ROS

REVIEW ARTICLE

A Review on the Antioxidative and Prooxidative Properties of Luteolin

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ABSTRACT | Luteolin is a natural flavonoid compound mainly present as glycosides in vegetables, fruits, and herbs including carrot, cabbage, artichoke, tea, celery, and apple. Evidence has revealed that luteolin possesses profound biological properties such as antimutagenic, antitumorigenic, antioxidative, immunomodulatory, anticarcinogenic, antibacterial, antiapoptotic, and anti-inflammatory capacities. In particular, luteolin helps preserve the oxidation and antioxidation balance, the disruption of which plays a crucial role in the pathophysiologic processes of various diseases including cardiovascular disorders, cancers, and neurodegenerative diseases. This mini-review briefly summarizes the established beneficial effect of luteolin with regard to the maintenance of balance between pro- and anti-oxidation. We will provide an overview of luteolin and its therapeutic application in cardiovascular diseases, cancers, and neurodegenerative diseases.

KEYWORDS | Antioxidative activity; Luteolin; Oxidative stress; Reactive oxygen species; Redox balance

ABBREVIATIONS | ATP, adenosine triphosphate; ER, endoplasmic reticulum; HCC, hepatocellular carcinoma; IMM, inner mitochondrial membrane; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinase; MPTP, mitochondrial permeability transition pore; NOX, NADPH oxidase; OMM, outer membrane of mitochondria; ROS, reactive oxygen species; SOD, superoxide dismutase

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1. INTRODUCTION

Reactive oxygen species (ROS) are commonly defined as a class of small and highly reactive chemical species resulting from incomplete one-electron reduction of oxygen (e.g., superoxide, hydroxyl radicals, and peroxides such as hydrogen peroxide) [1, 2]. In physiological settings, ROS are produced through both non-enzymatic and enzyme-catalyzed reactions. Electrons are transmitted through respiratory chain reactions to complete oxidative phosphorylation in mitochondria [3, 4]. An electrochemical gradient is established across the inner mitochondrial membrane (IMM) in this process for the production of adenosine triphosphate (ATP). Cellular ROS are commonly generated through mitochondrial respiratory chain in most tissues. Steady-state ROS are maintained at nontoxic levels through various antioxidant and repair enzymes [2, 5, 6]. The delicate balance between oxidative stress and antioxidant defenses appears to play an essential role in cell survival, proliferation, and cell death [7, 8]. In pathological conditions, accumulation of ROS can result in the oxidization and damage of proteins, DNA, and lipids, resulting in pathological sequelae such as oxidative stress, inflammation, apoptosis, and autophagy [2]. On the other hand, cells generate non-enzymatic or enzymatic anti-oxidative defensive factors including superoxide dismutase (SOD), catalase, glutathione, thioredoxin, catalase, and peroxidases to scavenge ROS and combat oxidative stress [2, 9]. This oxidative/anti-oxidative balance plays a crucial role in the physiological processes of various organisms and disruption of this balance can lead to a number of diseases including cardiovascular disorders, cancers, and neurodegenerative diseases [2, 4].

Flavonoids are a class of hydroxy derivatives found ubiquitously in various kinds of vegetables, fruits, and herbs. It has been well known that fruit and vegetable intake is closely related to risk reduction of various forms of cancers and cardiovascular diseases [10]. While this protection seems to be of primary benefit from β-carotene and ascorbate, flavonoid ingredients should not be discounted. Luteolin (3′,4′,5,7-tetrahydroxyflavone) is a natural, soluble flavone found in high concentrations in carrot, cabbage, artichoke, tea, celery, and apple (**Figure 1**). Accumulating evidence has depicted an important role for luteolin in an array of biological processes such as cardiovascular regulation, antimutagenic, antitumorigenic, antioxidative, immunologic, anticarcinogenic, antibacterial, and antiapoptotic activities, as well as anti-inflammatory properties [11]. Epidemiological research has validated that luteolin intake may play a vital role in the reduced prevalence of certain chronic diseases, while prospective clinical studies are still warranted to further consolidate its clinical benefit [12, 13]. This mini-review summarizes recent research discoveries on luteolin concerning primarily its regulatory role in the balance of antioxidative and prooxidative functions and its protective effects in cardiovascular disorders and other chronic diseases.

