Oral treatment of gastric ulcerated mice with ellagic acid at a dose of 7 mg/kg significantly reduced the levels of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 while inducing anti-inflammatory cytokines IL-4 and IL-10 [120]. In agreement with these studies conducted with various experimental models, ellagic acid treatment of mice with adjuvant-induced arthritis significantly decreased levels of IL-1β and TNF-α [121]. Although the precise mechanism by which ellagic acid decreases serum levels of these pro-inflammatory cytokines is unclear, it has been suggested that the compound acts through direct inhibition of the NF-κB pathway [122].

IL-17 is a pro-inflammatory cytokine known to stimulate the production of other inflammatory cytokines and chemokines [123,124]. To date, two studies have demonstrated ellagic acid's ability to decrease serum levels of IL-17 in different experimental mouse models. First, Allam et al. [121] showed that ellagic acid treatment (58.33 mg/kg b.w., i.p.) was effective in reducing serum levels of IL-17 in arthritic mice while more recently, Sanadgol et al. [125] found that ellagic acid (40 and 80 mg/kg/d i.p., 4 wk) decreases IL-17 expression at both the protein and mRNA level. These results are in harmony with a previous study demonstrating that pomegranate juice rich in ellagic acid (25 μg/mL) inhibited the synthesis of IL-17 from human peripheral blood mononuclear cells [126]. Taken together, these studies show that ellagic acid is capable of down-modulating pro-inflammatory mediators and stimulating the production of anti-inflammatory cytokines.

Activation of toll-like receptor (TLR)-2 and TLR4 facilitates the activation of the MAPK and IκB kinase (IKK) complex pathways, which transduce various upstream signals leading to activation of AP-1 and NF-κB transcription factors. In a study conducted by Lee et al. [127], pre-treatment with ellagic acid (50, 100, or 200 mg/kg b.w., p.o.) reduced TLR2 and TLR4 protein levels as well as mRNA expression in liver

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tissue. These new results are extremely relevant because the effect of ellagic acid on these receptors had not previously been reported. The authors proposed that ellagic acid may have general anti-inflammatory effects in diseases associated with TLR signaling.

Ellagic acid in metabolic syndrome

The term metabolic syndrome refers to a group of factors that raises the risk for heart disease and other health problems, including diabetes and stroke. This set of risk factors includes: abdominal obesity, high triglyceride levels, low high-density lipoprotein (HDL)-cholesterol levels, high blood pressure, and high fasting blood glucose levels [128]. Any one of these factors alone is a problem, such as high blood pressure or high fasting blood glucose, but when a patient presents with three of them, for example the two aforementioned factors plus abdominal obesity, a diagnosis of metabolic syndrome is likely [129]. Various studies have reported on the antihyperglycemic and antihyperlipidemic properties of ellagic acid, which were analyzed *in vitro* and *in vivo* [52, 130-132]. Indeed, the effect of this acid on glucose metabolism has been widely investigated and represents the principal target for the compound's potential effects against metabolic syndrome (with several studies focusing on glucose and lipid metabolism in diabetic rats), as well as its effects on obesity.

Working *in vitro*, Pinto Mda et al. [133] demonstrated that ellagic acid has good potential for the management of hyperglycemia and hypertension linked to type 2 diabetes. They studied the effects of ellagic acid, purified ellagitannins, and a strawberry extract (a good source of ellagic acid) as inhibitors of α-amylase, α-glucosidase, and angiotensin I-converting enzyme, and observed that purified ellagitannins and ellagic acid inhibited α-amylase and angiotensin-converting enzymes, but had a limited inhibitory effect on α-glucosidase.

Malini et al. [134] studied the antidiabetic effects of ellagic acid (50 and 100 mg/kg, for 35 d) in streptozotocin-induced diabetes in rats. The acid reduced the concentration of glucose in plasma, along with insulin, glycosylated hemoglobin (HbA1c), and hexokinase activity, while simultaneously decreasing glycogen (liver and muscle), as well as glucose-6-phosphatase and fructose-1,6-bisphosphophatase activity in the liver and kidney, all with respect to the increased values in diabetic rats. In other assays, Uzor and Osadebe [135] studied the antidiabetic activity of ellagic acid (20 mg/kg) in alloxan-induced diabetes in mice. The compound was isolated from the roots of

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Combretum comosum var. *dolichopetalum* (Engl. & Diels) Jongkind (Combretaceae, syn: *Combretum dolichopetalum* Engl. & Diels), a plant used as an antidiabetic remedy in Nigerian folk medicine. Ellagic acid (10 mg/kg, orally, 2 wk) reduced fasting blood glucose by 24% at 9 h [135]. It also improved the glucose/insulin balance, lipid profile, redox level, and the levels of liver enzymes, inflammatory cytokines, and adipokines in serum and tissues (liver, pancreas, adipose tissue, and brain), while enhancing insulin signaling, adiponectin receptors, glucose transporters, and inflammatory mediators. These experiments were performed in rats fed with a high-fat fructose diet for 2 months to induce insulin resistance and type 2 diabetes [136].

In a previous report, Ueda et al. [137] described the effects of ellagic acid on sorbitol accumulation *in vitro* and *in vivo*, reporting inhibitory activity for this compound on sorbitol accumulation in erythrocytes, the lens, and the sciatic nerve under incubation with glucose *in vitro*. The IC_{50} of ellagic acid against sorbitol accumulation in erythrocytes was 2.4 μM, whereas in the case of the lens and sciatic nerve, the effect was quite limited (at 400 μM, the inhibition was 14% and 32%, respectively). When the effect was analyzed on diabetic rats, ellagic acid at 50, 75, and 100 mg/kg/d reduced the elevated sorbitol accumulation in erythrocytes, the lens, and the sciatic nerve, with the middle dose (75 mg/kg/d) producing the best effect. Chao et al. [91] studied the protective effects of ellagic acid (2.5 and 5% in the diet for 12 wk) on the kidneys of diabetic rats. The intake of ellagic acid increased plasma insulin and decreased blood glucose levels at weeks 6 and 12; at the higher dose, it also decreased plasma levels of HbA1c. In addition, it reduced sorbitol and fructose levels in plasma and suppressed the aldose reductase mRNA expression in the kidney. Ellagic acid also lowered renal levels of IL-6, IL-1β, TNF-α, and MCP-1, down-regulating the mRNA expression of the latter proteins in the kidney. Taken together, these results provide clear evidence that ellagic acid possesses anti-glycation properties as well as anti-inflammatory effects; for this reason, it may aid in the prevention or attenuation of diabetic kidney disease.

Panchal et al. [138] analyzed the effects of ellagic acid (0.8 g/kg in food, 8 to 16 wk) on high-carbohydrate, high-fat diet-induced metabolic syndrome in rats. This experimental model produces impaired glucose tolerance, with increased protein levels of NF-κB and decreased protein levels of Nrf2 and carnitine palmitoyl transferase 1 in the heart and liver. After administration, ellagic acid attenuated the symptoms of metabolic syndrome provoked in this experiment, normalizing the protein levels of Nrf2, NF-κB, and

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carnitine palmitoyl transferase 1. As described above, Nrf2 is a regulator of cellular resistance to oxidants and plays a relevant role in oxidant stress resistance; its regulation is thus highly important in redox homeostasis [139]. The role of Nrf2 activation in the prevention of obesity, metabolic syndrome, nephropathy, retinopathy and neuropathy has only recently been described, with the results indicating that its activation can prevent the development of complications in type 2 diabetes mellitus [140]. For example, NF-κB is activated by a wide variety of cell-stress stimuli (including hyperglycemia) as well as in cases of renal fibrosis. Its inhibition produces a significant amelioration of diabetic nephropathy, thus making it an excellent therapeutic target for avoiding this serious associated effect [141]. The ability of ellagic acid to inhibit the renal NF-κB pathway can thus improve the multifactorial response due to its antihyperglycemic, antiglycative, antioxidant, and anti-inflammatory properties [122].

Pomegranate extract and its principal components, including ellagic acid, were found to suppress the formation of advanced glycation end-products (AGE) from bovine serum albumin and sugars [142]. Because hyperglycemia enhances the aldose reductase-related polyol pathway and increases AGE formation, these two processes may constitute good targets for counteracting their relevant roles in the complications of type 2 diabetes mellitus, such as cataracts [143]. Aldose reductase catalyzes the reduction of glucose to sorbitol, which is transformed into fructose by sorbitol dehydrogenase; consequently, increased fructose levels are a key factor in AGE formation. In diabetic patients, polyol levels rise and accumulate due to their poor penetration and metabolism, thus generating many of the complications of diabetes [144,145]. Taking this into account, Rao et al. [143] studied different plants and principles for their inhibitory activity against aldose reductase of different origin, as well as on the generation of AGEs. Among them, ellagic acid gave IC_{50} values of 16 μM (rat lens), 19 μM (rat kidney), 9 μM (human recombinant), and 18 μM (AGE formation). In addition, *in vivo* inhibition of lens galactitol accumulation by ellagic acid in galactose-fed rats was also studied, giving an IC_{50} value of 6.3 μM. Aslan and Beydemir [146] studied the ability of ellagic acid to inhibit the enzymatic activity of aldose reductase and sorbitol dehydrogenase from sheep livers, establishing IC_{50} values of 7.0 and 13.0 μM, respectively, for each enzyme.

The formation of AGEs is accelerated in the case of hyperglycemia, which alters the structure and function of proteins, contributing to long-term diabetic complications. In

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this context, Muthenna et al. [147] studied the effects of ellagic acid as an antiglycating agent on different proteins, including hemoglobin and various glycating agents such as fructose, among others. The mechanism proposed by these authors for ellagic acid involves the inhibition of N^{ϵ}-(carboxyethyl)lysine through scavenging of dicarbonyl compounds; they also demonstrated its effectiveness against loss of eye lens transparency through the inhibition of AGEs in the lens organ culture system. These results established the antiglycating effects of ellagic acid and its potential for controlling AGE-mediated diabetic pathologies, such as damage to the lens crystalline fibers, hemoglobin, and LDL, all of which are involved in type 2 diabetes mellitus and its associated complications [147]. The accumulation of AGEs has also been implicated in the pathogenesis of the vascular complications of diabetes, including diabetic nephropathy. In this case, ellagic acid was shown to prevent the accumulation of AGEs in streptozotocin-induced diabetes in rats. Indeed, addition of ellagic acid to food (0.2% or 2%, in the diet, 12 wk) prevented glycation-mediated red blood cell-immunoglobulin G cross-links and HbA1c accumulation while also inhibiting the accumulation of *N*-carboxymethyl lysine, a predominant AGE in the diabetic kidney. It also ameliorated AGE-mediated pathogenesis of diabetic nephropathy [148].

