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Review Article

Pharmacological Activities of Punicalagin: A Review

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Abstract

Traditional medicine was becoming more popular as a component of complementary and alternative medicine around the world. The ability to provide information on the pharmacological effects of plants requires a thorough understanding of the chemical components found in plants. One of the chemical compounds reported having pharmacological activity was punicalagin. Punicalagin was a polyphenol compound found in *Punica granatum*, *Lafoensia pacari*, and genus *Terminalia*. This review provides information on the pharmacological activities of punicalagin. The bibliographic databases used as the sources of information were PubMed, Google Scholar, and ScienceDirect. The pharmacological activities of punicalagin have been reported as anticancer, antioxidant, hepatoprotective, antimicrobial, antivirus, neuroprotective, antiinflammation, gastroprotective, pre-eclampsia, antidiabetic, and anti hyperlipidemia.

Keywords: Punicalagin, Antioxidant, Anticancer, Antivirus.

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INTRODUCTION

The use of natural or traditional medicine as complementary and alternative medicine increased worldwide. The most common reasons for using herbal therapy for treatment are dissatisfaction with conventional medicine, previous favorable experiences, positive aspects of herbal medicine, and family traditions [1]. Polyphenol compounds have been shown to provide health benefits in preventing various diseases, including cancer, metabolic syndrome, diabetes, non-alcoholic liver disease, and periodontal disease [2]. Traditionally, the health benefits of polyphenolic compounds have been attributed to their antioxidant activity [3]. Knowledge of chemical plant components is essential for quality control analysis of plants, extracts, or any formulation containing chemical plant components. Existing compounds or groups of compounds can serve as "biological markers." The presence and concentration of the biologically active ingredients will be used to decide the drug's authenticity or formulation [4].

Punicalagin was a polyphenol compound that is widely contained and important in pomegranates [5]. Pomegranate polyphenol compounds exhibit a variety of pharmacological activities, including anti-inflammatory, hepatoprotective, antigenotoxic, and anticoagulant properties [6]. Punicalagin was the main ellagitannin constituent of the leaves of *Lafoensia pacari*, a Brazilian medicinal plant widely used to treat gastric ulcers and wound healing [7].

Punicalagin also known as 2,3-(S)-hexahydroxydiphenoyl-4,6-(S,S)-gallagyl-D-glucose, 2,3-(S)-hexahydroxydiphenoyl-4,6-(S,S)-gallagyl-4,6-(S,S)-gallagyl-4,6-(S,S) punicalagin. It has a molecular weight of 1084.7 g/mol, with a total atomic weight of 78. Punicalagin has the chemical formula $C_{48}H_{28}O_{30}$.

Punicalagin is a yellow powder with a 5 mg/ml solubility in methanol and a clear [8]. Punicalagin is a polyphenol type found in the peel, aril, fruit, juice, husk of *Punica granatum, and Lafoensia pacari* leaves [7,9–14]. Punicalagin was discovered in alpha and beta forms in the Terminalia (T) genus, including *T. arborea* (fruit), *T. avicennioides* (bark), *T. arjuna* (bark), *T. brachystemma* (leaves), *T. brownie* (bark), *T. laxifora* (root), *T. calamansanai* (leaves), *T. catappa* (bark), *T. chebula* (leaves, fruit), *T. parvifora* (bark), *T. citrina* (fruit), T. ivorensis (bark), *T. macroptera* (bark), *T. muelleri* (leaves), *T. myriocarpa* (leaves), and *T. oblongata* (leaves) [15]. Punicalagin was also considered a promising multifunctional compound due to its health-beneficial pharmacological properties, bioavailability, and low toxicity. This article aims to provide information on the pharmacological activity of punicalagin.

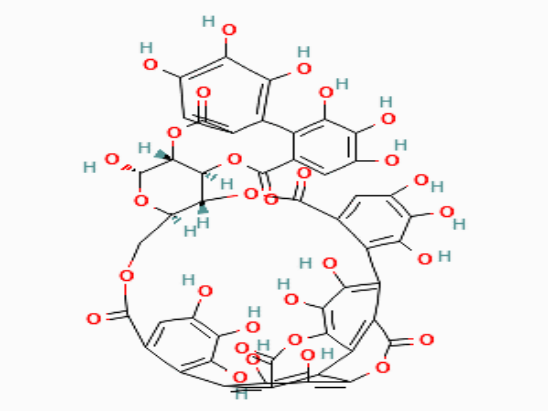

Figure 1. 2D Structure of Punicalagin [8]

METHODS

The method utilized to compile this review was a literature review based on data searches conducted in scientific literature databases such as PubMed, Google Scholar, and ScienceDirect. This review was undertaken to discover the pharmacological action of punicalagin compounds published in the last ten years (2011–2021). The keywords used in searching data from all databases were "pharmacological activity" AND "punicalagin." All abstracts and full-text publications were gathered, examined, summarised, and concluded. The selected articles were the most relevant, filtered, and included in this review.

