APOCYNIN, AN INHIBITOR OF THE NADPH OXIDASE COMPLEX

APOCININA, UM INIBIDOR DO COMPLEXO DE NADPH OXIDASE

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RESUMO

A apocinina é um composto fenólico isolado originalmente da planta *Picrorhiza kurroa* Royle (PK). Tal composto tem sido amplamente investigado pelo seu potencial terapêutico para doenças que envolvem processos inflamatórios ou estresse oxidativo devido à sua capacidade em inibir o complexo multienzimático NADPH oxidase. Este complexo consiste em duas proteínas transmembrana (Nox2 e p22phox) e quatro proteínas citosólicas reguladoras (p67phox, p47phox, p40phox e GTPase-Rac) e sua ativação ocorre após o estímulo de células fagocíticas pela mediação da enzima mieloperoxidase (MPO). O NADPH oxidase é um complexo enzimático que produz ânion superóxido a partir de oxigênio molecular. Em vias paralelas catalisadas por enzimas, o ânion superóxido é precursor de espécies reativas de oxigênio (EROs). Essas espécies oxidantes, quando em excesso, podem atuar modificando o estado redox de moléculas de DNA, proteínas ou lipídeos desempenhando um papel central no desenvolvimento de patologias crônicas e complicações diversas de saúde como por exemplo, problemas vasculares, hiperglicemia, diabetes, hipertensão, mal de Alzheimer e câncer. Apocinina, previamente ativada por MPO, bloqueia o complexo enzimático e impede a formação dessas espécies oxidativas. Nesse sentido, a função biológica central do composto consiste em modular a ação do NADPH oxidase promovendo um efeito positivo na prevenção/remediação de doenças de caráter inflamatório.

Palavras-chave: *antioxidante; neutrófilos, mieloperoxidase, anti-inflamatório, acetovanilona.*

ABSTRACT

Apocynin (C₉H₁₀O₃) is a phenolic compound isolated from the plant *Picrorhiza kurroa* Royle ex Benth. Such a compound has been extensively investigated for its therapeutic potential in diseases involving inflammatory processes or oxidative stress due to its ability to inhibit the NADPH oxidase multienzyme complex. This complex consists of two transmembrane proteins (Nox2 and p22phox) and four cytosolic regulatory proteins (p67phox, p47phox, p40phox, and GTPase-Rac) and their activation occurs after the stimulation of phagocytic cells by the mediation of the enzyme myeloperoxidase (MPO). NADPH oxidase is the only enzyme complex that is intended for the production of superoxide anion that is precursor of highly oxidizing substances classified as reactive oxygen species (ROS). NADPH oxidase is an enzyme complex that produces superoxide anion from molecular oxygen. At the same time, the superoxide anion is a precursor to reactive oxygen species (ROS) catalyzed by enzymes. These oxidative species, when in excess, can induce burst, causing irreparable tissue damage. They can act by modifying the redox state of DNA, protein or lipid molecules, playing a central role in the development of chronic pathologies and various health complications. One can cite vascular problems, hyperglycemia, diabetes, hypertension, Alzheimer's disease, and cancer, among others. Apocynin, previously activated by MPO, blocks the enzyme complex and prevents the formation of these oxidative species. Therefore, the central biological function of compound is to modulate the action of NADPH oxidase, promoting a positive effect in the prevention/remediation of inflammatory diseases.

Keywords: *antioxidant, neutrophils, myeloperoxidase, anti-inflammatory, acetovanillone*

1. INTRODUCTION:

The plant *Picrorhiza kurroa* royle ex Benth. is traditionally used by Ayurvedic medicine (Kapahi *et al.*, 1993; Katoshi *et al.*, 2011). It is a small perennial herb that belongs to the Scrophulariaceae family (Katoshi *et al.*, 2011) and grows in the Indian trans-Himalayas and at high altitude, being a rare and endangered medicinal plant (Kala, 2000; Gangola *et al.*, 2013). Because it is a species that lives at high altitudes, oxidative stress can be one of the main limiting factors in its growth and productivity (Polle and Rennenberg, 1992). Oxidative stress occurs when there is an imbalance between the generation of oxidizing compounds and the performance of antioxidant defense systems (Birben *et al.*, 2012). Thus, the production of a highly antioxidant system by the plant provides resistance against the biotic and abiotic stresses (Gangola *et al.*, 2013), eliminating the excess of reactive oxygen species (ROS) (Poljsak *et al.*, 2013). Among the compounds with recognized antioxidant activity is apocynin (Sun *et al.*, 2017; Tanriverdi *et al.*, 2017). Apocynin is a natural substance that was isolated from a root extract of *P. kurroa* in 1971 (Basu *et al.*, 1971) and gained extensive attention from researchers due to the medicinal properties attributed to its species of origin (Stefanska and Pawliczak, 2008). The same compound had previously been described by Schmiedeberg (1883) when it was isolated from the roots of the plant *Apocynum cannabinum* L.

Years later, Simons *et al.* (1990) found that *P. kurroa* root extracts decreased *in vitro* the inflammatory response of lymphocytes stimulated by *Phytolacca americana* L, and found that the incubation of non-activated neutrophils with apocynin did not significantly influence their ability to produce O²⁻. This fact suggests that cells that do not release ROS after activation are relatively insensitive to this compound so that apocynin would have selective action. The main conclusion of Simons *et al.* (1990) was that the methoxy-substituted catechols, which include apocynin, present action only when previously activated by myeloperoxidase (MPO).

These findings have led to several similar studies of interest (Kim *et al.*, 2011; Maiocchi *et al.*, 2017; Qin *et al.*, 2017) for the application of apocynin as an anti-inflammatory drug metabolically activated by neutrophils that would have action only at the site of inflammation. Given the importance of these studies, this review aims to provide an overview of the mechanism of action

and biological applications of apocynin.

2. MATERIALS AND METHODS:

2.1 Inflammatory process mediated by neutrophils

Neutrophils are abundant circulating leukocyte cells that make up the innate immune system (Kolaczowska and Kubes, 2013). They represent the mainline of defense of the body and constitute 40 to 60% of the population of white blood cells (Wright *et al.*, 2010). Neutrophils belong to a family whose hallmark is the presence of granules in their cytosol. The granules are lysosomal organelles with antimicrobial enzymes (Amulic *et al.*, 2012), with azurophilic granules due to the presence of the enzyme myeloperoxidase (MPO), which has the characteristic dark green color (Schultz and Kaminker, 1962).

