Abstract

Flavonoids exhibit various neuroprotective actions. They have a potential to protect neurons against injury induced by neurotoxins in brain. Flavonoids also suppress neuro-inflammation and promote memory, learning and cognitive function. Oxidative stress is the well accepted concept in the etiology and progression of neurodegenerative diseases. Thus the therapeutic agent is targeted against suppressing and alleviating the oxidative stress-induced cellular damage. Flavonoids are reported to possess neuroprotective properties. In the present paper we reviewed the literature on the neuroprotective mechanism of flavonoids in protecting the dopaminergic neurons. Various mechanisms like flavonoids as a mitochondrial target therapy, effect of flavonoids in suppressing the lipid peroxidation, activation of intracellular antioxidant enzymes are reviewed.

Keywords: Flavonoids, neuroprotective mechanism, neurodegenerative diseases.

Introduction

Flavonoids may act to protect the brain in a number of ways, including by protections of vulnerable neurons, the enhancement of existing neuronal function or by stimulating neuronal regeneration. Regular dietary intake of flavonoids-rich foods or beverages has been associated with 50% reduction in the risk of dementia, a preservation of cognitive performance with aging, a delay in the onset of Alzheimer’s disease and a reduction in the risk of developing Parkinson’s disease. Flavonoids are now becoming valuable pharmacological drugs, due to their low toxicity. Dementia is a serious degenerative disease affecting predominantly elderly people with the two most common forms of this illness being Alzheimer’s and vascular dementia. The factors affecting dementia are age, hypertension, arteriosclerosis, diabetes mellitus, smoking. There are evidence to suggest that flavonoids may be capable of preventing many forms of cerebrovascular disease, including those associated with stroke and dementia.

Parkinson’s disease results primarily from the loss of dopamine producing neurons in the nigrostriatal system. One theory for cause of Parkinson’s disease that is gaining attention is that of unstable free radicals contributing to nerve cell death. The radicals are a byproduct of oxidative stress, generated by normal chemical reactions in the body. A free radical is an atom, molecule, or compound that is highly unstable because of its atomic or molecular structure. Free radicals are often referred to as reactive oxidative species (ROS) and are byproducts of chemical reactions that mostly occur in the mitochondria. Under certain conditions, the number of ROS produced exceeds the capacity of the removal mechanisms. This process is termed oxidative stress. As a result of this failure, these very reactive oxidative species attempt to pair with other molecules, atoms, or individual electrons to create a stable compound.

Several studies provide evidence that one of the main targets of this process occurs with genetic material. Researchers revealed the increases of norsalsolinol, an endogenous neurotoxin present in dopamine-rich areas, affected cytochrome c release and caspase 3 activation.
in such a way that it induced ROS and resulted in apoptosis\(^8\). It is demonstrated in cell cultures and mice that a deficiency in MTH1, an oxidized purine nucleoside triphos-phatase, is strongly associated with the accumulation of 2-deoxy-8-oxoguanosine triphosphate in both nuclear and mitochondrial DNA, thus contributing to the increase of ROS from oxidative stress\(^9\). These findings have been supported in studies involving human Parkinson’s disease patients that show that MTH1 suppresses cell death caused by oxidative stress\(^8\)-\(^9\). It is evident that ROS from oxidative failures play a significant role in Parkinson’s disease.

**Neuronal Protective Activity of flavonoids**

Researchers\(^10\) investigated that flavonoid intake could be associated with a minor incidence of dementia. For that purpose, between 1991 and 1996 they used a group of individuals aged over 65. After statistical analysis of the data, it was observed that the relative risk of suffering dementia was significantly lower in those people who consumed the larger amount of flavonoids. Oxidative stress has been associated with neuronal loss in neurodegenerative diseases and during age related cognitive decline. Epicatechin and its 3’-O-methyl epicatechin metabolite inhibit neuronal toxicity in vitro induced by oxidised low density lipoproteins, thus inhibiting the activation of protein-kinases and the caspase-3-like protease activity in neurons. Thus\(^11\) they can be used as protective agents against neuronal apoptosis caused by oxidative stress. Flavonoids have been suggested to exert beneficial effects on the the central nervous system (CNS), such as anti-anxiety and cognitive enhancement, by stimulating of inhibiting enzyme activities /signal transduction pathways\(^12\).

