Morin: A Promising Natural Drug

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Running title: Nutraceutical properties of Morin: an overview

Abstract

Morin is a natural polyphenol,originally isolated from members of the *Moraceae* family, that can be extracted from leaves, fruits, stems and branches of numerous plants. Several evidence demonstrated that Morin could have a beneficial effect on several human diseases. In fact, Morin exerts antioxidant, antidiabetic, anti-inflammatory, antitumoral, antihypertensive, antibacterial, hypouricemic, and neuroprotective effects, by modulating the activity of many enzymes. In some cases, Morin shows a systemic protective action, reducing negative side effects of several drugs, without interfering with their functions. In addition, *in vitro* and *in vivo* studies demonstrated that Morin exhibits very low toxicity levels and its chronic administration is well tolerated. All these findings suggest that Morin could be used, either alone or in combination with other drugs, to prevent many human pathologies.

Keywords: Morin, flavonoid, polyphenol, natural drug; anti-oxidant, anti-diabetic; anti-inflammatory.

Introduction

Plants, yeast and bacteria are the most important source of bioactive compounds for pharmaceutical and medicine industry. In fact, several commercially available drugs, aimed to contrast bacterial infections or to treat a wide range of diseases including diabetes, cancer and neurodegeneration, have been directly derived from natural molecules or their semi-synthetic derivatives [1]. However, the procedures leading to isolation and identification of pharmacologically active molecules from natural sources are often expensive and complicated, due to their relative abundance and chemical complexity.

Nevertheless, in many cases, this strategy has been successful, and allowed the identification of new innovative drugsand bioactive compounds Well-known examples are acetylsalicylic acid and metformin, derived from salicylic acid isolated from *Salix alba***,** and galegine obtained from *Galega officinalis*, respectively. In the last decades, many natural compounds have been characterized for their pharmacological properties and have become drugs. It is not a coincidence that most of these substances are contained in the extracts used for centuries by traditional medicine [2].

Morin, 3,5,7,2',4'-pentahydroxyflavone, is a flavonol isolated as a yellow pigment from plants belonging to the *Moraceae* family. Morin is one of the principal constituents of many preparations of botanical origin, and is recommended by traditional medicine to treat several human pathologies [3]. Compelling evidence demonstrated that Morin is a bioactive compound, showing a broad range of pharmacological activities and very low cytotoxicity. However, the action mechanism of Morin remain to be clarified. Herein, we report a detailed analysis of biological properties and pharmacological activities of Morin.

N**atural sources of Morin**

Morin is present in several fruits and vegetables such as almond hulls [4], guava (*Psidium guajava L.*) leaves [5], old fustic (*Chlorophora tinctoria*), mill (*Prunus dulcis*) [6], osage orange (*Maclura pomifera*), *Acridocarpus orientalis* [7], onion, apple and in several beverages such as tea, red wine [8], seaweeds [9], coffee and cereal grains. In addition, it represents one of the constituent of several traditional herbal medicines [3].

Absorption and metabolism of Morin

Likewise other polyphenols, the free and glycosylated form of Morin can be detected in all its natural sources [10; 11]. After oral uploading, glycosylated, methylated or sulfated forms of polyphenols pass intact through stomach and reach the small intestine, where they are hydrolysed by pancreatic and intestinal enzymes, and converted to the aglycone form [12; 13]. The hydrolysis

step is essential to allow their intestinal absorption. In fact, most of glycosylated forms are not uploaded from the enterocytes of small intestine, but are transported into the large intestine, where they are metabolized from the resident bacteria. Specific shuttle proteins enable the hydrophilic morin aglycone to cross cellular membrane. Experiments carried out with human aortic endothelial cells confirmed the involvement of an energy-dependent transport system in the delivery of Morin from blood into the cells [14]. Likely, a similar mechanism contributes to transport Morin from the intestinal lumen into enterocytes. Nevertheless, studies with human Caco-2 cells, demonstrated that Morin possesses a very low intestinal permeability (P_{app} = 0.62 m/s) when compared with other polyphenols [15]. In line with these findings, tests on Wistar rats have confirmed that the plasma concentration of Morin does not exceed 1%, also after oral administration of high doses (200 mg/Kg) [16]. The poor bioavailability of Morin is mainly due to the activity of Multidrug Resistance-Associated Protein-1, a carrier protein widely present on the plasma-membrane of intestinal cells that is responsible for the extrusion of Morin [15]. The inhibition of the Multidrug Resistance-Associated Protein-1 by specific drugs significantly increases Morin upload, thereby confirming the role of this transporter in regulating intestinal absorption of Morin [17]. Once absorbed, specific enzymes convert Morin into its glucuronidated, methylated and sulfated forms , which are then poured into the bloodstream [18].

A significant increase of Morin aglycone in the blood, can be detected after high oral dose administration (>100 mg/Kg) of Morin, suggesting that activities of intestinal glucuronyl transferase/sulfotranferase enzymes are easily saturable [19]. Moreover, it has been demonstrated that hepatic enzymes, such as glucuronyl-transferase/sulfo-transferase [19], or non-hepatic cytochrome P450 and sulfotransferases, participate to Morin metabolism [20].

Physiological effects of Morin

Citotoxicity

Several studies demonstrated the low cytotoxicity of Morin on cellular cultures and animal models. *In vitro*, Morin showed weak cytotoxic effects (IC_{50}=250 ± 40 μM) on human promyelocytic leukemia cells [21]. Moreover, *in vivo* studies revealed no toxic effects of Morin on F344 rats. After 13 weeks treatment with high Morin doses (from about 300 to 2400 mg/Kg b.w.) the rats didn't show any adverse effects, displaying only a modest alteration of liver functionality or a moderate increase in liver or kidney weight. Based on these observations, authors calculated the no-observed-adverse-effect level of Morin at about 300 mg/Kg of body weight/day [22].

Antiperglycemic and antidiabetic activity

In an attempt to identify the herbal preparations with antidiabetic activity, some researchers have analysed more than 160 traditional medicinal plant extracts. The methanolic extract of *Psidium guajava L.,* which contains a high concentration of Morin, was one of the most active preparations [23]. This extract is able to inhibit the activity of PTP1B, one of the most important enzymes involved in the negative regulation of insulin receptor signalling pathway [24]. *In vivo* studies demonstrated that diabetic mice daily treated with *Psidium guajava* extracts, showed a significant reduction of glycaemia, as well as a decrease in lipid liver deposits [25]. Similarly, the *Psidium guajava Linn* extracts showed a significant antihyperglycemic and antioxidative effect in streptozotocin-induced diabetic rats [26]. Moreover, lower blood glucose levels has been detected in subjects affected by maturity-onset diabetes and in healthy volunteers after administration of *Psidium guajava* extracts. [27]. These evidence suggested that extracts containing high Morin concentration can complement orthodox anti-diabetic therapies.

