REVIEW ARTICLE

Microglia and regulation of inflammation-mediated neurodegeneration: Prevention and treatment by phytochemicals and metabolic nutrients

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Inflammation, a common denominator among the diverse list of neurodegenerative diseases, has recently been implicated as a critical mechanism responsible for the progressive nature of neurodegeneration. Microglias are the resident innate immune cells in the central nervous system and produce a barrage of factors (ILs, TNF α, NO, PGs, SOD) that are toxic to neurons. Evidence supports that the unregulated activation of microglia, in response to environmental toxins, endogenous proteins and neuronal death, results in the production of toxic factors that propagate neuronal injury. Herbal medicine has long been used to treat neural symptoms. Although the precise mechanisms of action of herbal drugs have yet to be determined, some of them have been shown to exert anti-inflammatory and / or antioxidant effects in a variety of peripheral systems. Now, as increasing evidence indicates that neuroglia-derived chronic inflammatory responses play a pathological role in the central nervous system, anti-inflammatory herbal medicine and its constituents are being proved to be potent neuroprotectors against various brain pathologies. Structural diversity of medicinal herbs makes them a valuable source of novel lead compounds against therapeutic targets that are newly discovered by genomics, proteomics and high-throughput screening. In the following review, we discuss the common thread of microglial activation across numerous neurodegenerative diseases, define current perceptions of how microglia are damaging neurons and explain how the microglial response to neuronal damage results in a self-propelling cycle of neuron death. This article synthesizes what we know about these destructive processes, while offering an insight into a new avenue of treatment involving phytochemicals and other nutrients.

Key words: Astrocyte, flavonoids, free radical scavengers, microglia, neuroinflammation, phytochemicals

INTRODUCTION

Over the last decade there has been an unexplained increase in the number of neurodegenerative diseases, especially prototypical neurodegenerative disorders, which include Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD) that appear to go beyond the normal ageing of the population.[1] Neurodegenerative disorders are a heterogeneous group of diseases of the nervous system, including the brain, spinal cord and peripheral nerves that have many different etiologies. Many are hereditary, some are secondary to toxic metabolic processes and others result from infections. Due to the prevalence, morbidity and mortality of the neurodegenerative diseases, they represent a significant medical, social and financial burden on the society. Neuropathologically, these are characterized by abnormalities of relatively specific regions of the brain and specific populations of neurons. The degenerating neuron clusters in the different diseases determine the clinical phenotype of that particular illness. Recent investigations in medical genetics have identified specific genes for various neurodegenerative disorders, and specially bred animal models have begun to be used to study the aetiological factors and underlying pathogenic mechanisms.[2]

Neurodegenerative disorders are characterized by progressive and irreversible loss of neurons from specific regions of the brain. Even though the pathology and the pathogenesis are distinctly different, they share a common pathogenic mechanism in the process of neuronal cell death and degradation. The common mechanisms include: (a) selective vulnerability, characterized by the exquisite specificity of the disease processes for particular types of neurons; (b) genetic predisposition, playing an important role in the aetiology of neurodegenerative disease; infectious

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.102807

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Received: 18-02-2012; **Accepted:** 13-06-2012

agents and environmental toxins have also been proposed as aetiological agents; (c) neuronal injury resulting from the presence of excess glutamate in the brain; (d) energy, metabolism and ageing; (e) oxidative stress, where neurons depend on oxidative metabolism for survival, a consequence of this process is the production of reactive compounds such as hydrogen peroxide and oxyradicals;[3] and (f) inflammation, due to increased levels of pro-inflammatory cytokines (Interleukins - IL-1, IL-2, IL-6), Interferon-γ (INF-γ), Proteases, Complement proteins, S100β, Tumour necrosis factor-α (TNF-α) and so on. These agents, other than the mediators, can lead to DNA damage, peroxidation of membrane lipids and neuronal death. Stress, hypoxia, ischaemia, metabolic alterations like atherosclerosis and diabetes and neuro-humoral changes, like hypertension, are also implicated in neurodegeneration.[1,4]