RESULT AND DISCUSSION

Punicalagin as anticancer

Punicalagin isolated from *Lafoensia pacari* concentration of 50 mg/kg showed a significant cytotoxic effect by micronucleus(MN) assay and concomitant treatment with cyclophosphamide (CPA). This cytotoxic effect was enhanced. The cytotoxic effect of punicalagin appears through the role of punicalagin by inducing DNA repair [7]. *Carneiro et al.* looked into the role of punicalagin in angiogenic activity and plays a role in tissue repair and wound healing [7].

Cheng et al. found that 100 µM punicalagin, similar to 25 mg/250 ml in humans, can produce therapeutic benefits and favorable responses to senescence thyroid cancer cells. Punicalagin treatment causes senescent growth arrest and senescence-associated secretory phenotype (SASP) by activating NF-κB. Punicalagin activates the DNA damage response in papillary thyroid cancer BCPAP cells, causing autophagic cell death [16]. *Li et al.* also looked into the anticancer impact of pomegranate peel extract on thyroid cancer in athymic BALB/c mice using a BCPAP tumor model in vitro and in vivo. Punicalagin can suppress proliferation and induce apoptosis by causing a decrease in mitochondrial membrane potential in thyroid cancer cells. Pomegranate peel extract inhibits thyroid cancer cell migration and invasion by suppressing the production of matrix metalloproteinase-9 (MMP-9) [17].

Pan et al. investigated the effect of punicalagin on breast cancer. The results showed that punicalagin concentrations more significant than 50 µM could suppress breast cancer cell viability and reduce migration and invasion of MCF-7 cells and MDA-MB-231 cells. Golgi phosphoprotein 3 (GOLPH3) was transfected into cells with or without punicalagin administration, and GOLPH3 expression levels were examined using quantitative real-time polymerase chain reaction (qRT-PCR). Punicalagin inhibited the expression of GOLPH3, MMP-2, MMP-9, and N-Cadherin, and increased the expression of E-Cadherin. Based on this mechanism, punicalagin can suppress cell viability and decrease the process of metastasis through the regulation of GOLPH3 in breast cancer [9].

Subkorn et al. investigated the anti-leukemic effects and molecular mechanisms of punicalagin using the leukemia cell lines acute promyelocytic leukemia cell line (NB4) and acute lymphocytic leukemia cell line (MOLT-4). Punicalagin was utilized to treat leukemia cells, and the cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Punicalagin inhibited NB4 and MOLT-4 cell viability in a dose-dependent manner. Punicalagin has a cytotoxic effect by activating the caspase cascade, changing Bax and Bcl-2, and regulating autophagy via the mTOR/ULK1 signaling pathway, suppressing proliferation and promoting apoptosis and autophagy [18].

Punicalagin protects the syncytiotrophoblast by inhibiting autophagy and thereby lowering apoptosis; according to *Wang et al*. Punicalagin suppresses the mTOR kinase pathway in cultured human primary syncytiotrophoblasts, increasing autophagic turnover and limiting death. Punicalagin reduces the expression of phosphorylated ribosomal protein S6, a downstream target of mTOR kinase, and the autophagy markers LC3-II and p62 in the syncytiotrophoblast. Punicalagin's apoptosis-inhibiting activity was reduced by inhibiting autophagy with bafilomycin or knocking down the autophagy-associated gene ATG16L1. Punicalagin enhances syncytiotrophoblast culture by modulating the interaction between autophagy and apoptosis [19].

Punicalagin (1-30 µg/ml) reduced cell viability by raising cyclin E levels while lowering B and A levels. Punicalagin also caused apoptosis in cells by cleaving PARP, activating caspase-9, and increasing caspase-3 activity in cells. Punicalagin (1-30 µg/ml) elevated the levels of phosphorus-AMPK and phosphorus-p27 at Thr198 in cells, which was associated with autophagic cell death induction. Punicalagin activates apoptotic and autophagic pathways in human U87MG glioma cells, causing them to die [20].