In vitro screening test, using in-house collection of 19 polyphenols, identified Morin as a potent non-competitive inhibitor of the PTP1B enzyme. HepG2 cells treated with 25-50 μM Morin exhibited increased insulin receptor phosphorylation, resulting in increased glycogen synthesis and in a decreased gluconeogenesis. In addition, it has been observed that Morin stimulates the insulin activity, suggesting that it can act as insulin mimetic and insulin sensitizer [28]. More recently, other experimental evidence confirmed that Morin is able to improve glucose metabolism control in animal models. Vanitha P. *et al.* showed that Morin administration (50 mg/kg b.w.) contributes to decrease the blood glucose levels at fasting in streptozotocin-induced diabetic rats [29]. The observed effects are comparable with those obtained following administration of glibenclamide, a well-known antidiabetic drug. The effectiveness of Morin is, at least in part, linked to its ability to protect pancreatic insulin-producing cells from death, thereby sustaining insulin release, blood glucose absorption, and glycogen synthesis in liver [29]. Interestingly, it has been observed that effectiveness of Morin in the control of the postprandial glycaemia in diabetic rats is strongly enhanced when Morin is administered as zinc complex [30]. In fact, in diabetic rats, the chronic treatment with low doses of the zinc-Morin complex (5 mg/kg b.w.) resulted in a glucose level decrease at fasting, in an improvement in insulin sensitivity,, in a reduction of the level of glycosylated haemoglobin, circulating lipids and lipoproteins with respect to untreated diabetic rats. No significant adverse effects or alterations in both carbohydrate and lipids metabolism were observed in the control rats following the administration of the zinc-Morin complex [30]. It is important to consider that zinc ions are also potent inhibitors of PTP1B enzyme [31], and that intracellular zinc fluctuations contribute to the modulation of activity of several phosphotyrosine protein phosphatases and to the regulation of specific signalling pathways [32]. Hence, Morin may

contribute to enhance insulin receptor signalling by two different mechanisms: i) direct inhibition of PTP1B, ii) by increasing transport of zinc ions into the cells (Figure 1).

Diabetes induces various complications including neuropathy, endothelial dysfunction, and retinopathy. These effects are partially due to the covalent modification of proteins and lipids by glucose. These glycosylated adducts, named "advanced glycation end products"are thought to contribute to the development of micro- and macrovascular diseases. Interestingly, it has been observed that Morin inhibits protein glycosylation in a concentration dependent manner, [33]. In diabetic rats, one week treatment with Morin (15-30 mg/Kg b.w.) significantly decreases the level of inflammatory markers (TNFα, IL1β, IL-6) in brain, , meanwhile increasing the circulating levels of neurotrophic factors, thereby exerting a neuroprotective action. These results suggest that Morin, besides to represent an effective anti-diabetic agent, could protect nervous cells from degeneration, avoiding the onset of the encephalopathy in subjects affected by diabetes [34].

Antioxidant activity Reactive oxygen species (ROS) are implicated in the regulation of several cell signalling pathways. When ROS production exceeds the ROS scavenging activity of antioxidant enzymes (i.e. superoxide dismutase, catalase and glutathione peroxidase), proteins, carbohydrates, lipids, and nucleic acids, can be damaged [35]. Polyphenols are natural antioxidants that can act as a first line of cellular defence toward ROS [36]. Antioxidant activity of Morin is mainly due to the presence of a double bond between C2-C3 atoms as well as to the presence of a hydroxyl group activating the double bond at the C-3 position. Moreover, anti-lipid-peroxidation activity of Morin seems to be strictly related with the presence of two hydroxyl groups on the 2' and 4' positions of the B ring [37-39]. While the hydroxyl group in 4' position of B ring is thought to be responsible for the antiradical activity of Morin, Myricetin, and Quercetin, the hydroxyl group in position 2' of B ring, exclusively present in Morin, seems to be related to its more effective antiradical activity. By *in silico* studies, Morales J. *et al*. [40; 41] demonstrated that the hydroxyl group in position 2' of B ring forms a hydrogen bond with the oxygen atom in position 1 of C ring, inducing rotation of the B ring, which acquires a planar configuration respect to ring C. This configuration favours the transmission of electronic effects from the B ring to the double bond of C ring, thereby making Morin a good natural radical scavenger [42]. *In vitro* Morin inhibits the oxidation of LDL induced by 2,2'-azo-bis-(2-amidinopropane) dihydrochloride and contributes to prevent oxidized LDL uptake by macrophages, reducing expression of CD36, the receptor responsible for their internalization. Furthermore, Morin can be useful to prevent negative effects of lipid peroxidation, contributing to atherosclerosis prevention [43; 44] and protecting cardiomyocytes, endothelial cells, and erythrocytes from the oxidative action of peroxyl radicals generated following treatment with pro-oxidant agents such as 2,2'-azo-bis (2-amidinopropane) dihydrochloride, xanthine oxidase