Neutrophils are produced by the myeloid stem cells of the red bone marrow and remain circulating in the bloodstream until its activation (Nathan, 2006). The action of neutrophils begins with the pathogen-associated molecular patterns (PAMPs), released by invading microorganisms, and damage-associated molecular patterns (DAMPs), derived from damaged cells (Nourshargh and Alon, 2014).

These factors stimulate the expression of selectins and the activation of integrins (Sun *et al.*, 2017), which are transmembrane proteins mediating cell adhesion and binding in the extracellular matrix, as well as cell-cell interaction (Amulic *et al.*, 2012). In this way, the integrins allow the transmigration of the neutrophils by the endothelial cells near the site of inflammation, a mechanism called diapedesis or homing (Filippi *et al.*, 2016; Gong *et al.*, 2017; Leach, Morton, and Sansom, 2017). Both sterile lesions (DAMPs) and infectious lesions (PAMPs) activate similar receptors, which result in phagocytosis, destruction, and subsequent elimination of the pathogen through clearance of debris by the phenomenon of exocytosis or clasmocytosis (Nourshargh and Alon, 2014; Marki *et al.*, 2015). Phagocytic recognition of microbial pathogens is mediated by pathogen recognition receptors (PRRs) (Kumar and Sharma, 2010). PRRs are genetically encoded proteins found mainly in the plasma membrane and in the cytoplasm of phagocytes that recognize conserved ligands in microorganisms, the PAMP's, and DAMP's (Panday, 2014).

At the site of infection, these membrane receptors recognize immunoglobulins and bind to opsonized microorganisms, leading to pseudopodia formation and onset of phagocytosis (Wright *et al.*, 2010) (Figure. 1).

(a)

(b)

(c)

Figure 1. (a) Neutrophil membrane receptors in recognition of opsonized pathogens (b). Formation of pseudopodia and onset of phagocytosis of the pathogen by the neutrophil (c). Establishment of the phagosome for later release of lysosomal enzymes and destruction of the pathogen. Source: (Lim, Grinstein and Roth, 2017). Images edited with the content of images made available in LES LABORATOIRES SERVIER.

Although PRRs are critical for immunological surveillance and to contribute to the increase of neutrophil functions, phagocytosis is more efficient in the presence of opsonins (Kobayashi, Malachowa and Deleo, 2017).

Opsonins are proteins that make up the complement system, in which IgM and IgG are prominent. They adsorb to the pathogen (Gordon, 2016) and function as an immunostimulant to promote bacterial phagocytosis, generate an oxidative burst and stimulate neutrophils to produce interleukin-8 (Katzenmeyer, Szott and Bryers, 2017). Interleukin-8 is a pro-inflammatory chemical mediator that exerts a significant regulatory effect on the development of inflammation (Baggiolini, Clark-Lewis, 1992), in which, at low concentrations, it stimulates the release of L-selectin and increases the expression of integrins and in higher levels results in the onset of oxidative burst (Amulic *et al.*, 2012; Qin, 2017).

However, interleukin-8 represents only one of the regulatory mechanisms of the complex cascade chemical signaling of mediators, fundamental in the recruitment and activation of neutrophils (Nauseef and Borregaard, 2014). PRRs include various receptor proteins such as Toll-like (TLRs), nucleotide-binding, and oligomerization domain (NOD)-like receptors (NLRs), RIG-I-type and C-type lectin (Panday, 2014). Receptors responsible for this regulatory activity mainly involve recruitment and activation of neutrophils and consequent assembly of the enzymatic complex NADPH oxidase and activation of the oxidative explosion in Nox2 during phagocytosis (Hogan and Wheeler, 2014).

2.2. NADPH oxidase system

NADPH (nicotinamide adenine dinucleotide phosphate) oxidase is a large multienzyme complex translocated in the neutrophil cell membrane (Jiang, 2014). These complexes are formed by a heterodimeric flavocytochrome immersed in a cytosolic membrane, known as cytochrome b 558 (consisting of Nox2 and p22phox) and four cytosolic regulatory proteins: p47phox, p67phox, p40phox and GTPase-Rac (Pick, 2014a). This protein, when disposed of as a monomer, is inactive (Figure 2), and requires activators to develop its physiological activity (Pick, 2014b). For the assembly of the massive complex, Nox2 interacts with the small transmembrane protein p22phox which has a shielding function for protein

folding (Brandes, Weissmann and Schröder, 2014).

Figure 2. Activated NADPH oxidase enzyme complex. Adapted from Brandes; Weissmann; Schröder, (2014). Source: Images edited with content of images made available in LES LABORATOIRES SERVIE.

In the early stages, p47phox translocates the ternary complex of cytosolic proteins to the newly formed phagosome, which translocates p67phox to the membrane (Pick, 2014b) (Figure 2). The p67phox subunit should interact with Nox2. For this, the regulatory protein RAC must be active (Brandes, Weissmann and SCHRÖDER, 2014). Rac involvement is expressed in the RacGDP-RhoGDI Complex Translocation to the membrane, subsequent RacGDP dissociation from RhoGDI, followed by RacGTP binding to p67phox, promoting interaction with Nox2 (Pick, 2014b). Once assembled, the electron transport in Nox2 (Chan *et al.*, 2009) and the electron flow along a redox gradient, from NADPH to oxygen, leads to the reduction of a molecular oxygen electron (O_2) in superoxide anion (O_2^-) (Pick, 2014a).

2.3 Reactive oxygen species (ROS)

Oxidative stress is the imbalance between oxidant species and antioxidants, in favor of oxidants, leading to a rupture of redox signaling and molecular damage (Sies, 2017). The oxidant species can act by modifying the redox state of DNA, protein, or lipid molecules (Schieber and Chandel, 2014) and play a central role in the development of chronic pathologies and various health complications. As an example, one can cite vascular problems (Munzel *et al.*, 2014; Montezano *et al.*, 2015); hyperglycemia, *diabetes mellitus* (Hur *et al.*, 2010; Rains and Jain, 2011;

Zhang *et al.*, 2016), hypertension (Chan and Chan, 2014; Wu and Harrison, 2014; Togliatto, 2017), Alzheimer's disease (Dumont and Beal, 2011); cancer (Glasauer and Chandel, 2014) among other inflammatory diseases (Schieber and Chandel, 2014; Marín, 2017; Rhaman, 2017).

Among the species responsible for causing this damage are the superoxide anion produced from molecular oxygen and the NADPH multienzyme system (Chan *et al.*, 2009). There are also other species, which originate from the superoxide anion through enzymatic reactions classified as reactive oxygen species (ROS) (Lugrin *et al.*, 2014; Sies, 2017). The formation of ROS by NADPH oxidase complex occurs in the sizeable transmembrane protein Nox2 (Brandes, Weissmann and Schröder, 2014) and is associated with phagocyte formation, pathogen death and activated inflammatory processes (Dupré- Crochet and Nüße, 2013).