A mechanism of great interest to researchers is the effect of ellagic acid on resistin, an adipocytokine considered to be the link between obesity and type 2 diabetes mellitus. In their work, Makino-Wakagi et al. [149] demonstrated that both ellagic acid and its source (pomegranate fruit juice) suppress resistin secretion by a novel mechanism involving the degradation of intracellular resistin protein in adipocytes, but had no effect on adiponectin secretion. For the *in vivo* experiments, they only used pomegranate fruit juice and ovariectomized mice, an animal model with elevated resistin levels in serum and up-regulated resistin mRNA expression in white adipose tissue. In this case, the treatment group presented a clear reduction in serum resistin levels versus the control group. These results, together with the *in vitro* data, gave rise to the hypothesis that ellagic acid is the active compound of the extract. In a second study, these same authors demonstrated that ellagic acid reduced serum resistin levels without altering mRNA expression in adipose tissue. They thus concluded that ellagic acid is a potent suppressor of resistin secretion *in vivo* [150].

Kam et al. [151] screened various pomegranate extracts and their major constituents and observed a poor inhibitory activity for ellagic acid against rat intestinal α-glucosidase

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(42% at 67 μg/mL, ~222 μM), with no effect on porcine pancreatic α-amylase. This effect was corroborated by Bellesia et al. [152], who observed a slight inhibitory activity for ellagic acid against α-glucosidase, but in this case with an IC_{50} of 381 μM. These results indicate that the effects of ellagic acid on these enzymes are not significant; however, its effects on glycogen degradation and β-cell physiology and functionality seem to be more important in the acid's pharmacological effects on diabetes, most likely because glycogen phosphorylase catalyzes the first step in the intracellular degradation of glycogen to yield α-D-glucose-1-phosphate. For this reason, this process may serve as an apt target for the discovery of specific inhibitors, which may then be used as antihyperglycemic agents [153]. In the case of ellagic acid, it is a significant inhibitor of this enzyme, with a K_i of 13.4 μM and 7.5 μM for glycogen phosphorylase-a and -b, respectively. It is a competitive inhibitor against the substrate, glucose-1-phoshate, and non-competitive with respect to the allosteric activator, AMP [154].

A direct mechanism on β-cells was proposed by Fatima et al. [155], who studied neonatal streptozotocin-induced non-obese type 2 diabetes in rats. Ellagic acid from *Phyllanthus emblica* L. (syn: *Emblica officinalis* Gaertn., Phyllanthaceae) was tested for its effects on glucose-stimulated insulin secretion and the glucose tolerance test. Indeed, this phenolic compound decreased glucose intolerance in non-obese type 2 rats (23% at 100 mg/kg, after 45 min), stimulated glucose-induced insulin secretion in isolated islets at 100 μM (5.8 ng insulin/islet/h vs. 2.1 ng insulin/islet/h for glucose alone). The authors concluded that ellagic acid exerts antidiabetic activity through its effects on pancreatic β-cells, increasing both their size and number, as well as on serum insulin and antioxidant status, all while decreasing blood glucose.

In addition to its effects on glucose homeostasis, other factors involved in metabolic disease have also been analyzed. For example, when ellagic acid was tested against nonalcoholic fatty liver disease and atherosclerosis, the results showed that it can regulate lipid metabolism and reduce certain obesity-mediated metabolic complications [156]. The co-administration of ellagic acid and coenzyme Q_{10} to hyperlipidemic rats which were fed a high-fat diet for 4 weeks improved their endothelial function and hyperlipidemic conditions, lowering cholesterol, glucose, and triglyceride levels. In other experiments on diabetic rats, the effect of ellagic acid on LDL-cholesterol was also described. Because the oxidation of LDL is implicated in the origin and

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development of atherosclerotic plaque formation through endothelial inflammation, its reduction or attenuation is of interest for preventing metabolic syndrome and cardiovascular disease. Not only can LDL be oxidized in the subendothelial space, but monocytes will attach to endothelial cells that express cell adhesion molecules and inflammatory cytokines. The uptake of oxidized-LDL via scavenger receptors leads to foam cell formation; the oxidized-LDL cholesterol taken up this way will then be subject to esterification and storage in lipid droplets, converted to more soluble forms, or exported to extracellular HDL acceptors [157]. In their study, Park et al. [158] demonstrated that ellagic acid reduced oxidized LDL uptake and cholesterol influx while suppressing both scavenger receptor class B1 (SRB1) induction and foam cell formation in murine oxidized-LDL-stimulated macrophages. At ≤5 μM, ellagic acid also up-regulated peroxisome proliferator-activated receptors (PPAR)γ and ATP binding cassette transporter-1 (ABCA1), all responsible for cholesterol efflux, in lipid-laden macrophages. It also accelerated the expression and transcription of the nuclear receptor of liver X receptor (LXR)-α. Several studies have demonstrated the central role of PPAR-γ in governing cholesterol homeostasis through the influence of LXR in macrophages, enhancement of the expression of ABCA1, and reduction of the membrane expression of SRB1 [159]. Because ellagic acid works as a PPARγ modulator, administration of this phenolic compound can transfer effluxed cholesterol onto lipid-poor apolipoproteins, initiating the formation of HDL particles. In this way, it acts as an anti-atherogenic agent, blocking foam cell formation and/or enhancing cholesterol efflux pertaining to reverse cholesterol transport [158]. In addition, Rani et al. [160] demonstrated the efficacy of ellagic acid in preventing platelet-derived growth factor-BB-induced proliferation of primary cultures of rat aortic smooth muscle cells, along with its ability to prevent atherosclerosis in streptozotocin-induced diabetes in rats. Indeed, ellagic acid (25 μM) blocked platelet-derived growth factor receptor-β (PDGFR-β), tyrosine phosphorylation, generation of intracellular ROS, and downstream activation of ERK1/2. In diabetic rats, ellagic acid (2% in the diet) blocked diabetes-induced lipid deposition in the arch of the aorta, reducing the atherosclerotic process by blocking the proliferation of vascular smooth muscle cells [160].

The principal anti-hyperglycemic effects of ellagic acid on glucose homeostasis which can modify the metabolic syndrome are summarized in Figure **3**. The effect of ellagic

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acid on lipid and cholesterol metabolism and the positive consequences this has on atherogenic formation are summarized in Figure **4**.

Fig. 3 here

Fig. 4 here

Ellagic acid as a potential neuroprotective agent

Various assays have been performed to determine the possible effect of ellagic acid as a neuroprotective agent. Many of these have focused on its antioxidant properties; however, other interesting features are also of interest. For example, in a recent review, de Oliveira [51] compiled both *in vivo* and *in vitro* studies on the neuroprotective activity of ellagic acid against different stressors, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, streptozotocin, the traumatic brain injury test, a transgenic model of Alzheimer's disease, and hypoxic ischemic brain lesion. It also cited the positive effects of ellagic acid on pro-oxidant and anti-inflammatory mediators, and described the acid's ability to decrease lipid peroxidation, superoxide radical, NO, IL-1β, IL-6, and TNF-α production, as well as to activate the nuclear factor of activated T-cells 1 and phosphorylated-IκB (p-IκB). In addition, ellagic acid increased catalase and paraoxanase-1 activities, neuronal and memory function, and decreased the amount of brain area lost. Mansouri et al. [161] assayed ellagic acid (30 and 100 mg/kg) in two tests of memory impairment induced by scopolamine (0.4 mg/kg, i.p.) or diazepam (1 mg/kg, i.p.), demonstrating the efficacy of the compound in preventing both scopolamine- and diazepam-induced cognitive impairment without altering the animals' locomotion. Bansal et al. [162] studied the effect of ellagic acid (17.5 and 35 mg/kg, orally) on streptozotocin (3 mg/kg) -induced dementia in rats after bilateral intracerebroventricle injection in rats. After 28 days, ellagic acid both prevented the damage produced by streptozotocin and ameliorated the symptoms of dementia produced by this agent, probably by restoring the balance between cellular pro-oxidants and antioxidants in rat brains [162].

In their research, Sanadgol et al. [125] used C57BL/6J mice in which a depletion of oligodendrocytes in the corpus callosum had been induced. They found that ellagic acid (80 mg/kg/d, i.p., 4 wk) effectively reduced lesions via reduction of neuro-inflammation and toxic effects on mature oligodendrocytes with respect to the non-treated group (control). Treatment significantly down-regulated the expression of IL-17 and up-

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regulated the expression of IL-11, but had no effect on the expression of stromal cell-derived factor 1. Previously, these same authors had demonstrated that ellagic acid not only decreased the number of activated microglia cells, but also restricted the proliferation of these cells, thus lowering the concentration of microglial pro-inflammatory chemokines in the corpus callosum. They concluded that ellagic acid may consitute a suitable therapeutic agent for ameliorating brain damage in neuro-inflammatory diseases [163].

Nejad et al. [164,165] proposed a model of global cerebral ischemia-reperfusion induced by bilateral vertebral and common carotid artery occlusion, which leads to disturbances in brain function. In this model, ellagic acid (100 mg/kg/10 d, orally) improved both heart [164] and renal [165] function impaired by the global cerebral ischemia-reperfusion. The authors hypothesized that the acid's antioxidant properties may be responsible for these beneficial effects and noted ellagic acid's potential benefits in stroke victims. However, more specific studies are necessary to elaborate on this fairly simple hypothesis.

Farbood et al. [166] demonstrated the positive effects of ellagic acid in a model of traumatic brain injury induced by dropping a 200 g weight from a 2 m height through a free-falling tube onto the head of an anesthetized rat with a steel disk attached to its skull. Animals that received prior treatment with ellagic acid (100 mg/kg/7 d, orally) showed lower levels of traumatic brain injury-induced memory loss and hippocampal long-term potentiation impairment. Pretreatment with ellagic acid also decreased the elevated content of IL-1β, IL-6, and blood-brain barrier permeability in the brain normally produced during traumatic processes.

Liu et al. [167] tested an experimental model based on oxygen-glucose deprivation and reoxygenation in primary cultured cortical neurons from rats. In this protocol, ellagic acid increased neuron viability, cell nuclear integrity, and the ratio of B-cell lymphoma 2/Bcl-2-associated X protein (Bcl-2/Bax) expression. In *in vivo* experiments, ellagic acid increased the number of Bcl-2-positive cells while significantly decreasing both the volume of cerebrum infarction and the neurological deficit scores in the rats. The authors hypothesized that ellagic acid provides neuroprotection and that it could be used for treating nerve dysfunction, neurodegenerative disease, and aging processes.