Punicalagin as antioxidant

Punicalagin is a bioactive chemical found in the pomegranate rind with powerful antioxidant capabilities. The antioxidant-rich pomegranate peel fraction was extracted using methanol, ethanol, and ethyl acetate as solvents. Compared to ethanol and ethyl acetate extracts, the methanol extract of pomegranate peel demonstrated the best antioxidant activity, inhibiting 78.23% of free radicals in the DPPH assay. Free radical scavenging was high in the most effective pomegranate peel methanol extract [21]. The results of *Oudane et al.* involved the characterization of punicalagin hydrolyzed tannins derived from the peel of a yellow pomegranate (*Punica granatum* from family Lythraceae). The results showed that the antioxidant activity of pure punicalagin (IC_{50} 1.9±0.2 µg/ml) was comparable to tannic acid (IC_{50} 1.3±0.2 µg/ml) as evaluated by the DPPH radical scavenger test. The punicalagin percentage obtained from the isolation of the yellow pomegranate peel extract is 23% punicalagin in 100 mg [22].

Punicalagin as hepatoprotective

Punicalagin's hepatoprotective activity in cyclophosphamide-induced rats was studied by *Fouad et al*. punicalagin was given in two doses, 15 and 30 mg/kg/day, peroral, starting on the same day as cyclophosphamide, for seven days. Punicalagin exerts a significant and dose-dependent hepatoprotective effect on cyclophosphamide toxicity in rats through its antioxidant activity, antiinflammatory, and anti-apoptotic properties. Punicalagin also reduced cyclooxygenase-2 expression and reduced histological liver tissue damage in rats given cyclophosphamide [23]. According to a study by *Yan et al.,* Punicalagin can protect HepG2 cells against palmitate-induced lipotoxicity via activating the ERK/Nrf2 pathway. Apoptosis is closely related to oxidative stress. Punicalagin decreases palmitate-induced ROS (Reactive Oxygen Species) generation and enhances mitochondrial function by increasing mitochondrial membrane potential. Punicalagin can also modulate cellular antioxidant systems via the ERK/Nrf2 pathway, protecting mitochondrial function [24].

Punicalagin as antimicrobial

Punicalagin's antibacterial activity was investigated by *Xu et al.* Punicalagin had a good antistaphylococcal effect with a minimum inhibitory concentration (MIC) of 0.25 mg/ml and produced cell membrane damage through its mechanism of action. When cells were treated with punicalagin at 2 times MIC, there was an increase in potassium efflux. Punicalagin

causes cell membrane morphological damage. Punicalagin also inhibited the production of *Staphylococcus aureus* biofilms quite well. According to these findings, Punicalagin exhibits antibacterial and antibiotic activity against *S.aureus* [25]. Pomegranate peel powder was extracted with water, water/ethanol (1:1; v:v), ethanol, acetone, heptane as solvents. Tests using the agar diffusion method on pomegranate peel extract with water and ethanol solvents showed significant antimicrobial activity with an inhibition zone diameter of up to 20 mm. The identification results by *Gosset-Erard et al.* also showed that the active forms of the anomer alpha and beta punicalagin had MIC values between 0.3 and 1.2 µg/ml. The minimum inhibitory concentration (MIC) was determined based on the smallest concentration that showed activity against the strain indicator. The strain with the highest sensitivity to pomegranate extract was selected as an indicator strain [26].