plus hypoxanthine system, menadione, 3-morpholinosydnonimine-N-ethylcarbamide (SIN-1), N-nitrosodiethylamine. Interestingly, in all cases Morin resulted more effective than classical antioxidant compounds such as Trolox (a vitamin E analogue), mannitol, and ascorbate [45-49]. Jonnalagadda J.V. *et al.* [50] demonstrated that Morin, due to its antioxidant and anti-inflammatory activity, protects rats from Gentamicin-induced nephrotoxicity. In fact, Gentamicincytotoxic effects are mainly mediated by ROS, which trigger and sustainchronic inflammatory response that is the cause of tubular necrosis [51]. Administration of Gentamicin in combination with Morin (from 50 to 200 mg/Kg b.w./day) for fifteen consecutive days, leads to a decrease of the antibiotic-induced nephrotoxicity, reduction of cells damage, and improvement of kidney functionality. *In vitro* experiments showed that Morin protects lung fibroblasts from DNA damage and from lipid peroxidation caused by exposition to H_2O_2 or γ-irradiation [52; 53]. Pre-treatment with Morin (100 mg/kg b.w.) in mice irradiated with γ-irradiation strongly reduces the intestinal mucosa deterioration, inhibits inflammation and cells death, by preventing the intracellular reducing agents depletion and the malondialdehyde production. These results suggested that Morin behaves as a potent radioprotective agent [54]. Moreover, Kapoor R. *et al* showed that Morin decreases the ROS levels in primary rat hepatocytes exposed to high glucose concentration (40 mM), thereby contributing to maintain mitochondrial integrity, to inhibit release of pro-apoptotic proteins, and to prevent DNA damages [55]. In keeping with these findings, *in vivo* studies showed that Morin treatment (100 mg/Kg b.w.) protects rat's liver exposed to cyclophosphamide from oxidative damage [56]. Similar results have also been obtained by Ray S. *et al* on rabbits treated with cyclophosphamide/flutamide Besides to protect from the oxidative stress, Morin contributes to prevent the cholesterol increase [57]. Morin minimizes the side toxic effect induced by some chemotherapy agents such as doxorubicin and mitomycin C. In fact, Kok L.D. *et al* [58], showed that Morin administration protects non-malignant cells from cytotoxic activity of drugs, without interfering with their effectiveness. Morin can also inhibit xanthine oxidase, one of the most important enzyme involved in the ROS production following ischemic-reperfusion events [59; 60]. In addition, it has been observed that Morin stimulates the expression of genes encoding proteins involved in the antioxidant response (such as superoxide dismutase, catalase, heme oxygenase-1, glutathione peroxidase and glutathione reductase) both *in vitro* and in animal models, preserving the intracellular levels of glutathione, ascorbic acid and α-tocopherol [52; 61-63] (Figure 2).

Metal ions are essential for cells, being cofactors of several proteins and enzymes. Nevertheless, when present in excess, they can contribute to elevate intracellular ROS levels through different mechanisms, such as the Fenton reaction. Several polyphenols are able to chelate metals, contributing to reduce their toxicity. Accordingly, it has been observed that *in vitro* Morin forms stable complexes with transition metal ions, such as iron, copper, chromium and cobalt, thereby

inhibiting free metal-catalysed ROS production [64; 65]. In some cases, metal-Morin complexes are more potent free radical scavengers than free Morin, mainly due to the acquisition of additional superoxide dismutating centers [66].

Antinflammatory and antiallergic activity

Chronic inflammation contributes to the development of pathologies such as cancer, diabetes, cardiovascular and chronic bowel diseases, as well as of neurodegenerative disorders [67; 68]. At the molecular level, numerous molecules and factors, including cytokines, chemokines, pro-inflammatory transcription factors and enzymes, and matrix metalloproteinases, regulate inflammation. Many data show that Morin is able to inhibit most of the effectors involved in inflammation acting as a potent anti-inflammatotry agent by inhibiting activated macrophage cells both *in vitro* [69; 70] and *in vivo* [71]. *In vivo* experiments demonstrated that Morin administration (from 10 to 200 mg/kg b.w.) reduces colitis triggered by treatment with trinitrobenzenesulfonic acid, preserving intestinal cells from damages. In fact, rats treated with Morin show a lower deterioration of bowel, a fewer granulocyte infiltration in the intestinal mucosa, and lower levels of leukotriene B4 and malondialdehyde with respect to untreated rats [72]. More recently, Sunil K. *et al* [73] demonstrated that Morin inhibits the activity of the transcription factor NF-kB, one of the most important effector in the inflammatory response [74]. Morin inhibits the IkBα kinase pathway, favouring the stabilization of IkBα, thus reducing the expression of inducible form of nitric oxide synthase, as well as of the COX-2, IL-6, IL-8, and TNF genes. Similar results were obtained using LPS-stimulated RAW 264.7 cells and macrophages derived from mice models [75]. Moreover, Morin is effective in reducing liver inflammation of rats fed with a high fructose diet. Excessive fructose consumption causes activation of the SphK1/S1P-NF-kB signalling pathway, which, in turn, triggers liver inflammation, insulin resistance, and the increase of fat depots [76; 77]. Treatment with Morin causes a down-regulation of SphK1 activity and blocks the NF-kB nuclear translocation, inhibiting secretion of IL-1β, IL-6 and TNF-α by hepatocytes [78]. Other *in vivo* studies demonstrated that pre-treatment with Morin, reduces NF-kB activation, the expression levels of TNF-α, IL-1β, IL-6, and iNOS, and protects mice from the hepatic damage [79]. Taken together, these data demonstrated that anti-inflammatory activity of Morin is, in part, attributable to its ability to inhibit the NF-kB activity [80].

Few data are available on the anti-inflammatory activity of Morin metabolites, but they are probably the main effectors of the observed phenomena. In keeping with this hypothesis, it has been observed that sulfates/glucuronides metabolites of Morin strongly reduce the production of NO, TNF-α, and IL-12 in macrophages activated with LPS. Interestingly, these metabolites show a potency 1000 fold higher than Morin. [81].

Studies on mast cells suggested that Morin possesses also anti-allergic activity. For example, Morin inhibits the release of IL-6, IL-8, and TNF-α from human umbilical cord blood-derived cultured mast cells [82; 83]. Analyses of signalling pathways showed that Morin-induced inhibition of mast cells degranulation is mediated by the inhibition of Syk kinase activation [84]. In fact, Morin is a reversible inhibitor (IC_{50} value of 5.7 μM) of Fyn kinase, the main positive effector of Syk kinase in mast cells following IgE-mediated activation [85]. *In vivo* experiments confirmed that Morin suppresses IgE-mediated PCA in mice and inhibits degranulation and production of TNF-α and IL-4 in antigen-stimulated mast cells. Thus, reversible inhibition of Fyn kinase is the main mechanism through which Morin exerts its anti-allergic activity [86].

Antitumoral activity

Cancer is the second leading cause of death in the western countries [87]. Carcinogenesis is a multistep process, encompassing several stages consistent with initiation, which elicits oncogenes activation or DNA damages, promotion, in which anomalous cells proliferate generating preneoplastic foci, and progression, the final stage in which preneoplastic cells shift toward an uncontrolled and invasive phenotype [88]. It has been suggested that numerous natural compounds, including Morin, exert an anticancer preventive activity, reducing DNA damages and modulating signalling pathways involved in proliferation and differentiation [89].

Chemopreventive activity

Morin inhibits carcinogenic activity and tumor-promoting activity of several chemical compounds. For instance, treatment with Morin (50 mg/kg for three days) protects rats treated with 7,12-dimethylbenz(a)-anthracene from oxidative stress, decreases the expression of tumor markers and inhibits tumor growth [90]. Moreover, it has been observed that Morin blocks liver cells transformation induced by 12-O-tetradecanoylphorbol-13-acetate [91].