2. BASIC CHEMICAL STRUCTURE AND FUNCTION OF LUTEOLIN

Luteolin is a natural flavonoid compound found mostly in the form of glycosides, among various medicinal plants (**Figure 1**). These plants have higher content of whole leaves of blue orchid, honeysuckle, and perilla [12]. It was originally isolated from leaves, stems, and branches of the genus *Resedaceae* and *Reseda odorata* L. Levels of luteolin are generally lower than some other flavonols such as quercetin and kaempferol [11]. However, the levels may be much higher in some plants including sage, artichokes, parsley, wild carrots, thyme, and peanut hulls [11]. The median consumption of luteolin is approximately 0.01–0.2 mg/day through daily diet [14]. Luteolin is generally absorbed through intestinal mucosa after oral administration. For example, with a quantity of 14.3 mg/kg luteolin taken orally, a maximal plasma concentration (Cmax) may be achieved within 1.02 ± 0.22 h, with a Cmax of 1.97 ± 0.15 μg/ml, and a half-life of 4.94 ± 1.2 h [15].

Since flavonoids possess large π bonds and super delocalization, their oxygen atoms are highly coordinated to form coordination complexes with ease. Moreover, the efficacy of diverse flavonoids varies according to its glycosidic moieties, hydroxylation pattern, conjugation between the aromatic rings, and methoxy groups [16]. For instance, the ability to scavenge peroxyl radicals of luteolin significantly exceeds kaempferol, which attributes to the 3′,4′-catechol structure in the B-ring. Consequently, lipid peroxidation inhibition of luteolin is greatly enhanced, although luteolin and kaempferol share identical hydroxyl configurations [17–19].

