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The Effects of Baicalein and Baicalin on Mitochondrial Function and Dynamics: a Review

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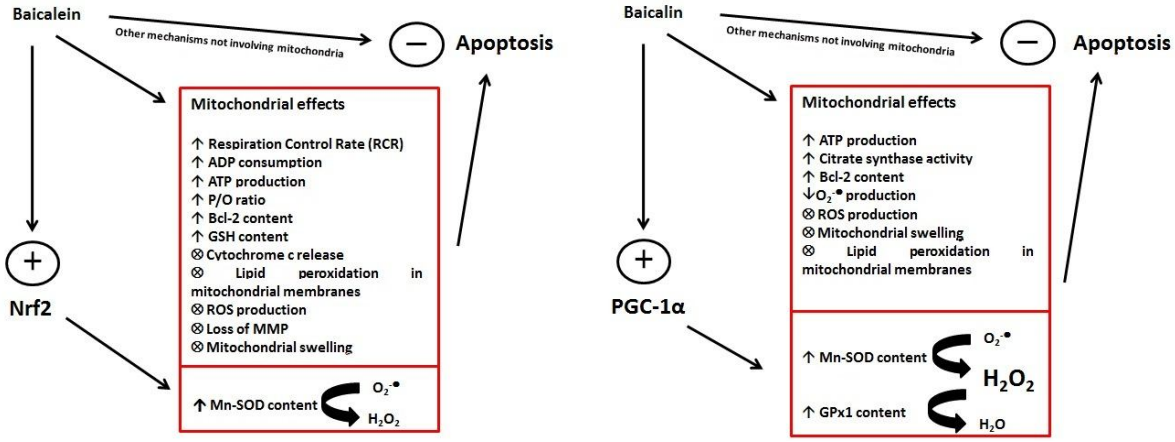
Graphical abstract



Abstract

Mitochondria play an essential role in cell survival by providing energy, calcium buffering, and regulating apoptosis. A growing body of evidence shows that mitochondrial dysfunction and its consequences, including impairment of the mitochondrial respiratory chain, excessive generation of reactive oxygen species, and excitotoxicity, play a pivotal role in the pathogenesis of different diseases such as neurodegenerative diseases, neuropsychiatric disorders, and cancer. The therapeutical role of flavonoids on these diseases is gaining increasing acceptance. Numerous studies on experimental models have revealed the favorable role of flavonoids on mitochondrial function and structure. This review highlights the promising role of baicalin and its aglycone form, baicalein, on mitochondrial function and structure with a focus on its therapeutic effects. We also discuss their chemistry, sources and bioavailability.

Keywords: Antioxidant, Baicalin, Baicalein, Flavonoid, Mitochondria.

1. Introduction

Baicalein (5, 6, 7-trihydroxyflavone) and baicalin (syn. baicalein 7-*O*-β-D-glucuronic acid) are the principal components found among 30 other flavonoid derivatives in the roots of *Scutellaria baicalensis* Georgi (*Scutellariae radix*) (skullcap), known as huangqin in Chinese

traditional medicine [1]. Baicalin and its aglycon Baicalein have been attracting growing interest from pharmaceutical, cosmetic, and food industries due to their excellent biological action. In particular, these two flavonoids have shown anti-inflammatory effects and improvement of mitochondrial dysfunction [2], while a combination strategy with baicalin or baicalein as chemotherapeutic adjuvants has been revealed to lead to favourable anticancer activity targeting assorted cancer lines and relevant signalling pathways [3].

Mitochondria, the cytoplasmic double-membraned organelles which take a fundamental role in cell physiology, produce energy through formation of adenosine triphosphate (ATP) by oxidative phosphorylation. This leads to the t .ransfer of electrons via the electron transport chain (ETC), consisting of approximately 80 different polypeptides structured into five trans-membrane protein complexes (I-V). In addition, mitochondria are involved in the apoptotic process and the production of reactive oxygen species (ROS) [4]. Therefore, adequate mitochondrial function is vital to many processes including energy homeostasis, apoptosis, and metabolic signalling pathways as well as cytosolic calcium homeostasis and lipid biosynthesis in cells [5,6]. Furthermore, the most recent research has disclosed that the role of mitochondria is much greater in cellular events and disease pathology than was previously known. The body of evidence suggests that mitochondrial dysfunction is associated with a large number of diseases, such as age-related neurodegenerative disorders, *e.g.* Parkinson's disease and Alzheimer's disease [7]; cancer [8], arrhythmia and cardiomyopathy [9]; inflammation-related diseases such as sepsis [10]; gastrointestinal disorders *e.g.* autism spectrum disorder (ASD) [11], obesity and diabetes, in which liver steatosis and insulin resistance is developed by mitochondrial damage [12,13]; Although the mechanisms underlying mitochondrial disorders are not entirely understood, there is a need for new treatment agents.

In this regard, natural compounds have always been an attractive target for the discovery of new drug candidates, and a number of flavonoid derivatives have been demonstrated to be effective in preventing mitochondrial damage. For instance; myricitrin, a flavonoid isolated from *Myrica cerifera*, demonstrated a protective effect on MPP(+)-induced mitochondrial dysfunction in SN4741 cells [14], while quercetin, luteolin, and epigallocatechin gallate were shown to prevent cellular apoptosis by restoring the mitochondrial membrane potential (MMP) as well as inhibiting caspase-3 activity [15]. Similarly proanthocyanidins, as polyphenolic bioflavonoid derivatives, were found to improve hydrogen peroxide (H_2O_2) induced mitochondrial dysfunction by means of endorsing the MMP and respiratory chain complex IV, and decreasing free radical generation by mitochondria [16]. Application of hesperidin, a main flavanone derivative in *Citrus* species, led to an increase in mitochondrial complex I-IV enzymatic activity [17]. Taking this information on flavonoids into account, the focus of this paper is to review baicalin and baicalein as bioactive flavonoid derivatives, referring to their chemistry, herbal sources, bioavailability, and effects on mitochondrial dysfunction. Within this frame, a general introduction to mitochondria and its functions will be also covered.

2. The Chemistry of Baicalein and Baicalin

Flavonoids are one of the most extensively studied groups of polyphenolic natural products [18, 19]. The first of their two major structural units is biosynthetically derived from the acetate pathway, giving rise to the phenolic ring moiety (ring-A) of their 15 carbon skeleton structure (**Fig 1**) [20]. The remaining 9-carbon skeleton is derived from the shikimic acid pathway, yielding an aromatic ring (ring-B) and a 3-carbon chain as in the common cinnamic or caffeic acid derivatives. Upon joining these two structural units , different structural possibilities emerge: in the first instance the three carbon linking chain may cyclise

to form the third ring (ring-C) (as found in flavones, flavonols, and flavanones) or remain in acyclic form to give rise to chalcones. A double bond may be introduced in the C2-C3 position of ring-C to form sub groups such as flavones and flavonols, the latter defined by a hydroxyl group at the olefinic C-3 position of ring-C which is absent in flavones.In the absence of the C2-C3 double bond other groups like flavans and flavanons emerge to represent other flavonoid sub groups.

The simplest flavone compound is represented by chrysin (**Fig 1**), where the basic *meta*-substituted hydroxyl groups on the A-ring are retained. Remarkably and unlike many flavonoids, chrysin has no hydroxyl substitution on ring-B. Certain biological activities have been attributed to this unique structural feature, such as the protecting non-cancerous cell from tumor necrosis factor-α [21]. If a hydroxyl group is added to the C-6 position of chrysin, baicalein (5,6,7-trihydroxyflavone, **Fig. 1**) is formed as a trihydroxy derivative. The biosynthetic conversion of chrysin to baicalein has been mimicked in the laboratory through a multiple-step synthetic approach [22]. Flavonoids also diversify through glycosylation, mostly through *O*-linkage as represented by baicalin which carries a glucuronic acid moiety at the C-7 hydroxyl position of baicalein (**Fig. 1**). The most prominent structural feature of baicalein and baicain is the presence of a *di-ortho* hydroxyl functional group on ring-A. This feature of polyphenolic compounds is the marker for efficient metal chelation and free radical scavenging properties [23, 24] as well as enzyme inhibition [21, 25, 26]. The reported antioxidant properties of baicalein and its chelation of divalent metal ions are attributed to these structural features [27].