These processes initiate the oxidative explosion that generates an amplification of the conversion of O_2 to O_2^- and the subsequent formation of other ROS catalyzed by enzymes also derived from neutrophils (Nauseef, 2017). The superoxide anion is a weak oxidant, but it is the precursor of an uncontrollable series of reactions with free radicals (Lushchak, 2014). As an example, the recombination reaction of superoxide anion radicals catalyzed by the enzyme superoxide dismutase (SOD), which has as a product the reactive oxygen species hydrogen peroxide (H_2O_2) (Sies, 2017). H_2O_2 , in turn, is a precursor to other ROS. The MPO enzyme catalyzes the oxidation of Cl^- and H_2O_2 to generate the oxidant hypochlorous acid (HOCl) (Winterbourn *et al.*, 2006). H_2O_2 is also responsible for damaging ferritin, releasing the ion Fe^{2+}/Fe^{3+} , which can induce Fenton reactions and the generation of hydroxyl radicals ($OH^\bullet$) and the peroxide radical ($OH_2^\bullet$), which are species with high oxidant potential (Yoon *et al.*, 2011). Besides, these species lead to inflammatory responses in H_2O_2 concentrations in the range of 0.1 to 1 μM , leading to growth arrest and cell death by several mechanisms, leading to an amplification of the inflammation (Sies, 2017).

Another route of ROS formation, parallel to those previously described, is the reaction of the O_2^- anion with nitric oxide ($NO^\bullet$), activated neutrophil pro-inflammatory cytotoxin, forming the peroxide nitrite radical ($ONOO^\bullet$) (Guzik, Korbout and Adamek-Guzik, 2003), a reactive nitrogen species (RNS). The nitrite peroxide is cytotoxic,

affects mitochondrial function, and triggers cell death through oxidation and nitration reactions (Radi, 2013). In addition to the above mentioned oxidative species, other reactive intermediates are formed through hydrogen peroxide, in which some use MPO catalysis (Figure 3). In the presence of hydrogen peroxide and bromide, thiocyanate, tyrosine or nitrite, it occurs the formation of hypobromous acid (HOBr) and hypothiocyanite (HOSCN) of the tyrosyl radicals and reactive nitrogen intermediates (ONOO•) (Odobasic, Kitching and Holdsworth, 2016).

Figure 3. Reactive species generated through the enzymatic complex NADPH oxidase during an oxidative explosion. Source: Adapted from (Sareila *et al.*, 2011; Odobasic, Kitching and Holdsworth, 2016).

This whole process of neutrophil activation and the production of ROS is a highly regulated process by peptides and regulatory proteins to trigger the antimicrobial action of neutrophils. Therefore, this process is vital to maintain immune homeostasis and host health (Kobayashi, Malachowa and Deleo, 2017). In inflammatory processes, in which many neutrophils are stimulated by activating Nox2, an excess of reactive species (ROS and RNS) is generated during the burst (Lugrin *et al.*, 2014). Thus, it is necessary to reestablish the redox equilibrium, because all the substances reported, have a high oxidizing capacity and are responsible for causing irreparable damage to the tissues of hosts (Sareila *et al.*, 2011; Odobasic, Kitching and Holdsworth, 2016; Sies, 2017). Damage ranges from molecular and cellular modifications to cell death through apoptosis and necrosis (Kobayashi, 2017).

Castor, Locatelli, and Ximenes (2010) reported apocynin metabolically activated by MPO during neutrophil activation, avoiding the exacerbated production of these oxidants during the burst.

2.4 Apocynin

After being isolated from the *P. kurroa* plant the same compound was isolated from several plant sources, such as, for example, *Corallodiscus flabellata* Craib) B. L. Burt (Feng *et al.*, 2004), *Sargentodoxa cuneata* (Oliv.) Rehder & E. H. Wilson (Tian *et al.*, 2005), *Allium cepa* L. (Xiao and Parkin, 2007), *Polygonum orientale* L. (Li, 2011), *Ziziphora clinopodioides* Lam. (Senejoux *et al.*, 2012), *Scutellaria ramosissima* Popov (Mamadalieva *et al.*, 2013), *Solanum erianthum* D. Don (Chen *et al.*, 2013), *Pelargonium endlicherianum* Fenz (Karatoprak *et al.*, 2017) and *Iris tectorum* Maxim. (Zhang *et al.*, 2017). The apocynin (C₉H₁₀O₃), CAS 498-02-2, is a phenolic compound belonging to the class of methoxy catechols, it is the 4-hydroxy-3-methoxyacetophenone compound (Figure 4a), commonly known as acetovanilone (RAM, 2013). It is indicated as a pro-compound, due to the need for a pre-activation mediated by the enzyme myeloperoxidase and has as a product the apocynin radical (Almeida *et al.*, 2012). In the work developed by Maiocchi *et al.* (2017) suprapharmacological concentrations of apocynin did not significantly affect the initial rate or extent of NO consumption catalyzed by MPO in diluted human plasma. They did not affect the NO• intake rate in protein-free buffer systems containing urate and tyrosine, which are peroxidase substrates and attenuated the initial rate of NO uptake in the presence of urate, tyrosine, and ascorbate. In contrast, apocynin significantly accelerated the consumption of NO• in the absence of physiological substrates of myeloperoxidase.

According to the author, this is due to the fact of its ability to increase the NO • consumption in the absence of competing peroxidase substrates, since it does not stimulate the competition for the consumption of the enzyme and, therefore, the MPO becomes available to the activation of apocynin (Maiocchi *et al.*, 2017). Consequently, it is evident the dependence relationship between the presence of the MPO enzyme and the inhibitory action of apocynin, establishing a direct correlation between the cellular MPO level and the inhibition of ROS generation through the apocynin radical

(Odobasic, Kitching and Holdsworth, 2016).

The mechanism proposed for this relationship justifies that the apocynin radical formed via enzymatic conversion (Figure 4b) is highly useful to oxidize thiolic groups (SH) (Castor, Locatelli and Ximenes, 2010).

Ximenes *et al.* (2007) suggest that the reactivity of the apocynin radical with thiol compounds may be involved in the inhibitory effect on the NADPH oxidase complex. The ability of apocynin to inhibit the aggregation of the NADPH oxidase complex in Nox2 is linked to the oxidation of these SH groups since such thiol groups are essential for the migration of the p47phox cytosolic fraction to the membrane (Kanegae *et al.*, 2007).