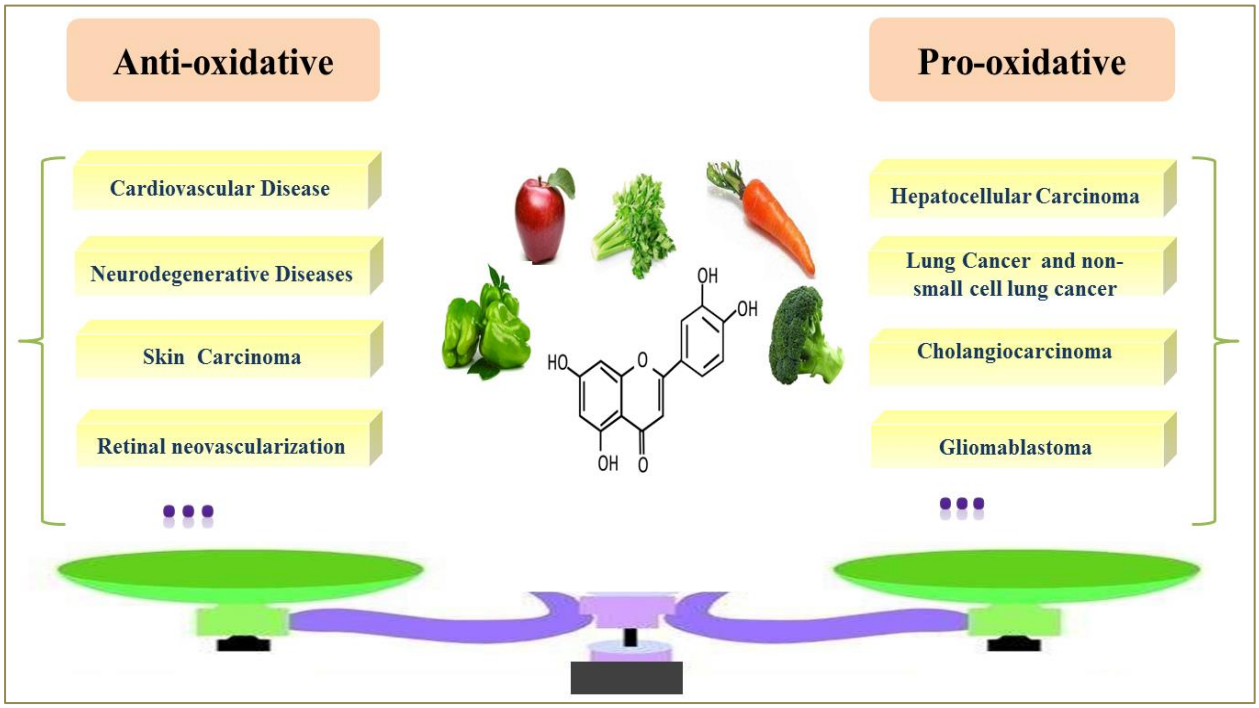

FIGURE 1. The chemical structure of luteolin and its role in maintaining the redox balance between antioxidative and prooxidative properties in protecting against various pathological processes. The major dietary sources of luteolin are also shown in the scheme. See text for detailed discussion on how luteolin protects against disease processes via its redox properties.

Luteolin is one of the most effective flavonoids, given its well-characterized structure and pharmacological properties [11]. Nonetheless, the pharmacokinetics of luteolin is less clear. It is reported that luteolin is hydrolyzed and absorbed in small intestines, where it undergoes hydrolysis to yield luteolin aglycone, and methyl- and sulfate conjugates [20]. Additionally, the absorption of luteolin may vary considerably among foods, due to the heterogeneity of sugars, dosages, vehicles of administration, gender, and microbial population of the colon [16]. Furthermore, bioavailability requires a quantitative estimate for its storage, after biotransformation to glucuronide. Although in vivo studies have revealed the beneficial effect of luteolin following intraperitoneal injection or oral intake, molecules of luteolin glycosides are cleaved during the process of uptake and are unable to be detected in the plasma. To this end, results from most in vitro studies should be considered with special caution.

3. ANTIOXIDATIVE PROPERTIES OF LUTEOLIN

Cumulative evidence has suggested that ROS play a crucial role in the pathogenesis of various diseases including cardiovascular disorders, neurodegenerative diseases, and cancer. The production and aggregation of ROS cause DNA damage, protein cross-linking, and lipid peroxidation, which contribute to mutagenesis, carcinogenesis, cellular aging [21], destabilization of cell membranes [17], and oxidation of low-density lipoprotein (LDL) [22–24]. These oxidative stress conditions are accompanied with the loss of mitochondrial transmembrane potential and the opening of mitochondrial permeability transition pores (MPTP) in the IMM. The increased opening of MPTPs leads to reduced ATP synthesis [25]. Simultaneously, the loss of mitochondrial transmembrane potential may result in the disruption of the outer membrane of mitochondria (OMM). Consequently,

the external leakage of apoptogenic factors, including cytochrome c and caspases, into the cytosol results in cell death [26].

In the past few decades, cellular oxidative and antioxidative balance has become an important factor for the maintenance of physiological functions of humans and other organisms. Novel cytoprotective treatment strategies geared towards the oxidative/antioxidative balance may effectively improve the pathological processes. Screening for drugs or compounds, such as luteolin, with a known oxidative/antioxidative balancing property or a multi-target activity would aid in providing an effective regimen for various diseases including cardiovascular diseases, neurodegenerative disorders, and cancer.

A plethora of experimental evidence has revealed the antioxidative properties for luteolin. As shown in **Figure 2**, luteolin was demonstrated to suppress oxidative damage and lipid peroxidation, and loss of antioxidant enzymes including catalase and SOD. It was also shown that luteolin attenuated lipopolysaccharide-induced pulmonary injury via decreasing NF-κB and mitogen-activated protein kinase (MAPK) expression [27]. Furthermore, luteolin possessed the highest DNA-protective effect against H_2O_2 [28]. Radio-protective studies revealed that luteolin possessed a remarkable protective property against gamma-ray-induced DNA damage [29]. In addition, an investigation in human melanoma HMB-2 cells demonstrated DNA protective potential of luteolin in scavenging free radicals and reducing damage caused by H_2O_2 in a dose-dependent manner. It was shown that luteolin decreased the incidence of chromosomal aberrations more effectively than quercetin [30]. Research has suggested that ROS generation drastically increased when human bronchial epithelial cells (BEAS-2B) were exposed to Cr(VI) after a short time. Luteolin could reduce lipid peroxidation, NADPH oxidase (NOX) activation, and glutathione depletion in a dose-dependent manner (**Figure 2**). Further studies also indicated that luteolin protected against carcinogenesis through scavenging ROS and impacting multiple cell signaling pathways related to ROS [31].