Other researchers have focused on the potential of ellagic acid as a protective agent against neural damage in Parkinson's disease, with several studies examining this

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protective effect against free radical-induced damage. For example, Sarkaki et al. [168] tested the effects of ellagic acid (50 mg/kg, orally) on locomotion, pallidal local electroencephalography and the power of its frequency bands, as well as on cerebral antioxidant contents in a rat model of Parkinson's disease induced by 6-hydroxydopamine (16 μg/2 μL) injected into the right medial forebrain bundle. The test compound restored the activity of glutathione peroxidase and superoxide dismutase in both the striatum and hippocampus tissues, significantly increasing malondialdehyde levels in both the striatum and hippocampus tissues in medial forebrain bundle-lesioned rats. It also ameliorated motor impairments and improved the electrophysiological performance in treated rats. These authors [166] had previously used the same experimental model to demonstrate that ellagic acid can improve induced motor impairments by reducing the higher levels of neuro-inflammatory biomarkers (TNF-α and IL-1β) in lesioned rats while protecting the brain against free radical-induced neural damage. Using the same experimental model, Baluchnejadmojarad et al. [169] tested ellagic acid (50 mg/kg/d, 1 wk) and obtained similar results; however, they also found that the compound lowered the striatal level of monoamine oxidase B (MAO-B) in the treated group with respect to the 6-hydroxydopamine group, but that both Nrf2 and HO-1 were increased at the striatal level. Treatment with ellagic acid also prevented loss of tyrosine hydroxylase-positive neurons within the substantia nigra pars compacta. These findings suggest that the neuroprotective effect of ellagic acid in this rat model of Parkinson's disease occurs via suppression of MAO-B, with its favorable influence being partly reliant on the estrogen receptor-β/Nrf2/HO-1 signaling cascade. Other studies on the effect of ellagic acid against MAO-B have determined its potency (IC_{50} = 412.24 nM) and the fact that its inhibitory activity against MAO is both competitive and noncompetitive [170].

Taken together, these results of both *in vitro* and *in vivo* experiments indicate that ellagic acid is of great interest for future studies, including on humans, with potential applications as a memory restorative agent in the treatment of dementia and other cognitive alterations observed in the elderly.

Hepatoprotective effects of ellagic acid

Several studies have shown that ellagic acid has the potential to prevent or reduce toxicity in the liver by inhibiting NF-κB activation and NO generation, and by enhancing the cellular antioxidant system. García-Niño and Zazueta [50] reviewed the

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pharmacological activities of ellagic acid related to liver protection against a number of toxins including alcohol, CCl_4, cisplatin, cyclosporine, rifampicin, isoniazid, mercury, and paracetamol, which impair both liver function and its architectural structure. The authors also described the molecular mechanisms involved, including free radical scavenging, regulation of cytokine production, phase I and II enzyme and lipid synthesis and degradation processes, as well as conservation of oligo element levels. Independently of this excellent review, we collected other relevant, but subsequently published, articles on this subject. For example, in their work, Keshtzar et al. [171] described ellagic acid's protective effects against liver toxicity induced by arsenic, a pro-oxidant hepatotoxic heavy metal and one of the most potent environmental toxins, as can be seen by its classification in group I of human carcinogens by the International Agency for Research on Cancer. Currently, exposure to arsenic is inevitable, contributing to chronic diseases through overproduction of free radicals and oxidative stress, mitochondrial dysfunction, ATP production impairment, and carcinogenicity. The effects of ellagic acid, a potent antioxidant, were tested at concentrations of 20-80 μM against the toxicity induced by arsenic in mitochondria isolated from rat livers. The results indicated that this phenolic compound was able to reverse ROS generation and mitochondrial membrane damage, disrupt arsenic toxicity, and protect the mitochondria either directly through its antioxidant effect or indirectly by means of conserving the activity of mitochondrial complex II [171].

Ellagic acid's ability to interfere with phase I enzyme-catalyzed reactions has also been evaluated. For example, Siah et al. [172] analyzed the potential effects of ellagic acid on aldehyde oxidase, a phase-I cytosolic molybdenum-containing hydroxylase enzyme which is mainly active in the liver and participates in the metabolism of several aldehydes and nitrogen-containing molecules with biological functions. Phenolic compounds are known to interfere with aldehyde oxidase catalyzed reactions, interacting with specific drug-metabolizing enzymes and drug-food interactions with implications for human health. Specifically, ellagic acid was shown to inhibit aldehyde oxidase from guinea pigs in a non-competitive mode of action and with an IC_{50} value of 14.5 μM, more potent than the specific inhibitor of aldehyde oxidase, menadione (IC_{50}=31.8 μM). With regard to herbal-drug interactions mediated by CYP, the isoform CYP2D6 is responsible for the metabolism of nearly 25% of drugs. Thus, the effects of ellagic acid on the oral bioavailability of metoprolol was assessed in an *in situ* single

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pass intestinal perfusion study. In rats pretreated with the acid, improvements in both the plasma concentration and the area under the serum concentration-time profile were observed, along with a reduction in clearance. Ellagic acid significantly improved the oral bioavailability of metoprolol by inhibiting CYP2D6-mediated metabolism in the rat liver, suggesting that adverse herbal-drug interactions may occur when products containing ellagic acid are taken together with drugs that are CYP2D6 substrates [173].

Cytotoxic, antitumor, and anticancer effects of ellagic acid

Ellagic acid is considered to be a promising new chemopreventive and/or chemotherapeutic agent. It has been shown to exert effects against human cancers, including prostate, colon, pancreatic, breast, ovarian, bladder, and glioblastoma cancers, as well as lymphoma. Its chemopreventative potential has been evaluated extensively, with results showing that ellagic acid exerts its anticarcinogenic effects through multiple pathways: by stopping tumor cell proliferation, inducing apoptosis, blocking DNA damage generated by oxidative stress and carcinogens, and interfering with inflammation, angiogenesis, and other process required for tumor progression and metastasis. Other indirect mechanisms associated with ellagic acid's anticancer properties include antivirus activity, heart and liver protection, radio-sensitizing and counter radio-resistance effects, and inhibition of glutathione *S*-transferase-induced drug resistance [174]. Liang et al. [175] evaluated the effects of ellagic acid on Ca^{2+} homeostasis in liver cells and found that it increases the intracellular Ca^{2+} concentration in HepG2 human hepatoma cells via a phospholipase C-dependent pathway. Moreover, ellagic acid (25-100 μM) exhibited concentration-dependent cytotoxicity, which was partially prevented by the intracellular Ca^{2+} chelator 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid-acetoxy methyl. Ellagic acid also killed HA22T and HA59T hepatoma cells, but had no effect on normal liver cells (AML12 mouse hepatocytes), providing evidence for its therapeutic potential in the treatment of hepatoma [175].

While some studies focus on the effects of ellagic acid, others analyzed its metabolites, which have been implicated in the compound's antiproliferative effect on different types of cancer cells. For example, the chemopreventive potential of ellagic acid and its metabolite urolithin A was studied in prostate cancer, which is initially dependent on androgens, but then over time evolves to an androgen-independent phenotype, which is more resistant to secondary endocrine treatment and chemotherapy. The effects of ellagic acid and its metabolite on cell proliferation, cell cycle, and apoptosis were

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evaluated in the androgen-independent DU145 and PC-3 prostate cancer cell lines, with both compounds inhibiting cell proliferation in a dose-dependent manner, albeit with differences in the two cell lines and through different mechanisms of action. Whereas treatment with ellagic acid gave an antiproliferative IC_{50} of 14.5 μM (at 96 h) against PC-3 cells and an IC_{50} of 23.0 μM in the case of DU145 cells, treatment with urolithin A gave an IC_{50} value of 74.8 μM (at 96 h), but had no effect on the PC-3 cells. The two compounds also had different effects on the modulation of cell cycle regulatory proteins, with ellagic acid decreasing cyclin B1 and D1 expression and urolithin A inducing cyclin-dependent kinase-1 (cdk-1, also called cell division control protein 2 or cdc2) phosphorylation at tyr-15 and increasing cyclin B1, which arrested the cell cycle in the S and G_2/M phases, respectively. Ellagic acid also exhibited pro-apoptotic activity via a caspase dependent pathway. These effects may explain the synergistically inhibitory interaction between ellagic acid and its metabolite against PC-3 cell proliferation [176,177]. Independently of these mechanisms, there is a growing body of evidence which indicates that the multifunctional cytokine IL-6 is involved in the transition of prostate cancer from an androgen-dependent to an androgen-independent state. Indeed, IL-6 interacts with different cellular regulatory signaling pathways, such as signal transducer and activator of transcription 3 (STAT3), protein kinase B (Akt), and pERK1/2, which can either inhibit or stimulate various cancer cell lines. Treatment of PC-3 cells with ellagic acid (30, 50 and 70 μM) led to increased IL-6 levels in culture supernatants in a dose-dependent manner and down-regulated the expression of phosphorylated cellular proteins (p-STAT3, p-Akt, and pERK1/2). Despite this increase in IL-6, which is probably due to heightened cancer cell resistance, and in agreement with previous studies, ellagic acid can be considered as a potent antiproliferative agent against PC3 through the reduction of ERK1/2, Akt, and STAT3 cellular signaling proteins [178].

The combination of the three pomegranate constituents — ellagic acid, punicic acid, and luteolin — as well as the juice of this fruit are considered to be an alternative treatment for prostate cancer. Clinical trials have shown that pomegranate juice inhibits prostate cancer progression and prolongs the prostate specific antigen doubling time in prostate cancer patients [179]. The three constituents together (64 μg each, i.p., once a day, 5 d/wk, 8 wk) exhibited inhibitory effects on prostate cancer cells, angiogenesis, and metastasis. When tumor progression was monitored with bioluminescence imaging

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in an immunodeficiency mouse model in which luciferase-expressing human prostate cancer cells were subcutaneously injected close to the prostate, the three compounds inhibited the metastasis by blocking the stromal cell-derived factor 1 (also known as CXCL-12)/CXC chemokine receptor type 4 (also known as fusin or CD184) axis, and interrupted the growth and metastasis of invasive *Pten*$^{-/-}$; *K-ras*^{G12D} prostate tumors. They also inhibited angiogenic factors IL-8 and vascular endothelial growth factor (VEGF) as well as their induced signaling pathways in endothelial cells [179]. Ellagic acid demonstrated anti-angiogenic activity *in vivo* in a hamster model of oral oncogenesis by inhibiting hypoxia inducible factor-1α, VEGF, and its receptor (VEGFR2). This was accomplished by blocking the phosphoinositide 3-kinase (PI3K)/Akt and MAPK signaling pathways and *in vitro,* by suppression of histone deacetylases in endothelial cell line ECV304 [180]. In the transgenic rat for adenocarcinoma of prostate model, ellagic acid suppressed tumor progression and induced apoptosis via caspase-3 activation after 10 weeks of treatment. This effect was confirmed in the human prostate cancer androgen dependent cell line LNCaP, where ellagic acid also induced apoptosis by augmenting the Bax/Bcl-2 ratio and increasing cell-cycle related proteins p21, p27, cdk-2, and cyclin E while decreasing cyclin D1 and cdk-1 [181].