**Flavonoid Therapy: Mitochondria Targeted**

In cellular aerobic respiration, in regulation of calcium ion homeostasis, in production of ROS\(^13\)-\(^14\), role of mitochondria is very vital. Onsets of age-related diseases (Parkinson’s disease and Alzheimer’s disease) are due to the dysfunctions in the physiological processes of this cytoplasmic organelle i.e., mitochondria. Accumulation and aggregation of amyloid – beta (Aβ) peptides\(^15\) results in mitochondrial dysfunction. In neurodegenerative diseases, occurrence of Aβ peptides in neuronal cell is a pathological hallmark. In neuronal cells soluble amyloid aggregates are formed. They penetrate the mitochondrial cells because they have the inherent capacity to do so. Thus, they induce neuronal death. The mitochondria-associated endoplasmic reticulum membranes is a physical connection between the membrane of the endoplasmic reticulum and the mitochondrial outer membrane, the penetration of the Aβ peptide occurs through it. So, in modulating mitochondrial dynamic function and biogenesis mitochondria targeted polyphenol therapy is an excellent approach\(^16\). Recent researches have shown that in a rotenone- induced rat model of Parkinsonism, flavonoid quercetin has the ability to repair the mitochondrial electron transport defect\(^19\).

Complex I, plays a very important role in mitochondria respiration chain\(^20\). Deficiency of this Complex I results in mitochondrial dysfunction. Neurotoxins such as 6-OHDA, MPP\(^+\) inhibit the activity of Complex I\(^21\) because when neuronal cells are exposed to these neurotoxins causes selective uptake of these toxins by the dopaminergic neurons. Excess of superoxide radical results due to decrease in Complex I and this excess are capable of overwhelming the natural antioxidant systems and causes oxidative stress and neurodegeneration. Mitochondrial ROS have ability to directly activate mediators of proinflammatory cytokine and MAPK\(^22\). This lead to various pathological conditions such as cardiovascular diseases, cancer and neurodegenerative diseases.

Genistein have protective effect on neuronal cells against oxidative damage\(^23\) and glutamate and Aβ amyloid toxicity\(^24\). Genistein exerts its protective mechanism via restoring mitochondrial membrane potential that was significantly decrease by 6-OHDA treatment in SK-N-SH neuroblastoma cells\(^25\). Naringin, a flavonoid glycoside, have antioxidant, ROS scavenging and metal chelating activities\(^26\)-\(^28\). It improves cognitive dysfunction and oxidative defense. Naringin can restore mitochondrial enzyme functions, specifically Complexes I and III activity in a murine model\(^29\). EGCG, a natural polyphenol derived from green tea, was reported to restore mitochondrial energy deficit in lymphoblast and fibroblasts from Down syndrome patients. The protective mechanism of EGCG is not clearly defined, but it is proposed that Complex I activity and ATP synthase catalytic activities have been activated beside promotion of cellular levels of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) dependent phosphorylation of Complex I. Treatment with EGCG effectively stimulated mitochondrial biogenesis in the lymphoblasts and fibroblasts Down syndrome patients via activation of the Siruitin 1 (SIRT1) dependent Peroxisome proliferator-activated receptor – γ-coactivator (PGC-1α), nuclear respirator factor-1 (NRF-1), and mitochondrial DNA content\(^30\).

**Intracellular antioxidant enzymes activation**

In neuronal cells flavonoid compounds have been found to activate the endogenous antioxidant status thus protecting them from undergoing neurodegeneration\(^31\). In upregulating the production of intracellular antioxidant enzymes such as SOD, GPx, CAT and glutathione in a 6-hydroxydopamine (6-OHDA) induced in PC-12 rat pheochromocytoma cells\(^32\)-\(^33\). Polyphenols such as quercetin glycosides, rutin and isoorquercetin have distinct features. Through elevation of intracellular glutathione level\(^24\) quercetin, fisetin, methyl gallate and propyl
gallate were also found to protect neuronal cells from oxidative stress.

Several studies have suggested that neurodegenerative diseases are due to free radical–induced oxidative stress. Free radicals in a normal state are usually detoxified by various internal antioxidant enzymes to less toxic molecules, which are then removed by various ways. Increased free radicals and oxidative stress in cells can culminate in damaging biological molecules including DNA, carbohydrates and proteins and cell death. The antioxidant enzymes are including glutathione peroxidase (GPx) superoxide dismutase (SOD), and catalase(CAT) that facilitate reactions that help to catalyze the ROS to less toxic molecules thus they play a very important role in preventing lipid peroxidation.