3. Sources of Baicalein and Baicalin

To date, by far the most thoroughly studied source of baicalein is the root of the well-known Chinese medicinal plant (Chinese skullcap, Huang Qin in Chinese), *Scutellaria*

baicalensis Georgi (Lamiaceae). While baicalein was found to be the major active principle of this traditional medicinal plant, baicalin was another active component. Baicalein, with its derivatives, exists as a principal constituent of another oriental medicinal plant, *S. radax* [28]. Since baicalein is also the main component of the American skullcap, *S. lateriflora*, [29] it is believed to be present in the various species of the genus *Scutellaria*. Baicalein, along with chysin and glucoside derivatives, has also been isolated from the seeds and various other parts of a popular Asian medicinal plant, *Oroxylum indicum* [30, 31]. Due to the popularity of baicalein and baicalin as potential therapeutic agents, a number of studies in recent years have focused on the development of suitable methods for the detection and quantification of these compounds in crude drug preparations. The most widely used methods range from simple thin layer chromatography [32, 33] to the various application modes of high performance liquid chromatography [34-37].

4. Bioavailability of Baicalein and Baicalin

One major hindrance in the clinical application of baicalein and baicalin is their low aqueous solubility and poor oral bioavailability. It has been reported that once baicalein is absorbed, it is quickly metabolized to give rise to baicalin and baicalein 6-*O*-sulfate in the blood [38, 39]. Given the 5-OH position of flavones in a chelated form with the closely located C-4 ketone functional group, the observed preference of baicalein metabolic products for the C-6 and C-7 positions is somehow expected. Numerous studies have also shown that baicalein undergoes extensive glucuronidation within the intestinal wall and liver following oral administration in both rats and humans [40-42]. Taiming and Xuehua [43] further note the variation in baicalein and baicalin absorption sites within the gastrointestinal tract of rats. While baicalin was found to be moderately absorbed in the stomach and poorly in the small intestine and colon, baicalein was well absorbed in stomach and small intestine and relatively

less in colon. Their study also indicated that bile could excrete baicalin while significantly promoting the absorption of baicalein. Even after intravenous administration of baicalein in rats, 75.7% of the dose was found to be circulating as its conjugated metabolites [44]. In this study, the absolute absorption of baicalein following an oral route was calculated to be 40% while the relative absorption for baicalin was 65% in comparison. Overall, this and other studies [45] clearly demonstrated that baicalin can be detected in the blood of animals immediately after administration of baicalein orally or intravenously. About 90% of baicalein administered to animals is now known to be metabolized to baicalin [46].

Tian *et al.* [47] have attempted to study the pharmacokinetic profiles of baicalein in monkeys by administering various does via oral and intravenous routes. The study revealed that the absolute bioavailability of baicalein ranges from 13.1% to 23.0% across different doses.

More recently, Li *et al.* [48] studied the pharmacokinetic properties, the safety and tolerability of baicalein and bacalin, after single-dose administration (doses of baicalein ranging from 100 to 2800 mg) in 72 healthy Chinese subjects included in a Phase I, randomized, double-blind trial. The results showed that pharmacokinetic profile was characterized by a median Tmax of 0.75-3.5 h and 0.5-3 h, respectively, followed by a multiphasic profile with a $t_1/_2$ of 1.90-15.01 h and 4.22-10.80 h, respectively. The total urinary clearance of baicalein and baicalin was <1%. Approximately 27% of baicalein was eliminated as unchanged drug in feces. Moreover, baicalein resulted to be safe and well tolerated. In fact, only 11 mild treatment-related adverse events were observed, which were resolved without further treatment and no serious adverse events occurred. In addition to these data, no signs of toxicity in the liver or kidney were registered. The authors concluded that the favorable safety profile and pharmacokinetic properties warrant further clinical studies for baicalein. These conclusions agree with those earlier obtained by Kim *et al.* that studied the *in vitro* antiallergic properties and the *in vivo* dermal application skin toxicity of

the aqueous extract of *S. baicalensis*, using β-hexosaminidase assay in rat basophilic leukemia cells (RBL-2H3), and BALB/c mice, New Zealand white rabbits (to perform the acute dermal irritation/corrosion test), and Hartley guinea pigs (to estimate the safety S. baicalensis for topical application), respectively. β-Hexosaminidase release in the cell model system was markedly decreased following the treatment. It also ameliorated antigen-induced ear swelling compared with the control group in BALB/c mice. In the toxicological studies, *S. baicalensis* extract did not induce any dermal irritation/corrosion in rabbits or skin sensitization in guinea pigs [49].

All these data, taken together, showed a safety profile of *S. baicalensis* extract and its components and warrant further investigations to improve dissolution and oral bioavailability of bacailein as the main bioactive component of *S. baicalensis* extract.

To effectively deliver baicalein, which is characterized by poor aqueous solubility, several solubility enhancement techniques have been developed as spray freeze drying and solvent evaporation method for preparing solid dispersions of baicalein with Pluronic F68 [50]. Specific brain targeting by intravenous injection was successful via incorporation of bacalein into tocol nanostructured lipid carriers [51]. Other approaches to enhance the bioavailability of both baicalein and baicalin have been attempted through formulation, including the use of nanocrystals [52,53]; self-microemulsifying drug delivery systems [54]; hydroxypropyl-β-cyclodextrin [55]; nanostructured lipid carriers [56]; the combined use of phospholipid complexes and self-emulsifying microemulsions [15]; and solid lipid nanoparticles [57].

5. Mitochondria: a Brief Overview

Mitochondria are the main production site for ATP in animal cells. This is due to the work of the electron transfer chain (ETC) and complex V (ATP synthase) enzyme activity in the inner mitochondrial membrane, which, through the process of oxidative phosphorylation,

produce an electrochemical gradient utilized in the synthesis of ATP from adenosine diphosphate (ADP) and inorganic phosphate (Pi). ETC deals with electrons from NADH and $FADH_2$, released in the tricarboxylic acid cycle (the so-called Krebs cycle). By accepting electrons each complex of the mitochondrial ETC (with exception of complex II) pumps protons from the matrix side to the mitochondrial intermembrane space where they accumulate, consequently decreasing pH and increasing the difference in charge between the matrix side and the intermembrane space (increasing the membrane potential, $\Delta\psi$). The protons mostly return to the matrix side through complex V, which utilizes the force generated by the proton flux to produce ATP from ADP and Pi [58-60]. Ubiquinone (coenzyme Q10) and cytochrome *c* (cyt *c*) are electron carriers from complexes I and II to complex III and from complex III to complex IV respectively [61, 62]. Ubiquinone is responsible for the transfer of two electrons from complexes I or II to complex III, and cyt *c* transfer one electron at a time to complex IV. Ubiquinone is free in the inner mitochondrial membrane and readily soluble in such apolar environments [61]. Cardiolipin is the phospholipid that binds cyt *c* to the inner mitochondrial membrane and is responsible for the movement of this protein between the complexes [63, 64]. Cardiolipin is necessary because cyt *c* is not soluble in that apolar membrane. In complex IV, oxygen (O_2) is converted to water (H_2O) by accepting an electron and proton from cardiolipin-associated cyt *c* [64]. The presence of O_2 is necessary to fulfill energetic demands for certain mammalian cells, for instance, neurons, muscular fibers, glandular cells, and hepatocytes. O_2 is responsible for the consumption of Krebs cycle products, and of those electrons carried from glycosis to the mitochondria by the electron shuttles (namely, malate-aspartate shuttle and glycerol-3-phosphate) [59-60]. Without O_2 (as observed during anoxia and at moderated levels during hypoxia), those NADH and $FADH_2$ molecules originating in the Krebs cycle and glycolysis

electrons would accumulate, decreasing the rate of Krebs cycle and, consequently, the production of ATP [60, 65, 66].

Even though the ETC and complex V are necessary to produce ATP, the ETC is the major site of reactive oxygen species (ROS) production, such as, the superoxide anion radical ($O_2^{-\bullet}$), which may give rise to H_2O_2 after dismutation by Mn-superoxide dismutase (the mitochondrial isoform) [67-71]. H_2O_2 is able to react with free iron (Fe^{2+}) or copper (Cu^{+}) ions (Fenton chemistry reaction) generating the unstable and reactive free radical, $^{\bullet}OH$ [72]. ETC produces ROS constantly and, together with other systems, such as the microsomal cytochrome P450 enzymes (CYP450), produces a considerable amount of $O_2^{-\bullet}$ which should be converted to H_2O_2 to avoid excessive damage to such biomolecules as proteins (some enzymes are very sensitive to $O_2^{-\bullet}$, including catalase (CAT), aconitase and α-ketoglutarate dehydrogenase from the Krebs cycle), lipids (mainly membrane phospholipids), DNA and RNA [72]. H_2O_2 is converted to H_2O by CAT or glutathione peroxidase (GSH) enzymes; however, it is capable of crossing biomembranes due to its solubility in lipids, spreading the pro-oxidant signal originating in mitochondria to other cellular compartments, and there giving rise to $^{\bullet}OH$ through a Fenton chemistry reaction [72, 73]. Actually, H_2O_2 is considered to be, among other ROS, a messenger that participates in the regulation of signaling pathways necessary for the maintenance of cellular homeostasis, including cell metabolism and proliferation [74-77].