These evidence suggest that Morin could act as an anticancer agent better than other classical cytotoxic drugs.

Inhibition of proliferation and induction of apoptosis

Morin inhibits proliferation of cancer cells by interfering with cell cycle. Brown J. *et al* [92] demonstrated that Morin induces the G2/M phase cell cycle arrest in human oral squamous carcinoma cells, without inducing apoptosis. Other evidence demonstrated that Morin is able to induce apoptosis in LNCaP prostate cancer cells and in Human Leukemia HL-60 cells. In HL-60 cells Morin promotes the activation of caspase-3 and Bax, stimulates expression of caspase-3 and -9, triggers cytochrome c release from mitochondria and decreases expression of antiapoptotic Bcl-2

expression. These data suggest that apoptosis induced by Morin might involve a mitochondria-dependent pathway and caspase-3 [8; 93]. Recently, studies conducted on multiple myeloma cells demonstrated that Morin increases apoptosis of cancer cells through the SHP1-mediated inactivation of STAT3 (signal transducers and activator of transcription 3) signalling pathway. Finally, it has been demonstrated that Morin increases apoptosis of the cancer cells by 30%, without affecting viability of normal cells [94].

Modulation of signalling pathways

In animals treated with pro-carcinogenic agent 7,12-dimethylbenz(a)-anthracene, Morin treatment inhibits tumor growth and decreases the expression of breast cancer specific tumor markers, thereby confirming its anti-carcinogenic activity [90]. In addition, Morin could prevent the growth and the dissemination of metastatic cancer cells by inhibiting the activity of NF-kB transcription factor. In fact, a low Morin dose (50 μM) is sufficient to revert mesenchymal phenotype of highly metastatic MDA-MB-231 breast cancer cells, reducing expression of N-cadherin, metalloproteinase-9 secretion, and inhibiting activation of Akt pathway [95].

Inhibition of pro-carcinogenic effects of metal compounds

Morin binds metal ions such as iron, copper, chromium, vanadium and cobalt, thus inhibiting ROS production through the Fenton or Haber–Weiss reactions. In addition, Morin prevents metal-catalyzed free radicals generation, thereby protecting biologically active molecules from oxidative stress. It is interesting to note that metal-Morin complexes are more effective than Morin alone in removing free radicals [96-98].

Effects on phase I enzymes and on P-glycoprotein

The therapeutic efficacy of many anticancer drugs depends in part on expression of drug-metabolising enzymes such as those belonging to cytochrome P450 family. These enzymes are mainly expressed in liver cells, but are also found in the cells of esophageal squamous mucosa, duodenum, and jejunum [99]. Many studies carried out on animal models, showed that Morin inhibits expression of Cytochrome P450-2C9 isoenzyme in liver [100] and of Cytochrome P450-3A4 [101] in intestinal mucosa. Cytochrome P450 enzymes play a key role in the metabolism of sterols, prostaglandins, drugs, or xenobiotic compounds. In some cases, these enzymes contribute to conversion of xenobiotic compounds in highly reactive metabolites able to react with proteins, lipids or nucleic acids, thereby initiating the carcinogenesis process. Polyphenols and Morin can: i) increase the plasma lifetime of chemotherapy drugs, thus improving their effectiveness; ii) inhibit

cytochrome P450-mediated carcinogen activation, reducing intracellular amount of pro-carcinogenic compounds [102].

Failure of chemotherapy is often due to high activity of P-glycoprotein, a an efflux membrane transporter over-expressed in several type of tumors. The P-glycoprotein contributes to the extrusion of many molecules, including anticancer drugs, immunosuppressants and antibiotics, thus preventing them from reaching their physiological targets. For this reasons P-glycoprotein is considered a key player in the induction of chemoresistance toward a large number of chemotherapy drugs such as taxanes, etoposide, and vinca-alkaloids [103]. Several studies demonstrated that Morin inhibits P-glycoprotein, thereby acting as a bio-enhancer that increases the bioavailability and bioefficacy of drugs [104-106]. This hypothesis is confirmed by independent studies showing that Morin increases the bioavailability of etoposide [107], tamoxifen [108], vinblastine [109], nicardipine [110], methotrexate [111], paclitaxel [112], daunomycin [113] and talinolol [114] in normal rats, as well as of etoposide in mammary tumor-bearing rats [115]. These findings suggest that Morin administration could increase the dose of drug absorbed by cells, thus increasing treatment efficacy [116].

Anti-hypertensive activity

Genetic factors, obesity and diabetes have a relevant role in the development of hypertension, which is one of the most important risk factors for cardiovascular diseases in the world [117]. Endothelial dysfunction, inflammation, and excessive ROS production contribute to the development of this pathological condition, suggesting that antioxidant agents could be used as adjuvants to the treatment of hypertension [118; 119]. Herrera M.D. *et al.,* reported that Morin contributes to relax vessels contracted by noradrenaline, KCl, and phorbol ester derivatives, and to enhance the effects of classical anti-hypertensive drugs, such as isoprenaline and sodium nitroprusside [120]. Morin administration (50 mg/kg for six consecutive weeks) improves the conditions of albino Wistar rats treated with deoxycorticosterone acetate-salt, a potent hypertension-inducing factor. In particular, it has been observed that Morin induces a considerable decrease of the systolic and diastolic blood pressure, and a decrease of hepatic and renal functional markers with respect to untreated hypertensive rats. Similar results have been confirmed in a recent study, showing that renal and cardiac damages induced by deoxycorticosterone acetate are reduced in animals pre-treated with Morin [121; 122]. Beneficial effects of Morin have been described also in hypertensive rats feeded with a diet rich in fructose. In this animals, Morin pre-treatment causes a reduction of blood pressure, of serum insulin, of triglyceride levels, and a downregulation of endothelin-1 expression, a vasoactive peptide that contributes to the development of hypertension [123]. In addition, in diabetic rats Morin promotes the production of the vasorelaxant nitric oxide, and the inhibition of

thromboxane A2, which acts as a vasoconstrictor. Taken together, these findings confirm the anti-hypertensive role of Morin [124].