It has been found that luteolin can form complexes with metal ions such as magnesium, iron, and zinc, and has potent free radical scavenging capacity. The ability to chelate metal ions of luteolin attributes to the possession of an *o*-di-OH catechol group in the B ring which is strongly associated with its antioxidative property [11]. Furthermore, luteolin may protect or enhance intracellular antioxidative enzymes, such as catalase, SOD, glutathione reductase, and glutathione *S*-transferase [32]. It was found that mice treated with luteolin exhibited significantly lowered incidence of rectal crypt lesions and upregulated levels of catalase, SOD, glutathione reductase, and glutathione *S*-transferase during the azoxymethane-induced colon carcinogenesis [33]. Moreover, luteolin may interfere with the oxidative production of some cellular components, probably through the inhibition of corresponding enzymes [34].

4. PROOXIDATIVE PROPERTIES OF LUTEOLIN

Luteolin primarily plays a preventative role in human nutrition and diseases due to its antioxidative properties through mechanism summarized in **Figure 2**. Meanwhile, luteolin may possess a prooxidative activity in protecting against some cancers. In normal conditions, luteolin as an antioxidant has weak prooxidative abilities and cannot undergo redox cycling. Some phytochemicals have displayed potent prooxidative action, leading to an oxidative stress environment that cannot be tolerated by cancer cells, ultimately resulting in subsequent cell death [35]. Similarly, the aforementioned study has found that luteolin also exerts its prooxidative effect on cholangiocarcinoma cells, lung cancer cells, glioblastoma cells, and melanoma cells [36–39]. This may mean that it exhibits selective cytotoxicity to cancer cells with no effect on normal cells in preclinical testing. Previous studies have revealed that luteolin plays its proapoptotic role by disrupting the oxidative/antioxidative balance, which leads to severe ROS accumulation, the loss of mitochondrial membrane potential, and endoplasmic reticulum (ER) stress, activating mitochondrial apoptotic pathways and causing cell death (**Figure 2**) [26].

The mechanism of the prooxidative capacity of luteolin is thought to be related to the number of hydroxyl groups [16, 40]. Hanasaki and colleagues once reported that a series of mono- and di-hydroxy flavonoids had no detectable prooxidative capacity, while the compounds with multiple hydroxyl groups, especially in the B-ring, could significantly increase the production of hydroxyl radicals in the Fenton system [41]. Other studies revealed that luteolin may