In addition to the enzymatic defenses mammalian cells possess non-enzymatic antioxidant defenses, for example reduced glutathione (GSH), vitamins, and bioactive molecules that may protect cells from reactive species and free radicals [72]. However, the amount of such defenses (both enzymatic and non-enzymatic) may vary according to several factors, including sex, age, diet, and exposure to pollutant chemicals [78, 79]. For example, GSH levels are significantly decreased by fasting [80].

Mitochondria also play a pivotal role in cell death regulation by releasing cyt *c* into the cytosol when exposed to certain deleterious conditions, for example, higher concentrations of pro-oxidant molecules, increased intracellular Ca^{2+} ions and free fatty acid concentrations [81, 82]. Likewise, mitochondria controlled cell death (the so-called intrinsic apoptotic pathway) is necessary during development and maintenance of tissue homeostasis throughout life [82]. The normal shape of the animal body is a consequence of the activation of physiological apoptosis in addition to other molecular aspects. Reduced mitochondrial cyt *c* present in the cytosol reacts with APAF-1 (Apoptotic Protease Activating Factor - 1), ATP (or dATP), and pro-caspase-9, which is autoactivated. The assembly of APAF-1, ATP/dATP, cyt *c*, and pro-caspase-9 constitutes the apoptosome, a multimeric complex able to activate procaspases and to regulate the onset of apoptosis [82-84]. Activated caspase-9 (initiator caspase) cleaves and activates caspase-3, which is known as the effector caspase due to be responsible for the breakage of specific cellular targets, such as cytoskeleton proteins and enzymes (PARP – Poly ADP Ribose Polymerase, for example) [82]. This is a *programmed cell death;* it is very controlled and does not lead to inflammation because there is no release of cytosol content to the extracellular environment [85]. The release of cyt *c* to the cytosol occurs by a specific stimulus dependent on a channel that is formed in mitochondrial membranes, the so-called mitochondrial permeability transition pore (mPTP) or mitochondrial outer membrane permeabilization (MOMP). This channel consists of the C subunit of the mitochondrial ATP synthase (in the inner mitochondrial membrane), the voltage-dependent anion channel-1 (VDAC1, outer membrane channel), the adenine nucleotide translocase-1 (ANT-1, inner membrane channel), cyclophilin D (Cyp D, in the mitochondrial matrix), among other molecules [82, 86]. Cyt *c* is released together with other proapoptotic factors, such as Smac/Diablo, endonuclease G, apoptosis inducing factor (AIF), and serine protease OMI/HtrA2 [86].

The role of mPTP has been demonstrated as being important during regulated necrosis, and the control of apoptosis depends on MOMP [86]. MOMP is regulated by both anti- and proapoptotic factors of the Bcl-2 (B-cell lymphoma 2) protein family. The proteins of this family can be classified according to the number and structure of their Bcl-2-homology domains (namely BH1-4). The BH3-only proapoptotic protein activation depends on a panoply of cell stress conditions. Once activated, they induce the oligomerization of proapoptotic proteins, *e.g.* Bax and Bak, and their insertion into the mitochondrial outer membrane, thereby triggering MOMP. On the other hand, multi-BH domain proteins, for instance Bcl-2, Bcl-XL, and Mcl-1, inhibit MOMP by binding to proapoptotic proteins [87].Extracellular signals may induce cell death through the extrinsic apoptotic pathway [88]. This is observed in the case of inflammation, in which the extracellular levels of chemicals such as the tumor necrosis factor-α (TNF-α), Fas ligand (FasL), and TNF-related apoptosis-inducing ligand (TRAIL) bind to membrane receptors and trigger cell death by activation of caspase-8 [89]. Such pro-inflammatory molecules belong to the TNF family, which bind to death receptors on the cell surface and activate them, leading to cell death through a mitochondria independent pathway [90].

When the mitochondrial damage is excessive (it may be observed by quantifying the amount of cyt *c* released to the cytosol, as well as through quantification of ATP and mitochondrial enzyme activities), the cells cannot sustain the apoptotic pathway due to lacking the ATP required to maintain apoptosome formation and activity [82,84,86]. Then, the cells die through necrosis, with the consequence of releasing cell components into the extracellular space and triggering inflammation [72]. Also, depending on the redox parameters of the environment, cyt *c* may be oxidized and thus unable to participate in the formation of apoptosome. Thus apoptosis fails and cells die by necrosis [91, 92].

Overall, the maintenance of the cellular bioenergetic state is of crucial importance and a challenge to be administrated — not only to maintain cellular functions, but also to allow cell death to occur without generalized damage to other tissue components.

6. The Effects of Baicalein and Baicalin on Mitochondrial Function and Dynamics

The exact mechanism by which baicalein exerts beneficial effects on human health is not completely understood yet, but as illustrated above it plays an apparent role in mitochondrial protection.

In an *in vitro* experimental model, baicalein (6.25 and 12.5µM) protected mitochondria from SH-SY5Y neuroblastoma cells against 6-hydroxydopamine (6-OHDA)-induced toxicity and also prevented loss of cell viability [93]. Additionally, pretreatment with baicalein (2h before 6-OHDA treatment) alleviated the increase in reactive oxygen species (ROS) production and alterations in cellular morphology. Interestingly, baicalein was effective in blocking the effect of 6-OHDA on the mitochondrial membrane potential (MMP; $\Delta\Psi m$). The authors suggested that baicalein protected mitochondria from loss of potential via a redox-dependent mechanism, since pretreatment with 1 mM N-acetylcysteine (NAC) induced a very similar effect. Zhang *et al.* [94] also demonstrated that pre-incubation of PC12 cells with baicalein at 5-40 µM for 12 h protected mitochondria from loss of MMP induced by H_2O_2, as well as caused an increased in the contents of Bcl-2 and a decrease in the levels of Bax. Furthermore, baicalein (10-40 µM) pretreatment (1 h) prevented the loss of MMP in immortalized human epidermal melanocyte cells (PIG1) exposed to H_2O_2 [95]. In another work, co-treatment with baicalein (10-40 µM) avoided loss of MMP induced by rotenone in PC12 cells [94]. Loss of MMP has been linked to caspase activation and triggering of cell death, as previously reviewed [82]. Indeed, baicalein blocked caspase activation in the 6-OHDA-treated SH-SY5Y cells and effectively protected cells from apoptosis [93]. Liu *et al.*

[95] reported that baicalein (10-40 µM) prevented caspase activation and death of PIG1 cells induced by H_2O_2 by exerting mitochondrial protection through a mechanism that depends, at least in part, on Bcl-2 protein and the inhibition of cytochrome c release to the cytosol. Hence, baicalein avoided mitochondrial damage consequently inhibiting cell death via the intrinsic apoptotic pathway.

In another study, baicalein (10 µg/mL) induced an increase in the immunocontent of Mn-superoxide dismutase (Mn-SOD) enzyme by a mechanism dependent on nuclear factor erythroid 2 [NF-E2]-related factor 2 (Nrf2) activation in Chinese hamster lung fibroblast (V79-4) cells exposed to H_2O_2 [95]. Additionally, baicalein increased Bcl-2 expression and caused a decrease in the levels of phosphorylated Bcl-2 and Bax. The phosphorylated form of Bcl-2 is in an inactive state and fails to inhibit apoptosis. On the contrary, Bax is activated under phosphorylation, and migrates to mitochondria to induce cytochrome c release from the organelle [82-88]. Mn-SOD is responsible for the conversion of $O_2^{-\bullet}$ to H_2O_2 in the mitochondrial matrix [98, 99]. The expression of Mn-SOD is regulated mainly by Nrf2 and the nuclear factor-κB (NF-κB) in situations of oxidative stress and/or inflammation [100-102]. Down-regulation of Mn-SOD is associated with cardiac disease, neonatal lethality, and neurodegeneration [103, 104]. On the other hand, up-regulation of Mn-SOD has been linked to cellular protection against several chemical challenges [105, 106]. Thus, Mn-SOD up-regulation through bioactive compounds would be an interesting strategy to prevent damage resulting from chemical stress on mitochondria. Indeed, authors described decreased ROS production levels in mitochondria from baicalein-treated V79-4 cells exposed to H_2O_2 [97].