Table 1: The pharmacological activity of Punicalagin

Pharmacological activity	Method	Dose/ Concentration	Reported activity	Ref
Anticancer				
Anticancer	Micronucleus (MN) Test	Punicalagin 50 mg/kg	Punicalagin at its highest dose of 50 mg/kg was found to have a considerable cytotoxic effect on rat bone marrow cells, especially when combined with cyclophosphamide.	7
Angiogenic activity	CAM models (chick embryo chorioallantoic membrane)	Punicalagin 12.5, 25, 50 µg/µL	Punicalagin showed angiogenic activity at all doses in the chick embryo chorioallantoic membrane (CAM) model, especially at the lowest concentration of 12.5 µg/L.	7
Thyroid anticancer activity	Subject/cell type: Papillary thyroid carcinoma BCPAP cells.	25, 50,100 µM	100 µM punicalagin, equivalent to 25 mg/250 ml in humans, can achieve clinical effects and respond well to senescent thyroid cancer cells.	16
Antitumor and anti-metastasis in thyroid cancer	Cells: BCPAP human papillary thyroid cancer cells. Cell viability: MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide)	0, 12.5, 25, 50, 100, 200µg/ml	By lowering cell proliferation and triggering apoptosis, pomegranate peel extracts significantly reduced tumor growth in a mouse model containing BCPAP.	17
Anticancer against breast cancer	Cells: MCF-7 and MDA-MB-231 breast cancer cells. Cell viability: CCK-8 assay Cell migration: wound healing. Cell invasion capacity: Transwell test GOLPH3 expression level: Test/assay with real-time polymerase chain reaction (qRT-PCR) and western blot.	0, 12.5, 25, 50, 100 µM	Punicalagin at a concentration of 50 µM or higher decreased the viability, migration, and invasion of MCF-7 and MDA-MB-231 cells considerably. Punicalagin inhibits cell viability and reduces metastatic processes in breast cancer through regulating GOLPH3.	9
Induces apoptosis and autophagy in acute leukemia	Cells: NB4 and MOLT-4 Cell viability: MTS assay. Apoptosis and autophagy: flow cytometry mRNA expression: reverse transcription-quantitative PCR.	25, 50, 75 and 100 µg/ml	Punicalagin suppresses proliferation while inducing apoptosis and autophagy via the mTOR/ULK1 signaling pathway by activating caspase cascades, altering Bax and Bcl-2, and regulating autophagy.	18
It inhibits autophagy as well as reduces apoptosis to protect syncytiotrophoblast	Human primary trophoblast isolated from the uncomplicated placenta of singleton pregnancies delivered by Cesar section at 39 weeks gestation	Punicalagin 33,8 mM (34 mM)	Punicalagin increases cell survival in cultured primary syncytiotrophoblasts by modulating apoptosis-autophagy crosstalk. Punicalagin's apoptosis-inhibiting activity was reduced by inhibiting autophagy with bafilomycin or knocking down the autophagy-associated gene ATG16L1.	19

Pharmacological activity	Method	Dose/ Concentration	Reported activity	Ref
Induces human U87MG glioma cell death via apoptotic and autophagic pathways.	Cell: U87MG Cell viability: MTT assay Cell cycle: flow cytometry Caspase-3 activity: spectrophotometer Bcl-2 level, cleaved caspase-9, cleaved poly (ADP-ribose) polymerase (PARP), phosphor-AMPK and phosphor-p27 in Thr198: immunoblot analysis	1-30 µg/ml	Punicalagin induces human U87MG glioma cells to die via inducing apoptosis and autophagy.	20
Antioxidant				
Antioxidant	DPPH method		The methanol extract of pomegranate peel reduced 78.23% of free radicals. The main ellagitannin found in pomegranate peel is punicalagin.	21
Antioxidant	DPPH method		The antioxidant activity of pure punicalagin was IC50 = 1.9±0.2 g/ml, which was close to that of tannic acid (IC50 1.3±0.2 g/ml) as assessed by the DPPH radical scavenger assay.	22
Hepatoprotective				
Hepatoprotective	Test animals: Sprague-Dawley male rats	15 and 30 mg/kg/day, p.o for 7 days	Punicalagin significantly and dose-dependent gave a hepatoprotective effect/protects the liver of rats against cyclophosphamide toxicity, namely by inhibiting oxidative/nitrosative stress, inflammation, and apoptosis	23
Hepatoprotective	Cells: HepG2 cells	2, 5, dan 10 µg/ml	At a dose of 10 µg/ml, punicalagin effectively attenuated free fatty acid (FFA)-induced lipotoxicity by activating the Keap1-Nrf2 cytoprotective signaling pathway	24
Antimicrobial				
Antimicrobial	Growth inhibition assay: agar diffusion	4, 2, 1, 0.5, 0.25, and 0.125 mg/ml	At a MIC value of 0.25 mg/ml Punicalagin showed a good antistaphylococcal effect. Punicalagin has good antimicrobial activity against *Staphylococcus aureus* by causing damage to microbial cell membranes.	25
Antimicrobial	Agar diffusion	10 µl	Punicalagin showed significant antimicrobial activity with an inhibition zone diameter of up to 20 mm. The active forms of the anomeric alpha and beta punicalagin have MIC values (minimum inhibitory concentration) between 0.3 and 1.2 µg/ml	26
Antivirus				
Antivirus against enterovirus 71	Media used: DMEM (Dulbecco's modified Eagle's medium), containing 10% Fetal Bovine Serum (FBS) Methods: qRT-PCR	1, 5, 10, 20, 50, 100 and 200 µg/ml (punicalagin in saline solution) doses of 0.4, 1, or 5 mg/kg body weight of mice	Punicalagin had an IC50 value of 15 µg/ml and reduced the virus's cytopathic effect on rhabdomyosarcoma (RD) cells. Punicalagin prevented enterovirus 71 (EV71) infection in RD cells and lowered EV71 infection mortality in mice.	27