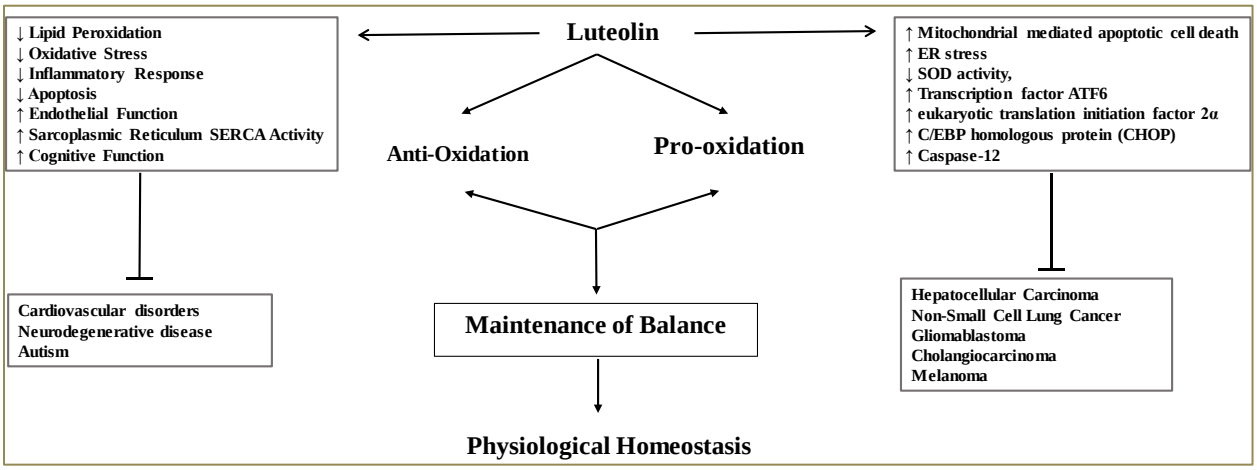

FIGURE 2. Possible mechanisms contributing to luteolin-mediated regulation of the balance of antioxidative and prooxidative functions and its protective role in cardiovascular and other chronic diseases. SERCA denotes sarco(endo)plasmic reticulum Ca^{2+}-ATPase. See text for detailed discussion.

potentiate the increase of ROS generation and the activation of caspase-9 and caspase-3, accompanied by the release of cytochrome c and the decreased Bcl-2 and Bcl-XL proteins in KKU-100 cholangiocarcinoma cells [26] (also see **Figure 2**). More in-depth work is needed to further define the mechanism behind the prooxidative properties of luteolin.

In addition to its participation in redox regulation, luteolin may exert an array of additional biological functions, including cardiovascular regulation, anti-inflammatory activity, antibacterial effect, antitumor action, and immune regulation. It is known that luteolin may suppress macrophage activation and inhibit the inflammation in endothelial cells [42, 43]. An independent study revealed that luteolin inhibited niacin-induced flush [44, 45], and increased insulin sensitivity in endothelial cells [42]. Luteolin also retarded peroxidation of low-density lipoprotein (LDL) in diet-induced obesity [46], as well as prevented against high fat-diet-induced cognitive deficits in mice [47].

5. APPLICATION OF LUTEOLIN IN CARDIOVASCULAR DISEASES

Cardiovascular diseases are known to be a widespread public health problem and a leading cause of morbidity and mortality. Considerable evidence revealed that oxidative stress plays a vital role in the pathogenesis and development of coronary heart disease, heart failure, and cardiac arrhythmias. The importance of antioxidants in the diet in the intervention and prevention of cardiovascular diseases has aroused considerable interest over the last few years. Several studies have shown that luteolin may protect against cardiovascular disorders via acting as an antioxidative agent.

A recent epidemiological study, involving individuals aged 65–99 years old with a 10-year follow-up, suggests that luteolin may have important influence on the reduced risk of acute myocardial infarction [48]. Another study, involving a cohort of 2,748 Finnish men and 2,385 Finnish women, showed that subjects with very low consumptions of flavonoids displayed higher risks of coronary heart disease. It is thought that luteolin might afford the protective effect through protection against oxidation of LDL [49]. In another population study of over 9000 Finnish residents with a 28-year follow-up, it was reported that the risk of cardiovascular disease risk could be related to apple intake, but not intake of quercetin which is the main flavonol component of apples [50]. A United States and European population cohort study also reported that the intake of flavonoids is strongly related to a lower incidence of mortality of coronary heart disease [51]. Another double-blind, randomized, placebo-controlled study showed that

the polyphenolic compound, pycnogenol, could improve the endothelial function in coronary heart disease [52]. Based on the aforementioned studies, it appears that nutritional intake of luteolin would contribute to the protective effect against cardiovascular disease due to its capacity of inhibiting lipid peroxidation, chelating redox-active metals, and attenuating other processes involving ROS.