Bioenergetic parameters associated to mitochondrial function were analyzed by He *et al*. [107]. The authors investigated the effect of subacute baicalein treatment (30 or 100 mg/kg for 27 days) on mitochondrial dysfunction induced by chronic cerebral hypoperfusion (CCR). Baicalein increased the respiration control ratio (RCR), the consumption of ADP, and

the production of ATP. However, baicalein did not alter O_2 consumption. Furthermore, baicalein improved MMP and decreased mitochondrial ROS production. Baicalein was also effective in avoiding morphological changes induced by CCR, as assessed through the analyses of the degree of mitochondrial swelling. On the other hand, baicalein only partially alleviated the effects induced by rotenone on mitochondria isolated from rat brain [96]. Baicalein was not effective in preventing the decrease in O_2 consumption induced by rotenone, and it did not improve the ATP produced/O_2 consumed ratio. However, the authors found that mitochondria incubated with baicalein (0.5 or 5 µM) for 30 min before rotenone exposure presented higher ATP levels than the control. Additionally, baicalein (5 µM) alleviated ROS production and blocked mitochondrial swelling induced by rotenone *in vitro*.

Taken together, this data demonstrates that baicalein may be an important protective agent regarding mitochondrial function. Nonetheless, the beneficial effects of baicalein depend on the nature of the toxic agent, as seen in the experimental model using rotenone as a mitochondrial stressor [96]. Rotenone is a specific complex I (NADH dehydrogenase) inhibitor and causes enhanced mitochondrial ROS production that leads cells to apoptosis [108]. The binding of rotenone to complex I is irreversible, and treatment with baicalein did not avoid the direct effects of this toxin on mitochondrial function (as for instance electron transfer, O_2 consumption, and ATP produced). Nevertheless, baicalein did act as an antioxidant by decreasing ROS detection, as assessed through DCFH-DA assay [96]. Therefore, baicalein may sustain cellular bioenergetics in the event of redox impairment by protecting mitochondrial systems involved in ATP production.

Baicalein was also tested in an experimental model of pulmonary carcinogenesis induced by benzo[a]pyrene [109]. The authors found that baicalein treatment at 12 mg/kg once a week for 16 weeks alleviated the effects of benzo[a]pyrene (BaP; 50 mg/kg twice a week for four weeks) on rat lung mitochondria with regards to ROS production,

morphological changes (swelling of the organelle), enzyme activities (isocitrate dehydrogenase - ICDH, α-ketoglutarate dehydrogenase – α-KDH, succinate dehydrogenase - SDH, malate dehydrogenase - MDH, NADH dehydrogenase, and cytochrome c oxidase), lipid peroxidation in mitochondrial membranes, and reduced glutathione (GSH) content. Additionally, the authors demonstrated that antioxidant enzymes in mitochondria were modulated by baicalein pre-treatment. Post-treatment with baicalein was only partially effective in protecting mitochondria from BaP-induced toxicity in that experimental model. This data demonstrates that baicalein protected mitochondrial function by maintaining bioenergetic homeostasis related to the Krebs cycle (CK, the so called tricarboxylic acid cycle – TCA) and mitochondrial electron transfer chain (METC) system. In the same work, baicalein blocked BaP-induced carcinogenesis by triggering apoptosis in lung cells through a mechanism dependent on mitochondrial integrity, since apoptosis is an ATP-dependent process. In this context, baicalein protected the lung against BaP-induced carcinogenesis by sustaining the apoptotic machinery associated with mitochondrial function and integrity.

The effects of baicalein alone (0.5-5.0 μM) on mitochondria isolated from rat brain were investigated by Li *et al*. [96]. The authors found that baicalein induced a decrease in the amount of O_2 consumed in state 4 (respiration occurring in the absence of ADP or inhibitory agents) without altering ATP production, consequently increasing RCR and the mitochondrial P/O ratio. This effect may be linked to decreased electron leakage from mitochondria, an event closely related to ROS production by the organelle [108]. Baicalein alone did not alter ATP levels, but decreased ROS production by isolated mitochondria. Additionally, baicalein alone did not change mitochondrial morphology according to analyses of the swelling of the organelles [96]. Taken together, these data demonstrate that baicalein has a potential role as an agent, which is able to amplify mitochondrial function, ensuring increased rates of ATP production in situations of stress.

Baicalin, another flavonoid isolated from *Scutellaria baicalensis* Georgi, presents antioxidant and anti-inflammatory properties. Baicalin (120 mg/kg for 30 days) decreased mitochondrial damage induced by streptozotocin in an animal model of diabetes, protecting mitochondria from morphological alterations associated with mitochondrial pathology (changes in the volume of the organelle, damaged membranes, and decreased number of cristae) induced by streptozotocin (STZ), as assessed through transmission electron microscope analyses [111]. Additionally, baicalin increased the number of mitochondria and citrate synthase enzyme activity in diabetic rats. Nonetheless, the mechanism by which baicalin induced mitochondrial biogenesis is not yet clear. The effect of baicalin was found to be stronger in the presence of metformin, indicating that this flavonoid may be utilized as a therapeutic agent in cases of diabetes.

In another study, pre-treatment with baicalin at 200 mg/kg (at 24h and 1h) protected the organelle from hepatic ischemia/reperfusion (I/R) in an experimental model in rats [112]. Baicalin was effective in preventing mitochondrial swelling induced by experimental I/R. Additionally, baicalin blocked caspase activation and avoided cell death in rat liver. Baicalin also decreased the inflammation that resulted from I/R by NF-κB activation. NF-κB has been implicated in both antioxidant and pro-oxidant events. However, the role of NF-κB in inflammation seems to be more associated with pro-oxidant effects closely related to production of pro-oxidant molecules, for instance nitric oxide ($NO^{\bullet}$) [113]. Therefore, baicalin exerted antioxidant and anti-inflammatory effects that either directly or indirectly prevented the impact of I/R on mitochondria. In fact, baicalin (1-100 mg/kg *i.p.* 30 min before induction of renal I/R) exerted anti-inflammatory effects in an animal model of renal I/R, by decreasing the expression of interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α), through a mechanism related to NF-κB down-regulation [114]. The authors also demonstrated that baicalin elicited an antioxidant effect by increasing SOD enzyme activity

418 and decreasing the levels of lipid peroxidation markers. Additionally, baicalin decreased
419 caspase-9 and caspase-3 activation and increased Bcl-2 expression. Therefore, baicalin was
420 capable of ameliorating the redox environment and reducing the inflammatory signs resulting
421 from renal I/R, inhibiting apoptosis of renal cells.

422 Baicalin also exerted beneficial effects on mitochondrial dynamics and function in an
423 experimental model of toxicity induced by antimycin A in rat L6 skeletal muscle cell line
424 [113]. Cells were treated with baicalin (50 µg/mL) for 1 h and then were exposed to
425 antimycin A (100 µg/mL) for 24 h. Baicalin improved cell viability, ATP production, and
426 MMP, whereas it led to a decrease in mitochondrial $O_2^{-\bullet}$ production in antimycin A-treated
427 cells. Furthermore, baicalin increased the immunocontent of peroxisome proliferator-
428 activated receptor gamma coactivator 1-alpha (PGC-1α) by 40%, which is a regulator of
429 mitochondrial biogenesis and is up-regulated in cases of pathological hypertrophy and heart
430 failure [116]. Moreover, PGC-1α increases the expression of superoxide dismutase 2 (SOD2;
431 Mn-SOD) and of glutathione peroxidase-1 (GPX1), enzymes responsible for removing $O_2^{-\bullet}$
432 and H_2O_2, respectively [117]. Overall, PGC-1α is a master regulator in mitochondrial
433 biogenesis and remodeling, as well as being involved in ROS detoxification, and baicalin acts
434 as a protective agent to mitochondria, at least in part, through up-regulation of PGC-1α and
435 its associated signaling pathway.

436 Yan and Liu [118] demonstrated that direct treatment of mitochondria isolated from
437 rat brain with baicalin (0.8-1.5 mM) decreased state 3 and RCR in an animal model of
438 hypoxia induced by hypobaric situations. On the other hand, baicalin did not exert any effect
439 on state 4 and on MMP. Thus, baicalein did not prevent the mitochondrial changes elicited by
440 hypoxia. The authors did not quantify ROS or RNS production to verify whether baicalin
441 would exert an antioxidant effect in a situation in which ROS and RNS may be generated at
442 higher rates. Such effects may be a result of exposing isolated mitochondria to the herbal

monomer baicalin. It is possible that different results would be obtained from treating the animals before induction of hypoxia. Interestingly, baicalin (1-25 µM) did not exert any protective effect against rotenone-induced loss of viability in RGC-5 cells (a cell line with some ganglion cell characteristics) [119].

7. The role of baicalin and baicalein in modulating cell signaling pathways and the impact on mitochondria

There are several reports demonstrating the effects of baicalein on different cell signaling pathways in a myriad of experimental models involving both *in vitro* and *in vivo* studies. However, some works did not demonstrate whether there is a causative link between signaling pathways modulation and mitochondrial physiology maintenance. Some research groups clearly described that by triggering a certain signaling pathway it would lead to mitochondrial alterations that will or will not culminate in cell survival. Therefore, we focused to discuss in this Section only reports in which the authors studied cell signaling pathways that are involved with mitochondrial function and/or quality and that is associated to cell fate.