Pharmacological activity	Method	Dose/ Concentration	Reported activity	Ref
Antivirus for coronavirus disease-19 (COVID-19)	The activity of the 3CL protease was assessed using an improved 3CL Protease Assay Kit. The cytotoxicity was evaluated using the tetrazolium dye, MTS, proliferation assay.	10 µg/mL punicalagin, 3 mg/ml Zn sulfate monohydrate, Combination of 10 µg/mL punicalagin with 3 mg/ml Zn sulfate monohydrate	Punicalagin (10 µg/ mL) significantly inhibited 3CL-protease activity. An extreme 3CL-protease activity was achieved when punicalagin was combined with zinc sulfate monohydrate (punicalagin/Zn-II). Punicalagin was also reported to have no significant Cytotoxic effect with an IC_{50} value of 6.192 µg/ml.	28
Coronavirus antiviral activity through inhibition of the main protease enzyme (Mpro)	An assay kit containing recombinant Mpro was used to assess the inhibitory effects. Using an MBP-tagged recombinant enzyme, this kit assays the activity of 3CL protease.	Punicalagin 2–50 µM.	At a concentration of 50 µM, punicalagin inhibited the activity of the main protease (Mpro) enzyme by 6.6–100.0% by binding directly to the Mpro protein.	29
Other Activity of Punicalagin				
Neuro-inflammatory	Cell: Glial cell culture from the Sprague-Dawley mouse's cerebral cortex. An enzyme immunoassay was used to assess the ability of punicalagin to suppress the production of TNF-alpha, IL-6, and prostaglandin E2 in culture conditions. Western blotting and PCR were used to look for Cyclooxygenase-2 and microsomal prostaglandin E synthase one protein and mRNA.	5–40 µM	Punicalagin inhibited neuroinflammation in LPS (Lipopolysaccharide)-stimulated microglia via impaired nuclear factor kappa B (NF-κB) signaling.	30
The protective effect of punicalagin on LPS-induced acute respiratory distress syndrome (ARDS).	Test animals: Male BALB/c mice Inducer: lipopolysaccharide (LPS) The levels of TNF-α, IL-6, and IL-1β in BALF were measured using ELISA kits. The myeloperoxidase (MPO) activity was determined using test kits	12.5, 25, and 50 mg/kg	Punicalagin suppressed LPS-induced increases in macrophage and neutrophil infiltration as well as myeloperoxidase activity in lung tissues. Punicalagin reduced lung edema and pulmonary histologic changes, suggesting that it could be beneficial in treating LPS-induced ARDS.	31
Neuroprotective		Punicalagin 15 and 30 mg/kg	Punicalagin effectively repairs oxidative damage caused by cerebral ischemia/reperfusion based on their antioxidant potential.	32
Antiulcerative/ Gastroprotective	Test animals: rats	Punicalagin 120 mg/kg, 150 mg/kg	Lyophilized water fractions of *Lafoensia pacari* and punicalagin reduced volume gastric secretion, free acidity, and total acidity in rats 4 hours after pyloric ligation.	14
Pre-eclampsia	Test animals: Sprague-Dawley rat Hypertension inducer: NG-	25, 50 and 100 mg/kg	Punicalagin lowers blood pressure and oxidative stress, restoring angiogenic balance in pregnant rats with induced pre-eclampsia.	33