Using colon cancer stem cells, Núñez-Sánchez et al. [182] evaluated the effect of a mixture of ellagic acid (5%) and the gut microbiota-derived urolithins A (85%) and C (10%), detected in human colon tissues after intake of products with a high amount of ellagitannin, such as pomegranates and walnuts. The mixture not only inhibited aldehyde dehydrogenase activity, but also decreased the number (26.3 ± 3.8%) and size 23.7 ± 7.4%) of colonospheres in primary tumor cells from a patient with colorectal cancer, providing evidence for its potential role against colon cancer chemoresistance and relapse. In the case of ellagic acid (30 or 100 µg/mL), the treatment of human colorectal carcinoma cells CaCo-2 and HCT-116 lowered cell proliferation through an apoptotic effect, arrested the cell cycle in the G_1 phase, inhibited Akt phosphorylation (at Thr308 and Ser473), with subsequent downstream effects on the PI3K/Akt pathway, which plays a central role in colon tumorigenesis. Silencing of the *K-ras* (an early mutation in colorectal carcinogenesis) and treatment of transfected HCT-116 cells with ellagic acid inhibited cell proliferation and Akt phosphorylation at Thr308, confirming the role of activated *K-ras* in activating the PI3K/Akt pathway [183]. Microarray profile

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analysis of HCT-116 cells treated with ellagic acid (100 μM) for 72 h aided in identifying differentially expressed genes affecting cellular functions such as proliferation, apoptosis, cell cycle, and angiogenesis [184]. In a leptin-enriched microenvironment (200 ng/mL), ellagic acid (25 or 50 μg/mL) inhibited the proliferation of HCT-116 and CaCo-2 cells, modulated the cell cycle, up-regulated Bax, activated caspase-8, and decreased the expression of proliferating cell nuclear antigen, indicating a potential effect on obesity-related colon carcinogenesis [185]. Treatment of HCT-15 colon adenocarcinoma cells with ellagic acid (up to 60 μM) decreased cell proliferation and induced ROS production and apoptosis. It diminished cell viability and induced G_2/M phase cell cycle arrest, and also modulated alkaline phosphatase and lactate dehydrogenase activities, all of which indicate an antiproliferative and cytotoxic effect. Ellagic acid reduced the expression of the proliferation-associated markers proliferating cell nuclear antigen and cyclin D1 and blocked the PI3K/Akt pathway. Moreover, ellagic acid up-regulated the expression of Bax, caspase-3, and cytochrome C while suppressing Bcl-2 [186].

In an experimental model of colon carcinogenesis induced by 1,2-dimethylhydrazine in rats, treatment with ellagic acid (60 mg/kg/d, orally) led to beneficial effects such as reparation of negative effects on biochemical indexes, restoration of mitochondrial and membrane-bound enzyme activities, and a decrease in lysosomal enzymes [187]. Ellagic acid also seems to inhibit the growth, ability to repair, migration, and invasion of human pancreatic carcinoma PANC-1 cells in a dose dependent manner. Thus, PANC-1 cell tumor-bearing mice treated with ellagic acid had an increased survival rate, with inhibition of tumor growth through cell cycle arrest in the G_1 phase; down-regulation of COX-2, NF-κB, and vimentin; and up-regulation of E-cadherin [188].

In human breast cancer MCF-7 cells, ellagic acid (10 to 40 μg/mL) exerted its anti-proliferative effects by arresting the cell cycle the in G_0/G_1 phase via modulation of the transforming growth factor (TGF)-β/Smads signaling pathway [189]. Ellagic acid improved the efficacy of the PI3K inhibitor GDC-0941, inhibiting cell growth, migration, and invasion in breast cell lines as well as reducing tumor induction and metastasis *in vivo*. In addition, the combination of ellagic acid and GDC-0941 induced apoptosis and reduced the Akt/mammalian target of rapamycin (mTOR) activation in breast cancer cells [190]. Ellagic acid also has potential as a drug adjuvant for enhancing cancer radiotherapy due to its ability not only to improve apoptotic

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sensitivity on γ-irradiated human breast cancer MCF-7 cells, but also to up-regulate Bax and down-regulate Bcl-2, leading cells to undergo apoptotic death. While it was found to have a radio-protective effect on normal cell lines, combined treatment with ellagic acid (10 μM) and doses of 2- and 4-Gy γ radiation on MCF-7 exhibited synergistic tumor cytotoxicity [191].

Ellagic acid enhances the sensitivity of cytostatic drugs by modulating various pathways in different ovarian cancer cell lines. Indeed, the use of ellagic acid (3.2 μM) in the short-term treatment of the ovarian cancer A2780 cell line and its cisplatin-resistant subtype A2780CisR, obtained by intermittent treatment with cisplatin for 26 weekly cycles, led to a moderate reversal of cisplatin-chemoresistance [192]. Treatment of human endometrial cancer cells with ellagic acid (20 μM, 48 h) significantly inhibited ROS formation and regulated cytosolic pH and glycolytic flux. It down-regulated sodium-hydrogen antiporter 1 expression, and likewise decreased Na^+/H^+ exchanger activity, cytosolic pH, glucose uptake, and lactate release, all of which led to reprogramming and growth inhibition of tumor cells [193]. Ellagic acid has also been demonstrated to inhibit cervical cancer HeLa cells in a dose dependent manner (2.5-10 μM) by blocking the Akt/mTOR signaling pathway through up-regulation of insulin-like growth factor-binding protein 7(IGFBP7) [194]. Treatment of HeLa cells with a combination of ellagic acid and curcumin (25 μM, they do not declare the proportion of each) enhanced their potential anticancer and antihuman papilloma virus properties, as evidenced by decreased levels of the human papilloma virus HPV-E6 oncoprotein [195].

The activity of mitomycin C, commonly used for treating bladder cancer, is enhanced by ellagic acid, which could thus serve as adjunct therapy for this type of cancer. Indeed, ellagic acid (1.25-40 μM, *in vitro*, and 40 mg/kg, i.p., daily/15 d, *in vivo*) reduced the growth rate, infiltrative behavior, and tumor-associated angiogenesis of human bladder cancer xenografted mice. In addition, it inhibited both the tumor invasion and chemotaxis induced by VEGF-A. This phenolic acid also down-regulated the expression of the receptor VEGFR-2, as well as the programmed cell-death ligand 1 [196]. In addition to these effects, ellagic acid may also be beneficial for the management of glioblastoma cancer, as demonstrated by Wang et al. [197]. These authors found that at 50 and 100 μM, the acid suppressed the cell viability of U251 glioblastoma cells and affected cell cycle progression by inducing cell cycle arrest in the

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S phase. Ellagic acid markedly inhibited the anti-apoptotic proteins Bcl-2 and survivin while enhancing caspase-3 and the pro-apoptotic protein Bax. It up-regulated MAPKs (JNK, ERK1/2 and p38) and the expression of death receptor (DR)4, DR5, and cytosine-cytosine-adenosine-adenosine-thymidine-enhancer-binding protein (C/EBP) homologous protein (CHOP) an endoplasmic reticulum stress-regulated protein, indicating the involvement of ROS-JNK/ERK signaling in cell death [197]. In addition, this phenolic compound (50 and 100 μM) also inhibited the viability and proliferation of U87 and U118 human glioblastoma cell lines, increasing the proportion of cells in the S phase. This activity was confirmed *in vivo* (40 μg/g b.w., p.o. gavage daily, 5 d/wk, 4 wk) in glioblastoma xenografted mice. Ellagic acid suppressed tumor growth, up-regulated E-cadherin expression, and inhibited the expression of Snail, matrix metalloproteinase (MMP)-2 and MMP-9, Bcl-2, cyclin D1, cdk-2, and cdk-6, and also blocked Akt and Notch signaling pathways [198].

Chronic lymphocytic leukemia is characterized by failed apoptosis which, in turn, plays an important role in its resistance to conventional therapies. Pro-apoptotic signals such as oxidative stress, DNA damage, and mitochondrial membrane alterations all induce apoptosis. Salimi et al. [199] demonstrated that ellagic acid (25 μM) was selectively able to induce ROS-mediated apoptosis in B-lymphocytes obtained from patients with chronic lymphocytic leukemia via the mitochondrial pathway. It did so by inducing failure of mitochondrial membrane potential, increasing mitochondrial swelling and cytochrome c release, all of which point to the acid's anticancer potential.

Protein kinase C regulates many cellular processes, including apoptosis. Recent studies have reported novel and atypical isozymes of the protein kinase C subfamily to be mainly involved in cell proliferation, apoptosis, and differentiation; in fact, ellagic acid has been found to exhibit its anticarcinogenic activity through modulation of these isozymes. Using Dalton's lymphoma mice, Misra and Vinayak [200] demonstrated the anti-carcinogenic effects of ellagic acid, which increased longevity and survival while decreasing tumor size, viability, and the proliferation of ascites cells. Treatment of lymphoma-bearing mice with 40, 60, or 80 mg/kg daily for 15 consecutive days induced apoptosis in the liver by promoting expression and activation of protein kinase Cδ and caspase-3, and also by inhibiting energy metabolism.

The low bioavailability of ellagic acid has inspired a number of studies focused on drug delivery systems in order to reach therapeutic concentrations in the systemic circulation

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and to increase efficacy. Wei et al. [201] used a nanomedicine against fibrotic stroma and tumor-promoting pancreatic stellate cells. It consisted of 9 nm human serum albumin-ellagic acid and human serum albumin-paclitaxel complexes co-encapsulated into thermosensitive liposomes that improved drug perfusion and led to tumor growth inhibition and apoptosis. When ellagic acid (up to 100 μM) was encapsulated into the polymer-based nanoparticles (diameter average 150-300 nm) poly D-L-lactide-co-glycolide decorated with chitosan and polyethylene glycol, the resulting poly D-L-lactide-co-glycolide-chitosan-polyethylene glycol potentiated apoptosis–mediated cell death in HepG2 human hepatoma cells [202]. Ellagic acid-encapsulated (2 μM) nano-sized metalla-cages exerted anticancer activity by inhibiting the growth of cancer cells through modulation of the granulocyte-colony stimulating factor at gene and protein expression levels and in macrophages regulated by activation of normal T cell expressed and secreted protein [203].

