

Review

# Neuroprotective Potential of Punicalagin, a Natural Component of Pomegranate Polyphenols: A Review

Peng Chen<sup>1,†</sup>, ZhiLei Guo<sup>2,†</sup>, Benhong Zhou<sup>1,\*</sup>

<sup>1</sup>Department of Pharmacy, Renmin Hospital of Wuhan University, 430060 Wuhan, Hubei, China

<sup>2</sup>Department of Pharmacy, Wuhan Fourth Hospital, 430033 Wuhan, Hubei, China

\*Correspondence: [benhongzh@whu.edu.cn](mailto:benhongzh@whu.edu.cn) (Benhong Zhou)

<sup>†</sup>These authors contributed equally.

Academic Editor: Gernot Riedel

Submitted: 7 April 2023 Revised: 11 May 2023 Accepted: 16 May 2023 Published: 9 August 2023

## Abstract

Neurodegenerative diseases (NDs), such as Alzheimer's disease (AD) and Parkinson's disease (PD), are major health problems worldwide. To date, available remedies against NDs are limited. In fact, current treatment options include drug intervention and nutritional therapy, which mainly focus on the repair of neuronal damage and functional monitoring. However, these treatments do not completely alleviate disease symptoms. Recently, eliminating harmful molecules, such as reactive oxygen species, and inhibiting neuroinflammation have become potential strategies recommended by many researchers. Accordingly, remarkable interest has been generated in recent years regarding natural products, including polyphenols, that provide neuroprotective effects. In this review, we aimed to provide experimental evidence of the therapeutic potential of punicalagin (PUN), a prevailing compound in pomegranate polyphenols with antioxidant activity. Overall, the chemistry, methods of determination, characteristics of metabolism, transformation mechanisms of action, and neuroprotective effects of PUN on NDs are summarised to provide a scientific basis for elucidating the therapeutic mechanisms and targets of NDs.

**Keywords:** punicalagin; neurodegenerative disorders; therapy; evidence

## 1. Introduction

Increased consumption of fruits or nuts has beneficial effects on human health and can prevent various diseases [1]. Pomegranate (*Punica granatum*) is an ancient fruit that has been used since biblical times and is considered to be of high nutritional value and a folkloric medicine mainly based on anecdotal evidence of its benefits in treating a number of ailments and diseases [2]. Pomegranate has been traditionally used as a blood tonic in Asian countries; however, it is also popular in South America and Europe owing to its broad-spectrum health-beneficial properties [3].

According to previous studies, pomegranate juice is associated with many positive health functions, such as reducing inflammation, inhibiting and preventing cancer development, alleviating diabetes, and promoting neuroprotective actions *via* antioxidant activity [4]. These biological effects are attributed to bio-polyphenols, which are primarily composed of hydrolyzable tannins [5]. Based on extensive evidence, punicalagin (PUN) is the most abundant component of pomegranate peels. It has been that [6] reported that more than two-thirds of the antioxidant activity of pomegranate juice is attributed to the high proportion of PUN and its hydrolyzed tannins. The concentration of PUN in juice can reach approximately 2 g/L and is composed of high levels of glucose, which is located in the center of PUN [7]. Glucose exists in  $\alpha$ - and  $\beta$ -anomeric forms and is esterified with ellagic acid (EA), gallic acid dimers, gallagic acid, and EA dimers.

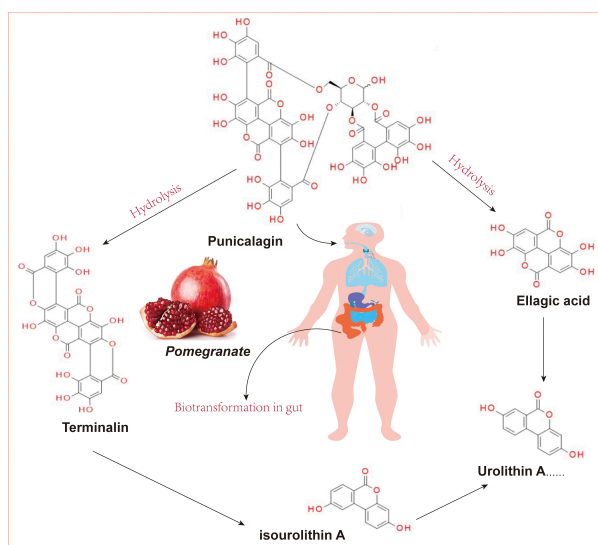
Extensive evidence shows that PUN has different biological activities, such as in cancer, cardiovascular diseases, liver diseases, and inflammation [8–10]. Recently, a pharmacological study found that this substance and its polyphenols had significant neuroprotective potential against Alzheimer's disease (AD), Parkinson's disease (PD), stroke, and stress [11–14]. Neurodegenerative diseases (NDs) have common characteristics that are associated with oxidative stress (OS) and inflammation. Therefore, oral polyphenols, such as PUN, which have strong antioxidant and anti-inflammatory properties, may be feasible and effective as preventive and therapeutic strategies for NDs. In this review, the therapeutic potential and mechanisms of action of PUN in neurological diseases are systematically summarised to provide new insights into the treatment of NDs.

## 2. Chemistry of PU and its Content in Food Products

PUN ( $C_{48}H_{28}O_{30}$ ), which has a molecular weight of 1084.72 Da, is a phenolic substance with the largest known molecular weight. PUN is a brown-yellow amorphous powder that is strongly polar and soluble in water, methanol, ethanol, acetonitrile, and other organic solvents [15]. The structure of PUN (Fig. 1) contains one Galloyl-Hexahydroxydiphenoyl (HHDP) unit, one galloyl unit, and one glucose unit, in which C2 and C3 of glucose are connected to the HHDP structural unit, and C4 and C6 are con-



nected to the galloyl unit [16]. Each molecule contains 16 phenolic hydroxyl groups, whereas the other phenolic substances only contain 3–4 phenolic hydroxyl groups, making them the most antioxidant phenolic substances. PUN exists as  $\alpha$ - and  $\beta$  isomers that can be converted. The ratios of  $\alpha$  and  $\beta$ -PUN increased with an increase in pH value when the pH was between 2 and 3.5; the maximum was achieved at pH = 3.5; however, the ratios decreased as the pH increased in the pH range of 3.5 to 8 [17]. Light, heat, acids, bases, and strong oxidants can affect the stability of PUN and  $\text{Fe}^{3+}$  and  $\text{Cu}^{2+}$  can be complexed with it.



**Fig. 1. The structure of punicalagin and its derivatives.** (Ellagic acid and terminalin were produced from PUN derived from pomegranate through hydrolysis and then metabolized into urolithins *via* biotransformation-gut microbiota). PUN, punicalagin.

PUN was isolated from the fruits and leaves of *Elmema japonica* and Chekhwa. PUN is a specific component of pomegranate fruit [18]. It has been reported that the PUN content in pomegranate peel can reach 10–50 mg/g, which is the highest in common fruits. In addition, the concentration process was not found to affect the composition, properties, or content of PUN, indicating that concentrated pomegranate juice could provide health benefits to the body [19].

### 3. Progress in PUN Determination

Currently, many mature chemical analysis methods, such as gas chromatography, high-performance liquid chromatography (HPLC), and ultra-performance liquid chromatography-MS-MS (UPLC-MS<sup>n</sup>), are used for the detection and validation of PUN and related substances in pomegranates [20]. HPLC was applied to determine the presence and concentration of the components in the sample; this technique is used to separate the isolated individual components.