The apocynin radical further establishes a bond with a second radical, forming a dimeric product, the diapocynin (Figure 4c). Several authors indicate the apocynin dimer as the real inhibitor of NADPH oxidase (Castor, Locatelli and Ximenes, 2010; Dranka *et al.*, 2013; Potje, 2017; Pérez *et al.*, 2017).

According to Pérez *et al.* (2017), the inactivation of p47phox occurs more strongly with the apocynin dimers since the bonds between the aromatic rings of diapocynin allow the interactions with the aromatic residues W193, W263, F209, W204 and Y274 in p47phox, which are the terminals where p22phox turns on. The same author further justifies the increased inhibition of NADPH oxidase due to the increased hydrophobicity of the apocynin dimer over the parent compound. These studies corroborate with that reported by Potje (2017), in which the levels of nitric oxide, ROS, and calcium in aortic endothelial cells stimulated by diapocynin by apocynin were evaluated. The results presented by the author indicated that diapocynin showed a higher antioxidant capacity than apocynin, concerning the promotion of hypotensive effects without alteration of heart rate. Also, both compounds were effective in increasing NO• and also decreased RO levels in endothelial cells.

(c)

Figure 4. (a) Apocynin Structure, (b) Structure of the Apocynin radical, (c) Structure of the dimeric compound formed as the product of the interaction of two apocynin radicals. Source: (Castor, Locatelli and Ximenes, 2010; Pérez *et al.*, 2017).

In the study reported by Marín *et al.* (2017), diapocynin was chemically synthesized and tested in macrophages stimulated by lipopolysaccharide in an ulcerative colitis model. In this case, diapocynin showed inhibitory activity similar to apocynin, but more expressive. Dimer reduced levels of ROS production and inflammation mediation, such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-1β), as well as inhibited the enzymatic expression of nitric oxide synthase (iNOS) and cyclooxygenase (COX-2), which act in the synthesis of nitric oxide and prostaglandins, intensifying inflammation. The results reported suggest that oxidation products, or transient species generated during the oxidation of apocynin, prevent migration of the p47phox component to the membrane, avoiding the assembly of NADPH. In this way, the operation of this complex is prevented (Dranka *et al.*, 2013; Potje, 2017; Pérez *et al.*, 2017; Marín, 2017).

Since the pre-activation of apocynin occurs through neutrophil myeloperoxidases that are recruited and activated during the occurrence of inflammation, the anti-inflammatory activity of apocynin makes this compound active in the locus of action of inflammation (Castor, Locatelli and Ximenes, 2010; Aratani, 2018). In according Stefanska *et al.* (2010), anti-inflammatory activity was demonstrated in a variety of cellular and animal models with a significant reduction of all inflammation parameters. Besides, the importance of advances in studies that refer to the development of apocynin-based drugs is due to the central role of Nox2 activity in inflammation. This, therefore, is closely linked in the modulation

of redox signaling pathways (Kim *et al.*, 2012), acting in the prevention and treatment of the most diverse diseases, such as neuronal dysfunction and degeneration and neuroinflammation. Diseases range from strokes, Alzheimer's disease, and Parkinson's disease, to psychiatric disorders (Simonyi, 2012). Due to the characteristics listed, many health problems can be improved by the action of apocynin. Some of the results of studies that have been conducted in recent years with the compound apocynin/ radical/ dimer in disease models encompassing oxidative disorders and/or inflammation are set out in Table 1.

3. RESULTS AND DISCUSSION:

3.1 Apocynin against the effects of ischemia injuries

Oxidative stress is one of the first biochemical changes after ischemia injury; such a factor contributes to the deterioration of the injury leading to other secondary damage (Sun *et al.*, 2017). In this sense, apocynin supplementation has shown essential benefits regarding the mitigation of oxidative stress and its consequent effects on post-injury in ischemic accidents. In vivo, apocynin application studies show the significant reduction in NADPH oxidase activity and the resulting reduced generation of ROS in pulmonary ischemia (Chiang, Chuang and Liu, 2011), ischemic myocardial injury (LUO *et al.*, 2012) for ischemia/reperfusion (I / R) and torsion / destruction (T / D) in testis (Şener *et al.*, 2015; Ozbek *et al.*, 2015), ischemic kidney injury (Choi *et al.*, 2015; Hu *et al.*, 2017), spinal cord injury (Sun *et al.*, 2017) and cerebral ischemia (Kapoor *et al.*, 2018).

Other significant effects of apocynin on ischemia is that the compound has been shown to significantly reduce the activity of the enzyme myeloperoxidase (MPO) which has catalytic activity in the production of reactive oxygen species (Şener *et al.*, 2015; Ozbek *et al.*, 2015; Sun *et al.*, 2017), and also present significant decrease in malonaldehyde (MDA) levels, the end product of lipid peroxidation (Choi *et al.*, 2015; Şener *et al.*, 2015; Ozbek *et al.*, 2015; Hu *et al.*, 2017; Sun *et al.*, 2017). Besides, several studies point to a significant increase in glutathione peroxidase (GSH) and superoxide dismutase (SOD), enzymes responsible for free radical scavenging (Şener *et al.*, 2015; Ozbek *et al.*, 2015; Hu *et al.*, 2017 ; Sun *et al.*, 2017; Kapoor *et al.*,

2018).

Corroborating these reported biochemical indices and proving the effectiveness of in vivo application of apocynin in attenuating biochemical alterations after apocynin injury proved to be efficient in hemodynamics, lung injury indices, and activation of attenuated apoptotic pathways (Chiang, Chuang and Liu, 2011), myocardial infarction size and significantly smaller apoptosis ratio of cardiomyocytes (Luo *et al.*, 2012), reversal of histopathological changes (Ozbek *et al.*, 2015), serum and renal zinc levels, urea nitrogen in the reduced blood (BUN) and serum creatinine (Scr) (Hu *et al.*, 2017) and restoration of mitochondrial membrane potential and reduction of behavioral deficits in ischemic animals for post cerebral ischemia (Kapoor *et al.*, 2018).

Furthermore, a recent study has shown that the up-regulation of NADPH / NOX2 expression and activity is likely a molecular mechanism responsible for the developmental origins of cardiovascular disease in the offspring of diabetic mothers. In this research, the compound apocynin acted in the prevention and reduction of apoptosis rates, ROS generation and infarction providing a new hypothesis that could be tested in future clinical studies (Zhang *et al.*, 2018).