Genistein and naringenin elevate the antioxidant enzymes, namely superoxide dismutase and glutathione peroxidase. Antioxidant effects of quercetin and catechin are mediated by direct interaction with the GPx enzyme. These flavonoids cause modulation in the structure activity of GPx and thereby enhanced its antioxidant activity. In a similar study it was also found that addition of quercitin, rutin, kaempferol and myricetin to catalase had a direct effect of activation of catalase and this effect was attributed to the binding of these polyphenols to heme moiety of protein region of this enzyme.

**Suppression of Lipid Peroxidation in Parkinson’s Disease**

Reactive oxygen species such as the hydrogen peroxide (H$_2$O$_2$), nitrogen species (NO), hydroxyl ion(OH$^-$), superoxide anion(O$_2^-$)and alkoxyl radicals are hazardous to cells, if it is not fully catabolized to its less toxic substance by the natural antioxidant enzyme systems. As by-products of several normal cellular functions such as the mitochondrial oxidative phosphorylation system, phagocytosis and the arachidonic acid metabolism pathway free radicals and ROS are generated. Oxidative stress and lipid peroxidation which causes cellular damage are due to if the constituent build-up of ROS and free radicals cannot be supported by the various antioxidant enzyme systems. The presence of neurotoxic substances in human brain may augment the ROS induced oxidative damage. In Parkinson’s disease prolonged exposure to neurotoxins such as paraquat and 1-methyl-4phenyl-1, 2, 3, 6- tetrahydropyridine (MPTP) leads to increased generation of ROS in brain neurons as these toxic substances could not be effectively removed by the natural antioxidant enzymes in the brain. This inhibited the mitochondrial complex I system, oxidation of polyunsaturated fatty acid (PUFA), protein aggregation and DNA damage in neuronal cells. Lipid peroxidation of certain polyunsaturated fatty acids (PUFA) produces HNE(4-hydroxy-2-nonenal) as one of the by-products. HNE is cytotoxic and it is involved in various degenerative diseases. In Parkinson’s disease HNE is found to be an effective protein modifier that induces cross-linking of the monomeric $\alpha$-synuclein molecules, there by converting these proteins into high molecular weight $\beta$-sheet–rich oligomers. The $\alpha$-synuclein is usually located at the presynaptic region of neurons and is a soluble protein consisting of 140 amino acid molecules. $\alpha$-synuclein is involved in neurotransmitter secretion as well as in the regulation of synaptic vesicle pool and plasticity. By inducing lipid peroxidation in the pathogenesis of Parkinson’s disease it has been proposed that oxidative stress triggers a vicious cycle and accumulation of $\alpha$-synuclein aggregates, which forms Lewy bodies, which are associated with neuronal dysfunction that triggers the onset of Parkinson’s disease. Inhibition of lipid peroxidation in neuronal cells could help to delay the ongoing neurodegeneration process in Parkinson’s disease.

By attenuating lipid peroxidation induced by 6-OHDA on PC12 neuronal cells, quercetin glycoside derivatives, rutin, and isoquercitrin have shown potent antioxidant potential. Black tea which contains epigallocatechin (EGCG) polyphenol, was also reported to suppress lipid peroxidation in a 6-OHDA induced rat model of PD. The polyphenol theaflavin was reported to inhibit xanthine oxidase (XO), an enzyme involved in producing superoxides, hence protecting the neuronal cells from undergoing lipid peroxidation. These evidences markedly support the ability of flavonoids to exert neuroprotective roles via scavenging ROS generated during oxidative stress and subsequently suppress lipid peroxidation in neuronal cells or in animal models of Parkinson’s disease.

**Cerebral Blood Flow**

Efficient cerebral blood flow is also vital for optimal brain function, with several studies indicating that there is decrease in cerebral blood flow (CBF) in patients with dementia. Brain imaging techniques such as “functional magnetic resonance imaging” (fMRI) and “trans – cranial Doppler ultrasound” (TCD) has shown that there is correlation between cerebral blood flow and cognitive functions in humans. For example - cerebral blood flow velocity is significantly lower in patients with Alzheimer’s disease. Flavonoids have been shown to exert a positive effect on cerebral blood flow in humans. After a consumption of flavonoid rich cocoa drink, the ‘flow oxygenation level dependent’ fMRI showed an increase in blood flows in certain regions of the brain, along with a modification of the response to the task switching. Furthermore, ‘arterial spin- labeling sequence magnetic resonance imaging’ (ASL-MRI) also indicated that cocoa flavonoids increase cerebral blood flow up to a maximum of two hours after ingestion of the flavonol-
rich drink. In support of these findings, an increase in cerebral blood flow through the middle cerebral artery has been reported after the consumption of flavonol-rich cocoa using TCD.