Qu *et al.* [21] developed an HPLC method for ellagic, gallic acid, and PUN determination. These results indicate that the HPLC method has good stability and reproducibility, a high recovery rate, and low limits of detection and quantification. In comparison to existing methods, this method significantly improves the permeability of the sample and can achieve content detection of four polyphenols, including pomegranate glycoside, pomegranate, ellagic acid (EA), and pyrogallol acid, in one operation. Other analytical methods, such as Raman spectrum and nuclear magnetic resonance (NMR) spectroscopy, have been reported, which have certain advantages in the qualitative and isomeric analysis [6].

## 4. Role of Oxidative Stress in Neurodegeneration

Oxidative stress in the body has now drawn much attention for its potential role in various diseases and is believed to be responsible for the increase in disease incidence in Western societies [22]. Numerous studies have shown that OS is a key link in cancer, inflammation, cardiovascular and neurodegenerative diseases, and aging [23,24]. The toxicity of reactive oxygen species (ROS) depends on associated and sensitive biological substrates, such as nucleic acids, proteins, and membrane lipids. The biologically relevant ROS include superoxide anion radicals, lipid peroxides, hydrogen peroxide, and hydroxyl radicals [25]. Hence, the activated antioxidants have been demonstrated to be beneficial for health through scavenging these oxygen free radicals.

According to Harman's free radical theory of aging, age-related chronic NDs such as AD, PD and multiple sclerosis (MS), are the consequence of damage to macromolecules by ROS in the mitochondria [26]. The role of OS in neurodegeneration has gained momentum in recent years as excessive ROS generation has been implicated in several neurodegenerative pathologies [23]; this can be explained by the fact that the mammalian brain is one of the most metabolically active organs in the body. Despite a relatively small size (2% of total body weight) the brain requires 20% of the oxygen consumption and energy generation from oxidation of glucose in the human body [27]. More importantly, the enhanced neuronal vulnerability to OS aggravated such fuel overconsumption. Nervous degeneration is a progressive age-related process. As aging intensifies, the mitotic renewal potential of neurons is compromised by long-term pro-oxidative environment [28]. The accumulation of ROS-damaged macromolecules, such as lipid peroxidation, oxidatively modified proteins, and DNA, can result in a serious decline in cell viability.

According to previous studies, ROS disrupts the blood-brain barrier (BBB) by destroying tight junction proteins [29]. Furthermore, studies have confirmed that OS is an important mechanism underlying amyloid  $\beta$ -protein ( $A\beta$ ) neurotoxicity in which excessive ROS production

triggers A $\beta$  high loading by changing A $\beta$  peptide kinetics [30]. In addition, excessive lipid peroxidation and the accumulation of reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), can significantly increase the risk of AD and PD [24]. As OS plays an indispensable role in neurodegeneration, antioxidants have attracted the attention of researchers in the prevention and treatment of NDs and other fields and have been the focus of current research.

## 5. PUN as an Antioxidant in Neurodegenerative Diseases Based on *in Vitro* Evidence

PUN has potent anti-inflammatory, anti-carcinogenic, and antioxidant properties [31]. PUN protects endogenous organs and cells from OS-induced damage by directly scavenging free radicals and inducing Nrf-2 expression and the hundreds of antioxidant response element-dependent genes it regulates to counter the physiological and pathophysiological outcomes of oxidant exposure. Table 1 (Ref. [32–39]) displays the protective effect of PUN as an antioxidant in NDs based on *in vitro* experimental data.

Clementi *et al.* [32] pretreated PC12 cells with PUN (0.5, 1, 5, 10, and 20  $\mu$ M) for 24 h and then exposed them to H<sub>2</sub>O<sub>2</sub> to produce oxidative damage. Based on their result, the H<sub>2</sub>O<sub>2</sub>-induced decrease in the viability of PC12 cells was improved by pretreatment with PUN. Particularly, the neuroprotective effect might be related to the decreased production of radical oxygen species and improved mitochondrial function. The apoptotic cascade was found to be modulated by PUN and the expression of apoptosis-related proteins such as Bax and caspase 3 were reduced. Numerous studies have reported that excitotoxicity is an important mechanism of neuronal death in central nervous system diseases, including AD. Pathakoti *et al.* [33] investigated endogenous metabolic changes and the protective effects of PUN against glutamate-induced oxidative toxicity in HT22 cells. Based on the results, PUN prevented glutamate-induced death of HT22 cells, possibly by reducing intracellular ROS levels and restoring mitochondrial membrane depolarization. Metabolite profiling using HPLC and Gas chromatography–mass spectrometry (GC-MS) spectrometry revealed that amino acids, fatty acids palmitic acid and stearic acid are potential metabolite biomarkers in HT22 cells after PUN treatment. PUN was also found to decrease cellular ROS and upregulate the expression and enzymatic activity of methionine sulfoxide reductase A (MsrA) in human neuroblastoma IMR-32 cells with A $\beta$  peptide treatment. These results suggest a possible preventive role of PUN in neurodegenerative diseases related to OS [34].

Interestingly, PUN has been shown to be beneficial in cell models of PD. Chu and Han [35] treated human neuroblastoma SH-SY5Y cells with 6-hydroxydopamine (6-OHDA), a synthetic organic compound, *in vitro* to mimic PD. Pretreatment of SH-SY5Y cells with PUN (50, 100,

and 200  $\mu$ M) for 2 h significantly alleviated the 6-OHDA-induced decline in cell viability and apoptosis. PUN treatment effectively restored the mitochondrial function and enhanced AMP-activated kinase (AMPK) phosphorylation. Thus, a theoretical basis may exist for the application of PUN in the clinical treatment of PD.

According to considerable evidence, neuroinflammation plays a significant role in AD and other NDs. Olajide *et al.* [36] examined PUN's effects on activated microglia in lipopolysaccharide (LPS)-induced model of neuroinflammation. Rat primary microglia were pretreated with PUN (5–40  $\mu$ M) before stimulation with LPS (10 ng/mL) and it was found to result in significant reductions in TNF- $\alpha$ , IL-6 and prostaglandin E2 production. Other similar reports suggested that PUN has neuroinflammatory protective activity [37–39]. Overall, these results indicate that PUN can relieve neuroinflammation mediated by LPS-activated microglia, suggesting its potential as a nutritional preventive strategy against NDs.

## 6. Preclinical Evidence for the *in Vivo* Neuroprotective Roles of PUN

Owing to preclinical evidence, the *in vivo* effects of PUN on the treatment of NDs were evaluated, and the results are displayed in Table 2 (Ref. [38–50]).

### 6.1 AD

AD is an age-related progressive neurodegenerative disorder and the most prevalent cause of dementia [51]. Despite some pathological features, such as the accumulation of A $\beta$  oligomers and plaques, and subsequent hyperphosphorylation of the tau protein in the cortex and hippocampus, the molecular mechanism of AD has not been fully defined, and the development of disease-modifying therapies for AD has been unsuccessful to date [40]. Recently, many studies have demonstrated that PUN may be beneficial in experimental models of AD. For example, Xu *et al.* [41] evaluated an APP/PS1 double transgenic mouse model administered 12.5, 25, and 50 mg/kg PUN for 45 days and found that this flavone could improve motor coordination, anxiety psychology, and working memory based on the rotating rod and Y Maze testings. The researchers also found that senile plaque deposition and tau hyperphosphorylation were decreased in PUN-treated APP/PS1 mice based on immunohistochemistry and western blot analysis. Protein-protein interaction (PPI) network construction and core target analysis revealed that PUN might act on serum albumin (ALB), epidermal growth factor receptor (EGFR), insulin-like growth factor 1 (IGF-1), and catalase (CAT), thereby reducing the over-expression of neurotoxic proteins and OS in the AD brain. According to Rojanathammanee *et al.* [39], PUN enhances learning and memory functions in 12-month-old APP/PS1 mice, decreases NFATc1 activity, and reduces the oxidative damage-related protein markers, nitrotyrosine and HNE protein (Michael) adducts.