The aetiology of neurodegenerative diseases remains enigmatic; however, for defects in energy metabolism, excitotoxicity and for oxidative damage it is increasingly compelling; it is likely that there is a complex interplay between these mechanisms. A defect in energy metabolism may lead to neuronal depolarization, activation of N-methyl-D-aspartate (NMDA) excitatory amino acid-receptors and increase in intracellular calcium, which are buffered by mitochondria. Mitochondria are the major intracellular source of free radicals, and increased mitochondrial calcium concentrations enhance free radical generation. Mitochondrial DNA is particularly susceptible to oxidative stress, and there is evidence of age-dependent damage and deterioration of respiratory enzyme activities with normal ageing. This may contribute to the delayed onset and age dependence of neurodegenerative diseases. Potential therapeutic approaches include glutamate release inhibitors, excitatory amino acid antagonists, strategies to improve mitochondrial functions, free radical scavengers, and tropic factors. All these approaches appear promising in experimental studies and are now being applied to human studies.[5]

Inflammation occurs in multiple neurodegenerative diseases, where each disease has a unique pathology and symptoms. It has become increasingly evident that there are diverse triggers through which microglia are activated to exert their neurotoxicity. Interestingly, while these diverse toxins elucidate several mechanisms of microglial activation, Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation is also a common pathway through which microglia exerts neurotoxicity that is shared across these toxins. These diverse triggers of microglial activation include immunological insult, such as LPS,[6-8] environmental toxins,[9-11] endogenous disease proteins[8,12] and neuronal injury.[13-16]

In neuroinflammation, microglia and astrocytes play a critical role. Microglial cells are ubiquitously distributed in the central nervous system (CNS) and comprise up to 20% of the total glial cell population in the brain.[17,18] Although the ontogeny of microglial cells has long been debated, recent studies using monoclonal antibodies specific for microglial cells indicate that these cells are closely related to monocytes and macrophages.[19] As the primary immune effectors cells in the CNS, microglial cells migrate to the site of tissue injury or inflammation, where they respond to the invading pathogens or other inflammatory signals.[13,20] Similar to monocytes / macrophages, they also secrete inflammatory cytokines and toxic mediators, which may amplify the neuroinflammatory responses.[21,22] Astrocytes form an intimately connected network with neurons in the CNS, and they provide mechanical and metabolic support for the neurons.[23] The critical role of these cells in ion buffering and clearance of neurotransmitters is also well-established.[24,25] Upon inflammatory stimulation, astrocytes proliferate and produce diverse intercellular mediators such as nitric oxide (NO) and TNF-α.[26-28] There is growing evidence that the inflammatory mediators produced by activated astrocytes may be involved in the pathogenesis of various neurodegenerative diseases.[25-29] Thus, the activation of astrocytes and the ensuing production of toxic inflammatory mediators may need to be tightly regulated. Activation of inflammatory cells in CNS (microglia or astrocytes) may be intended to initially protect neurons. More frequently, however, activation of these neuroglial cells and inflammatory products derived from them has been implicated in neuronal destruction, commonly observed in various neurodegenerative diseases.[22] Thus, our understanding of the pathogenesis of neurodegenerative diseases may be enhanced by the elucidation of a molecular mechanism that is underlying the regulation of neuroglial activation. Among many endogenous or exogenous factors that regulate neuroglial activation and result in neuroinflammation,[30] herbal medicine has recently drawn much attention, due to its potent inhibitory effects on inflammatory responses and neuroprotective activity.[31,32] A central role of microglia and astrocytes in neuroinflammation (and potentially neurodegeneration) and a regulatory effect of herbal medicine on the inflammatory activation of the neuroglia will be discussed in this review.