3.2 Apocynin in cancer treatment

Anticancer agents derived from natural products activate or inhibit various pathways leading to suppression of tumor growth, apoptosis induction, and antimetastatic and antiangiogenic activity. These include prevention of DNA damage, inhibition of microtubule assembly, antimigration, anti-invasion, inhibition of cell growth, tumor tissue proapoptosis as well as enzymatic inhibition of topoisomerase for cell replication.

As shown in table 1, apocynin has been reported as an agent with antioncongenic properties with measurement of biological markers directly attributed to the proposed mechanism of action for compound, as the decrease in generation of ROS (Suzuki, 2013) and inflammatory signaling such as nuclear-κB factor (Komyia *et al.*, 2015; Byun *et al.*, 2016). As activities attributed to tumor growth suppression and metastatic activity factors (Suzuki *et al.*, 2013; Komyia *et al.*, 2015; Jantaree *et al.*, 2017).

In researching suppression of prostate cancer in rats, Suzuki *et al.*, (2013) focus on

clusterin precursor, belonging to deregulated genes, which is known to be related to tumorigenesis in many locations. The decreased clusterin expression in the ventral prostate occurred in the apocynin-treated rats' group. In this research, apocynin reduced Ki-67 labeling index and cyclin D1 expression, indicating that ROS generation is related to cell proliferation in rat prostate lobes. Also, NADPH oxidase regulates plasma adiponectin, the concentration of which may be negatively correlated with prostate cancer progression.

According to Suzuki *et al.* (2013), in rats with induced prostate cancer (TRAP), the activation of p38 MAPK (P38 mitogen-activated protein kinases) and ERK1/2 (extracellular signal-regulated kinase) in prostate tissue and their inactivation by prostate cancer were previously detected. Apocynin reduced cell proliferation and induced apoptosis in the prostate tissue, being active in suppressing prostate cancer.

Horemans *et al.* (2017) investigated the action of apocynin on the suppression of oncogenic activity induced by the presence of *H. pylori*. As a result, the histopathological analysis showed high infectious burden and severe gastritis after infection in control animals and immunohistochemistry confirmed the presence of *H. pylori* in all infected stomachs. The untreated and uninfected group had normal gastric histology, indicating that apocynin has no direct effect on stomach tissue. For infected animals, the administration of apocynin in stearic acid showed a significant decrease in abscess formation in the stomach cavity compared to the control group, thus suggesting that apocynin can positively modulate the process by reducing tissue damage induced by *H. Pylori*.

According to the results reported by Byun (2016), administration of apocynin has been investigated as a prevention of non-melanoma skin cancer, to provide new ways to prevent skin damage caused by UVB rays. The NADPH oxidase inhibitor suppressed UVB-induced skin cancer training. While UVB irradiation-induced the expression of critical carcinogenic factors in skin cells, or apocynin treatment reduced serious effects, thus inhibition of NADPH oxidase activity by apocynin was considered responsible for attenuation of signaling pathways during infection of the skin and carcinogenesis, such as signaling Akt, which regulates survival and tumor growth, and the expression factor NF-kB of inflammation.

In work reported by Jantaree *et al.* (2017)

with HepG2 cells from human hepatocellular carcinoma, apocynin and diapocynin were effective in inhibition of migration and invasion, thus effective in antimetastatic effect. During attack and movement, the cell surface binds to the extracellular matrices of cancer cells (ECM) by a family of cell surface receptors, integrins. Integrin-ECM interactions activate downstream signaling including the FAK, PI3K / Akt, ERK and NF-kB signaling pathways. The results showed that the invasion assay induced the phosphorylation of FAK, Akt and ERK proteins in HepG2 cells, while both the apocynin monomeric compound and its dimer specifically suppressed FAK and Akt phosphorylation. These results indicate that diapocynin and its monomeric form act on the same targets with differential power, inhibiting FAK and cell-ECM activated PI3K / Akt signaling, and these compounds are active in preventing metastasis (Jantaree *et al.*, 2017).

Paul *et al.* (2020) investigated the action of apocynin in suppressing the progression of pulmonary carcinoma in vitro and in vivo. Along with the identification of targets and the underlying mechanisms, the property of the compound to bind to tubulin protein and stabilization of cellular microtubules was investigated avoiding mitosis and, consequently, angiogenic activity. As a result, apocynin showed selective cytotoxicity relative to lung cancer cells rather than normal fibroblast cells.

Apocynin also inhibited oncogenic properties, including growth, proliferation, polyp formation, invasion, and spheroid formation in lung cancer cells. In addition, in other established features, apocynin is a novel and potent component for binding to tubulin and depolymerizing that of cellular microtubules. Depolymerization was the mechanism that triggered autophagy-mediated apoptotic cell death, which in turn slowed the progression of lung cancer. Moreover apocynin has shown tumoricidal characteristics inhibit pulmonary tumorigenesis in mice as well. Tubulin-microtubule balance with apocynin may be the primary regulator of catastrophic cellular catabolic processes to mitigate lung carcinoma (Raul *et al.*, 2020).

Considering all the apocynin-listed anticancer activity pathways in the articles cited, it is evident that reactive generation of oxygen species is found to be associated with tissue damage and DNA damage, with neoplastic transformation, aberrant growth and tumor proliferation. In this sense, apocynin deserves

more attention as a chemopreventive drug and also promising for cancer treatment.

3.3 Apocynin in the treatment of dysfunctions from *Diabetes mellitus* (DM)

Increasing reactive oxygen species by activating NADPH oxidase contributes to the development of diabetic complications (Olukam *et al.*, 2018). Diabetes-induced hyperglycemia causes damage to various organs and is associated with vascular and heart disease, responsible for increased morbidity and mortality (Gimenes *et al.*, 2018). Several mechanisms are involved in these effects, including increased or increased production of reactive oxygen species (ROS) produced by NADPH oxidase. In this context, the action of apocynin as an inhibitor of this enzyme complex is a compound with the potential to detect the adverse effects caused by this metabolic syndrome.

According to the results proposed by Gimenes *et al.*, 2018, apocynin showed a modulation activity of systemic antioxidant enzymes by decreasing SOD and GSH activity in control rats and restoring SOD activity in diabetic rats. Therefore, the compound had an influence on streptozotocin-induced cardiac remodeling in diabetic rats, attenuating systolic and diastolic dysfunction in diabetic rats. Also, Apocynin did not alter cardiac structures or myocardial and ventricular functions in normoglycemic rats.

The study reported by Qiu *et al.*, (2016) was developed to focus on unraveling the role of NADPH oxidase in the *diabetes mellitus* setting in addition to the potential benefits of apocynin in diabetic atrial remodeling. The inducibility of atrial fibrillation was increased in the DM group and significantly reduced by apocynin. In addition, the apocynin caused atrial structural remodeling in diabetic rabbits. Western blot analysis indicated that apocynin reduced the increased protein expression of TGF- β , NF- κ B, P-p38, P-JNK, ERK, and P-ERK, which are expressions induced by metabolic syndrome.