Liu et al. showed that baicalein (20 mg/kg i.p., 30 min before and 2 and 4 h after onset of ischemia) did not alter infarct volume in permanent middle cerebral artery occlusion (MCAO) induced in rats, but was effective in protecting rat brain regions (total brain, cortex, and subcortex) against transient MCAO [120]. Additionally, baicalein did prevent caspase-3 activation in MCAO experimental model. In the same work, authors demonstrated that baicalein (0.035 – 3.5 µM, 2h before induction of oxygen and glucose deprivation, OGD – an experimental model utilized to mimic ischemia *in vitro*) preserved neuronal viability and blocked cytotoxicity induced by OGD. Baicalein at 35 µM was not effective in protecting

primary cultured cortical neurons against OGD. Baicalein (3.5 μM, 2h before induction of OGD) prevented the increased in ROS in cultured neuronal cells. However, a treatment with 10 μM LY294002 (an inhibitor of PI3K) 30 min before OGD partially suppressed the effects of baicalein on ROS production in that experimental model. Furthermore, baicalein pretreatment inhibited the increase in 3-nitrotyrosine content in neurons exposed to OGD. However, LY294002 treatment abolished the protective effects of baicalein regarding nitrosative stress. Authors found that baicalein did induce phosphorylation of Akt, GSK-3β, and PTEN (the phosphatase and tensin homolog deleted on chromosome 10). Akt becomes activated after phosphorylation, but PTEN is inactivated after being phosphorylated. PTEN, when activated, is a negative regulator of Akt pathway. Activated PTEN would lead to apoptosis by a mitochondria-related mechanism that accounts with release of cytochrome c from the organelle [121-123]. Then, baicalein protected cultured neuronal cells by activation of the PI3K/Akt pathway and inactivation of PTEN, resulting in increased phosphorylation of Bad at Ser136 (a pro-apoptotic protein) and, consequently, decreased release of cytochrome c from mitochondria, since dephosphorylated Bad plays a role in activating the MPTP during early apoptosis. Actually, baicalein pretreatment decreased OGD-induced cytochrome c release from mitochondria through a PI3K/Akt axis-dependent fashion. Thus, a protective effect of baicalein involved the activation of protein kinases that mediate cell survival through maintenance of mitochondrial function and quality. The mechanism by which baicalein reduced infarction volume *in vivo* was not addressed in that work.

In another study, Pallast et al. showed that baicalein (300 mg/kg, i.p. just before MCAO induction in mice) prevented the increase in the amount of apoptosis-inducing factor (AIF) in cell nucleus [124]. AIF translocation from mitochondria to nucleus is related to apoptosis induction through a caspase-independent cell death [125-127]. AIF translocation plays a role in triggering cell death through apoptosis in MCAO experimental models, as previously

492 demonstrated [126,128,129]. Furthermore, authors observed that the translocation of AIF to
493 the nucleus was somewhat associated to the increase in the expression of 12/15-LOX (12/15-
494 lipoxygenase) enzyme. Indeed, 12/15-LOX colocalizes with AIF in the nucleus of neuronal
495 cells after MCAO induction, as demonstrated by that research group. However, baicalein
496 treatment did inhibit 12/15-LOX and AIF expression *in vivo*. To better analyze the
497 mechanism by which baicalein exerted neuronal protection, authors performed *in vitro* assays
498 utilizing HT22 cells (a neuronal cell line derived from murine hippocampus). Authors found
499 that baicalein (10 μM) co-administration protected HT22 neuronal cells from glutamate-
500 induced toxicity by inhibiting the interaction of 12/15-LOX with membranes of mitochondria
501 and endoplasmic reticulum (ER). Additionally, baicalein treatment suppressed the effect of
502 glutamate in causing leakage of luminal ER proteins to the cytosol, indicating a role for
503 baicalein as an inhibitor of disturbances in the membrane of ER in this experimental model.
504 Baicalein also inhibited translocation of AIF from mitochondria to the nucleus in cultured
505 HT22 neuronal cells. The aggregation of 12/15-LOX near the cell nucleus (the so called
506 perinuclear region, which is rich in ER and mitochondria) seems to play a role in the leakage
507 of proteins commonly found in the lumen of ER, since blockade of 12/15-LOX aggregation
508 lead to decreased levels of ER luminal protein in the cytosol, as demonstrated in that
509 experimental model and by other researchers [130]. A clear role for 12/15-LOX in causing
510 mitochondrial damage was not addressed in that work, and the authors did state that more
511 investigations are needed to better comprehend the exact mechanism by which baicalein
512 counteracted the 12/15-LOX-mediated organelle damage in neurons. A previously published
513 work by van Leyed et al. also demonstrated that baicalein (300 mg/kg, i.p. 5 min before
514 induction of MCAO) protected mice from the deleterious effects elicited by ischemia by a
515 similar way when compared to animals in which *ALOX15* gene was knocked down,
516 demonstrating a role for 12/15-LOX in the mechanism of neuronal damage triggered by

MCAO [129]. Recently, Cui et al. found that baicalein (30 mg/kg, intravenous injection) co-treatment alleviated the effects of MCAO in rat brain (striatum and cortex) [132]. Baicalein decreased the contents of 12/15-LOX, p38 (phosphorylated form), and cytosolic phospholipase A2 (cPLA2, a pro-inflammatory enzyme that releases arachidonic acid from biomembranes increasing its bioavailability to the LOX pathway, as previously published by Farooqui and Horrocks [133]. However, a link with mitochondrial function was not analyzed in those works.

Baicalein modulated the ERK1/2 pathway in an *in vitro* experimental model of Parkinson's disease (PD) utilizing SH-SY5Y neuroblastoma cells. Song et al. reported that pretreatment with baicalein (25 – 100 μM for 1h) prevented ERK1/2 activation induced by rotenone [134]. Prolonged ERK1/2 activation may play a role in apoptosis induction, as described elsewhere [135-137]. Furthermore, baicalein reduced Bax levels in rotenone-treated cells. Consequently, baicalein blocked loss of MMP and suppressed caspase-3 activation, causing inhibition of apoptosis in SH-SY5Y cells exposed to rotenone. By blocking loss of MMP induced by rotenone, baicalein prevented the increase in mitochondrial permeability that would lead to cytochrome c release to cytosol, which will culminate in cell death through apoptosis. Zhang et al. demonstrated that baicalein (50 – 200 μM for 12h) pretreatment was able to increase cell viability of PC12 cells exposed to 6-OHDA [138]. Baicalein activated Nrf2 leading to increased expression of HO-1 and causing cytoprotection. In addition, authors described that baicalein increased the activity of antioxidant enzymes, as for instance catalase (CAT) and SOD in 6-OHDA-treated PC12 cells. Nonetheless, a role for baicalein as a mitochondrial protective agent was not demonstrated in that work.

Recently, Qi et al. described that baicalein (2 and 4 mg/kg, i.p. once a day during 7 weeks) was effective in protecting rat hippocampus in an experimental model of diabetes

(induced with STZ) [139]. STZ treatment did lead to increased acetylcholinesterase (AChE) activity and decreased choline acetylase (ChAT), but baicalein significantly alleviated the alterations in such enzymes in that experimental model. Exposure to STZ induced a decrease in the levels of phosphorylated PI3K and Akt protein kinases, as well as increased the contents of phosphorylated GSK-3β (glycogen synthase kinase-3β). Baicalein suppressed the effects of STZ on such protein kinases, causing a pro-survival signal that culminates with decreased activation of both caspases-9 and -3. Baicalein also ameliorated cognitive deficits elicited by STZ in that experimental design. Therefore, baicalein exerted a protective effect *in vivo* by activating protein kinases that trigger pro-survival effects by inhibiting apoptosis probably by a mitochondria-related pathway, since the mediators of the intrinsic apoptotic pathway were modulated by baicalein treatment.

8. Baicalin *versus* baicalein

Baicalein is an aglycone derivative from baicalin. Then, there are structural similarities between these two flavonoids. However, baicalin and baicalein may exert different effects on mammalian cells, as will be discussed here considering only the authors that analyzed baicalin and baicalein in the same manuscript.