In general, dying or damaged neurons have the potential to activate microglia, regardless of how the neurons were damaged (environmental toxin, endogenous disease protein, or reactive microgliosis) or the neurodegenerative disease in question. Figure 1 depicts the relationship between neuronal damage and microglial activation, and characterises how the damaged neurons activate

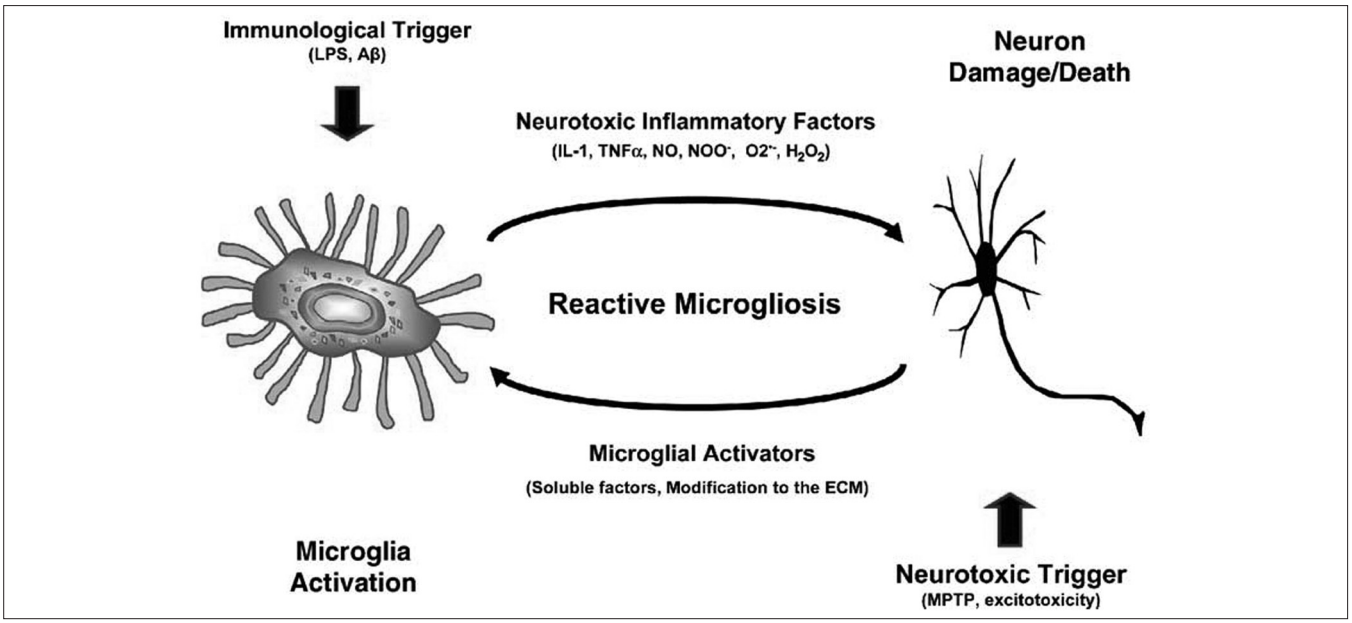

Figure 1: Reactive microgliosis is a self-propelling cycle of neuronal damage. Regardless of the initial toxic insult (immunological insult from microglia or direct neuronal toxicity), dying or damaged neurons activate microglia to produce neurotoxic factors, which are toxic to the surrounding neurons, resulting in perpetuating toxicity. As neuronal death is a common denominator across multiple neurodegenerative diseases, microgliosis may be the common thread responsible for the ongoing microglial activation and the progressive nature of many neurodegenerative diseases

microglia to initiate a self-propelling cycle of neuron-death. Previously, inflammation was viewed only as a passive response to neuronal damage. However, increasing reports demonstrate that inflammation is capable of actively causing neuronal death and damage, which then fuels a self-propelling cycle of neuronal death. Thus, while the triggers of various neurodegenerative diseases are diverse, inflammation may be a basic mechanism driving the progressive nature of multiple neurodegenerative diseases. Several cell types have been listed as contributors to inflammation-mediated neurodegeneration, but microglia are implicated as critical components of immunological insult to neurons. In the following review, we discuss the role of microglia in neuronal death and describe the evidence implicating microglia as a critical mechanism driving the self-propelling nature of neurodegenerative disease.