Data from preclinical studies were consistent with the above-mentioned epidemiological studies. Xia and colleagues reported that luteolin may attenuate the development of atherosclerosis by protecting endothelial cells from TNF-α-induced oxidative stress and inflammatory response through NF-κB and MAPK signaling. This research revealed that luteolin decreased the production of ROS and the expression of NOX4 and $p22^{phox}$ expression. While the exact mechanism needs further investigation, luteolin treatment led to down-regulation of NOX4 and decreased BCL-2, ICAM-1, and VCAM-1 [53]. In another study, luteolin was found to possess potent protective actions in cardiomyocytes subjected to simulated ischemia/reperfusion injury. Several mechanisms, including inhibiting oxidative stress, improving cardiomyocyte contractile function, and activating PI3K/Akt pathways, were speculated to participate in luteolin-afforded cardiac benefits [54]. It was also found that luteolin exerted protection against the prooxidant pyrogallol-induced superoxide accumulation and subsequent apoptosis in cardiac capillary endothelial H5V and human umbilical vein endothelial cells. It was suggested that luteolin might be capable of up-regulating Bcl-2 expression and suppressing the expression of caspase-3 and caspase-8, and reducing apoptosis via MAPK signaling pathway [55].

Based on the above discussion, luteolin appears to play a cardioprotective role. The protective effect may stem from the maintenance of oxidative/antioxidative balance and the interaction with mitochondrial function by suppressing the phosphorylation of JNK and p38 MAPK and up-regulating the expression of MnSOD [56]. In addition to the antioxidative action, luteolin also promotes sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) activity and improves systolic or diastolic function during cardiac ischemia/reperfusion injury and heart failure in rats [57, 58]. Several mechanisms have been suggested for luteolin-induced cardiac responses, including phosphorylation of protein kinase B (Akt), phospholamban, and p38 MAPK [59, 60]. Thus, luteolin could be considered as a candidate for therapeutics against cardiovascular diseases.

6. APPLICATION OF LUTEOLIN IN CANCER

Investigation has been ongoing for several decades regarding the biological functions of ROS and their potential involvement in the development and disease progression of cancer [61]. Cancer cells are known to be in a more active metabolic condition and undergo increased oxidative stress, which is possibly related to the uncontrolled cell proliferation and metabolic regulation dysfunction. Since the overproduction of ROS disrupts the balance between oxidation and antioxidative defense systems, it is possible that the apoptotic induction of cancer cells is one of the direct approaches to attenuating the development and progressing of cancers [62].

The prooxidative properties of flavonoids have been observed in various cell types [63, 64]. A recent study demonstrated that both luteolin and kaempferol could induce apoptosis in cells isolated from hepatocellular carcinoma (HCC), but not normal cells. The apoptosis of HCC cells was subsequent to the reduction of cytochrome c and caspase-3 activation. Luteolin was suggested as a potential candidate for the complementary treatment of HCC [36]. Another research also showed that luteolin inhibited cancer cell growth and induced cell death in HepG2 hepatocarcinoma cells via the release of ROS and AMPK-NF-κB signaling [65].

Another study also found that luteolin played a prooxidative and proapoptotic role in KKU-100 cholangiocarcinoma cells [66], which could be attributed to its ability to promote intracellular ROS production and subsequently activating the mitochondria-mediated apoptotic cell death. These effects were related to the suppression of Nrf2, heme oxygenase-1 (HO-1), and gamma-glutamylcysteine ligases, mitochondrial depolarization, release of cytochrome c, and the decrease of Bcl-2 and Bcl-xl [26]. In addition, the mechanism of ROS accumulation induced by luteolin has also been investigated in lung cancer cells, which seem to be involved in the decrease of SOD activity [37]. A proteomic study in human hepatoma Huh-7 cells revealed that several proteins were associated with the anti-cancer effects of luteolin. Interestingly, these proteins included

peroxiredoxin 6 (PRDX6) and prohibitin, which are implicated in ROS metabolism and apoptosis [66].

A study examined the sensitivity to the ionizing radiation in non-small cell lung cancer cells and revealed a radio-sensitizing role for luteolin due to its induction of apoptotic cell death through p38-ROS-caspase cascade, an effect independent of activation of p53 and phosphatase and tensin homolog (PTEN) [67]. Furthermore, luteolin was found to promote ROS accumulation, which led to ER stress in human melanoma cells through up-regulated expression of ER stress-associated factors, including protein kinase RNA-like ER kinase, activating transcription factor (ATF) 6, phosphorylation of eukaryotic translation initiation factor 2α, C/EBP homologous protein (CHOP), and cleaved caspase-12 [39]. Work in cultured glioblastoma cells and in vivo suggested similar activities of luteolin through an ERK-eIF2α-ATF4-CHOP-caspase 12-meditaed mechanism [38]. These studies indicated that luteolin promoted ROS accumulation to cause apoptosis, ER stress, and mitochondrial dysfunction in various carcinoma cells. To this end, luteolin may have the potential to become an antitumor drug through its prooxidative characteristics.