Antibacterial activity

Antimicrobial properties of many plant extracts have long been known [125; 126]. Kang S.S. *et al* reported that Morin inhibits both sortase A and B, two enzymes expressed in *Staphylococcus aureus*. In Gram-positive bacteria, Sortase A plays a critical function, modulating the adhesion of bacteria to the host tissue. Inhibition of sortase A by Morin prevents the establishment of infections, without affecting microbial viability [127]. DNA helicase is another enzyme essential for cell growth of bacteria, virus, and eukaryotic cells being involved in DNA metabolism . *In vitro* tests showed that Morin can inhibit ATPase activity of DNA helicase RepA, with an IC_{50} value of 45 μM. These results suggested that Morin could be useful to contrast growth of both Gram-positive and Gram-negative bacterial species [128]. Looking for natural antibacterial compounds, Arima H. and Danno G. found that Morin-3-O-α-L-lyxopyranoside and Morin-3-O-α-L-arabopyranoside isolated from leaves of guava (*Psidium guajava L.*) behave as antibacterial agents, showing a minimum inhibitory concentration of 300 and 150 μg/ml for *Bacillus cereus* and *Salmonella enteritidis*, respectively [11]. Further studies demonstrated that Morin inhibits growth of *E.coli* and *S.aureus* [129]. More recently, it has been highlighted that Morin-3-O-arabinoside and Morin-3-O-lyxoside extracted from *P. guajava laves* show antibacterial activity against several strains of spoilage and foodborne pathogenic bacteria, including *B. stearothermophilus*, *B. thermosphacta*, *E. coli* , *L. monocytogenes*, *P. fluorescens*, *S. enterica*, *S. aureus*, and *V. cholerae* (Figure 3). Taken together, these findings suggest that Morin acts as food preservatives and can be useful to improve the shelf-life and the safety of foods, thereby protecting humans from foodborne diseases.

Anti-uricemic activity

Uric acid is the final metabolite of purine catabolic pathwayand is one of the main antioxidant agents of blood. The excess of uric acid is eliminated *via* the urine. When its synthesis is increased, or its excretion is impaired, it rushes causing pain, chronic inflammatory, and kidney failure [130]. Recent studies demonstrated that Morin can be useful to reduce serum levels of uric acid in patients suffering of hyperuricemia . It has been observed that Morin acts through two different mechanisms: i) by inhibiting liver xanthine oxidase (Ki=7.9 μM), thus reducing conversion of xanthine into uric acid; ii) by inhibiting human urate anion transporter-1, the main responsible for the urate reabsorption,present on the brush-border membrane of renal proximal tubule [131]. *In vivo* studies showed that in hyperuricemic animal models, the treatment with Morin leads to a significant reduction of the plasma uric acid levels, without impairing total serum antioxidant

capacity or causing others undesirable side effects [132; 22]. Morin administration (50 and 100mg/kg b.w. for 3 days) may also contribute to restore normal levels of uric acid in a hyperuricemic mice model [133]. Moreover, Morin behaves as a competitive inhibitor of human urate transporter *in vitro* with a Ki of about 6 μM, resulting more potent, but much less toxic, than some classical urate-lowering agents (including probenecid and sulfinpyrazone) [134; 135]. Interestingly, Shi Y.W. *et al* reported that administration of ethanol extract of *Ramulus Mori*, which contains mulberroside A, oxyresveratrol, 4-hydroxycinnamic acid, resveratrol, 7- hydroxycumarin and Morin, contributes to regulate renal organic ion transporters, thereby reducing the levels of uric acid and protecting the kidney function [136]. Taken together, these data suggest that Morin could be considered an excellent alternative drug to the treatment of hyperuricemic patients.

Neuroprotective and anti-amyloidogenic activity

Neuronal cells are highly sensitive to environmental conditions, and may suffer severe damages when exposed to oxidative stress conditions. Chronic ROS production triggers and feeds inflammatory processes, thereby inducing apoptosis of nervous cells [137-138]. Compelling evidence demonstrated that Morin exerts a relevant neuroprotective effect on cells and on animal models, and it may be used as a new therapeutic agent for the treatment of neurodegenerative diseases. Morin exerts its activity through different mechanisms:

i) It attenuates ROS formation in differentiated PC12 cells treated with 1-methyl-4-phenylpyridinium ion, inhibits caspase-3 activation and apoptosis. In addition, in animal models, Morin treatment relieves symptoms of Parkinson's disease, and prevents dopaminergic neuronal death and the striatal dopamine depletion in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [139]. Ibarretxe G. *et al* [48] showed that nanomolar concentrations of Morin protect oligodendrocytes and cortical neurons from ROS accumulation mediated by AMPA receptors overstimulation.

ii) It inhibits glycogen synthase kinase 3, a pivotal enzyme involved in the onset of the Alzheimer disease. It has been demonstrated that dysfunction of glycogen synthase kinase 3 induces alterations in the choline metabolism, impairs axonal transport, microtubule dynamics and neurogenesis, triggers apoptosis, blocks the differentiation of newborn neurons, induces morphological alteration in neuronal cells and impairs their connectivity [140]. Inhibition of glycogen synthase kinase 3 contributes to improve condition of patients suffering of Alzheimer disease. Low Morin concentrations (1-10 μM) *in vitro* inhibits glycogen synthase kinase 3 activity, thereby reducing Aβ-induced tau hyperphosphorylation, without affecting cell viability. Comparable results were obtained with animal models of Alzheimer disease. In these animals, Morin treatment (10 mg/kg b.w. for 7 days) results in a

reduction of total levels of tau hyperphosphorylation, confirming the hypothesis that Morin may have a potential role as therapeutic agent for treatment of tauopathies [141].

iii) It inhibits NF-kB activity, thereby behaving as an anti-inflammatory agent.

iv) It inhibits assembling and/or acts as disintegrating agent of amyloid fibers, the well organized proteinaceous structures appearing in the late stages of degenerative pathologies such as Alzheimer, Parkinson's disease or type-2 diabetes. [142]. Similar results were obtained with peptide β-amyloid. *In vitro*, Morin inhibits β-amyloid peptide fibrillogenesis with IC_{50} value of about 25 μM and protects HT22 murine neuroblastoma cells from the oxidative stress triggered by amyloid fibrils, confirming that this flavonoid, differently from many other natural molecules, has a double action mechanism [143]. Nevertheless, the exact mechanism by which Morin exerts its inhibitory activity on fibrillogenesis has not been yet completely elucidated [144]. *In silico* analyses carried out using atomistic explicit-solvent molecular dynamics simulations, showed that Morin binds to the ends of the β-amyloid growing fibrils, blocking the protein polymerization process [145]. Morin modifies both tertiary and quaternary structure of newborn protofibrils, thereby inhibiting their cytotoxicity and their conversion in long mature amyloid fibrils [146].

v) It inhibits acetylcholinesterase activity, preventing the loss of acetylcholine, a typical pathological event that characterizes patients affected by Alzheimer's disease [147].

vi) It inhibits β-Secretase 1, the enzyme involved in the abnormal production of the amyloidogenic peptide Aβ42. *In silico* docking analysis revealed that Morin targets active site of β-Secretase 1, interacting with the catalytic residue Asp228. Morin is a good inhibitor of β-Secretase 1 and shows an IC_{50} value of about 20 μM [148].

vii) It inhibits the membrane destructuring ability of wild type, and of α-synuclein mutants, proteins implicated in the pathophysiology of both familial and sporadic Parkinson's disease. Caruana M. *et al* showed that Morin and others polyphenols, such as baicalein and apigenin, protect membrane against perturbations induced by aggregates obtained from wild-type and some α-synuclein mutants.