INFLAMMATION AND TISSUE INJURY

Injury, trauma, or infection induce a series of complex and interconnected reaction sequences, initiated at the site of tissue damage.[33,34] This sequence of reactions serves to contain and destroy the infection or damaging agents, and to prevent continued tissue damage and initiate repair processes to restore normal function. This rapid response is known as acute inflammation.[35] The toxic reactions, which are employed to destroy infectious organisms or protect the host, also paradoxically have the capacity to injure the host tissues. If these toxic responses are not tightly regulated, tissue injury may predominate over tissue protection and repair, thereby, leading to inflammatory diseases. The characteristics of the inflammatory response include localized changes within the damaged tissue, such as: (1) the release of preformed inflammatory mediators from the intracellular stores; (2) the initiation of a reaction cascade through the activation of soluble plasma components; (3) the new synthesis of inflammatory mediators such as eicosanoids and cytokines and (4) resolution of the inflammatory response. The acute inflammatory response is beneficial to the organism, in that, it helps to deal with potentially dangerous microorganisms. However, inflammation does cause some degree of damage to the surrounding tissues. Reactive oxygen species (ROS), reactive nitrogen species (RNS), prostanoids, leukotrienes and hydrolytic enzymes produced by neutrophils, macrophages and monocytes may all play a role in mediating inflammation. Persistence of infection or defective resolution of inflammatory reaction results in chronic inflammation, where severe tissue damage may occur. Although inflammation is normally a self-limiting event and its benefits outweigh the minor tissue damage it causes, abnormal activation of the immune or inflammatory system has the potential to provoke a devastating response.[36] Other striking consequences of the abnormal inflammatory response are autoimmune diseases, such as, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), autoimmune vasculitis (AV), dermatomyositis, chronic autoimmune gastritis and myasthenia gravis. Tissue-damaging chronic inflammatory response may also occur in CNS, where the main inflammatory cells are microglia and astrocytes,

instead of monocytes / macrophages or neutrophils, in the periphery.[37-39]

NEUROGLIA (MICROGLIA, ASTROCYTES), NEUROINFLAMMATION AND NEURODEGENERATION

Microglia and astrocytes are essential for ensuring the proper functioning of the neurons. They are quick to intervene when neurons become injured or stressed. As they are sentinels of the neuron well-being, pathological impairment of microglia or astrocytes could have devastating consequences on the brain function. Nevertheless, there is still a debate over neuroprotective and neurotoxic functions of these neuroglial cells.[37,40] It is assumed that neuroglial activation is largely determined by neuronal signals. Acute injury causes the neurons to generate signals that inform the neuroglia about the neuronal status. Depending on how severe the degree of neuronal injury is, the neuroglia will either nurse the injured neurons into regeneration or kill them if they are not viable. These types of neuroglial responses are considered to represent normal physiological and neuroprotective responses. In contrast, some processes that are chronic in nature persistently activate neuroglia, eventually causing a failure in their physiological ability to maintain homeostasis. This could have detrimental consequences and may lead to bystander damage due to neuroglial dysfunction. In this scenario, the neuroglia exerts neurotoxic effects through the secretion of a variety of toxic inflammatory mediators. Thus, although activation of neuroglial cells may be intended to protect neurons, inflammatory products derived from activated neuroglia may also be implicated in neuronal injury, potentially leading to neurodegenerative diseases.[22] These deleterious effects of neuroglial activation may be exacerbated by failure of the auto-regulatory mechanisms of neuroglia. In recent times, activated macrophages, whose functions are closely related to microglia, have been shown to undergo apoptosis.[41-43] It has been suggested that the apoptosis of activated macrophages is one mechanism, whereby, an organism may regulate immune and inflammatory responses involving macrophages.[43] It has been recently demonstrated that a similar regulatory mechanism also exists for microglial cells[44,45] and astrocytes[46]. Microglial cells and astrocytes undergo apoptosis upon inflammatory activation, in a manner similar to activation-induced cell death (AICD) of lymphocytes.[45,46] AICD is an active process. The T and B lymphocytes that undergo AICD as an autoregulatory mechanism for the body to remove unwanted activated cells, after making appropriate use of them,[47] are not well studied in this respect. Now, as results in this and other laboratory studies have indicated that neuroglial cells may be under the control of a similar regulatory mechanism,[44-46,48-52] further investigation is warranted, to better understand the molecular mechanism(s) of neuroglial AICD and its physiological significance.