Another finding of apocynin action for the treatment of cardiac dysfunction caused by DM, the study proposed by Liang *et al.* (2018), sought to explore the mediated mechanism of ROS production and events in atrial fibrillation induced by diabetes. Western blotting was performed to detect the expression of NADPH oxidase subunits, including Rac1 (regulatory subunit), p22phox and gp91phox (structural subunits). As a result,

apocynin markedly suppressed expression of Rac1 activator but did not affect the expression of the p22phox and gp91phox structural subunits in NADPH stimulated by high glucose levels and H₂O₂ deferment.

In addition, Liang *et al.* (2018) report on the expression and activity of key factors involved in mitogen-activated protein kinase (MAPK) signaling, in which high glucose and H₂O₂ stimulation induced an increase in MMP9 expression. The addition of apocynin markedly reduced the expression of this marker of cellular reproduction. Also, apocynin addition did not induce low MMP2 expression while combined treatment of glucose and H₂O₂ induced increased MMP2 expression. Corroborating these results, glucose and H₂O₂ stimulation promoted cell proliferation and apocynin addition inhibited this increase in atrial fibroblast proliferation induced by high glucose and H₂O₂ stimulation. Therefore, the promoter effects of high glucose and H₂O₂ stimulation were attenuated by apocynin. These results led the author to the conclusion that hyperglycemia and oxidative stress-induced increased proliferation of atrial fibroblasts, leading to cardiac tissue fibrosis and cardiac function dysfunction, including atrial fibrillation. All these effects are alleviated by apo administration, further stating that such results provide the basis for the antioxidative therapy of atrial fibrillation caused by DM.

In addition to the factors mentioned referring to cardiac dysfunctions caused by diabetes mellitus, other consequences generated by this syndrome also presented improvements in apocynin administration. The work reported by Olukam *et al.* (2018) investigated the potential of the protective effect of apocynin on diabetic neuropathy in rats and its precise mechanism of action at the molecular level. Treatment with apocynin increased the dose-dependent pain threshold, showed increased expression of catalase and NOX-p47phox protein in the spinal cord, and was able to increase sciatic nerve conductance and blood flow in diabetic rats.

Another health complication that is a consequence of diabetes Mellitus is retinopathy. Retinopathy is a common complication of diabetes that affects the retina due to the high blood sugar content. Recent studies have shown that the presence of advanced oxidative species is the cause of retinal damage leading to the leakage of tiny blood vessels or act as signaling molecules to trigger neovascularization and apocynin is

presented as an NADPH oxidase inhibitor in various Nox isoforms corresponding to a potential compost for the treatment of this complication (Peng *et al.*, 2019).

In the development of diabetic retinopathy, expression of the inflammatory markers NF- κ B and toll-like receptor 4 occurs. Treatment of rat models with streptozotocin-induced diabetes retinopathy has improved the rates of biochemical markers as well as histological markers. After twelve weeks of apocynin treatment, cell apoptosis, oxidative stress and retinal inflammation were alleviated. Apocynin treatment improved the adverse effects of retinopathy in rats, decreased TLR4 / NF- κ B marker expression by apocynin are involved in this process (Wang *et al.*, 2019).

After proving apocynin as an adjunct in the treatment of many effects caused by metabolic syndrome, the results presented by Chan *et al.*, (2017) show apocynin as an active compound in preventing the generation of type 2 diabetes mellitus. As presented by the author, elevations in C-reactive protein (CRP) levels are positively correlated with the progress of type 2 diabetes mellitus. Purified human CRP impairs insulin secretion in isolated mouse islets and insulin-secreting NIT-1 cells from dose and time dependent ways. CRP increased the production of NADPH oxidase-mediated ROS, which simultaneously promoted the production of nitrotyrosine (indicator of reactive nitrogen species) which decreases cellular viability and insulin secretion in islets and secretory insulin cells. In this research, the apocynin compound considerably reversed the PCR-stimulated RNS ageration, indicating the action of apo on redox signaling and inactivation of RNS and NF- κ B and the reversal in the production of proinflammatory cytokines that cause cell damage.

4. CONCLUSIONS:

The *in vivo* results of apocynin has a broad spectrum of beneficial effects against various biological markers or pathophysiological processes. These include the markers used to monitor the inhibition of NADPH complex activity, such as increased activity of antioxidant enzymes GSH and SOD, decreased MDA content, decreased cell death by necrosis and apoptosis, as well as decreased expression of inflammatory biomarkers TNF- α , IL-6 e IL-1 β , beyond other markers specific to each disease.

The present results are of evaluation in various diseases, such as degenerative diseases, cancer, ischemia, hypertension, diabetes, among many other health problems. Thus, apocynin has been describing as active in the prevention and stabilization of diseases and in the reduction of secondary damage rates in cases of chronic diseases. These notes highlight the potential of apocynin as an excellent compost of its pharmacological properties. However, despite all these promising results that highlight the future potential of this substance, there are controversies about the real mechanism of action *in vivo* that occurs with this compound to obtain these beneficial biomarker indices. Some studies indicate the pro-oxidant activity of apocynin (Castor, Locatelli and Ximenes 2010; Wong *et al.*, 2015). That, once oxidized by MPO enzymes, they are activated and become capable of inducing oxidation of the NADPH enzyme complex, but also of various classes of biomolecules. Concomitantly inhibiting the NADPH complex, apocynin can act as an oxidant by initiating a deleterious intracellular oxidative process.

The same authors also reported that the apocynin radical is capable of oxidizing the endogenous substrate of the NADPH oxidase enzyme complex, and this culminates in necessary implications for the apocynin mechanism as an NADPH oxidase inhibitor. The depletion of its substrate would block NADPH oxidase from superoxide anion production. However, it is known that chemical interaction may also contribute to redox cell imbalance and that NADPH oxidation involves the NADP $\cdot$ intermediate (NADP radical), which is also capable of initiating a prooxidant process, reducing molecular oxygen to the superoxide anion (Castor, Locatelli and Ximenes, 2010).