**Table 1. *In vitro* neuroprotective effects of PUN.**

No.	Cell line	Models	PUN dose	Duration (hrs)	Effects	Suggested mechanisms	References
1	PC12 cells	H <sub>2</sub> O <sub>2</sub> -induced cells oxidative damage	0.5, 1, 5, 10, 20 $\mu$ M	24	cell viability $\uparrow$ ; cell apoptosis $\downarrow$ ; mitochondrial membrane potential $\downarrow$	ROS $\downarrow$ ; Bax and Caspase 3 $\downarrow$	[32]
2	HT22 cells	Glutamate-induced oxidative toxicity	6.25 and 50 $\mu$ M	24	cell viability $\uparrow$ ; cell cytotoxicity $\downarrow$ ; mitochondrial membrane potential $\downarrow$	ROS $\downarrow$ ; Metabolic alterations	[33]
3	IMR-32 cells	A $\beta$ -induced neurotoxicity	20 $\mu$ M	48	cell viability $\uparrow$	ROS $\downarrow$ ; MsrA $\uparrow$	[34]
4	SH-SY5Y cells	6-OHDA-induced 7-oxidative damage	50, 100, 200 $\mu$ M	2	cell viability $\uparrow$ ; cell apoptosis $\downarrow$ ; mitochondrial membrane potential $\downarrow$	ROS $\downarrow$ ; SOD $\uparrow$ ; ATP $\uparrow$ ; p-AMPK $\uparrow$	[35]
5	Primary microglia	LPS-induced neuroinflammation	5–40 $\mu$ M	24	cell viability $\uparrow$ ; PGE2 $\downarrow$ ; TNF- $\alpha$	COX-2 $\downarrow$ ; mPGES-1 $\downarrow$ ; NF-kB $\downarrow$ ; p38, JNK and p42/44 MAPKs $\downarrow$	[36]
6	BV2 cell line	LPS-induced neuroinflammation	25, 50, 75, 100 $\mu$ M	24	cell viability $\uparrow$ ; NO $\downarrow$ ; PGE2 $\downarrow$	IL-6 $\downarrow$ ; IL-18 $\downarrow$ ; IL-1 $\beta$ $\downarrow$ ; STAT3 $\downarrow$ ; MAPK $\downarrow$ ; NF-kB $\downarrow$	[37]
7	BV2 cell line	LPS-induced neuroinflammation	10, 20, 50 $\mu$ M	24	COX-2 and iNOS $\downarrow$	IL-1 $\beta$ , IL-6 and TNF- $\alpha$ $\downarrow$ ; NF-kB $\downarrow$	[38]
8	Primary microglia	A $\beta$ <sub>1–42</sub> -induced neuroinflammation	10 $\mu$ M	24	TNF- $\alpha$ $\downarrow$	NFATc2 $\downarrow$ ; p-IkB $\downarrow$	[39]

ROS, Reactive oxygen species; SOD, Superoxide dismutase; LPS, Lipopolysaccharide; PGE2, Prostaglandin E2; COX-2, Cyclooxygenase-2.

**Table 2. *In vivo* neuroprotective effects of PUN in various disorders.**

Condition	No.	Animal model	Dosage	Findings	Ref.
AD	1	APP/PS1 transgenic mice; 2-month-old	50, 25 and 12.5 mg/kg daily; Oral administration for 45 days	Alleviated learning and memory impairment and ameliorated A $\beta$ deposition	[40]
	2	Male APP/PS1 transgenic mice; 12-month-old	1561 mg/L daily; Oral administration for 3 months	Improve cognitive deficits and reduced neuroinflammation	[41]
	3	Male Institute of Cancer Research (ICR) male mice; LPS-induced neuroinflammation model	2.5 mg/kg daily; Oral administration for 4 weeks	Inhibited neuroinflammation, OS and memory impairment	[39] [38]
PD	4	Male Sprague Dawley (SD) rats; Manganese-induced Parkinson's disease;	2.5 mg/kg daily; Oral administration for 35 days	Enhanced animal motor functions and decreased their catalepsy score	[42]
	5	Male Wistar rats; ACR ip injection-induced toxicity;	10, 20, 40 mg/kg daily; Ip injection for 11 days	Recovered movement disorders, changed OS and reduced apoptosis	[43]
Stroke	6	Male Wistar rats; Middle cerebral artery occlusion (MCAO)-induced ischemia-reperfusion (I/R) injury model	15 and 30 mg/kg daily; Oral administration for 7 days	Presented a dose-dependent reduction in infarct volume and substantial improvement in behavioral deficits	[44]
	7	Male Wistar rats; MCAO-I/R injury model	15 and 30 mg/kg daily; Oral administration for 7 days	Improved neurologic deficits, brain water content (BWC), histopathology changes	[45]
	8	Male Sprague Dawley (SD) rats; Intracerebral haemorrhage (ICH)-induced brain inflammatory damage	25, 50, 75 mg/kg daily; Oral administration for 2 weeks	Reduced inflammatory cell infiltration and cell damage, improved brain tissue architecture and BBB integrity	[46]
Diabetes-induced neurology	9	C57BL/6 male mice; HFD (60% kcal fat content, D12492)-induced diabetes	50 and 100 mg/kg daily; Oral administration for 8 weeks	Ameliorated the diabetes-associated cognitive dysfunction and improved the neuronal apoptosis	[47]
	10	C57BL/6J mice; Embryos were cultured for 24 or 36 hours with 100 mg/dL glucose	0, 10 and 20 $\mu$ mol/L; Whole-embryo cultured with 24 h	Protected against high Glucoseinduced cellular stress and neural tube defects	[48]
	11	Male Sprague-Dawley rats; Exposed to restraint stress on days 14–20 of pregnancy, three times each day	50 and 100 mg/kg daily; Oral administration for 3 days	Protected the neurodevelopment and cognitive functions <i>in vivo</i> in rats offspring exposed to prenatal restraint stress	[49]
Sleep deprivation memory deficits	12	Male Wistar rats; Lateral ventricular injection penicillin-G to induced neurotoxicity	0.001, 0.01, or 0.1 $\mu$ g/rat; Received a lateral ventricular injection	Alleviated total sleep deprivation and impaired memory processes	[50]

AD, Alzheimer's disease; PD, Parkinson's disease; OS, Oxidative stress; BBB, Blood brain barrier.

Based on accumulating evidence, glia-mediated neuroinflammation is a pathological hallmark of many central nervous system (CNS) disorders, including AD and brain aging [52]. Kim *et al.* [38] evaluated the anti-inflammatory activity of PUN in mice co-treated with LPS. To examine the memory-improving effects of PUN in an LPS-induced neuroinflammatory injury model, PUN (1.5 mg/kg) was continuously administered to mice through drinking water for 4 weeks, followed by daily injections of LPS for 1 week. PUN was found to ameliorate these memory-impaired effects and reduce hippocampus levels of IL-1 $\beta$ , IL-6 and TNF- $\alpha$ . The mechanism underlying the efficacy of PUN in AD management is the potential improvement of microglial activation and OS in the brain by the NF- $\kappa$ B inhibitor, thereby causing neuronal loss. PUN has been confirmed to be a natural inhibitor of advanced glycation end products (AGEs) and has huge potential in the prevention and treatment of NDs through anti-glycosylation [53]. The T cells and microglia activation was inhibited after PUN treatment in AD transgenic mice [39]. These findings provide evidence of the role of PUN in the alleviation of neuroinflammation in AD.