Pharmacological activity	Method	Dose/ Concentration	Reported activity	Ref
	nitro-L-arginine methyl ester (L-NAME)			
Antidiabetic (Increased insulin release)	Test animals: C57BL/6 male mice Cell: β-Tumor cell line (βTC3) Cell viability: MTT colorimetric assay Insulin release/ insulin levels: ELISA.	50 µM punicalagin	Punicalagin at a concentration of 50 µM had the effect of boosting insulin secretion in both in vivo and in vitro testing	34
Antihyperlipidemic	Test animals: rat Diabetes inducer: high-fat diet (HFD) The cellular Triglyceride (TG) and cholesterol levels: were analyzed using a commercial kit (Beyotime).	50, 150 mg/kg/day	At a 150 mg/kg/day dose, pomegranate extract effectively reduced high-fat diet (HFD)-induced hyperlipidemia and hepatic lipid deposition. Punicalagin was the main active ingredient in pomegranate extract when decreasing triglyceride and cholesterol levels in HepG2 cells.	35

Punicalagin as Antivirus

Yang et al. investigated the antiviral activity of punicalagin in vitro and in vivo. Punicalagin reduced the effect of the cytopathic virus on rhabdomyosarcoma cells (RD cells) with an IC_{50} value of 15 µg/ml. Punicalagin treatment in mice infected with enterovirus 71 (EV71) resulted in a reduction in mortality and relief of clinical symptoms by inhibiting viral multiplication. Punicalagin also increases survival time and decreases mortality in mice, with a dose of 1 mg/kg BW being the most effective treatment for EV71 infection [27].

Treatment for the 2019 Coronavirus (COVID-19) is critical because of the spread of the virus. COVID-19 is speedy and is causing an ever-increasing death rate around the world. Coronavirus 2019 (COVID-19) has been declared a worldwide pandemic. *Saadh et al* investigated the activity of punicalagin as an antiviral agent against the Coronavirus 2019 (COVID-19) virus. The coronavirus requires SARS-CoV-2 3CL-Protease (3CL-protease) for its polyprotein cleavage to produce a helpful protein in disease progression. Punicalagin showed inhibitory action against 3CL-protease in a dose-dependent manner, with an IC_{50} value of 6.192 µg/ml. The combination of punicalagin (10 µg/ml) with zinc sulfate monohydrate (3 µgram/ml obtained) resulted in a significant decrease in the activity of the 3CL-protease enzyme [28]. Administration of hydrolyzed tannins, including ellagitannin (punicalagin) and galotanin (tannic acid and pentagalloyl glucose) at concentrations of 10 and 50 µM exerted an inhibitory effect on the enzyme activity of severe acute respiratory syndrome coronavirus (SARS-CoV-2) main protease (Mpro). SARS CoV-2 Mpro is a non-structural protein that breaks down viral polyproteins to produce other non-structural proteins: RNA-dependent, RNA polymerase, and helicase, which plays an essential role in viral replication. The inhibitory effect of tannins and other metabolites on the Mpro enzyme was influenced by the number of hydroxyl groups (−OH) in the aromatic ring [29].

Other activity of punicalagin

Punicalagin inhibits NF-κB signaling in microglia via attenuating NF-κB-driven luciferase production and inhibiting NF-κB phosphorylation and nuclear translocation of the p65 subunit. Punicalagin reduces neuroinflammation in microglia that activates Lipopolysaccharide (LPS) by interfering with NF-κB signaling, implying that it could be used as a nutritional therapy to avoid neurodegenerative diseases [30].

The effect of punicalagin in lipopolysaccharide-induced acute respiratory distress syndrome (ARDS) in mice was examined by *Peng et al.* Punicalagin was given to male BALB/c mice with ARDS produced by intranasal lipopolysaccharide (LPS) 1 hour before lipopolysaccharide (LPS) induction. Punicalagin treatment reduced LPS-induced pulmonary edema and increased TNF-α, IL-6, and IL-1 levels in bronchoalveolar lavage fluid (BALF). Punicalagin suppressed LPS-induced increases in macrophage and neutrophil infiltration as well as myeloperoxidase activity in lung tissue. Punicalagin protects mice against LPS-induced ARDS. Punicalagin suppresses the expression of Toll-like receptor 4 (TLR4) and LPS-induced NF-κB activation. TLR4-mediated suppression of the NF-κB signaling pathway could be one of the underlying mechanisms [31].

Punicalagin reduces nerve damage in cerebral ischemia by reducing malondialdehyde levels, sodium-potassium adenosine triphosphatase activity, nitric oxide, protein carbonyl content, and reactive oxygen species produced by mitochondria and upregulation of superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione, glutathione reductase activity. The administration of punicalagin effectively repairs oxidative damage caused by cerebral ischemia/reperfusion based on its antioxidant potential [32].