Ikemoto et al. tested the ability in baicalin and baicalein in inducing antitumor effects on bladder cancer cell lines (KU-1, EJ-1, and MBT-2) and found that baicalin exerted a stronger antitumor activity when compared to baicalein, since the concentration of baicalin necessary to cause 50% inhibition of tumor growth was 3.4 µg/mL and the concentration of baicalein was 30 µg/mL [140]. Mitochondrial parameters were not assessed in that work, but the success of baicalin and baicalein in inducing tumor cell growth inhibition may be associated to induction of cell death, as demonstrated by others and discussed in the present work. Evidently, it is necessary to perform more analyses comparing the efficiency of these

565 flavonoids in inducing cancer cell growth inhibition and to examine the mechanism by which
566 baicalin and baicalein exerted antitumor activity. Indeed, Zhou et al. reported that baicalin or
567 baicalein treatment induced apoptosis in human breast cancer cells (MCF-7 and MDA-MB-
568 231 cell lines) by activating the ERK/p38 MAPK signaling pathway and triggering the
569 intrinsic apoptotic pathway associated to mitochondria PTP opening [141]. The combination
570 of the flavonoids (50 µM baicalin more 25 µM baicalin for 24h or 48h) exerted a stronger
571 effect in the induction of cell death in that experimental model. The combination of baicalin
572 and baicalein induced an increase in the expression of Bax, a pro-apoptotic protein that is
573 involved in the triggering of cytochrome c release from mitochondria [82]. The combination
574 of the two flavonoids also caused caspase-9 and caspase-3 activation, clearly demonstrating a
575 role for mitochondria in the process of ongoing apoptosis in that experimental model. The
576 levels of the anti-apoptotic protein Bcl-2, which inhibits MPTP and, consequently, the release
577 of cytochrome c, were decreased by the combination of baicalin and baicalein. Then, baicalin
578 and baicalein, when combined, induce a stronger antitumor effect on breast cancer cell lines
579 by activating the intrinsic apoptotic pathway through a mechanism that depends, at least in
580 part, on the activation of MAPK signaling pathway. A role for NF-κB (which is regulated by
581 MAPK, among other protein kinases) or another transcription factor associated to apoptosis
582 was not described in that work. The investigation regarding the involvement of transcription
583 factors in the apoptotic event would be very useful to better analyze the exact mechanism by
584 which the flavonoids baicalin and baicalein elicit antitumor activity.

585 Gao et al. found that baicalin and baicalein (10 µM for 10 min) were efficient in
586 protecting SH-SY5Y neuroblastoma cells against H_2O_2-induced toxicity. Baicalin and
587 baicalein similarly prevented the decreased in cell survival, in cell viability, and in membrane
588 integrity elicited by H_2O_2 [142]. Nonetheless, baicalein induced a stronger protective effect
589 regarding inhibition of lipid peroxidation, as assessed through measurement of

malondialdehyde (MDA) levels, when compared to baicalin-treated cells. Authors did not analyze mitochondrial parameters, but found that both baicalein and baicalin similarly prevented the increase in intracellular Ca^{2+} levels triggered by H_2O_2. The increase in intracellular Ca^{2+} levels may lead to augmented mitochondrial concentrations of this ion, which favors mitochondrial dysfunction (*i.e.* resulting in increased ROS production and oxidative and nitrosative damage to mitochondrial components) and cell death by activation of the MPTP, as previously reviewed by others [82]. Interestingly, Kyo et al. have previously demonstrated that baicalein reduced intracellular Ca^{2+} ions concentration with a greater potency than baicalin (> 10 µM to each flavonoid) [143]. The effects of the flavonoids on intracellular Ca^{2+} were due to the inhibition elicited by baicalein and baicalin on phospholipase C (PLC) activity. Then, by inhibiting PLC, baicalin and baicalein decreased the intracellular Ca^{2+} concentration in C6 glioma cells. Thus, this may be considered an indirect effect of baicalin and baicalein that may participate in mitochondrial protection against, for example, chemically induced oxidative stress *in vitro*. In the work by Gao et al., the antioxidant effects of baicalin and baicalein were attributed to its structure, since baicalin presents an o-di-hydroxyl and baicalein contains an o-tri-hydroxyl group [142]. Then, the antioxidant effects elicited in such experimental model may be a result from its chemical structure. Additionally, the incubation with such flavonoids were very short (10 min), leading to the conclusion that the effects seen in that work were not due to the activation of transcription factors and the resulting increase in the expression of antioxidant enzymes.

A comparison regarding the effects of baicalin and baicalein on mitochondria was made by Chang et al. [144]. Authors found that baicalin or baicalein alone decreased cell viability on hepatoma cell lines (Hep G2, Hep 3B, and SK-Hep1) and caused MMP disruption at a similar way. Baicalin (50 µM for 48h) induced a stronger effect on impairing MMP in Hep G2 cells when compared to baicalein at the same concentration. On the other

hand, baicalein (50 µM for 48h) was more effective in causing loss of MMP in Hep 3B cell line than baicalin in that experimental model. Surprisingly, baicalin or baicalein decreased cellular GSH content in hepatoma cell lines, indicating a pro-oxidant role for such flavonoids. Additionally, it may be a consequence of the active metabolism of xenobiotics by glutathione S-transferase (GST) enzymes. However, the authors did not analyze such parameters in that work. In this context, the differences among the effects elicited by baicalin and baicalein may be associated to the cell type. Moreover, it remains to be analyzed whether these two flavonoids would exert different effects regarding mitochondrial protection in future works.

Takahashi et al. reported that baicalin or baicalein treatment induced cell death in human pancreatic cells (BxPC-3, HPAF-II, MIAPaCa-2, and Panc-1 cell lines) [145]. However, the effect elicited by baicalein was stronger than that induced by baicalin at the same dose tested. Baicalein induced a dose-dependent (1 – 50 µM) effect inhibiting tumor cells growth and decreasing cell viability. Baicalein was especially stronger in inhibiting cell proliferation in BxPC-3 cell line, in which baicalein at 1 – 5 µM exerted an inhibitory effect on cell proliferation that was only partially achieved by baicalin at 50 µM. Authors did not compared these two flavonoids in another parameters, as for instance mitochondrial function, but demonstrated that baicalein exerted its antitumor activity by activating apoptosis through a mechanism that involved down-regulation of Mcl-1, an anti-apoptotic protein that may bind Bak (a pro-apoptotic protein), causing increased resistance to apoptosis, as demonstrated by the authors. Bak interacts with mitochondria causing opening of the MPTP and consequent release of cytochrome c to cytosol, triggering the intrinsic apoptotic pathway, as reviewed elsewhere [82]. Reducing Mcl-1 levels is an important step towards apoptosis in tumor cells because it was previously described that the down-regulation of Bcl-2 or Bcl-xL anti-apoptotic proteins is not sufficient to elicit apoptosis [146,147]. Then, Mcl-1 regulation by

baicalein is an interesting strategy to induce cell death in tumor cells by activation of the mitochondria-related apoptotic mechanism.

Recently, Wang et al. published that baicalein was more efficient than baicalin in decreasing cell proliferation at the same concentrations tested (10, 20, and 50 µM for HCT-116, and 20 and 50 µM for HT-29 colorectal cancer cell lines) [148]. Moreover, authors found that the aglycone-rich fraction (ARF) of *Scutellaria baicalensis* extract exerted a stronger effect in inducing loss of MMP in such cell lines when compared to the baicalin fraction (BF). ARF contains both baicalein and wogonin flavonoids, but not baicalin. Additionally, ARF decreased Bcl2 expression more intensely than BF. Other parameters associated to mitochondrial function or related to the role of this organelle in apoptosis were not investigated in that work.

Utilizing human leukocytes, Shen et al. observed that baicalein ($IC_{50} \cong$ 2-3 µM) exerted a stronger antioxidant effect than baicalin ($IC_{50} \cong$ 5-26 µM) in suppressing extracellular ROS accumulation induced by N-formyl-methionyl-leucyl-phenylalanine (fMLP) or phorbol-12-myristate-13-acetate (PMA) depending on the cell type (neutrophils or mononuclear cells) [149]. Baicalein also presented a higher ability in inhibiting the accumulation of intracellular ROS when compared to baicalin at the same concentrations tested (1 – 100 µM). Baicalein (100 µM) also prevented the increase in cytosolic Ca^{2+} ions concentration induced by fMLP and AlF_4^- (an activator of G-protein), but failed when thapsigargin was utilized to trigger Ca^{2+} release from intracellular store. Baicalin (10 – 100 µM) was not effective in preventing the increase in intracellular Ca^{2+} ions levels in this experimental model. Even though the authors did not investigate whether there is a causative link between the decrease in ROS accumulation and the reduced levels of cytosolic Ca^{2+} ions in that work, one may argue that the decreased concentration of Ca^{2+} ions in the cytosol may

prevented an increase in ROS production and its posterior release for the extracellular environment, since mitochondria (among other cellular compartments and protein systems) produce more ROS in conditions in which the levels of intracellular Ca^{2+} ions are augmented. The mechanism by which baicalein prevented both ROS and Ca^{2+} ions accumulation in leukocytes remains to be fully understood, but may account with a role for mitochondria in buffering Ca^{2+} ions and an antioxidant role for baicalein in blocking ROS accumulation by a direct (chemical structure-related antioxidant capacity) or indirect (through the activation of transcription factors associated to the antioxidant defenses in mammalian cells) manner.