## 6.2 PD

PD is an incurable neurodegenerative disorder characterized by motor and nonmotor deficits, which are caused by the death of dopaminergic (DA) neurons [54]. The hallmark of PD is related to the depletion of dopamine in the striatum, which leads to symptoms such as bradykinesia and resting tremors. Hence, the main goal of PD therapy is to prevent damage to dopaminergic neurons, despite the availability of few effective therapeutic agents for PD.

Environmental pollutants, such as manganese chloride (MnCl<sub>2</sub>) and acrylamide (ACR), can aggravate the occurrence of PD [55]. Abu-Elfotuh *et al.* [42] investigated the neuroprotective activity of PU against MnCl<sub>2</sub>-induced PD in Sprague Dawley (SD) rats. PUN was found to improve locomotor activity in a novel open-field test and significantly decrease acetylcholinesterase levels in rat brains. Moreover, the researchers found that PUN displayed significant neuroprotective effect and exhibited anti-inflammatory activity (an obvious decrease in the brain levels of cyclooxygenase-2 (COX2), interleukin-18 (IL-18), and interleukin-1 $\beta$  (IL-1 $\beta$ ) was observed) in a dose-dependent manner. Of note, the glutamate/gamma-aminobutyric acid (GABA) balance was restored and abnormal glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ ) protein expression levels were pronouncedly decreased after PUN treatment. The beneficial effects of PUN on ACR-induced neurotoxicity *in vivo* have been recently reported. Foroutanfar *et al.* [43] administered a continuous intraperitoneal injection of 50 mg/kg ACR for 11 days to rats to induce PD neurotoxicity; the movement disorders were recovered, and OS markers and apoptosis in the cerebral cortex were reduced. The expression of myelin basic protein

(MBP), which can maintain the stability of the CNS myelin structure and function to reduce ACR toxicity, was significantly rescued.

As mentioned above, PUN has strong ability to mitigate PD progression, which is associated with its satisfactory activities in improving redox homeostasis and neuroinflammation.

## 6.3 Stroke

Stroke is one of the leading causes of death and disability worldwide, affecting millions of individuals each year [56]. Of these patients, 15% experience haemorrhagic, while 85% experience an ischaemic stroke. Cerebral ischaemia (CI) is considered one of the most disabling cerebral events. CI can cause motor, sensory, visual, speech, cognitive and other neurological dysfunction and forgetfulness, spatial learning, and memory disorders [57]. The conventional clinical drug such as alteplase can be applied for acute stroke; however, its therapeutic application is limited to the first 3 h of the occurrence of the stroke [58]. Owing to their limited therapeutic applications, remarkable research has focused on the evolution of traditional medicinal plants, nutritional supplements, and plant compounds.

Caspases are cysteine proteases that mediate apoptotic death in various cellular systems, including neurons [59]. Yaidikar *et al.* [44] demonstrated a high expression of activated caspase-3 after ischemia/reperfusion (I/R) injury in rat brains. In addition, the relative expression of caspase-3 was found to be significantly downregulated, thereby alleviating apoptosis in middle cerebral artery occlusion (MCAO) model rats treated with PUN. In neurological disorders, the BBB plays a central role in the homeostatic regulation of the brain microenvironment in the case of ischaemia, irreversible tissue damage, and BBB breakdown, leading to the extravasation of serum proteins and development of vasogenic brain oedema. In another study performed by this group, it was found that there was a significant reduction in neurological deficit scores as well as brain water content (BWC), an indicator used to evaluate altered BBB permeability was observed in MCAO rats after treating with 15 and 30 mg/kg PUN [45]. Interestingly, inflammatory cell infiltration and neuronal damage were reported to be markedly reduced, and antioxidant enzyme activities, such as superoxide dismutase (SOD) and catalase (CAT), were increased through activation of the Nrf-2/ARE/HO-1 signaling pathway in rats with spontaneous intracerebral haemorrhage (ICH) after PUN intragastric therapy [46]. Taken together, PUN supplementation can effectively improve the neuronal damage caused by cerebral I/R, and its main mechanism may involve enhanced antioxidant function.

## 6.4 Diabetes-Induced Neuropathy and Other Diseases

In addition to its anti-inflammatory and antioxidant activities, PUN has been widely reported to ameliorate diabetes. Notably, PUN has been found to prevent diabetes-

related cognition dysfunction in recent years. He *et al.* [47] reported that PUN effectively promotes the expression of 5-hydroxymethylcytosine and attenuates neuronal apoptosis induced by a high-fat diet (mouse). The potential mechanism of action of PUN is related to AMPK activation and Krebs homeostasis. These findings further confirm the neuroprotective effect of PUN and provide new ideas for further elucidation of the mechanisms underlying diabetes-related nervous lesions and drug development.

Maternal diabetes-induced birth defects occur in 6–10% of babies born to mothers with pregestational diabetes, representing an important genetic issue. Exploring the effects of natural polyphenols with significant antioxidant properties and low toxicity on diabetic embryonic diseases can promote the development of new and safe dietary supplements [60]. Zhong *et al.* [48] determined whether PUN could reduce high glucose-induced neural tube defects (NTDs) in embryonic day 8.5 (E8.5) mouse embryos and whether this rescue occurs through the blockage of cellular stress and caspase activation. Surprisingly, 20 mM PUN significantly inhibited high-glucose-induced NTD formation. Moreover, experimental evidence revealed that PUN supplementation could abrogate endoplasmic reticulum stress and suppress the high glucose-induced caspase 3 and caspase 8 cleavage. These observations suggest that PUN supplementation protects against the teratogenicity of hyperglycemia in developing embryos and may prevent diabetes-induced NDs.

Dementia and cognitive impairment are leading causes of disability and death worldwide and pose a global health challenge as the population ages. An abnormal increase in mitochondrial homeostasis is considered the main cause of cognitive impairment. Cao *et al.* [49] evaluated the effects of PUN on the expression of AMPK in the offspring of rats subjected to prenatal restrictive stress. Based on the results, PUN protected neurodevelopment and cognitive function in mice by inducing mitochondrial biogenesis and phase II enzymes. Furthermore, PUN can protect mice from memory impairment induced by 24 h of total sleep deprivation (TSD) in the passive avoidance test [50]. Considering the beneficial effects of PUN reported in these studies, the possible mechanism of action of PUN on memory alteration should be further explored.

## 7. PUN Application in Anti-NDs Therapy

### 7.1 What Happens after PUN Ingestion?

Currently, most existing studies have opted to explore the potential of PUN to protect neurons using *in vitro* cell line models. Notably, an extensive amount of time will be required to translate the obtained data into an *in vivo* model. For the application of PUN in neurotherapy, it is important to consider its processing in the human body. First, PUN passes through the intestinal tract after being taken orally and is easily affected by low stomach pH. Notably, digestive hydrolases are active [31].