Taken together, all these data suggest that Morin can be considered an interesting starting compound for the development of new generation drugs useful to the treatment of Parkinson's and Alzheimer's disease [149].

Inhibitory activity of Morin on enzyme activity and protein function

Morin acts as inhibitor of key regulatory enzymes involved in the control of many intracellular signalling pathways, showing IC_{50} or Ki values near to μM range (Table I). Morin binds into the active site, acting as competitive inhibitor of the cytochrome P450-2C9, of monocarboxylate

transporter-1, of multidrug resistance proteins 1 and 2, and of the fatty acid synthase. Nevertheless, in other cases, the docking site of Morin remain to be identified. It has been reported that Morin behaves as a non competitive inhibitor of DNA helicase RepA and PTP1B, or as a mixed type inhibitor of urate-anion transporter. Finally, it has been suggested that some polyphenols, including Morin, could bind to specific sites on the enzymes surface, modulating theiractivity. For example, X-ray analysis of the ATP synthase-Morin complex has actually identified a "polyphenol binding pocket" [150]. Iglesias *et al* [151] demonstrated that Morin is able to act as a potent inhibitor of phospholipase A2 from *Crotalus durissus cascavella venom*, showing an IC_{50} value of about 5 μM. Morin interacts with the hydrophobic catalytic pocket of the enzyme, thereby inducing a strong change in the secondary structure. Nevertheless, interaction with Morin does not suppress inflammatory and neurotoxic effects of phospholipase A2. Interestingly, independent works showed that Quercetin, a molecule structurally similar to Morin, has a similar behaviour and is able to inhibit both F1-ATPase from bovine heart mitochondria [152] and phospholipase A2 from *C. durissus terrificus venom* [153]. Finally, Morin is able to interact with human serum albumin with a dissociation constant value of about 9 μM. The bind of Morin to albumin causes significant alteration of protein secondary structure content, suggesting that Morin could acts as a modulator of albumin physiological functions [154].

Taken together, these evidence confirm that several enzymes and proteins possess binding sites for polyphenols, thereby reinforcing the hypothesis that these natural compounds can participate to the regulation of several human physiological functions.

Other activities of Morin

In vitro studies on rat chondrocytes, have demonstrated that Morin is able to inhibit metalloproteinases release induced by IL-1β stimulation, thereby preventing breakdown of the cartilage matrix. *In vivo* investigation on a rat model of anterior cruciate ligament transection (ACLT)-induced osteoarthritis, demonstrated that Morin administration (50 mg/kg/d for four weeks) inhibits cartilage degradation. These results suggest that Morin could be used as a possible therapeutic agent for the treatment of osteoarthritis [155].

Morin has been reported to possess protective effect on hepatic fibrosis induced by dimethylnitrosamine in rats. Oral administration of Morin leads to reduction of hepatic expression of collagen type I, TGF-β1, and α-SMA, some of most important hepatic fibrosis-related factors. In addition, in dimethylnitrosamine treated rats, Morin administration normalizes the level of serum alanine transaminase (ALT), aspartate transaminase (AST), and total bilirubin. These results suggest that Morin may be useful also to prevent the development of hepatic fibrosis and cirrhosis [156].

Finally, flavonoids such as Morin show inhibitory activity against a variety of virus, including herpes simplex virus (HSV), respiratory syncytial virus, poliovirus and Sindbis virus.

Conclusions

Despite the improvement of knowledge and technologies, the war against pathologies such as diabetes, cardiovascular diseases, cancer and neurodegenerative diseases is far from over. This is due to a deficit of knowledge about the actual molecular mechanism contributing to the onset and development of diseases and to the lack of innovative drugs. Many lines of evidence indicate that development of new drugs is a key objective for the future.

To date, natural extracts or medicinal foods, recommended by traditional medicines to prevent or treat several human pathologies, are the major font of innovative bioactive molecules.

In the last years, the concept of dietary chemoprevention is gaining increasing attention and numerous natural compounds have recently been suggested as potential therapeutic agents. In this context, Morin is a very interesting molecule with healing properties. One of the peculiarity of Morin is its strong antioxidant and antiradical activity. Differently to other polyphenols, that can generate ROS by undergoing auto-oxidation reactions [157], Morin retains its antioxidant character also at high concentration. Morin is not toxic for animals for doses up to 300 mg/Kg of body weight/day [158], and it is highly effective in protecting cells from oxidant insults generated by oxidants, xenobiotics, excess of metals or radiations. ROS can modulate the function of several transcription factors, influencing the expression of genes involved in the synthesis of pro-inflammatory cytokines, and can regulate cell survival and proliferation. This evidence can explain why Morin may act also as a potent anti-inflammatory and antitumoral agent. Since chronic oxidative stress contributes to onset of diabetes, kidney failure, hypertension and neurodegenerative diseases, we can suppose that Morin could exert a preventive activity toward such pathologies. Thus, the ROS scavenger activity of Morin can alone justify many of the observed beneficial pharmacological activities.

Nevertheless, the direct interaction of Morin with protein targets or enzymes, strongly contributes to the modulation of signalling pathways involved in the regulation of cells physiology or metabolism. The high number of protein targets identified, suggest that Morin can simultaneously modulates the activity of different signalling pathway, thereby influencing several cellular functions. Finally, Morin is able to inhibit some cellular membrane efflux transporters, as well as some enzymes belonging to cytochrome P450 family. The use of Morin to increase absorption and effectiveness of drugs has been proposed and beneficial effects have been demonstrated.

In summary, to date a systemic analysis of Morin effects on human patients is still lacking. The few tests performed on human patients confirm the data obtained with animal models [27] and, despite

they are still insufficient to elect Morin as a true natural drug, they are an excellent starting point for further investigations.