However, the presence of NO-independent cytotoxic mechanism has been also suggested.[53,54] Elimination of activated neuroglial cells by apoptosis could be an important mechanism, whereby, undesirable effects of long-term neuroglial activation can be minimized. Inflammatory mediators that are produced by activated neuroglia in CNS may have harmful effects on the neurons or other neuroglial cells that they originally intended to protect.[21,22] Thus, in various neurodegenerative diseases involving chronic neuroglial activation, the neuroglial functions seem to play a more significant role in mediating diseases than in the protection of neurons. According to the model of activation-induced apoptosis of neuroglial cells, inflammatory signals that activate neuroglia may also initiate an internal death program.[53,55] One interesting question that can be raised then is how neuroglial cells can survive inflammatory activation. It should be kept in mind that neuroglial cells *in vivo* are heterogeneous and interact with other neuroglial cells as well as neurons. There is also growing evidence that activated neuroglial cells proliferate *in vivo* as one way of replenishment.[17] Thus, not all neuroglial cells may respond to the inflammatory signals in the same fashion. Upon inflammatory activation, individual neuroglial cells in the heterogeneous population may either undergo AICD or return to the resting state via other regulatory mechanisms, depending on the specific microenvironment under which they react to the signals. Although many of the activated neuroglial cells may be eliminated, some would survive to be deactivated. Whatever the mechanism of downregulation may be, this may be an excellent auto-regulatory system for neuroglial activation. One can easily imagine pathological situations where this type of auto-regulatory mechanism goes wrong. Failure of auto-regulation of the 'over-activated' neuroglial cells may result in the pathological destruction of the bystander cells (neurons and other neuroglial cells) exposed to toxic mediators, produced by activated neuroglia. Recently, upregulated Bcl-x_L expression has been detected in the reactive microglia of patients with neurodegenerative diseases.[56] Authors have proposed that a high level of Bcl-x_L protein may render microglia more resistant to cytotoxic environment, such as, areas of neurodegeneration. Expression of anti-apoptotic Bcl-2 protein has also been associated with an aged brain and neurodegenerative diseases.[57] The importance of the physiological regulation of neuroglial activation by AICD is supported by these previous reports.

Recent studies have focused on the possible role of neuroglia in causing neurodegeneration. Convincing evidence from *in vitro* studies point to the neurotoxic role of neuroglia during traumatic or ischemic brain injury[13] and AD pathogenesis.[58] Supernatants obtained from neuroglial cell cultures kill the cultured neurons. Such supernatants contain various neurotoxic substances, which include, glutamate, NO,

ROS, inflammatory cytokines, as well as, yet unidentified neurotoxins.[59,60] Production of these neurotoxins by neuroglia is enhanced by treatment with inflammatory stimuli such as LPS and / or IFN-γ. Paradoxically, other investigators have shown that neuroglia-conditioned media promote neuronal survival.[61] Thus, the balance of the neurotoxic and neurotrophic effects of neuroglia appears to depend on the nature of the experimental paradigm used. In traumatic brain injury where neuronal regeneration may occur, neuroglial secretory products may help to promote regenerative efforts by injured, but surviving, neurons. However, the situation may be different in neurodegenerative diseases such as AD or human immunodeficiency virus (HIV)-associated dementia, where functionally compromised neuroglia may produce neurotoxins, thereby resulting in neuronal damage. There is considerable evidence from postmortem examinations of AD brains that auto-destructive mechanisms are at work, which may in part be responsible for the neurodegeneration.[62,63]