### 7.2 Metabolism of Ellagitannins by Microbiota

PUN is a phenolic compound that is a hydrolyzable tannin that forms the same subgroup as gallotannins [61]. Although the water solubility of hydrolyzed tannins is satisfactory, their high molecular weight (generally >500 Da) inhibits their efficient transport across biological membranes [62]. In addition, studies have shown that structural components, such as their aromatic rings and phenolic and carbonyl groups, can interact with amino acids in free proteins and peptides. Under the action of intestinal flora, hydrolyzable monobaric acid undergoes biotransformation and then hydrolyses and releases phenolic acids, such as ingallic acid and ellagic acid (EA) [63]. The phenolic acids formed by this reaction have limited bioavailability and poor water solubility. On the one hand, these substances can provide nutrients for some microbes, and on the other hand, microbes can convert them into small molecules, such as urolithins and isourolithins [64].

EA, a precursor compound of urolithins, can be released from ellagitannins, and the gut microbiota can convert these large structures into metabolites that are more bioavailable than the precursor compounds [65]. There are different metabolisms of hydrolyzable tannins in different individuals owing to different microorganisms in the gut [66]. According to the differences in metabolic phenotypes, urolithins can be divided into the following metabolic phenotypes: Uro-A (producing only uro-A conjugates), Uro-B (producing uro-A, isouro-A, and/or uro-B), and Uro-0 (no urolithins) [67]. Based on numerous studies, pomegranate fruit preparations intake can generate urolithin A, isourolithin A, and urolithin B in the body, and these substances are most likely responsible for the neuroprotective activity of pomegranate in the brain [68–70].

### 7.3 Punicalagin Biotransformation

Similar to other ellagitannins, PUN has been confirmed to undergo analogical transformations in the human body. When PUN is hydrolyzed and degraded in the stomach, the products are processed into dibenzopyranone-type urolithin and isourolithin by the intestinal microflora [71,72]. Compared to PUN, these released life-active molecules are not convergent but have many health benefits, including neuroprotective activity [73]. Iglesias-Aguirre *et al.* [74] summarised the neuroprotective functions of ellagitannin derivatives and discussed the neuroprotective effects of various urolithin types on brain health and their associated molecular mechanism. For instance, urolithin A has been demonstrated to cross the BBB and attenuate *D*-galactose-induced brain aging and cognitive function in mice *via* the activation of the miR-34a-Mediated SIRT1/mTOR signaling pathway [75]. Urolithin B has been demonstrated to have anti-apoptotic effects during brain aging, with an improvement in cognitive deficits by inhibiting Cyt C-mediated apoptosis and promoting the survival

of neurons through the PI3K pathway in aging mice [76]. In summary, urolithins might be the main substances responsible for the neuroprotective activity of PUN *in vivo*.

#### 7.4 Urolithin Clinical Trials

Clinical trials are beginning to be undertaken using urolithin A, or ellagitannin rich foods such as raspberry, walnuts or pomegranate [77–79]. These studies have demonstrated improved mitochondrial and endothelial cell function, muscle strength and endurance countering the decline in these components with aging. Urolithin A is a natural dietary metabolite that improves muscle health in old animals and in preclinical models of aging. Urolithin A induces beneficial alterations in fatty acid metabolism and intestinal cellular tight junctions which regulate intestinal permeability, improvement in gut bacterial biodiversity has also been observed. Urolithin A intake alters the gut microbiota, improving bacterial diversity and also improves endothelial cell function (UMIN-CTR, trial number: UMIN000042014) [80], improves fatty acid metabolism and increases the proliferation of the beneficial symbionts *Clostridiales* including, *Ruminococcus lactaris*, and *Gemmiger formicilis* in the microbiome [81]. *R. lactaris* is an acetate producer, *G. formicilis* is a carbohydrate fermenting Gram-negative anaerobic bacterium; both of these bacteria improve gut health. The *Clostridiales* is a key bacterial group that restricts gut colonization by potentially damaging Enterobacteriaceae pathogens thus ensuring a healthy gut environment is maintained for beneficial symbionts. Urolithin A also results in improvement in mitochondrial function with aging [82] and improved muscle performance suggesting urolithin A may counteract age-associated muscle decline and reduces age-related inflammation [83–85]. A double-blind randomized controlled trial [ClinicalTrials.gov NCT02734901] has shown that consumption of dietary achievable amounts of red raspberries which are rich in Urolithin A acutely improves endothelial function suggesting that punicalagin metabolites could also induce similar effects if therapeutic levels can be achieved [86].

## 8. Conclusions

According to available data, PUN might be a promising neuro prophylaxis for AD, PD, stroke, and other types of neurological diseases, regardless of the actual pharmacodynamic material basis [87]. Administering PUN with other components of pomegranate preparations as well as hydrolyzed conversion to EA, urolithins, and other components may prevent neurological defects [88]. In addition to the well-known antioxidant activity of PUN, the observed neuroprotective effects might be closely related to improvements in neuroinflammation in AD and PD. However, as most of the reported results are from animal and cell model studies, they must be considered in-depth before translation to practical applications. For example, to overcome the low bioavailability of PUN after oral administration, other

routes of administration should be designed, such as the application of nanotechnology, sustained and controlled-release preparations, liposomes, and targeted therapy [89]. Finally, PUN might be a necessary factor in these neuroprotective effects; however, more research is needed to investigate the optimal therapeutic dose, timing of administration, and optimal PUN-containing substrates (purified compounds, extracts, juices, or fruits) for PUN to exert neuroprotective effects [90]. Moreover, whether PUN is responsible for its direct neuroprotective activity remains unclear, as increasing evidence suggests that derivatives of urolithins, produced by the intestinal metabolism of ellagitannins, may be the ultimate bioactive neuroprotective metabolites.

## Author Contributions

PC, ZLG and BHZ conceptualized and designed the study, analyzed and interpreted data. PC wrote the manuscript. PC and ZLG designed the figure. PC and ZLG acquired the data. PC and BHZ reviewed the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Not applicable.

## Acknowledgment

Not applicable.

## Funding

This work was supported by grants from the National Natural Science Foundation of China (31770381) and the Open Project of Hubei Key Laboratory of Wudang Local Chinese Medicine Research (Hubei University of Medicine) (WDCM2022006).

## Conflict of Interest

The authors declare no conflict of interest.

## References

- [1] Cena H, Calder PC. Defining a Healthy Diet: Evidence for The Role of Contemporary Dietary Patterns in Health and Disease. *Nutrients*. 2020; 12: 334.
- [2] Vučić V, Grabež M, Trchounian A, Arsić A. Composition and Potential Health Benefits of Pomegranate: A Review. *Current Pharmaceutical Design*. 2019; 25: 1817–1827.
- [3] Akaberi M, Boghrati Z, Sahebkar A, Emami SA. Therapeutic Potential of Pomegranate in Metabolic Disorders. *Advances in Experimental Medicine and Biology*. 2021; 1328: 421–440.