The principal studies *in vitro* of effect of ellagic acid on different cancerous cell lines are summarized in Table **2**.

Table 2 here

Ellagic acid and skin protection

UV radiation causes oxidative stress through production of ROS, which disrupt the endogenous antioxidative system of the skin cells and may lead to skin inflammatory disorders, depigmentation, photoaging, and carcinoma. Several studies have described the potential photoprotective effects of ellagic acid, suggesting its promising potential as a food supplement and/or in the preparation of skin care products for the prevention or treatment of skin disorders. Indeed, the antioxidative effect of ellagic acid against UVA- and UVB-induced oxidative stress on human keratinocyte (HaCaT) cells and human dermal fibroblasts has already been demonstrated. Ellagic acid (1-10 μM) increased in a dose-dependent manner the viability of UVB-exposed keratinocytes and fibroblasts, attenuated MMP secretion, and raised collagen levels in dermal fibroblasts. Ellagic acid thus exhibited photoprotective effects on skin wrinkle formation resulting from collagen breakdown through increasing MMP production. Moreover, topical application of the acid (10 μM) to the dorsal skin of SKH-1 hairless mice exposed to chronic UVB radiation (100 mJ/cm^2, 8 wk) attenuated wrinkle formation and epidermal thickness while also decreasing the accumulation of inflammatory cytokines such as IL-

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1β and IL-6 and the expression of ICAM-1 [119]. Ellagic acid (5 μM) also reduced pro-inflammatory mediators and significantly increased IL-10 expression in HaCaT under UVB-radiation [204]. In addition, the photoaging protection was confirmed in cultured fibroblasts when cells were exposed to ellagic acid (5, 15, 30 μM prior to UV-B irradiation (70 mJ/cm^2). It decreased both ROS levels and MMP-2 production and also restored total glutathione levels and superoxide dismutase activity in a concentration-dependent manner, partly by up-regulating Nrf2 [82]. Pre-treatment of irradiated (UVA, 20 J/cm^2) HaCaT cells with ellagic acid (25-75 μM) inhibited cytotoxicity and suppressed ROS production and lipid peroxidation. It also inhibited UVA-induced apoptosis by blocking DNA strand breaks, down-regulating activation of caspase-3, and dysregulating Bcl-2 and Bax expression. These effects were associated with a notable rise in HO-1 or superoxide dismutase via up-regulation of the oxidative stress marker Nrf2 and down-regulation of the Kelch-like ECH-associated protein-1. These findings add further support for ellagic acid's protective effects against UVA-induced skin damage [83].

Due to ellagic acid's poor biopharmaceutical properties, low solubility, and low permeability, various formulations have been developed. As a plausible agent for manufacturing anti-photoaging cosmetics, for example, the acid should be incorporated into a topical formulation because it permeates the skin barrier to reach the viable epidermis and dermis layers, thus helping to avoid or delay UV radiation damage. Pomegranate peel polyphenols including ellagic acid were delivered to the deeper skin layers by applying nanoemulsions of the ethyl acetate fraction prepared with pomegranate seed oil onto the skin [205]. Previously, a topical ointment prepared with polyethylene glycol and 5% standardized pomegranate rind extract containing ellagic acid (13%) was developed for release and skin permeation studies and was found to exhibit acceptable physicochemical properties [206]. When the wound healing activities of this ointment were compared with the equivalent amount of ellagic acid (0.65%), the latter was less effective in inhibiting neutrophil infiltration and collagen augmentation in rat skin [207]. However, both products applied topically exhibited similar anti-inflammatory effects against a mouse model of contact dermatitis [92]. Another dermal delivery system that was developed involved ellagic acid-loaded niosomes. Those prepared with the mixture Span 60 and Tween 60 (2:1), with 15% polyethylene glycol

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400 as solvent, exhibited the highest percentage of both entrapment efficacy as well as delivery of ellagic acid to human epidermis and dermis [208].

Although melanin protects from UV damage, its excessive production causes hyperpigmentation. While hydroquinone is a well-known benchmark product for treating hyperpigmentation, its adverse effects make the search for alternative agents necessary. Ellagic acid is considered to be a useful depigmentation agent in the treatment of hyperpigmentation disorders because it interferes with the melanin biogenesis pathway in which tyrosinase catalyzes the hydroxylation of monophenols to *o*-diphenols and their subsequent oxidation to the unstable *o*-quinones, which are then converted to melanins. In their research, Ito and Wakamatsu [209] determined the differences between leukoderma-inducing phenols and phenolic skin whitening tyrosinase inhibitors using spectrophotometric (420 nm) and HPLC analyses after reduction with $NaBH_4$ for detecting the corresponding catechols. They demonstrated that while the leukoderma-inducing phenols were readily oxidized by tyrosinase to form *o*-quinones, the latter were not. Thus, rather than being a tyrosinase inhibitor, ellagic acid can act as an alternative tyrosinase substrate to be oxidized to form *o*-quinones and semiquinones, which may then react with nucleophilic compounds. As a powerful antioxidant, ellagic acid is capable of modifying the redox status of the cell and may thus reduce these reactive molecules (the ratio of the antioxidant concentration necessary to decrease the initial concentration of the ABTS to 50%, is 20 for ellagic acid, five times greater than that of ascorbic acid), inhibiting the melanogenesis process [210]. This should be taken be into account when ellagic acid is used as an ingredient in whitening creams and other cosmetics. The skin-lightening ability, tolerability, and safety profile of a novel alternative formulation containing ellagic acid was assessed against other active compounds in a single-blind study (n = 82). The results were similar for the ellagic acid formulation and the standard cream (hydroquinone + tretinoin), prompting the authors to claim that the former could be used as a benchmark to give dermatologists a frame of reference for expected efficacy [211]. Previously, the same efficacy and tolerance were observed in comparing a topical formulation containing ellagic acid (0.5%) and salicylic acid (0.1%) versus hydroquinone (4.0%), with similar results on skin depigmentation [212].

Cardiovascular effects

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In their review of the effects of ellagic acid on vascular health, Larrosa et al. [8] noted the extreme difficulty in establishing the systemic potential of the compound due to its low bioavailability. This property can only be justified either by an effect *in situ* or as a consequence of the acid's antioxidant activity, which hampers the oxidation of other bioactive compounds, such as vitamins or fatty acids. However, recent studies have been conducted using various *in vivo* experiments to elucidate the activity of ellagic acid in cardiovascular disease. For example, ellagic acid (10 μM) accelerated the rate of relaxation and the rate of Ca^{2+} transient decay in streptozotocin-treated mice, with the former effect being completely inhibited by the sarco-endoplasmic reticulum Ca^{2+}-ATPase inhibitor cyclopiazonic acid. This indicates not only that diabetes mellitus-induced myocardial diastolic dysfunction is partly caused by reduction of sarco-endoplasmic reticulum Ca^{2+}-ATP_{ase} function, but also that it can be ameliorated by ellagic acid and other activators [213]. Ellagic acid exerts a cardioprotective effect against As_2O_3 toxicity, a consequence of its antioxidant properties, which in this case enhance the endogenous antioxidant system [214]. It also protects against doxorubicin-induced cardiotoxicity in mice [215]. Indeed, intake of ellagic acid (0.25, 0.5, and 1%, in feed, 8 wk) dose-dependently increased the content of this compound in cardiac tissue and preserved glutathione content while lowering ROS and malondialdehyde levels; it also reduced xanthine oxidase activity. Ellagic acid (0.5, and 1%) lowered some of the experimental results that had been increased by administration of doxorubicin, such as lactate dehydrogenase activity, creatine phosphokinase activity, caspase-3 activity, and cleaved caspase-3 formation, while suppressing both p-p38 expression as well as the activity and protein levels of NF-κB. At 1% ellagic acid down-regulated p-ERK 1/2 expression. These findings suggest that ellagic acid is a potent cardiac protective agent against doxorubicin [215].

At the higher dose, it also down-regulated p-ERK1/2 expression [215]. Ellagic acid was also shown to have cardioprotective effects in rats treated with isoproterenol to induce myocardial infarction. Administration of ellagic acid (7.5 and 15 mg/kg, orally) modified various biochemical parameters, including serum iron, plasma iron binding capacity, uric acid, glycoprotein, and electrolytes. It also returned the various hematological parameters to near normal levels, down from the increased levels brought on by the administration of isoproterenol (100 mg/kg, 2 d) [216]. Using the same doses, administration method, and experimental protocols, these authors also described ellagic

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acid's protective effects against isoproterenol-induced arrhythmias, hypertrophy, and lipid peroxidation during myocardial infarction in rats [217]. In addition, ellagic acid (15 mg/kg, 10 d, orally), exhibited cardioprotective effects on $CaCl_2$-induced arrhythmias in a rat stress model, reducing the incidence rates of premature beats, fibrillation, and ventricular tachycardia induced by $CaCl_2$ (140 mg/kg, i.v.) [218].

The effects of ellagic acid on hypertension have been analyzed by many different research groups. For example, Berkban et al. [69] studied its effect on the oxidative stress and hypertension induced by *N*ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME) in male Sprague-Dawley rats. In these experiments, ellagic acid (7.5 or 15 mg/kg, orally, 5 wk) attenuated hypertension, prevented oxidative stress, and restored NO bioavailability by reducing NADPH oxidase subunit $p47^{phox}$ expression, which is responsible for increased vascular superoxide radical production in L-NAME hypertensive rats via up-regulation of the NADPH oxidase subunit $p47^{phox}$. Ellagic acid reduced both the systolic and diastolic pressures elevated by L-NAME (40 mg/kg/day, 5 w) from 199/140 mmHg to 168/114 and 165/111 mmHg at doses of 7.5/15 mg/kg, respectively [69]. In an *in vitro* study, Olgar et al. [219] had previously demonstrated that ellagic acid can modify ionic and mechanical properties of isolated rat ventricular myocytes, starting at nanomolar concentrations. It dose-dependently reduced Ca currents with an EC_{50} value of 23 nM and exerted negative inotropic effects through activation of the nitric oxide synthase-guanylyl cyclase-cGMP pathways, all without affecting the inactivation and reactivation parameters [219].