Abbreviations:

PTP1B, phosphotyrosine protein phosphatases 1B; TNFα, alpha-tumor necrosis factor; IL1β, interleukin 1 beta; IL-6, interleukin 6; ROS, reactive oxygen species; CD36, thrombospondin receptor; Bcl-2, B-cell lymphoma-2; COX-2, cyclooxygenase-2; CYP, cytochrome P450; H_2O_2, hydrogen peroxide; IkBα, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; LPS, Lipopolysaccharides; MMPs, matrix metalloproteinases; NFkB, nuclear factor kappaB; PI3K, phosphatidylinositol-3-kinase; PPAR, peroxisome proliferator-activated receptor; STAT, signal transducers and activators of transcription; LDL, Low Density Lipoprotein; AMPA receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; DNA, Deoxyribonucleic acid IL8, interleukin 8; RAW 264.7, macrophage cell line from Mus musculus, mouse; Sphk1, sphingosine kinase 1; iNOS, Inducible nitric oxide synthase; Syk, Spleen tyrosine kinase; IgE, immunoglobuline E; PCA, passive cutaneous anaphylaxis; Fyn, Src family tyrosine kinase; LNCaP, androgen-sensitive human prostate adenocarcinoma cells; Akt/PKB, protein kinase B; KCl, Potassium Cloride; ATPase, adenosine 5'-triphosphatase; PC12, pheochromocytoma of the rat adrenal medulla cell line; Aβ42, amyloid beta 1-42; Ki, inhibition constant; RepA, replicase A; TGF-β1, Transforming growth factor beta 1; α-SMA, Alpha Smooth Muscle Actin; ALT, Alanine transaminase; AST, aspartate aminotransferase

Conflict of interest statement

The authors declare no conflicts of interest.

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Table I: Inhibitory effect of morin on potential physiological substrates

Enzyme or protein	IC_{50} (μM)	Ki (μM)	Reference
Fyn kinase	5.7		[86]
Trypsin	27		[159]
Topoisomerase I	139		[160]
Topoisomerase II	135		[160]
Fatty acid synthase	2.3		[161]
Xantine oxidase		7.9	[132]
ATP synthase (E.coli)	70		[150]
Esterase	1.8		[162]
Beta-site APP Cleaving Enzyme-1, β-secretase	21.7		[148]
ATP synthase (rat)	60		[163]
Monocarboxylate transporter 1	6.4		[164]
Glycogen synthase kinase β	1-10		[141]
Aldose reductase	~ 1		[165]
Peroxisome proliferator-activated receptor-α	8.6		[166]
Peroxisome proliferator-activated receptor-β	16		[166]
Peroxisome proliferator-activated receptor-γ	43		[166]
PTP1B		5.9	[28]
TC-PTP	19		[28]
YopH	5		[28]
IF1	3.6		[28]
IF2	66		[28]
LTP1	99		[28]
Acetylcholinesterase	210		[147]
Sortase A	37.4		[127]
Sortase B	8.5		[127]
Cytochrome P4502C9		1.8	[101]
URAT1		17	[134]
Glutathione S-transferase P1-1	> 50		[167]
Glutathione Reductase	118.7		[168]
HIV-1 Proteinase	24		[169]

Multidrug resistance proteins 1	49		[170]
Multidrug resistance proteins 2	> 50		[170]
Cytochrome P4502C9 (CYP1A2)	9.5		[100]
DNA helicase RepA	45	18.1	[128]
Human serum albumin	9		[154]
Phospholipases A_2	~ 5		[171]

Figure Legends

Fig. 1. Molecular mechanism of insulin sensitizing action of Morin. Morin, in the free form or complexed with Zn^{2+} ions, passes through the plasma membrane. Morin and Zn^{2+} ions interact with enzymes involved in the negative regulation of insulin receptor, such as PTP1B and/or other PTPs .

Fig. 2. Antioxidant, anti-inflammatory and antitumoral activity of Morin. Morin contributes to neutralize the negative effects of ROS. Besides to inhibit ROS production, Morin stimulates expression of several antioxidant enzymes (*), decreasing the intracellular ROS levels and NF-kB transcriptional activity. Morin contributes to also inhibit P-glycoproteins activity enhancing the effectiveness of chemoterapy drugs. As a consequence, Morin sensitizes cancer cells against apoptosis and inhibits cell migration and invasion. Finally, through inhibition of Cytochrome P450 family enzymes Morin, inhibits activation of pro-carcinogenic molecules.

Fig. 3. Antibacterial and neuroprotective effects of Morin.

Figure 1

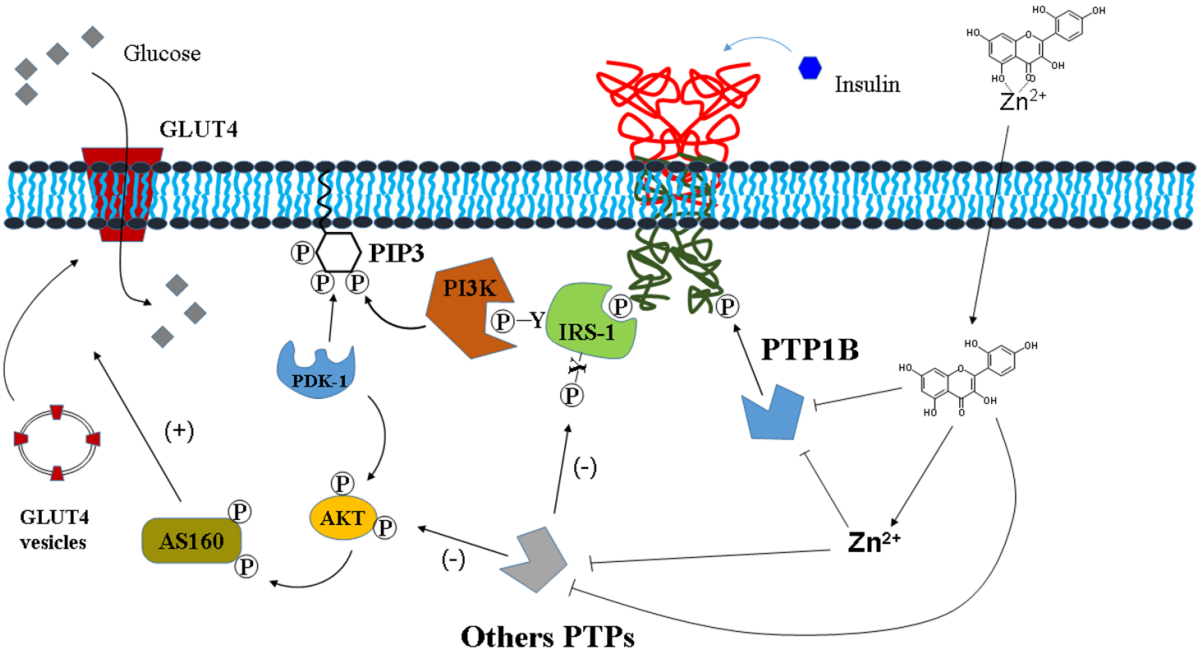

Glucose
GLUT4
Insulin
Zn2+
PIP3
PI3K
IRS-1
PTP1B
PDK-1
(+)
(-)
GLUT4
vesicles
AS160
AKT
Zn2+
(-)
Others PTPs