- [4] Saeed M, Naveed M, BiBi J, Kamboh AA, Arain MA, Shah QA, *et al.* The Promising Pharmacological Effects and Therapeutic/Medicinal Applications of Punica Granatum L. (Pomegranate) as a Functional Food in Humans and Animals. Recent Patents on Inflammation and Allergy Drug Discovery. 2018; 12: 24–38.
- [5] Sahebkar A, Ferri C, Giorgini P, Bo S, Nachtigal P, Grassi D. Effects of pomegranate juice on blood pressure: A systematic review and meta-analysis of randomized controlled trials. Pharmacological Reports. 2017; 115: 149–161.
- [6] Danesi F, Ferguson LR. Could Pomegranate Juice Help in the Control of Inflammatory Diseases? Nutrients. 2017; 9: 958.
- [7] Wu S, Tian L. Diverse Phytochemicals and Bioactivities in the Ancient Fruit and Modern Functional Food Pomegranate (*Punica granatum*). Molecules. 2017; 22: 1606.
- [8] Xie X, Hu L, Liu L, Wang J, Liu Y, Ma L, *et al.* Punicalagin promotes autophagic degradation of human papillomavirus E6 and E7 proteins in cervical cancer through the ROS-JNK-BCL2 pathway. Translational Oncology. 2022; 19: 101388.
- [9] Yu LM, Dong X, Xue XD, Zhang J, Li Z, Wu HJ, *et al.* Protection of the myocardium against ischemia/reperfusion injury by punicalagin through an SIRT1-NRF-2-HO-1-dependent mechanism. Chemico-Biological Interactions. 2019; 306: 152–162.
- [10] Liu H, Zhan Q, Miao X, Xia X, Yang G, Peng X, *et al.* Punicalagin Prevents Hepatic Steatosis through Improving Lipid Homeostasis and Inflammation in Liver and Adipose Tissue and Modulating Gut Microbiota in Western Diet-Fed Mice. Molecular Nutrition and Food Research. 2021; 65: 2001031.
- [11] Seo EJ, Fischer N, Efferth T. Phytochemicals as inhibitors of NF- $\kappa$ B for treatment of Alzheimer's disease. Pharmacological Research. 2018; 129: 262–273.
- [12] Kujawska M, Jourdes M, Kurpik M, Szulc M, Szaefer H, Chmielarz P, *et al.* Neuroprotective Effects of Pomegranate Juice against Parkinson's Disease and Presence of Ellagitannins-Derived Metabolite-Urolithin A-In the Brain. International Journal of Molecular Sciences. 2019; 21: 202.
- [13] Yaidikar L, Thakur S. Punicalagin attenuated cerebral ischemia-reperfusion insult *via* inhibition of proinflammatory cytokines, up-regulation of Bcl-2, down-regulation of Bax, and caspase-3. Molecular and Cellular Biochemistry. 2015; 402: 141–148.
- [14] Shao J, Wang P, Liu A, Du X, Bai J, Chen M. Punicalagin Prevents Hypoxic Pulmonary Hypertension *via* Anti-Oxidant Effects in Rats. American Journal of Chinese Medicine. 2016; 44: 785–801.
- [15] Li P, Du R, Chen Z, Wang Y, Zhan P, Liu X, *et al.* Punicalagin is a neuraminidase inhibitor of influenza viruses. Journal of Medical Virology. 2021; 93: 3465–3472.
- [16] Mathon C, Chater JM, Green A, Merhaut DJ, Mauk PA, Preece JE, *et al.* Quantification of punicalagins in commercial preparations and pomegranate cultivars, by liquid chromatography-mass spectrometry. Journal of the Science of Food and Agriculture. 2019; 99: 4036–4042.
- [17] Barbieri M, Heard CM. Isolation of punicalagin from *Punica granatum* rind extract using mass-directed semi-preparative ESI-AP single quadrupole LC-MS. Journal of Pharmaceutical and Biomedical Analysis. 2019; 166: 90–94.
- [18] Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG, *et al.* *In vitro* antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. The Journal of Nutritional Biochemistry. 2005; 16: 360–367.
- [19] Magangana TP, Makunga NP, Fawole OA, Opara UL. Processing Factors Affecting the Phytochemical and Nutritional Properties of Pomegranate (*Punica granatum* L.) Peel Waste: A Review. Molecules. 2020; 25: 4690.
- [20] Kraszni M, Marosi A, Larive CK. NMR assignments and the acid-base characterization of the pomegranate ellagitannin punicalagin in the acidic pH-range. Analytical and Bioanalytical Chemistry. 2013; 405: 5807–5816.
- [21] Qu W, Breksa Iii AP, Pan Z, Ma H. Quantitative determination of major polyphenol constituents in pomegranate products. Food Chemistry. 2012; 132: 1585–1591.
- [22] Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biology. 2015; 4: 180–183.
- [23] Tan BL, Norhaizan ME, Liew WP. Nutrients and Oxidative Stress: Friend or Foe? Oxidative Medicine and Cellular Longevity. 2018; 2018: 9719584.
- [24] Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nature Reviews Drug Discovery. 2021; 20: 689–709.
- [25] Peoples JN, Saraf A, Ghazal N, Pham TT, Kwong JQ. Mitochondrial dysfunction and oxidative stress in heart disease. Experimental and Molecular Medicine. 2019; 51: 1–13.
- [26] Chen Z, Zhong C. Oxidative stress in Alzheimer's disease. Neuroscience Bulletin. 2014; 30: 271–281.
- [27] Filomeni G, De Zio D, Cecconi F. Oxidative stress and autophagy: the clash between damage and metabolic needs. Cell Death and Differentiation. 2015; 22: 377–388.
- [28] Jaganjac M, Milkovic L, Zarkovic N, Zarkovic K. Oxidative stress and regeneration. Free Radical Biology and Medicine. 2022; 181: 154–165.
- [29] Chen S, Li L, Peng C, Bian C, Ocak PE, Zhang JH, *et al.* Targeting Oxidative Stress and Inflammatory Response for Blood-Brain Barrier Protection in Intracerebral Hemorrhage. Antioxidants and Redox Signaling. 2022; 37: 115–134.
- [30] Butterfield DA, Boyd-Kimball D. Oxidative Stress, Amyloid- $\beta$  Peptide, and Altered Key Molecular Pathways in the Pathogenesis and Progression of Alzheimer's Disease. Journal of Alzheimer's Disease. 2018; 62: 1345–1367.
- [31] Venusova E, Kolesarova A, Horky P, Slama P. Physiological and Immune Functions of Punicalagin. Nutrients. 2021; 13: 2150.
- [32] Clementi ME, Pani G, Sampaiolese B, Tringali G. Punicalagin reduces H<sub>2</sub>O<sub>2</sub>-induced cytotoxicity and apoptosis in PC12 cells by modulating the levels of reactive oxygen species. Nutritional Neuroscience. 2018; 21: 447–454.
- [33] Pathakoti K, Goodla L, Manubolu M, Tencomnao T. Metabolic Alterations and the Protective Effect of Punicalagin against Glutamate-Induced Oxidative Toxicity in HT22 Cells. Neurotoxicity Research. 2017; 31: 521–531.
- [34] Clementi ME, Sampaiolese B, Lazzarino G, Giardina B. Effect of Punicalagin and Resveratrol on Methionine Sulfoxide Reductase: A Possible Protective Contribution against Alzheimer's Disease. The Journal of Prevention of Alzheimer's Disease. 2015; 2: 33–37.
- [35] Chu J, Han W. Punicalagin Exerts Beneficial Functions in 6-Hydroxydopamine-Treated SH-SY5Y Cells by Attenuating Mitochondrial Dysfunction and Inflammatory Responses. Medical Science Monitor. 2018; 24: 5905–5913.
- [36] Olajide OA, Kumar A, Velagapudi R, Okorji UP, Fiebich BL. Punicalagin inhibits neuroinflammation in LPS-activated rat primary microglia. Molecular Nutrition and Food Research. 2014; 58: 1843–1851.
- [37] Lo J, Liu CC, Li YS, Lee PY, Liu PL, Wu PC, *et al.* Punicalagin Attenuates LPS-Induced Inflammation and ROS Production in Microglia by Inhibiting the MAPK/NF- $\kappa$ B Signaling Pathway and NLRP3 Inflammasome Activation. Journal of Inflammation Research. 2022; 15: 5347–5359.