Other properties of interest of ellagic acid

Ellagic acid has been described as antibacterial [220], antiviral [221], and antimalarial [222]. The antibacterial properties of ellagic acid were described in various reviews, such as that by Howell and D'Souza [223], which cited the activity of pomegranate juice and established ellagic acid as a potential active principle, a claim that was bolstered by Shaygannia et al. [224]. A selective review was carried out by Chinsembu [225], who reviewed the effects of natural products against tuberculosis and included ellagic acid as a putative active compound against mycobacteria.

Tran et al. [226] described the effect of ellagic acid (from *Aronia melanocarpa* (Michx.) Elliott, Rosaceae) against the influenza virus in the cytopathic effect reduction assay with an EC_{50} value between 0.14-0.27 μM against different virus strains. It inhibited

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hepatitis C virus protease activity, with an IC_{50} of 56.3 μM [227,228] and inhibited virus replication with an EC_{50} ~60 μM [218]. When Park et al. [229] studied the effect of ellagic acid against influenza virus (H1/K09) in a replication inhibition assay in MDCK cells, they observed that it reduced virus replication in the lungs of infected mice (about 50%) with respect to non-treated mice [229].

As commented above, ellagic acid showed activity *in vitro* against different *Plasmodium falciparum* strains, with an IC_{50} range of 105-330 nM. It also exhibited *in vivo* activity against *Plamodium vinckei petteri*, showing suppressive, curative, and prophylactic murine properties. Ellagic acid has a high therapeutic index when administered intraperitoneally, but when administered orally, its antimalarial efficacy is limited. For this reason its pharmacokinetic properties should be enhanced [222]. Other studies have described the synergy between ellagic acid and several antimalarial drugs, which could allow for dose reduction in the treatment of malaria, with the concomitant reduction in potential side effects [230].

Ellagic acid showed neither acute toxicity nor chronic effects after its administration to mice. Indeed, treatment with ellagic acid up to 5000 mg/kg induced no toxic signs and, after repeated oral administration (1000 mg/kg/d for 28 d), no obvious toxic symptoms affecting vital organs (liver and spleen) were observed [228]. Likewise, doses of 100 mg/kg/d administered intraperitoneally did exhibited no toxicity in mice [222].

Clinical trials with implications for ellagic acid treatment

A limited number of relevant clinical studies with ellagic acid have been conducted. Some were carried out with medicinal plants containing this compound as well as ellagitannins and their metabolites, urolithins, which are produced by the gut microbiota after metabolizing ellagitannins and ellagic acid. Núñez-Sánchez et al. [231] studied the possible effect of these metabolites in colorectal cancer patients (n = 52) after they had been given pomegranate extract (900 mg/d, 15 d), analyzing the presence of these metabolites in the urine or tissue of normal and malignant colons. Ellagic acid was detected in colon tissue both in free form and as conjugates. Samples from colorectal cancer patients who had received 291 mg/g of free ellagic acid showed 649 ng/g in normal tissue and 195 ng/g in malignant tissue.

In addition, various clinical trials have been conducted on cancer and human papillomavirus infection, but these have mostly been carried out with mixtures or

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supplementation together with other substances, making it difficult to establish the active principle responsible for the specific pharmacological effect. For example, in a randomized, controlled trial (NCT02263378), the authors evaluated the effects of a supplement with ellagic acid plus *Annona muricata* L. (Annonaceae) on the immune response against papillomavirus infection, but no study results were posted [232]. Other clinical trials analyzed dietary intervention in follicular lymphoma using various agents in which pomegranate juice with ellagic acid was included [233], while others examined the effect of pomegranate extract supplementation in colorectal cancer patients [234]. While no results were posted for either trial, the effects can most likely be assigned to the metabolites (urolithins) of ellagitannins and ellagic acid.

Another series of studies focused on skin hyperpigmentation in humans and the potential of ellagic acid to protect against different agents. For example, Ertam et al. [235] analyzed the effect of synthetic ellagic acid (n = 10) and plant extracts containing ellagic acid on thirty patients with melasma in a randomized, prospective, open-label study. Of the ten patients who received treatment with synthetic ellagic acid, nine completed the study; of these, eight showed decreases in melanin levels after treatment with the acid. In addition, formulations prepared with plant extracts containing 1% ellagic acid + 1% plant extract demonstrated the same efficacy against melasma as the formulations prepared with synthetic ellagic acid (1%). Previously, Kasai et al. [236] had conducted a double-blind, placebo-controlled trial for evaluating the protective and ameliorative effects of a pomegranate extract rich in ellagic acid on skin pigmentation after ultraviolet irradiation. Healthy female volunteers (n= 30 × 3 groups) were given either ellagic acid at a high dose (200 mg/d), a low dose (100 mg/d), or a placebo control (0 mg/d). The results demonstrated that oral administration of ellagic acid-rich pomegranate extract inhibits the effects caused by UV on pigmentation in human skin. In 2013, Dahl et al. [212] carried out a double-blind clinical study lasting 12 weeks to compare the effect of a topical product containing ellagic acid (0.5%) and salicylic acid (0.1%) with another containing hydroquinone (4%), both applied twice daily. They randomly assigned 54 multi-ethnic subjects into two groups and found that the effect of ellagic acid was comparable to that of the standard drug used for skin depigmentation, but with better physical and esthetic characteristics.

A single-center, investigator-blinded, 12 week study was developed by Draelos et al. [211], who divided 82 subjects (7 male, 75 female) between 25 and 60 years of age into

2 balanced groups of 41 subjects each. They compared the skin-lightening ability, tolerability, and safety profile of a novel formulation containing ellagic acid, hydroxyphenoxy propionic acid, yeast extract, and salicylic acid (formula percentages were not given) and compared the results with those of a standard treatment (cream with 4% hydroquinone and 0.025% tretinoin) applied nightly. The groups were balanced for age, severity of dyspigmentation, and Fitzpatrick skin types. The facial dyschromias deemed appropriate for inclusion were mottled hyperpigmentation and lentigines, but not melasma. The results were similar for both formulas, but use of the novel preparation avoided administration of more aggressive compounds, such as tretinoin, thus suggesting the valuable potential of this new formulation in the treatment of skin dyspigmentation [211].

Pharmacokinetic properties of ellagic acid

The intake of ellagic acid in humans around the world is varied and depends on both the region and the life-style. It can usually be obtained directly in its free form or as ellagitannins, which are hydrolyzed by the enzyme ellagitannase (ellagitannin acyl hydrolase) to release ellagic acid and other relevant metabolites [237]. With respect to the pharmacokinetic properties of ellagic acid, very few studies have been carried out, especially in humans. For example, in order to elucidate the acid's pharmacokinetic properties, Lei et al. [238] used HPLC to analyze the presence of ellagic acid after oral administration of pomegranate leaf extract (0.8 g/kg). They observed an open, two-compartment system with a lag time and a plasmatic C_{max} of 213 ng/mL (0.55 h) after oral administration of the extract, with poor absorption and rapid elimination.

Murugan et al. [239] performed an *in vivo* study with Wistar rats to investigate the pharmacokinetics of an ellagic acid-phospholipid complex (equivalent to 80 mg/kg of ellagic acid) and observed that the serum concentration of ellagic acid obtained from the complex was higher (C_{max} = 0.54 μg/mL) than when the equivalent dose of the free form (80 mg/kg) was used (C_{max} = 0.21 μg/mL); moreover, the plasmatic concentration of the complex was maintained over a long period of time [239]. In 2014, Yan et al. [240] analyzed the pharmacokinetics and tissue distribution of ellagic acid in Sprague-Dawley rats. The compound was separated, detected, and quantified in plasma using a solid phase extraction step prior to reversed-phase ultra-performance liquid chromatography. Mass spectrometric detection was carried out with heated electrospray ionization (negative mode) and multiple ion monitoring. After oral administration of

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ellagic acid (50 mg/kg), plasma levels peaked at about 30 min, with a C_{max} value of 93.6 ng/mL (0.31 μM). The area under the curve ($AUC_{0-\infty}$) of the concentration-time profile was 457.2 ng/mL × h, indicating that this compound exhibits extremely poor absorption after oral administration. Ellagic acid followed a pharmacokinetic profile fitted to a two-compartment model with a $t_{1/2\alpha}$ = 0.25 h and $t_{1/2\beta}$ = 6.86 h. Other relevant parameters were: CL = 109.3 L/h/kg; AUC_{0-t} 252.0 ng/mL ×h, K_{10} = 0.54 h^{-1}, K_{12} = 1.90 h^{-1}, K_{21} = 0.47 h^{-1}, and Ka = 14.52 h^{-1}. Ellagic acid was detected in all the various tissues examined, including kidneys, liver, heart, lungs, and brain, with the highest levels found in the kidneys (about 250 ng/g at 0.5 h and 180 ng/g at 2 h) and liver (about 45 ng/g at 0.5 h and 70 ng/g at 2 h). Although the values observed in this study differed from those of prior reports, it is also true that the doses and the experimental protocols were different.

The literature contains several clinical trials, but the protocols and number of patients are usually limited. For example, Seeram et al. [241] administered 180 mL pomegranate juice containing 25 mg of ellagic acid and 318 mg of ellagitannins (expressed as punicalagins) to one sole male subject. The maximum plasmatic concentration (31.9 ng/mL) was obtained after 1 h post-ingestion, but was completely eliminated at 4 h; however, this preliminary study is hardly conclusive due to the fact that only one case was analyzed. A year later, Stoner et al. [242] carried out a clinical trial with eleven subjects to determine the safety/tolerability of ellagic acid and other phenolic compounds after administration of black raspberries (45 g/d for 7 d). Samples of blood and urine were collected on days 1 and 7, with analyses showing that the maximum concentration of ellagic acid in plasma occurred at 1-2 h, while in urine it appeared from 0-4 h; nevertheless, upon quantification, it was demonstrated that less than 1% was absorbed and excreted in urine.