The intense interest in the development of drugs capable of enhancing brain function means that flavonoids may represent many important precursor molecules in the quest to develop of new generation of brain enhancing drugs.

**Induction of Autophagy**

An additional mechanism for flavonoid neuroprotection relates to the modulation of autophagy. Autophagy (from the Greek “to eat oneself”) refers to the cellular degradative pathway that involves delivery of the cytoplasmic cargo to the lysosomes. Autophagy (macroautophagy) is a multistep process involving the formation of double membrane structures, the autophagosomes which fuse with lysosomes. The content of resulting autophagolysosomes (misfolded proteins, cellular metabolic waste) is then degraded by hydrolytic enzymes. Autophagy is also important for removal of damaged mitochondria and of normal mitochondria undergoing turn over, in a process known as mitophagy. The integrity of the CNS is highly dependent on normal basal autophagy, as damaged organelles and misfolded proteins would accumulate in neurons unless they are successfully removed. Rapamycin, an inhibitor of mTOR (mammalian target of rapamycin) activity, is a potent inducer of autophagy and act as neuroprotector. In contrast, deletion of key autophagy genes (Atg5, Atg7) causes severe neurodegeneration. Stimulation of autophagy in the CNS would thus lead to neuroprotection as has been shown for various compounds. Flavonoid (quercetin) has been shown to alleviate cell damage caused in Schwann cells by high glucose by inducing autophagy. Similarly in *C. elegans* the neurotoxicity of amloid beta 1-42 is antagonized by quercetin through induction of autophagy.

**Modulation of Sirutins**

An additional field of interest with regard to the mechanisms of neuroprotection provided by quercetin is that of sirutins. These proteins (in mammals there are seven, named SIRT1 to SIRT7) are involved in variety of cellular and molecular processes pathways, with distinct cellular localization and molecular targets. Of the SIRT1 predominantly localizes in the nucleus and acts as a deacetylase for histones and other targets. SIRT1 protects cells from apoptosis and promotes differentiation of stem cells. SIRT2 is prevalently in the cytoplasm has been found to accumulate in neurons, while other SIRT1s localize primarily in the mitochondria. The neuroprotective effects of flavonoids, may also involve activation of SIRT1 which would lead to suppression of Bax-dependent apoptosis and repression of multiple proapoptotic transcription factors. A recent example of the effects of quercetin on the pathway is represented by findings showing that quercetin inhibits simplex virus type 1- induced neurodegeneration by activating SIRT1.

**Conclusion**

Various researches suggested that flavonoids can exert neuroprotective effects. These results clearly indicated the antioxidant nature of flavonoids in arresting free radical – induced oxidative damage, which is known to be central to many degenerating diseases. In terms of neuroprotection ability of flavonoids appear to impede the progressive neuronal loss in neurodegenerative diseases. Flavonoids such as quercetin, rutin isoqueretin were found to increase the levels of the natural antioxidant enzymes in the cellular compartment as a bid to suppress the free radical- induced lipid peroxidation .There is an increasing interest for the potential neuroprotective effects of flavonoids and other nutraceuuticals. Thus we came to the conclusion that flavonoids may undergo two common processes. Flavonoids interact with critical protein and lipid kinase signaling cascades in the brain leading to an inhibition of apoptosis triggered by neurotoxic species and to a promotion of neuronal survival and synaptic plasticity. In the same way flavonoids induce beneficial effects on the vascular system leading to changes in cerebrovascular blood flow capable of causing angiogenesis, neurogenesis and change in neuronal morphology.

Through these mechanisms, the consumption of flavonoids rich food throughout life holds the potential to limit neurodegeneration and to prevent or reverse age dependent loses in cognitive performance. Flavonoids exerting neuroprotective properties are found in many plants. This brief review has focused on mechanisms related to the ability of flavonoids of neuroprotection. In addition, the potential role played by particular flavonoids metabolites should be examined more systematically as only limited information is available.

**Conflict of interest statement**

We decline that we have no conflict of interest.

**Acknowledgments**

We are very grateful to University Grants Commission, New Delhi, India for their financial assistance (Grant No. F.15-39/12(SA-II)). We are also very thankful to Principal, Agra College, Agra for their valuable support.

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