- [38] Kim YE, Hwang CJ, Lee HP, Kim CS, Son DJ, Ham YW, *et al.* Inhibitory effect of punicalagin on lipopolysaccharide-induced neuroinflammation, oxidative stress and memory impairment *via* inhibition of nuclear factor-kappaB. *Neuropharmacology*. 2017; 117: 21–32.
- [39] Rojanathammanee L, Puig KL, Combs CK. Pomegranate Polyphenols and Extract Inhibit Nuclear Factor of Activated T-Cell Activity and Microglial Activation in Vitro and in a Transgenic Mouse Model of Alzheimer Disease. *Journal of Nutrition*. 2013; 143: 597–605.
- [40] Scheltens P, De Strooper B, Kivipelto M, Holstege H, Chételat G, Teunissen CE, *et al.* Alzheimer's disease. *The Lancet*. 2021; 397: 1577–1590.
- [41] Xu P, Xu L, Huang S, Li D, Liu Y, Guo H, *et al.* Analysis of the Molecular Mechanism of Punicalagin in the Treatment of Alzheimer's Disease by Computer-Aided Drug Research Technology. *ACS Omega*. 2022; 7: 6121–6132.
- [42] Abu-Elfotuh K, Hamdan AME, Abbas AN, Alahmre ATS, Elewa MAF, Masoud RAE, *et al.* Evaluating the neuroprotective activities of vinpocetine, punicalagin, niacin and vitamin E against behavioural and motor disabilities of manganese-induced Parkinson's disease in Sprague Dawley rats. *Biomedicine and Pharmacotherapy*. 2022; 153: 113330.
- [43] Foroutanfar A, Mehri S, Kamyar M, Tandishepanah Z, Hosseinzadeh H. Protective effect of punicalagin, the main polyphenol compound of pomegranate, against acrylamide-induced neurotoxicity and hepatotoxicity in rats. *Phytotherapy Research*. 2020; 34: 3262–3272.
- [44] Yaidikar L, Thakur S. Punicalagin attenuated cerebral ischemia-reperfusion insult *via* inhibition of proinflammatory cytokines, up-regulation of Bcl-2, down-regulation of Bax, and caspase-3. *Molecular and Cellular Biochemistry*. 2015; 402: 141–148.
- [45] Yaidikar L, Byna B, Thakur SR. Neuroprotective effect of punicalagin against cerebral ischemia reperfusion-induced oxidative brain injury in rats. *Journal of Stroke and Cerebrovascular Diseases*. 2014; 23: 2869–2878.
- [46] Zhang FC, Wu K, Wu XL, Xin C, Zhou MH, Lei J, *et al.* Punicalagin alleviates brain injury and inflammatory responses, and regulates HO-1/Nrf-2/ARE signaling in rats after experimental intracerebral haemorrhage. *Tropical Journal of Pharmaceutical Research*. 2020; 19: 727–737.
- [47] He X, Pei S, Meng X, Hua Q, Zhang T, Wang Y, *et al.* Punicalagin Attenuates Neuronal Apoptosis by Upregulating 5-Hydroxymethylcytosine in the Diabetic Mouse Brain. *Journal of Agricultural and Food Chemistry*. 2022; 70: 4995–5004.
- [48] Zhong J, Reece EA, Yang P. Punicalagin exerts protective effect against high glucose-induced cellular stress and neural tube defects. *Biochemical and Biophysical Research Communications*. 2015; 467: 179–184.
- [49] Cao K, Lv W, Hu S, Gao J, Liu J, Feng Z. Punicalagin Activates AMPK/PGC-1 $\alpha$ /Nrf2 Cascade in Mice: The Potential Protective Effect against Prenatal Stress. *Molecular Nutrition and Food Research*. 2020; 64: e2000312.
- [50] Jin D, Zhang B, Li Q, Tu J, Zhou B. Effect of punicalagin on multiple targets in streptozotocin/high-fat diet-induced diabetic mice. *Food and Function*. 2020; 11: 10617–10634.
- [51] Chen P, Guo Z, Zhou B. Insight into the role of adult hippocampal neurogenesis in aging and Alzheimer's disease. *Ageing Research Reviews*. 2023; 84: 101828.
- [52] Leng F, Edison P. Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? *Nature Reviews Neurology*. 2021; 17: 157–172.
- [53] Liu W, Ma H, Frost L, Yuan T, Dain JA, Seeram NP. Pomegranate phenolics inhibit formation of advanced glycation endproducts by scavenging reactive carbonyl species. *Food and Function*. 2014; 5: 2996–3004.
- [54] Kalia LV, Lang AE. Parkinson's disease. *The Lancet*. 2015; 386: 896–912.
- [55] Marwah PK, Paik G, Issa CJ, Jemison CC, Qureshi MB, Hanna TM, *et al.* Manganese-stimulated redox cycling of dopamine derivatives: Implications for manganese. *Neurotoxicology*. 2022; 90: 10–18.
- [56] Barthels D, Das H. Current advances in ischemic stroke research and therapies. *Biochimica Et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2020; 1866: 165260.
- [57] Dietz RM, Dingman AL, Herson PS. Cerebral ischemia in the developing brain. *Journal of Cerebral Blood Flow and Metabolism*. 2022; 42: 1777–1796.
- [58] Lim S, Kim TJ, Kim YJ, Kim C, Ko SB, Kim BS. Senolytic Therapy for Cerebral Ischemia-Reperfusion Injury. *International Journal of Molecular Sciences*. 2021; 22: 11967.
- [59] Franke M, Bieber M, Kraft P, Weber ANR, Stoll G, Schuhmann MK. The NLRP3 inflammasome drives inflammation in ischemia/reperfusion injury after transient middle cerebral artery occlusion in mice. *Brain, Behavior, and Immunity*. 2021; 92: 223–233.
- [60] Moon JH, Jang HC. Gestational Diabetes Mellitus: Diagnostic Approaches and Maternal-Offspring Complications. *Diabetes and Metabolism Journal*. 2022; 46: 3–14.
- [61] Yamada H, Wakamori S, Hirokane T, Ikeuchi K, Matsumoto S. Structural Revisions in Natural Ellagitannins. *Molecules*. 2018; 23: 1901.
- [62] Aguilar-Zarate P, Wong-Paz JE, Buenrostro-Figueroa JJ, Ascacio JA, Contreras-Esquivel JC, Aguilar CN. Ellagitannins: Bioavailability, Purification and Biotechnological Degradation. *Mini-Reviews in Medicinal Chemistry*. 2018; 18: 1244–1252.
- [63] Tejada S, Setzer WN, Daglia M, Nabavi SF, Sureda A, Braidyn N, *et al.* Neuroprotective Effects of Ellagitannins: a Brief Review. *Current Drug Targets*. 2017; 18: 1518–1528.
- [64] Djedjibegovic J, Marjanovic A, Panieri E, Saso L. Ellagic Acid-Derived Urolithins as Modulators of Oxidative Stress. *Oxidative Medicine and Cellular Longevity*. 2020; 2020: 194508.
- [65] Ríos JL, Giner RM, Marín M, Recio MC. A Pharmacological Update of Ellagic Acid. *Planta Medica*. 2018; 84: 1068–1093.
- [66] Tomás-Barberán FA, González-Sarriás A, García-Villalba R, Núñez-Sánchez MA, Selma MV, García-Conesa MT, *et al.* Urolithins, the rescue of "old" metabolites to understand a "new" concept: Metabotypes as a nexus among phenolic metabolism, microbiota dysbiosis, and host health status. *Molecular Nutrition and Food Research*. 2017; 61: 1500901.
- [67] Lu C, Li X, Gao Z, Song Y, Shen Y. Urolithins and intestinal health. *Drug Discoveries and Therapeutics*. 2022; 16: 105–111.
- [68] Xian W, Yang S, Deng Y, Yang Y, Chen C, Li W, *et al.* Distribution of Urolithins Metabotypes in Healthy Chinese Youth: Difference in Gut Microbiota and Predicted Metabolic Pathways. *Journal of Agricultural and Food Chemistry*. 2021; 69: 13055–13065.
- [69] Núñez-Sánchez MA, García-Villalba R, Monedero-Saiz T, García-Talavera NV, Gómez-Sánchez MB, Sánchez-Álvarez C, *et al.* Targeted metabolic profiling of pomegranate polyphenols and urolithins in plasma, urine and colon tissues from colorectal cancer patients. *Molecular Nutrition and Food Research*. 2014; 58: 1199–1211.
- [70] Piwowarski JP, Stanisławska I, Granica S, Stefańska J, Kiss AK. Phase II Conjugates of Urolithins Isolated from Human Urine and Potential Role of  $\beta$ -Glucuronidases in Their Disposition. *Drug Metabolism and Disposition*. 2017; 45: 657–665.
- [71] Seeram NP, Henning SM, Zhang Y, Suchard M, Li Z, Heber D. Pomegranate juice ellagitannin metabolites are present in human plasma and some persist in urine for up to 48 hours. *Journal of Nutrition*. 2006; 136: 2481–2485.