Various hypotheses can be made with the data known to date [156]. After ingestion of ellagic acid, there is a small proportion of free compound that can be absorbed in the stomach while the rest of it is absorbed in the small intestine [243]. In contrast, ellagitannins are resistant to gastric metabolism; their hydrolysis occurs in the small intestine at a neutral to slightly basic pH, giving free ellagic acid which can be taken up in the small intestine. As there are no specific transporters across the gut epithelium for this compound, this process must occur through passive diffusion due to a concentration gradient [244]. For this reason, the presence of ellagic acid in plasma depends on its

ratio to ellagitannins present in the specific food or medicinal plant [156]. In the case of pomegranate juice with 318 mg ellagitannins and 25 mg free ellagic acid, the plasmatic values of the free form reached a C_{max} of 32 ng/mL (0.106 µM) at 1.0 h [241]. When the same dose of pomegranate juice with same amount of ellagitannins (318 mg) but only 12 mg of free ellagic acid was assayed, the result was a C_{max} = 18 ng/mL (0.06 µM) [245], whereas a similar study with a pomegranate extract containing 330 mg ellagitannins and 22 mg free ellagic acid gave a C_{max} = 33 ng/mL (0.11 µM) at 1 h for this compound [246]. González-Sarrías et al. [247] described the absorption saturation in the small intestine when high doses of ellagic acid were used. Indeed, in a crossover study with humans who received either 130 mg punicalagin plus 524 mg free ellagic acid (high dose) or 279 mg punicalagin plus 25 mg free form (low dose), the authors observed that the high dose of the free form showed no enhanced bioavailability with respect to the low dose [247]. Another interesting hypothesis about the data obtained from pharmacokinetic studies on ellagic acid is that the primary absorption occurs in the stomach and the upper part of the small intestine (short T_{max}) and that the rapid elimination is due to an efficient first-pass metabolism and a weak enterohepatic recirculation [156]. The unabsorbed ellagic acid and ellagitannins are metabolized by gut microbiota in the colon to urolithins [248], whereas the absorbed ellagic acid is converted to methyl esters, dimethyl esters, and glucuronides, which are eliminated through urine 1–5 h after ingestion [156,245,246,249].

Several derivatives or new formulations have been proposed for increasing the pharmacokinetic properties and, in parallel, the pharmacological activity of ellagic acid, as seen in the study mentioned above. For example, whereas Wei et al. [201] used a nanomedicine (thermosensitive liposomes with co-encapsulated 9 nm human serum albumin-ellagic acid + human serum albumin-paclitaxel complexes) in their research, Abd-Rabou and Ahmed [202] encapsulated ellagic acid into the polymer-based nanoparticles (150-300 nm) poly D-L-lactide-co-glycolide, while Dubey et al. [203] used capsules of ellagic acid in nano-sized metalla-cages. In all these cases, there was a clear increase in activity which prompted the various authors to propose that these modifications be employed to increase ellagic acid's therapeutic utility as an anticancer agent. In the case of skin protection, some authors proposed the use of nanoemulsions [205], ointment (polyethylene glycol) [92,206,207], or niosomes (Span 60 - Tween 60, 2:1 and 15% polyethylene glycol 400) [208]. Again, all these novel formulations led to

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clear advantages, such as access to the deeper skin layers [204] or better delivery of ellagic acid through human epidermis and dermis [208].

Summary, future perspectives, and conclusions

Ellagic acid is present in different medicinal plants and vegetables, as well as in edible fruits and seeds. It can be present as complex polymers called ellagitaninns or in free form, which is hydrolyzed in the digestive tract to give higher plasmatic levels of ellagic acid after its digestion. A great number of authors have described this phenolic acid's various effects, many of which are known from folk medicine and the use of medicinal plants. Ellagic acid's antioxidant properties, for example, are of great interest as they are implicated in most of its pharmacological properties, including anti-inflammatory activities, neuroprotection, hepatoprotection, and protection against diabetes, cardiovascular disease, and cancer. However, other mechanisms are also relevant.

The acid's antioxidant capacity has been attributed to its free radical scavenging activity, which is due to the presence of four hydroxyl and two lactone functional groups that enable ellagic acid to scavenge an extensive variety of ROS, such as hydroxyl, hydroperoxyl, and peroxyl radicals, as well as nitrogen dioxide and peroxynitrite. Thus, ellagic acid has a protective effect against free radical-induced damage, such as gastric lesions, hepatic injury and hyperlipemia. In addition, the inhibition of lipid peroxidation through the scavenging of hydroxyl and peroxyl radicals can protect various vital organs including the liver, lungs, and brain from oxidative injury. Ellagic acid has also been shown to inhibit the pro-oxidative action of metals such as nickel and ferrous ion through a chelation process and can decrease oxidative DNA damage. Moreover, ellagic acid can protect against oxidative injury through the expression of Nrf2 and HO-1.

Because inflammation and oxidative stress are closely linked, the antioxidative effect of ellagic acid is relevant for its anti-inflammatory properties. However, other mechanisms collaborate in the reduction of inflammation, such as the reduction of pro-inflammatory cytokines (IL-1β, IL-6TNF-α), the increase of anti-inflammatory cytokines (IL-10), and the inhibition of transcription factors (AP-1) and various kinases (MAPK, ERK1/2, JNK). A reduction of the expression of proteins such as VCAM-1 and E-selectin on the surface of endothelial cells was also reported while ICAM-1 did not seem to be affected. Secretion of pro-inflammatory mediators such as MIF and MCP-1 was also

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reported, as well as the reduction of TLR2 and TLR4 protein levels and mRNA expression in liver tissues. These data are probably the most relevant as they had not previously been reported. They indicate that the compound could be of high interest against inflammatory diseases associated with TLR signaling.

In the case of metabolic syndrome, inflammation, together with heart disease, diabetes, and stroke, are clear negative factors, as are abdominal obesity, high triglyceride and HDL-cholesterol levels, high blood pressure, and a high fasting blood glucose. The effect of ellagic acid on inflammation have been described above, but its antihypertensive, antihyperglycemic, and anti-hyperlipidemic properties are also of great interest. There have thus been reports, in the first case, of the acid's ability to inhibit angiotensin I-converting enzyme. In the second case, researchers have described its inhibitory effects on α-amylase, HbA1c, hexokinase, glucose-6-phosphatase, and fructose-1,6-bisphosphophosphatase activities, as well as its ability to reduce glycogen and increase insulin. Independent of the effects of glucose metabolism, ellagic acid can reduce the collateral negative effects of hyperglycemia due to sorbitol accumulation and formation of AGEs, thus preventing them from contributing to the complications of type 2 diabetes mellitus, such as cataracts. Ellagic acid also suppresses resistin secretion by a novel mechanism involving the degradation of intracellular resistin protein in adipocytes, a link between obesity and type 2 diabetes mellitus. As for its effects on hyperlipidemia, ellagic acid reduces the oxidation of LDL-cholesterol, oxidized-LDL uptake, and cholesterol influx, all while suppressing foam cell formation. In addition, ellagic acid acts as a PPARγ modulator and transfers effluxed cholesterol onto lipid-poor apolipoproteins, initiating the formation of HDL particles and, in consequence, blocking foam cell formation.

Ellagic acid also acts as a potential neuroprotective agent in experimental models of both Alzheimer's and Parkinson's diseases. Indeed, it has positive effects on pro-oxidant and anti-inflammatory mediators, decreases lipid peroxidation, and acts as an ROS scavenger. In addition, ellagic acid has been shown to increase neuronal and memory functions and decrease the amount of brain area lost. Treatment with this phenolic compound down-regulates the expression of IL-17 and up-regulates the expression of IL-11, decreases the number and proliferation of activated microglia cells, and as a result reduces microglial pro-inflammatory chemokine concentration and moderates brain damage in neuro-inflammatory processes. Ellagic acid also has

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protective effects against the neural damage caused by Parkinson's disease and has been shown to improve motor impairments and electrophysiological performance in animals. These effects can all be explained by the reduction of the neuro-inflammatory biomarkers TNF-α and IL-1β as well as the protection of the brain against free radicals, the reduction of MAO-B, and the increase of Nrf2 and HO-1 at the striatal level. These findings indicate that ellagic acid has potential for memory restoration in the treatment of dementia and other cognitive alterations observed in the elderly.

The hepatoprotective effects of ellagic acid are principally due to its free radical scavenging and its regulation of cytokine production, phase I and II catalyzed enzyme reactions, and lipid synthesis and degradation processes. By means of similar mechanisms and other specific pathways, ellagic acid exerts positive effects against various human cancers, including prostate, colon, pancreatic, breast, ovarian, and bladder cancer, as well as glioblastoma and lymphoma. It induces apoptosis, blocks DNA damage generated by oxidative stress and carcinogens, and interferes with inflammation, angiogenesis, and other processes required for tumor progression and metastasis. With regard to its chemopreventive potential, both ellagic acid and its metabolites (principally urolithin A) are of great interest because they have anti-proliferative effects, modulate cell cycle regulatory proteins, decrease cyclin B1 and D1 expression, induce cdk-1 phosphorylation, and arrest the cell cycle in S and G_2/M phases. These compounds have also been shown to exhibit pro-apoptotic activity via a caspase-dependent pathway. Ellagic acid has also been shown to down-regulate the expression of p-STAT3, p-Akt, and pERK1/2, which could be considered a relevant mechanism for its anti-proliferative effects. In addition, it reduced cyclin D1 expression and inactivated the PI3K/Akt pathway, up-regulated the expression of Bax, caspase-3, and cytochrome C, and suppressed Bcl-2.

The principal problem of ellagic acid is its poor solubility in water, which has a relevant effect on its pharmacokinetic properties. Various researchers have described an open two-compartment system with poor absorption and rapid elimination. For this reason, various systems and formulations have been created to improve its bioavailability. These include an ellagic acid-phospholipid complex, a nanomedicine (thermosensitive liposomes), polymer-based nanoparticles, and nano-sized metalla-cages. In the case of skin protection, the use of nanoemulsions has been proposed. However, the relatively low number of clinical studies, with varied protocols and an extremely low number of

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study subjects, poses a problem for establishing the real potential of this phenolic compound as a possible medicinal agent. However, the experimental data to date, along with the limited results observed in human clinical trials, has piqued a great amount of interest in this metabolite, both as a therapeutic drug and as as component of medicinal plants/food extracts.

Conflict of interest

The authors state no conflict of interest

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Legends

Figure 1. Chemical structure of ellagic acid

Figure 2. Effect of ellagic acid on different metals. Proposal mechanism of chelation (adapted from Dalvi et al. [75]).

Figure 3. Anti-hyperglycemic effects of ellagic acid on glucose metabolism. Advanced glycation end-products (AGE); glycosylated hemoglobin (HbA1c); nuclear factor-kappa B (NF-κB); nuclear factor erythroid 2–related factor 2 (Nrf2).

Figure 4. Effects of ellagic acid on lipid and cholesterol metabolism and their positive consequences on atherogenic formation. ATP binding cassette transporter-1 (ABCA1); extracellular signal-regulated kinase 1/2 (ERK1/2); liver X receptor (LXR); platelet-derived growth factor receptor (PDGFR)-β; peroxisome proliferator-activated receptor (PPAR-γ); reactive oxygen species (ROS); scavenger receptor class B1 (SRB1).