Figure 2

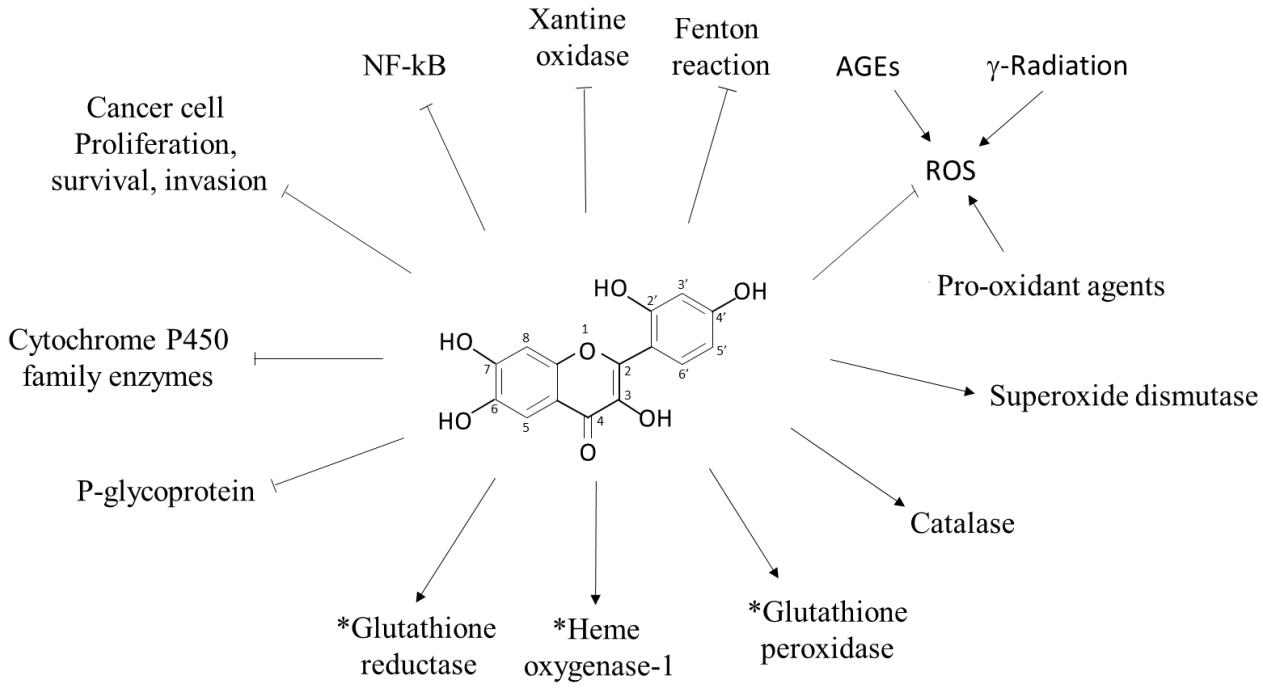
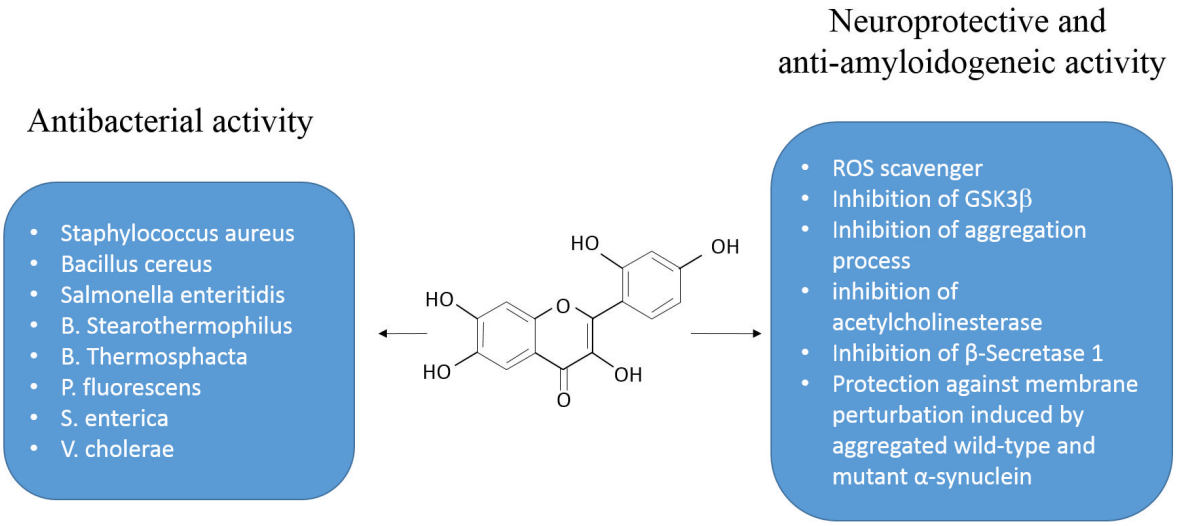

Xantine oxidase
Fenton reaction
NF-kB
AGEs
γ-Radiation
Cancer cell Proliferation, survival, invasion
ROS
Pro-oxidant agents
Cytochrome P450 family enzymes
Superoxide dismutase
P-glycoprotein
Catalase
*Glutathione reductase
*Heme oxygenase-1
*Glutathione peroxidase

Figure 3


Antibacterial activity
Staphylococcus aureus
Bacillus cereus
Salmonella enteritidis
B. Stearothermophilus
B. Thermosphacta
P. fluorescens
S. enterica
V. cholerae
HO
OH
HO
O
HO
OH
O
Neuroprotective and anti-amyloidogeneic activity
ROS scavenger
Inhibition of GSK3β
Inhibition of aggregation process
inhibition of acetylcholinesterase
Inhibition of β-Secretase 1
Protection against membrane perturbation induced by aggregated wild-type and mutant α-synuclein