- [72] Totiger TM, Srinivasan S, Jala VR, Lamichhane P, Dosch AR, Gaidarski AA 3rd, *et al.* Urolithin A, a Novel Natural Compound to Target PI3K/AKT/mTOR Pathway in Pancreatic Cancer. *Molecular Cancer Therapeutics*. 2019; 18: 301–311.
- [73] García-Villalba R, Tomás-Barberán FA, Iglesias-Aguirre CE, Giménez-Bastida JA, González-Sarriás A, Selma MV, *et al.* Ellagitannins, urolithins, and neuroprotection: Human evidence and the possible link to the gut microbiota. *Molecular Aspects of Medicine*. 2023; 89: 101109.
- [74] Iglesias-Aguirre CE, Cortés-Martín A, Ávila-Gálvez MÁ, Giménez-Bastida JA, Selma MV, González-Sarriás A, *et al.* Main drivers of (poly)phenol effects on human health: metabolite production and/or gut microbiota-associated metabolotypes? *Food and Function*. 2021; 12: 10324–10355.
- [75] Chen P, Chen F, Lei J, Li Q, Zhou B. Activation of the miR-34a-Mediated SIRT1/mTOR Signaling Pathway by Urolithin A Attenuates D-Galactose-Induced Brain Aging in Mice. *Neurotherapeutics*. 2019; 16: 1269–1282.
- [76] Chen P, Chen F, Lei J, Wang G, Zhou B. The Gut Microbiota Metabolite Urolithin B Improves Cognitive Deficits by Inhibiting Cyt C-Mediated Apoptosis and Promoting the Survival of Neurons Through the PI3K Pathway in Aging Mice. *Frontiers in Pharmacology*. 2021; 12: 768097.
- [77] Cortés-Martín A, Iglesias-Aguirre CE, Meoro A, Selma MV, Espín JC. Pharmacological Therapy Determines the Gut Microbiota Modulation by a Pomegranate Extract Nutraceutical in Metabolic Syndrome: A Randomized Clinical Trial. *Molecular Nutrition and Food Research*. 2021; 65: e2001048.
- [78] Selma MV, González-Sarriás A, Salas-Salvadó J, Andrés-Lacueva C, Alasalvar C, Örem A, *et al.* The gut microbiota metabolism of pomegranate or walnut ellagitannins yields two urolithin-metabolites that correlate with cardiometabolic risk biomarkers: Comparison between normoweight, overweight-obesity and metabolic syndrome. *Clinical Nutrition*. 2018; 37: 897–905.
- [79] Jarrard D, Filon M, Huang W, Havighurst T, DeShong K, Kim K, *et al.* A phase II randomized placebo-controlled trial of pomegranate fruit extract in men with localized prostate cancer undergoing active surveillance. *Prostate*. 2021; 81: 41–49.
- [80] Nishimoto Y, Fujisawa K, Ukawa Y, Kudoh M, Funahashi K, Kishimoto Y, *et al.* Effect of urolithin A on the improvement of vascular endothelial function depends on the gut microbiota. *Frontiers in Nutrition*. 2023; 9: 1077534.
- [81] Meroño T, Peron G, Gargari G, González-Domínguez R, Miñarro A, Vegas-Lozano E, *et al.* The relevance of urolithins-based metabotyping for assessing the effects of a polyphenol-rich dietary intervention on intestinal permeability: A post-hoc analysis of the MaPLE trial. *Food Research International*. 2022; 159: 111632.
- [82] Lippi L, Uberti F, Folli A, Turco A, Curci C, d'Abrascia F, *et al.* Impact of nutraceuticals and dietary supplements on mitochondria modifications in healthy aging: a systematic review of randomized controlled trials. *Aging Clinical and Experimental Research*. 2022; 34: 2659–2674.
- [83] Singh A, D'Amico D, Andreux PA, Fouassier AM, Blanco-Bose W, Evans M, *et al.* Urolithin A improves muscle strength, exercise performance, and biomarkers of mitochondrial health in a randomized trial in middle-aged adults. *Cell Reports Medicine*. 2022; 3: 100633.
- [84] Liu S, D'Amico D, Shankland E, Bhayana S, Garcia JM, Aebischer P, *et al.* Effect of Urolithin A Supplementation on Muscle Endurance and Mitochondrial Health in Older Adults: A Randomized Clinical Trial. *JAMA Network Open*. 2022; 5: e2144279.
- [85] Andreux PA, Blanco-Bose W, Ryu D, Burdet F, Ibberson M, Aebischer P, *et al.* The mitophagy activator urolithin A is safe and induces a molecular signature of improved mitochondrial and cellular health in humans. *Nature Metabolism*. 2019; 1: 595–603.
- [86] Istas G, Feliciano RP, Weber T, Garcia-Villalba R, Tomas-Barberan F, Heiss C, *et al.* Plasma urolithin metabolites correlate with improvements in endothelial function after red raspberry consumption: A double-blind randomized controlled trial. *Archives of Biochemistry and Biophysics*. 2018; 651: 43–51.
- [87] Xu J, Cao K, Liu X, Zhao L, Feng Z, Liu J. Punicalagin Regulates Signaling Pathways in Inflammation-Associated Chronic Diseases. *Antioxidants (Basel)*. 2021; 11: 29.
- [88] Gregory J, Vengalasetti YV, Bredesen DE, Rao RV. Neuroprotective Herbs for the Management of Alzheimer's Disease. *Biomolecules*. 2021; 11: 543.
- [89] Mansoor K, Bardees R, Alkhawaja B, Mallah E, AbuQatouseh L, Schmidt M, *et al.* Impact of Pomegranate Juice on the Pharmacokinetics of CYP3A4- and CYP2C9-Mediated Drugs Metabolism: A Preclinical and Clinical Review. *Molecules*. 2023; 28: 2117.
- [90] Khomich LM, Perova IB, Eller KI. [Pomegranate juice nutritional profile]. *Voprosy Pitaniia*. 2019; 88: 80–92.