Table 1. Presence of ellagic acid on different medicinal plants. This review compiles only the more recent papers in which ellagic acid was isolated or identified.

Table 2. Effects of ellagic acid on various cancer cell lines.

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Table 1. Presence of ellagic acid on different medicinal plants. This review compiles only the more recent papers in which ellagic acid was isolated or identified.

Plant species	Family	References
Acalypha hispida Burm.f.	Euphorbiaceae	[9]
Acca sellowiana (O.Berg) Burret *	Myrtaceae	[10]
Baccharis inamoena Gardner *	Compositae	[11]
Camellia nitidissima C.W.Chi *	Theaceae	[12]
Campomanesia adamantium (Cambess.) O.Berg	Myrtaceae	[13]
Canarium album (Lour.) DC. *	Burseraceae	[14]
Carpobrotus edulis (L.) N.E.Br.	Aizoaceae	[15]
Castanea crenata Sieb. & Zucc.	Fagaceae	[16]
Clematis ispahanica Boiss.	Ranunculaceae	[17]
Clematis orientalis L.	Ranunculaceae	[17]
Clerodendrum infortunatum L. *	Lamiaceae	[18]
Cornus officinalis Siebold & Zucc.	Cornaceae	[19]
Elaeagnus rhamnoides (L.) A.Nelson *	Elaeagnaceae	[20]
Euterpe edulis Mart.	Arecaceae	[21]
Eugenia uniflora L.	Myrtaceae	[22]
Euphorbia pekinensis Rupr.	Euphorbiaceae	[23]
Geum urbanum L.	Rosaceae	[24]
Gymnanthes lucida Sw. *	Euphorbiaceae	[25]
Juglans regia L.	Juglandaceae	[26]
Lafoensia pacari A. St.-Hil.	Lythraceae	[27]
Myrciaria floribunda (H.West ex Willd.) O.Berg	Myrtaceae	[28]
Myrtus communis L.	Myrtaceae	[29]
Nephelium lappaceum L.	Sapindaceae	[30]
Pandiaka angustifolia (Vahl) Hepper	Amaranthaceae	[31]
Phyllanthus acuminatus Vahl	Phyllanthaceae	[32]
Pleurotus eryngii (DC. ex Fr.) Quel	Pleurotaceae	[33]
Plinia cauliflora (Mart.) Kausel *	Myrtaceae	[34]
Plinia coronata (Mattos) Mattos *	Myrtaceae	[35]
Plinia peruviana (Poir.) Govaerts	Myrtaceae	[36]
Potentilla anserina L.	Rosaceae	[37]
Psidium brownianum Mart. ex DC	Myrtaceae	[38]

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Quassia undulata (Guill. & Perr.) D.Dietr.	Simaroubaceae	[39]
Salacia chinensis L.	Celastraceae	[40]
Sambucus lanceolata R.Br.	Adoxaceae	[41]
Sanguisorba officinalis L.	Rosaceae	[42]
Sedum roseum (L.) Scop. *	Crassulaceae	[43]
Sterculia striata A. St.-Hil. & Naudin	Malvaceae	[44]
Syzygium calophyllifolium (Wight) Walp.	Myrtaceae	[45]
Syzygium cumini (L.) Skeels	Myrtaceae	[46]
Terminalia chebula Retz.	Combretaceae	[47]
Tetrapleura tetraptera (Schum. & Thonn.) Taub.	Leguminosae	[39]
Tocoyena formosa (Cham. & Schltdl.) K.Schum.	Rubiaceae	[48]
Zanthoxylum armatum DC. *	Rutaceae	[49]

* These plants are cited with the present name according to 'The plant list. A working list of all plant species': http://www.theplantlist.org/

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Table 2. Resume of ellagic acid effects on different kind of cancer cell lines

Cancer type	Cell line	Mechanism of action	References
Breast	MCF-7	Cell cycle arrest G_0/G_1 via TGF-β/Smads pathway Up-regulate Bax and down-regulate Bcl-2 ↑ synergistic cytotoxicity and apoptotic sensitivity on γ-irradiated cells ↓ Akt/mTOR activation	[189-191]
Cervical	HeLa	Up-regulate IGFBP7and block Akt/mTOR pathway	[194]
Colon	Stem cells	↓ Number and size colonospheres	[182]
	Caco-2 HCT-116	Cell cycle arrest G_1 Up-regulate Bax, ↑caspase 8 ↓ PI3K/Akt pathway	[183,185]
	HCT-15	↓Cell proliferation and induces cycle arrest G_2/M Up-regulate Bax, caspase 3, cytochrome C Down-regulate Bcl-2 and cyclin D1, Blocked PI3K/Akt pathway	[186]
Endometrial	Ishikawa	↓ ROS, cytosolic pH and glycolytic flux	[193]
Glioblastoma	U251	Up-regulate Bax and caspase 3 Up-regulate MAPKs, and expression DR4, DR5, CHOP ↓ Bcl-2 and survivin	[197]
	U87 U118	Cell cycle arrest S	[198]
Liver	HepG2	↑ $[Ca^{2+}]i$ via phospholipase C	[175]
	HA22T HA59T	Cytotoxicity. No effect on normal liver cells (AML12)	[175]
Ovarian	A2780	↓ Cisplatin chemoresistance	[192]
Pancreas	PANC-1	↓ Cell growth, migration, invasion Cell cycle arrest G_1 Down-regulate COX-2, NF-κB and vimentin Up-regulated E-cadherin	[188]
Prostate	DU145	Anti-proliferative IC_{50} = 23 μM (96 h) Cell cycle arrest S ↓ Cyclin B1 and D1 expression	[176,177]
	PC-3	Anti-proliferative IC_{50} = 14.5 μM (96 h) Cell cycle arrest S ↓ Cyclin B1 and D1 expression ↑ IL-6 levels Down-regulate p-STAT3, p-Akt, pERK1/2	[176-178]
	ECV304	↓ Histone deacetylases	[180]
	LNCaP	↑ Bax, p21, p27, cyclin E, cdk-2 ↓ Bcl-2, cyclin D1,cdk-1	[181]

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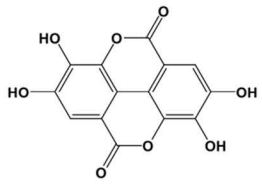

Figure 1. Chemical structure of ellagic acid

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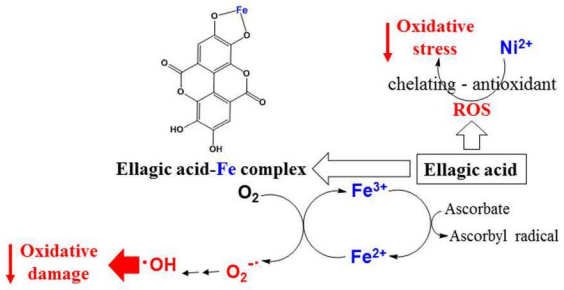

Figure 2. Effect of ellagic acid on different metals. Proposal mechanism of chelation (adapted from Dalvi et al. [75]).

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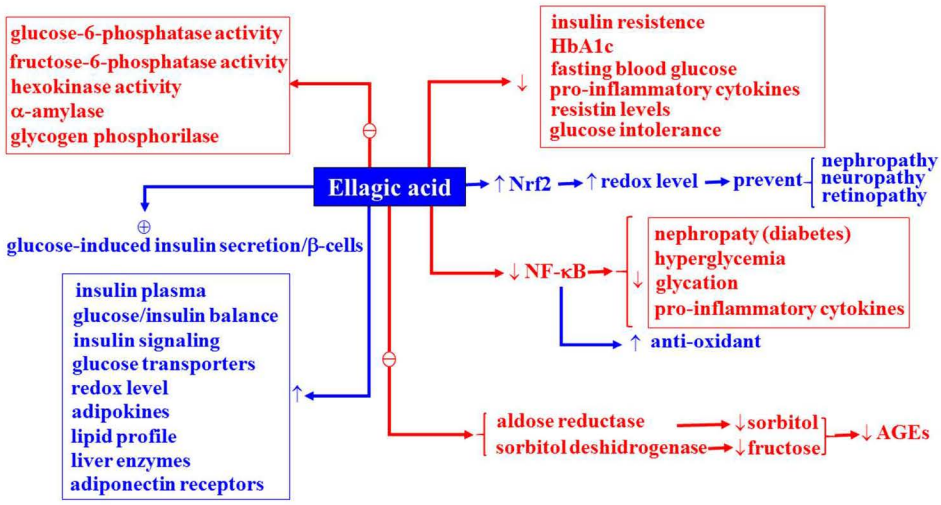

Figure 3. Anti-hyperglycemic effects of ellagic acid on glucose metabolism. Advanced glycation end-products (AGE); glycosylated hemoglobin (HbA1c); nuclear factor-kappa B (NF-kB); nuclear factor erythroid 2–related factor 2 (Nrf2).

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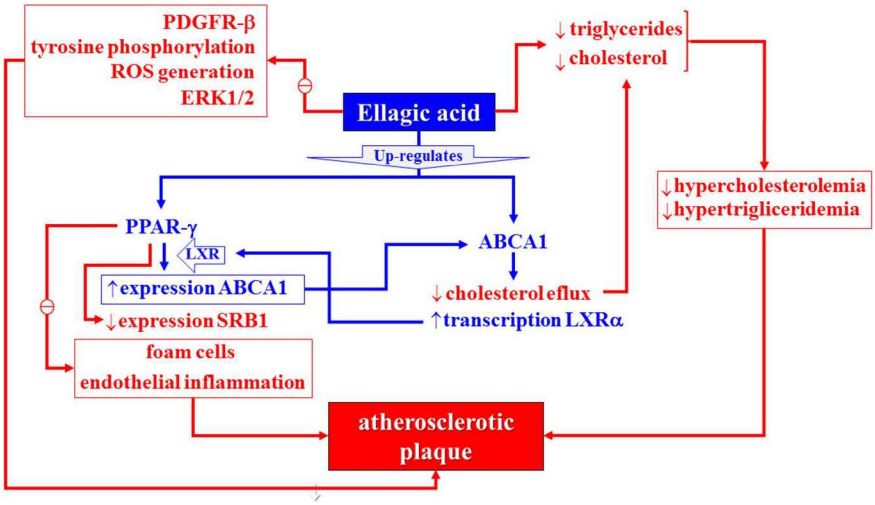

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