Chaibub et al. investigated the gastroprotective effects of punicalagin compounds. In the indomethacin delivery paradigm, the lyophilized water fraction of *L. pacari* and punicalagin can reduce gastric secretion volume, free acidity, and total acidity in mice 4 hours after pyloric ligation well as the rate of a stomach injury. Because of their acid antisecretory activity, the lyophilic water portion of *L. pacari* and punicalagin have antiulcerogenic properties. After treatment, acute gastric lesions were dramatically decreased with lyophilized water fraction and punicalagin [14].

Pre-eclampsia (PE) is a pregnancy disorder characterized by severe hypertension and increased fetal and maternal death risk caused due to maternal nutrition and oxidative stress. NG-nitro-L-arginine methyl ester (L-NAME) was used as an inducer of hypertension. Punicalagin treatment at doses (25, 50, or 100 mg/kg) significantly reduced diastolic systolic blood pressure. Punicalagin also restores angiogenic balance by increasing vascular endothelial growth factor expression and downregulating vascular endothelial growth factor receptors-1/fms such as tyrosine kinase-1. Punicalagin significantly increases placental nitric oxide levels. The punicalagin dose of 100 mg showed a higher protective effect. Punicalagin lowers blood pressure and oxidative stress and restores angiogenic balance in pregnant rats with induced pre-eclampsia [33].

Koren et al. did a study on punicalagin's antidiabetic properties. Punicalagin at a concentration of 50 μM had the effect of boosting insulin secretion in both in vivo and in vitro testing. This effect is the same as the effect of the enzyme POX1 activity (paraoxonase-1). PON1 is a potent antidiabetic enzyme that protects against diabetes through its antioxidative and insulin stimulation properties on β-cells [34]. The effect of punicalagin on non-alcoholic fatty liver disease (NAFLD) caused by a high-fat diet (HFD) was studied by *Zou et al.* The administration of pomegranate extract at a dose of 150 mg/kg/day affected the form of inhibition of hyperlipidemia and hepatic lipid deposition. Punicalagin was the predominant active component of pomegranate extract about lowering triglyceride and cholesterol content in HepG2 cells. Punicalagin may be a beneficial nutrient for the treatment of NAFLD because it promotes mitochondrial activity, reduces oxidative stress, and reduces inflammation [35].

Punicalagin can help to prevent neural tube abnormalities caused by glucose (NTDs). High glucose-induced NTD development was significantly reduced by 20 μM punicalagin. Punicalagin inhibits the lipid peroxidation marker 4-hydroxynonenal, nitrotyrosine-modified proteins, and lipid peroxides in high glucose-induced lipid peroxidation. Punicalagin protects the endoplasmic reticulum from stress by blocking the phosphorylation of ribonucleic acid by protein kinase (RNA). Punicalagin decreases the formation of NTDs caused by high glucose levels by inhibiting cellular stress and caspase activation. Punicalagin can help prevent diabetes-related NTDs by reducing the teratogenic effects of hyperglycemia in developing embryos. Punicalagin can protect rat embryos from oxidative stress caused by high glucose levels. Punicalagin reduces oxidative stress caused by high glucose levels by decreasing lipid peroxidation and nitrosative stress. [36].

CONCLUSIONS

Punicalagin was a polyphenol widely contained in *Punica granatum, Lafoensia pacari,* and genus *Terminalia*. The pharmacological activities of punicalagin have been reported as anticancer, antioxidant, hepatoprotective, antimicrobial, antivirus, neuroprotective, antiinflammation, gastroprotective, pre-eclampsia, antidiabetic, and antihyperlipidemic. The anticancer activity of punicalagin is through the mechanism of inhibiting autophagy, reducing apoptosis, suppressing proliferation, inhibiting cell viability, migration, and invasion. Hepatoprotective activity inhibits oxidative/nitrosative stress, inflammation, and apoptosis and activates cytoprotective signaling pathways. Antimicrobial activity of punicalagin by causing damage to microbial cell membranes. Antiviral activity of punicalagin compounds by inhibiting viral multiplication and enzyme inhibition. Inhibitors of this enzyme can reduce viral replication and transcription without causing cell toxicity. Punicalagin was reported to have strong antioxidant activity. Through this antioxidant activity, punicalagin can overcome oxidative stress, reduce inflammation, and have anticancer properties due to its antioxidant activity.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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