Apocynin has therapeutic potential against diseases involving inflammation or oxidative stress. Its antioxidant activities, however, are not well characterized and vary according to the experimental context, cell type, and NADPH oxidase system. In cells treated with a pro-oxidative and cytotoxic protein aggregate, the first evidence that apocynin has a more significant inhibitory effect on reactive nitrogen species levels compared to hydrogen peroxide levels (Wong, Fong, and Vieira, 2015). In addition, apocynin does not have a high free radical scavenging capability. However, it has a high scavenging capacity for non-radical oxidizing species, such as HOCl and H₂O₂. Thus, if a peroxidase is expressed or added to cells, depletion of H₂O₂ by

apocynin may represent an additional pathway for its biological effects (Petrônio *et al.*, 2013).

The results obtained for research on the action of apocynin in humans are scarce. It reinforces the need for further studies for this natural compound because, although there are controversies about its mechanism of action, the anti-inflammatory and antioxidant activities in the models presented are significant. However, the tremendous potential of apocynin to inhibit NADPH oxidase has been described in the literature based on results from *in vivo* applications. Apocynin can decrease superoxide anion production of activated neutrophils, while phagocytosis capacity is not affected. Also, it is vital to highlight the regulatory role of neutrophil myeloperoxidase enzyme in the activation of apocynin and, consequently, in the inhibition of the generation of reactive oxygen species that mediated inflammatory processes and cause intense and irreversible cell damage. Thus, apocynin has the potential for the prevention and treatment of several health complications presented in this review. Therefore, the importance of further studies with these compounds is emphasized.

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Table 1. Beneficial effects of apocynin and its radical and dimeric on disease models.

Medical complication	Biological markers and decreased effects of the disease	References
Models of ischemia		
Pulmonary ischemia	Reduction of NADPH oxidase activity; Suppress the generation of ROS; Hemodynamic monitoring indices of attenuated lung lesions; Attenuated apoptotic indices.	(Chiang and Chuang, 2011)
Myocardial ischemia	Reduction of NADPH oxidase activity; Significantly smaller myocardial infarction size; The proportion of cardiomyocyte apoptosis reduced; Reduction of myocardial mRNA, expression of VOP1 and NOX2 proteins, serum CK, myocardial NOX, and caspase-3 activity.	(Luo <i>et al.</i> , 2012)
Injury by ischemia / reperfusion (I/ R) and torsion / distortion (T/ D) in testicles	Inhibits the generation of free radicals and increases antioxidant defense; Reduction of NADPH oxidase activity; Reduction of MPO activity and malonaldehyde levels (MDA); Significant increase in glutathione (GSH) and SOD activity.	(Şener <i>et al.</i> , 2015; Ozbek <i>et al.</i> , 2015)
Renal Ischemia	Decrease in MDA levels; Significant increase of GSH and SOD; Lower levels of zinc in serum and renal tissues; Reduction of blood urea nitrogen (BUN) and serum creatinine (Scr).	(Choi <i>et al.</i> , 2015; Hu <i>et al.</i> , 2016)
Ischemic injury in the spinal cord	Reduction of NADPH oxidase activity; Reduction of MPO activity and MDA levels; Significant increase in GSH and SOD.	(Sun <i>et al.</i> , 2017)
Stroke induced by ischemia/ reperfusion (I/ R)	Significant attenuation in Nox2 and ROS levels; It avoids increases in cyclooxygenase (COX2) and inducible nitric oxide synthase (iNOS) in the cortex induced by I / R; Suppression of expression of inflammatory expression proteins, including NLRP3 ASC, caspase-1, IL-1 β and IL-18 in the ischemic cortex; Inhibits the activation of NF-kB; Significant blockade of increases in NOx2 and NOX4 protein levels induced by I / R; Reduction of infarct volume, Improvement in post-stroke survival; Recovery of neurological functions.	(Qin <i>et al.</i> , 2017)
Cerebral ischemia	Reduction of NADPH oxidase activity; Increased GSH and SOD; Restoration of mitochondrial membrane potential; Reduction of behavioral deficits in post cerebral ischemia; Mitochondrial injury attenuation; Restoration of MMP and mitochondrial enzymes.	(Kapoor <i>et al.</i> , 2018)

Cancer		
Prostate cancer	<p>Reduction of ROS;</p> <p>The number of carcinomas in the ventral and lateral prostates were significantly reduced;</p> <p>Inhibition of the production of reactive oxygen species in the human prostate cancer cell line (LNCaP);</p> <p>Blockage of cell growth by inducing G0 / G1 stop with negative regulation of clusterin and cyclin D1.</p>	(Suzuki <i>et al.</i> , 2013)
Intestinal cancer	<p>Reduction of loci of colorectal aberrant crypts in KK-A (and) obese mice;</p> <p>Reduction in the number of intestinal polyps in Min mice;</p> <p>Decreased induced levels of nitric oxide synthase mRNA in polyp tissues;</p> <p>Suppression of nuclear-κB factor transcriptional activity <i>in vitro</i>.</p>	(Komiya <i>et al.</i> , 2015)
Skin cancer	<p>Apocynin significantly reduced expression of major carcinogenic factors in skin cells;</p> <p>Suppression of UVB-induced COX-2 promoter activity;</p> <p>Inhibition of UVB-induced phosphorylation of p38, ERK, JNK and Akt in a dose-dependent manner;</p> <p>Attenuation of signaling pathways involved in skin inflammation and carcinogenesis.</p>	(Byun <i>et al.</i> , 2016)
<i>Helicobacter pylori</i> stomach-Induced cancer	<p>Reduction of <i>H. pylori</i>-activated gastritis;</p> <p>Reduced neutrophil infiltration in the colon mucosal region;</p> <p>Decreased <i>H. pylori</i>-induced well abscess formation;</p> <p>No indication of drug toxicity.</p>	(Horemans <i>et al.</i> , 2017)
Metastasis	<p>Regulation of the invasion process by inhibiting phosphorylation of FAK and Akt and consequent suppression of metastasis in a model of hepatocellular carcinoma by apocynin dimer.</p>	(Jantaree <i>et al.</i> , 2017)
Consequential effects of <i>Diabetes mellitus</i>		
Atrial fibrillation in <i>Diabetes mellitus</i>	<p>A marked reduction in the inducibility of atrial fibrillation (AF);</p> <p>Atrial structural remodeling attenuation in diabetic rabbits;</p> <p>Reduction of DM-induced protein expression of TGF-β, NF-κB, P-p38, P-JNK, ERK, and P-ERK.</p>	(Qiu <i>et al.</i> , 2016)
Vascular and myocardial dysfunction in the <i>Diabetes mellitus</i>	<p>Reduction of serum NO, MPO and MDA levels;</p> <p>Reduced protein expression levels of rac1 and nuclear factor-κB;</p> <p>Transforming growth factor-β, p38, P-p38, and metalloproteinase-9;</p> <p>Increased levels of SOD.</p>	(Qiu <i>et al.</i> , 2017)
Cardiac remodeling in <i>Diabetes mellitus</i>	<p>The increased diastolic function of the left ventricle;</p> <p>Restoration of serum antioxidant enzyme activity: glutathione, peroxidase, SOD, and catalase;</p> <p>Improvement of ventricular diastolic function and myocardial contractile function.</p>	(Gimenes <i>et al.</i> , 2018)