Interestingly, Lee et al. described that baicalein and baicalin exerted protection in primary cultured rat brain neurons against MK-801 or glucose deprivation, but failed to prevent an increase in $NO^{\bullet}$ at any concentration tested (0.35 – 10 µM) [150]. Actually, baicalein at 35 µM induced neurotoxic effects and increased $NO^{\bullet}$ production in glucose-deprived cells. Furthermore, baicalin slightly increased LDH (lactate dehydrogenase) release from primary neurons maintained under normal glucose concentration (33 mM). Also, baicalin (10 µM) was not effective in preventing the increase in LDH release induced by glucose deprivation in that experimental model. On the other hand, baicalein and baicalin alone did attenuate the increase in intracellular Ca^{2+} ions induced by glutamate/NMDA treatment. As discussed in this Section, by preventing an augmentation in cytosolic Ca^{2+} ions concentration, baicalein and baicalin may exert an indirect antioxidant role on mitochondria by blocking, for example, loss of MMP and mitochondrial ROS production.

Baicalin was not efficient counteracting the toxic effects of 6-OHDA or rotenone in PC12 (pretreatment with baicalin at 12 – 200 µM for 12h) [138] and SH-SY5Y (pretreatment with baicalin at 10 – 100 µM for 1h) [134] neuronal cells.

9. Conclusion

A growing body of evidence demonstrates that mitochondrial dysfunction plays an important role in the pathogenesis of different diseases, such as Parkinson's disease and Alzheimer's disease, cardiovascular diseases, cancer, and metabolic disorders. With respect to this, much attention has been paid to find new therapeutic agents for mitochondrial dysfunction-related disorders. It is well-known that oxidative stress plays an important role in these disorders. Abundant scientific evidence shows that natural polyphenolic antioxidants have beneficial effects on mitochondrial damage; among them, much attention has been paid to baicalin and baicalein. This review has demonstrated that baicalin and baicalein mitigate mitochondrial damage through redox-dependent mechanisms. In addition, it has shown that baicalin and baicalein protect mitochondria from oxidative stress both *in vitro* and *in vivo*. It has also revealed that the presence of hydroxyl moieties in the chemical structure of baicalin and baicalein play a pivotal role in their protective effects against oxidative stress in mitochondria. In conclusion, baicalein and baicalin exert protective (or preventive) effects on mitochondria in different biological systems. This action depends on several factors, such as concentration and duration of treatment, the chemical characteristics of the toxic agent/stressor, and the biological conditions of the target. However, a search on the Clinical Trials Gov database [1] with keywords “baicalein” and “baicalin” has shown that there are only two recruited clinical studies concerning these natural compounds and, therefore, it can be difficult to make a clear decision about their clinical impacts.

Finally, we recommend that future studies should be performed to:

- Enhance the bioaccessibility and bioavailability of baicalein and baicalin through new delivery forms, such as nanocrystallization, nanoemulsion, baicalin-loaded liposomes, and solid lipid nanoparticles of baicalin.

[1] Clinical Trials. Gov. A service of the U.S. National Institutes of Health. https://clinicaltrials.gov/ (accessed on June 25, 2015).

- Carry out toxicity studies for ascertaining the most effective and non-toxic doses of these compounds for future studies and the potential side and adverse effects
- Ascertain the most effective doses for future clinical studies, regarding the beneficial effects of baicalein and baicalin against mitochondrial dysfunction-related disorders.

Conflict of interest

Authors declare no conflict of interest.

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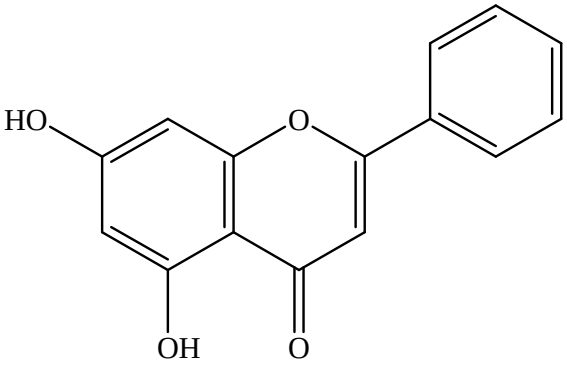
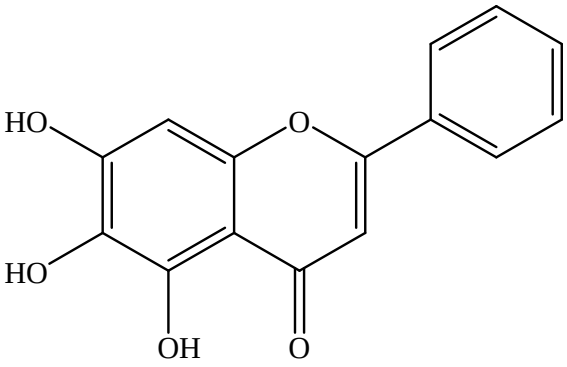
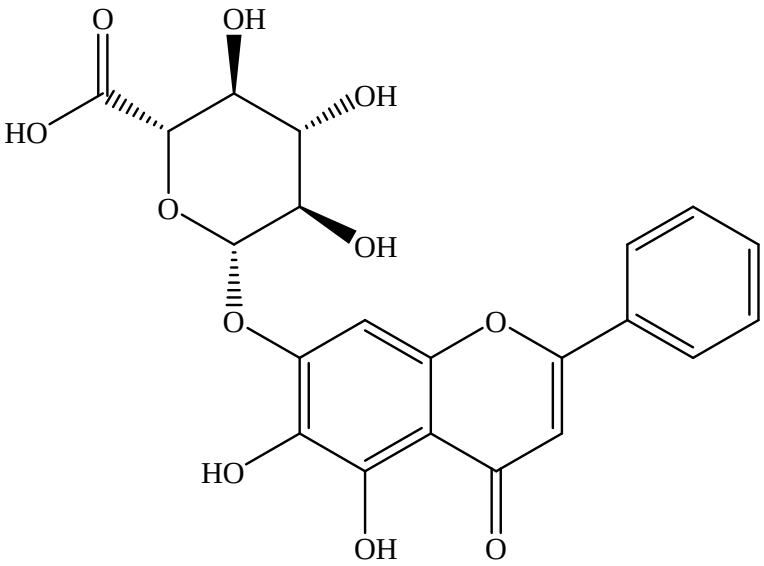


Chrysin

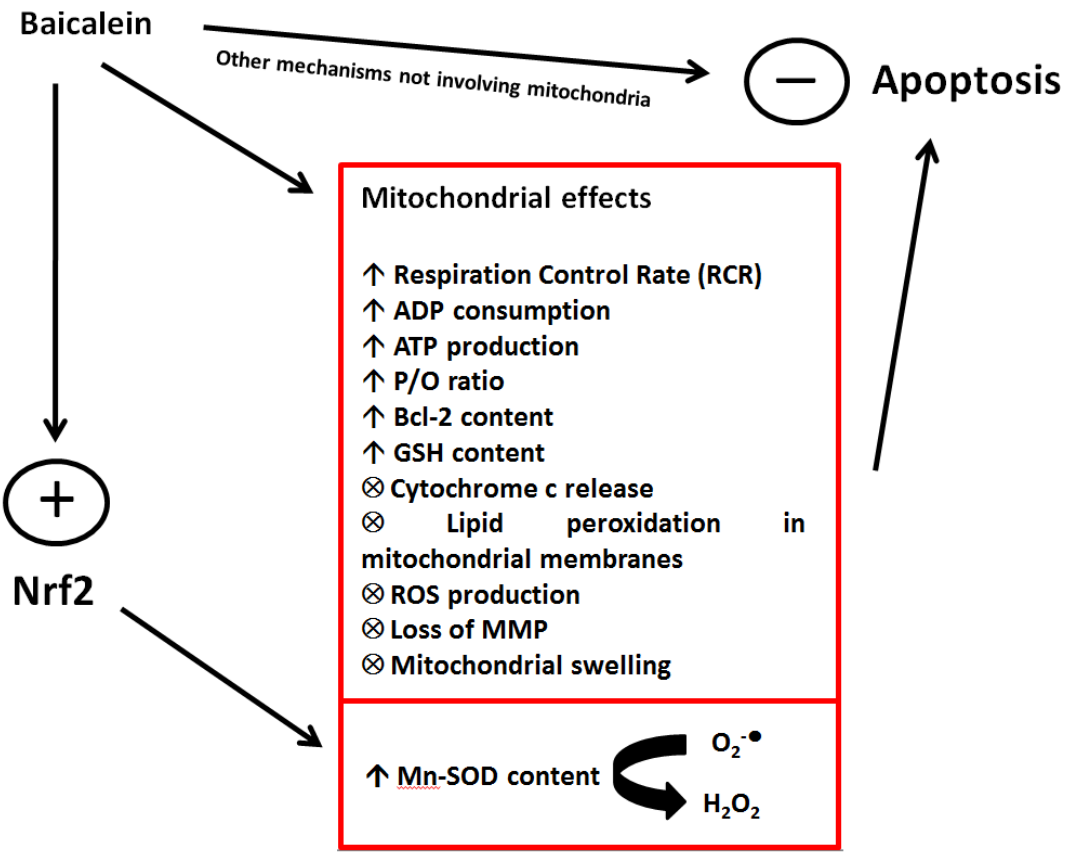


Baicalein



Baicalin

Fig 1. Structures of flavones chrysin, baicalein and baicalin.