7. APPLICATION OF LUTEOLIN IN NEURODEGENERATIVE DISEASES

Under normal metabolic conditions, there are levels of ROS generated in the mitochondria which are rapidly scavenged by enzymatic and non-enzymatic antioxidants. The maintenance of oxidative and antioxidative balance prevails in various tissues and cells [68]. Nonetheless, the capacity of scavenging ROS in the brain is relatively weak, making the tissue more sensitive to oxidant stress [69, 70]. In certain pathological conditions, the cerebral tissue is susceptible to attack of increased ROS accumulation, which inflicts the peroxidation of membrane polyunsaturated fatty acids and proteins and also increases membrane permeability [71]. Therefore, the increased oxidative stress may be one of the major pathogenic causes in the development and progression of neurodegenerative diseases, including cerebral ischemia/reperfusion injury, Alzheimer's disease, and Parkinson's disease, as well as aging [72, 73]. The antioxidative property of luteolin provides a basis for its use in treating neurodegenerative diseases.

Some studies have shown a great capacity of luteolin to protect neurons against oxidative stress. Luteolin could exert protective effects on oxidative attack-related events including increased formation of endogenous free radicals, loss of mitochondrial membrane potential, and mitochondrial dysfunction [74]. Under chronic cerebral hypo-perfusion conditions, luteolin administration also protected rats against the cognitive dysfunction and increased the neuron survival via decreasing intracellular ROS accumulation and up-regulation of Nrf2-ARE pathway [75]. Another research also suggested that luteolin protected against the high-fat diet-induced cognitive dysfunction in mice possibly through its antioxidative function [47]. Additionally, luteolin treatment improved the cognitive function and neuronal damage in both cortex and hippocampus due to its antioxidative activity in a streptozotocin-induced diabetes model [76].

Alzheimer's disease is a complicated disease associated with multiple genetic and environmental factors. In addition to the formation of senile plaques and neurofibrillary tangles due to amyloid-β and tau proteins, a growing body of evidence suggests that oxidative stress and mitochondrial dysfunction are possibly involved in Alzheimer's disease. Zhou and colleagues reported that luteolin efficiently attenuated zinc-induced tau hyperphosphorylation and decreased oxidative stress in Alzheimer's disease. Luteolin was considered as a nontoxic and highly bioavailable dietary flavonoid for protecting against chronic neurological damage induced by zinc toxicity [77, 78].

8. APPLICATION OF LUTEOLIN IN OTHER DISEASES

A number of studies have indicated that ultraviolet light induces generation of harmful ROS burst, contributing to the development of human skin carcinoma. Considering luteolin's capacity to penetrate into deep skin layers, it may be useful in counteracting the oxidative stress as well as affording anti-inflammatory effects in skin carcinoma [34]. Another potential protective effect of luteolin was shown in children with autism, where a formulation containing luteolin significantly improved children's attention and behavior [79]. It should be mentioned that luteolin is marketed and advertised as a dietary supple-

ment in humans. Moreover, luteolin is a potential antiangiogenic treatment for retinal neovascularization, which is associated with its antioxidative capacity to decrease ROS and subsequently proangiogenic function of vascular endothelial growth factor [80].

9. SUMMARY AND CONCLUSION

Luteolin exerts various beneficial biological actions through its antioxidative properties. These effects are likely attributable to a maintenance of oxidative and antioxidative balance. In order to ensure the safety and efficacy of luteolin, more research is warranted prior to the full validation of luteolin as an antioxidative agent. The beneficial effects of luteolin, either alone or in combination with another medicinal compound, should be directed to treat diseases associated with profound oxidative stress. It is likely that luteolin may possess a therapeutic value for multiple pathological conditions.

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