Neuropathy in <i>Diabetes mellitus</i>	<p>Relief of diabetic neuropathic pain;</p> <p>Increased conductance of sciatic nerve and blood flow in diabetic rats;</p> <p>Decreased expression of Nox-p47phox;</p> <p>Reduction of nNOS and iNOS immunoreactivity;</p> <p>Increased immunoreactivity S-100 (Schwann cell marker);</p> <p>Increased expression of Nox and catalase in the spinal cord.</p>	(Olukman <i>et al.</i> , 2018)
Nephropathy in <i>Diabetes mellitus</i>	<p>Reduction of renal damage and improvement of renal function;</p> <p>Negative regulation of NLRP3 expression in the renal cortex;</p> <p>Negative regulation of XIAP expression in the renal cortex;</p> <p>Attenuation of renal fibrosis.</p>	(Xin <i>et al.</i> , 2018)
Vascular diseases		
Hypertension	<p>Significant reduction of mean arterial pressure in spontaneously hypertensive rats (SHR);</p> <p>Improvement of the hypotensive effect of impaired acetylcholine (ACh) in SHR;</p> <p>Decreased levels of oxidative stress;</p> <p>Normalization of the overexpression of Nox2 and its p47phox subunit in SHR aortas.</p>	(Perassa <i>et al.</i> , 2016)
Hypotensive effect	<p>Promoting hypotensive effects without altering heart rate;</p> <p>Induction of independent and endothelium-dependent relaxation;</p> <p>Decreased levels of ROS in endothelial cells.</p>	(Potje <i>et al.</i> , 2017)
Hypertension	<p>Reduction of blood pressure;</p> <p>Prevention of the development of endothelial dysfunction;</p> <p>Reduction of Ang II pressure effects;</p> <p>Increased plasma antioxidant capacity;</p> <p>Decreased production of Nox-dependent aortic and mesenteric oxidants;</p> <p>Overexpression of Nox2 and p47phox;</p> <p>Increased levels of plasma and aortic nitrate / nitrate;</p> <p>Increased endothelial modulation of Ang II vasoconstriction in arteries of resistance to SHR.</p>	(Graton <i>et al.</i> , 2019)
Neurological diseases		
Alzheimer's disease	<p>Improvement in cerebrovascular behavior and function;</p> <p>Reduction of oxidative stress;</p> <p>Reduction of carbonyl levels in the cerebral cortex.</p>	(Dumont <i>et al.</i> , 2011)
Rescue of reduced neurogenesis after seizures	<p>Reduction of oxidative injury induced by seizures;</p> <p>Reduction of neuronal death;</p> <p>Increased hippocampal neuronal survival newly generated by reduced superoxide production following seizures;</p> <p>Increased neuroblast production following seizures.</p>	(Lee <i>et al.</i> , 2018)
Parkinson's disease	<p>Significant improvement of learning and memory deficits;</p>	(Hou <i>et al.</i> , 2019)

	Improvement in hippocampal neurodegeneration and alpha-synuclein pathology in the hippocampus; Reduction of microglial activation; Inhibition of activation of signal transducers and activators of transcription pathways 1 (STAT1) and nuclear factor kappa B (NF-κB), two key regulatory factors for microglial M1 inflammatory response.	
Cardiac diseases		
Cardiac remodeling induced by pressure overload	Inhibition of collagen deposition; Level reduction of metalloproteinase (MMP-2); Reduction of NADPH oxidase activity; Reduction of the apoptotic index of cardiomyocytes; Reduction of ROS production.	(Liu <i>et al.</i> , 2017)
Cardiac hypertrophy	Blockade of the hypertrophic responses evidenced by the HW / BW ratio, HW / TL ratio, echocardiogram, and histopathology; Reduction of transcription levels of p22-phox; Restoration of glutathione level; Prevention of increased mRNA levels of various markers of hypertrophy, including ANP, BNP, β-MHC, and ACTA-1; Reduction of oxidative stress in the heart.	(Saleem <i>et al.</i> , 2018)
Other diseases of inflammatory character		
Bronchial asthma	Inhibition of the expression of transforming growth factor beta (TGF-β) and arginase I induced by rhinovirus; Significant increase in mRNA expression of tissue inhibitors of TIMP-1 metalloproteinases.	(Wieczfinska and Pawliczak, 2017)
Potential for osteogenesis	Reversal of the aging process in mesenchymal stem cells; Increased bone mineral density and total bone volume; Reduced expression of p53; Partial inversion of the aging process and the increase of the osteogenic potential.	(Sun <i>et al.</i> , 2015)
Proliferative vitreoretinopathy (PVR)	Significant decrease in the formation of PVR in histopathological and ophthalmoscopic examinations.	(Ozer <i>et al.</i> , 2016)
Colitis	Protection against inflammatory bowel disease; Reduction of ROS production levels and TNF-α, IL-6 and IL-1β flags; Inhibition of expression of nitric oxide synthase (iNOS) and cyclooxygenase (COX-2); Decreased NO production and prostaglandins (PGE2).	(Marín <i>et al.</i> , 2017)
Nephrotoxicity	Improvement in tissue morphology; A significant decrease in 24-h urine volume, renal somatic index (RSI), urine protein, lactate dehydrogenase (LDH), creatinine (Cr), blood urea nitrogen (BUN), nitric oxide (NO) and malondialdehyde (MDA). Significant elevation in SOD and renal CCr activity;	(Abdelrahman, 2017)
Liver dysfunction	Decreased activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase;	(Rahman <i>et al.</i> , 2017)

	<p>Considerable reduction of oxidative stress markers MDA, MPO, NO, and apoptotic levels;</p> <p>Restoration of antioxidant enzymes catalase and SOD;</p> <p>Prevention of infiltration of inflammatory cells and fibrosis;</p>	
Osteoclastogenesis	<p>Decrease in the number of cells positive for tartrate resistant acid phosphatase (TRAP) and the area of osteoclasts;</p> <p>Inhibition of osteoclastogenesis by RANK-RANKL-related signaling pathways;</p> <p>Decrease in osteoclast markers;</p> <p>Reduces intracellular calcium concentration.</p>	(Ribeiro <i>et al.</i> , 2019)