1173

1174 **Figure 2. A summary of the effects of baicalein on mitochondrial function and dynamics**
1175 **and apoptosis. Baicalein is a polyphenol that may exert mitochondrial protection**
1176 **leading to prevention of chemically induced apoptosis in several experimental models.**
1177 **Baicalein increases mitochondrial activity and improves redox-related aspects in the**
1178 **organelle. Furthermore, baicalein inhibits changes in mitochondrial function and**
1179 **dynamics that would lead to apoptosis, as for instance cytochrome c release and loss of**
1180 **mitochondrial membrane potential (MMP). Baicalein also activates Nrf2, the master**
1181 **regulator of the redox environment in mammalian cells, causing an increase in the**
1182 **expression of the antioxidant enzyme Mn-SOD, which converts O2-• to H2O2 in the**
1183 **mitochondrial matrix. There are several efforts to elucidate the complete mechanism by**
1184 **which baicalein exerts its cytoprotective effects, but it clearly involves an improvement**
1185 **of mitochondrial function and quality, as discussed in the text.**

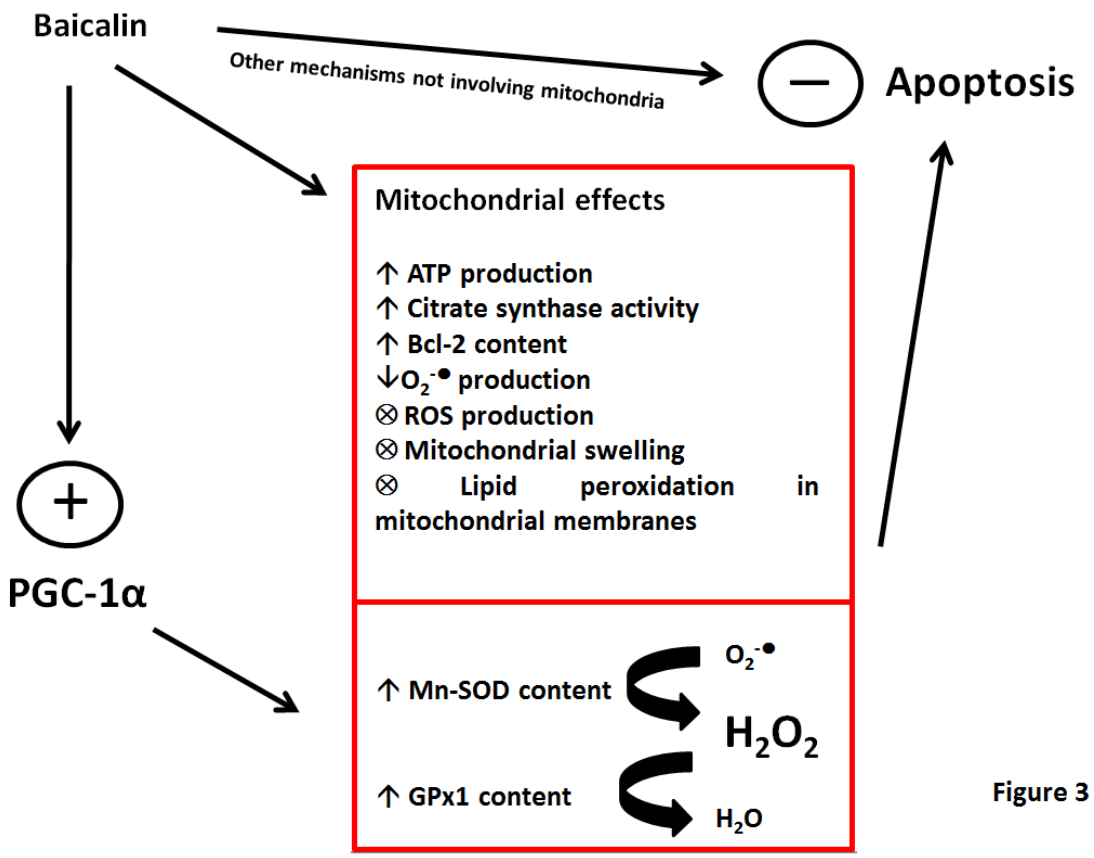


Figure 3

Figure 3. A summary of the effects of baicalin on mitochondrial function and dynamics and apoptosis. Baicalin improves mitochondrial function, i.e. augments ATP production and citrate synthase activity (which belongs to the tricarboxylic acid cycle – the so called Krebs cycle). Baicalin exerted antioxidant effects on mitochondria by decreasing ROS production and lipid peroxidation in mitochondrial membranes, as well as activating PGC-1α and up-regulating Mn-SOD and GPx1 expression.

Table 1. Summary of the in vitro effects of baicalein and baicalin on mitochondrial function and dynamics

Flavonoid	Concentration	Cell line	Experimental model	Effects	Reference
Baicalein	6.25 - 12.5 µM	SH-SY5Y	2h before exposure to 6-OHDA	↓ ROS production, ↑ MMP*, ↓ apoptosis	93
Baicalein	5 - 20 µM (40 µM**)	PC12	12h before exposure to H_2O_2	↑ MMP*, ↓ Bax content, ↑ Bcl-2 content	94
Baicalein	10 - 40 µM	PIG1	1h before exposure to H_2O_2	↑ MMP*, ↓ cytochrome c release, ↑ Bcl-2 content, ↓ apoptosis	95
Baicalein	10 - 40 µM	PC12	co-treatment with rotenone	↑ MMP*	96
Baicalein	10 µg/mL	V79-4	1h before exposure to H_2O_2	↓ mitochondrial ROS production, ↓ cytochrome c release, ↓ p-Bcl-2 content, ↓ p-Bax content, ↑ Nrf2 activity, ↑ Mn-SOD content and activity	97
Baicalin	50 µg/mL	L6	1h before antimycin A	↑ MMP*, ↓ O_2^- production, ↑ ATP production, ↑ PGC-1α content	115
Baicalin	1 - 25 µM	RGC-5	1h before exposure to rotenone	No alterations detected.	119
* Baicalein or baicalin were effective in preventing loss of MMP. ** Baicalein at 40 µM did not affect Bax levels in that experimental model.					

Table 2. Summary of the in vivo effects of baicalein and baicalin on mitochondrial function and dynamics

Flavonoid	Experimental model	Effects	Reference
Baicalein	Rat CCR model; Baicalein 30 or 100 mg/kg.day^{-1} for 27 days post-CCR induction, oral route	↑ respiration control rate, ↑ MMP*, ↑ ADP consumption, ↑ ATP production, ↓ mitochondrial ROS production	107
Baicalein	Mice pulmonary carcinogenesis model	↓ mitochondrial ROS production, ↓ mitochondrial sweeling, ↑ VDAC expression, ↑ activity of CK	109

	(50 mg/kg b.w. B(a)P); Baicalein 12 mg/kg once a week	enzymes (ICDH, α-KDH, SDH, and MDH), ↑ activity of METC enzymes (NADH dehydrogenase and cytochrome c oxidase)	
Baicalin	Rat diabetes model (STZ); Baicalin 120 mg/kg for 30 days	Baicalin protected mitochondria from STZ-induced morphological changes. ↑ number of mitochondria, ↑ citrate synthase activity	111
Baicalin	Rat hepatic I/R model; Baicalin 200 mg/kg 24h and 1h before I/R induction	↓ mitochondrial swelling, ↓ NF-kB activation, ↓ caspase activation	112
Baicalin	Rat renal I/R model; Baicalin 1-100 mg/kg i.p. 30 min before I/R induction	↑ Bcl-2 content, ↓ Bax content, ↓ caspase-9 and caspase-3 activation	114
* Baicalein or baicalin were effective in preventing loss of MMP.			

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