NEUROGLIA AS A TARGET OF PHARMACOLOGICAL INTERVENTION

Considering neuroglial activation as a common feature in many neuropathologies, and keeping in mind that over-activation of neuroglia can have neurotoxic outcomes, it is reasonable to assume that manipulation of neuroglial activation could serve future clinical approaches. Although the treatment of the primary events in neurodegenerative diseases would still be the preferred intervention, this may not always be possible. Brain or spinal cord injury is a sudden event that is followed by secondary cascades of destruction. Invading macrophages and intrinsic neuroglia in the brain may carry a significant portion of these cascades of reaction. Thus, it is of great interest to find a means to modulate neuroglial activation and CNS inflammatory responses for therapeutic interventions against these neurodegenerative diseases. Based on the understanding of intracellular signaling pathways that are specific for activated neuroglia, a temporary inhibition of signaling molecules or protein–protein interaction associated with signaling pathways, may probably allow for a rather selective effect on the activated neuroglia, while the respective functions in other cell types are unaffected. Elucidation of the intracellular key events that drive neuroglial activation could provide new routes for drug development.[64] Alternatively, the potentially harmful products of neuroglia could be neutralized, to limit the undesired consequences to CNS cells and tissues. Whether it is a direct inhibition of neuroglial activation or indirect suppression of neuroglia-derived toxic inflammatory mediators, a better understanding of neuroglial biology and selective manipulation of neuroglial activation processes represent a promising goal for developing novel neuroprotective strategies.

HERBAL MEDICINE AGAINST CNS DISORDERS: AS NEUROPROTECTIVE AGENTS

Over the last decade, renewed interest has been shown toward these bioflavonoid compounds, with some 4000 having been isolated so far.[65] Only a handful have been extensively studied for their medicinal properties. In the plant kingdom, they function as special pigments designed to prevent sunlight and toxin-induced free radical damage. Recent studies have shown some rather remarkable medicinal properties of flavonoid compounds. For example, some have been shown to have anticancer properties,[66] antiviral and antibacterial activity,[67,68] and immune stimulating qualities,[69] as well as, offering protection against strokes and heart attacks.[70,71] In this article, we will explore a new property of the flavonoids: Protection of the nervous system from neurodegeneration. It is estimated that nearly 25% of the modern drugs directly or indirectly originate from plants.[72] There are more than 120 traditional medicines in use for the therapy of CNS disorders, in Asian countries. Some of their therapeutic effects have been confirmed by recent clinical studies. An ethno pharmacological approach has provided a potentially rich source for drug discovery and development.[73] Many drugs currently available in Western medicine were originally isolated from plants. Although a large number of compounds have been isolated, most of these resources have not yet been fully characterized for pharmacological purposes.

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity, and economic viability. Ayurveda has a clinical specialty called *rasayana*, which prevents diseases and counteracts the ageing process by means of optimization of homeostasis. It has been reported that the *rasayanas* are rejuvenators, nutritional supplements, and strong antioxidants.

In Ayurveda, many herbs have been reported as nerve tonics or memory enhancers. On this basis, a number of herbs have been studied and validated for their neuroprotective properties. The most promising medicinal plants with CNS-activity in traditional Indian medicine are the following: *Acorus calamus*, *Azadirachta indica*, *Acanthopens radix*, *Bacopa monniera*, *Butea frondosa*, *Camellia sinensis*, *Centella asiatica*, *Celastrus pahiculatus*, *Clitoria ternatea*, *Convolvulus pluricaulis*, *Eclipta alba*, *Emblica officinale*, *Mucuna pruriens*, *Sida cordifolia*, *Vitis vinifera*, *Wadelia calandulacae*, *Withania somnifera*, *Ocimum sanctum*, and so on.[74-77] *Ginkgo biloba* and *Panex ginseng* used traditionally in Chinese medicine have been extensively screened and reported to possess diverse therapeutically beneficial properties.[78] While some of these need to be investigated in depth,

some have already been studied extensively. More recently, drug discovery techniques have been applied to the standardization of herbal medicines, to elucidate analytical marker compounds.

THE ROLE OF PLANT FLAVONOIDS IN NEURODEGENERATION

There has been a recent explosion of interest by research scientists in the flavonoid compounds, witha multitude of medically useful properties having been demonstrated in experimental, as well as, clinical studies of flavonoids. For instance, flavonoids have been shown to act as powerful free radical scavengers for a multitude of free radical species, even the powerful peroxynitrite radical.[79] In addition, several flavonoids have shown powerful metal-chelating properties, especially for iron and copper, two of the most potent-free radical catalysts.[80] Of equal importance are several studies that have shown that flavonoids interact with cell membranes, improving their fluidity, thereby protecting them from lipid peroxidation.[81,82] Along these same lines is the protection of microvessels in the nervous system by specific flavonoids from free radical damage.[83] This not only prevents leakage of such vessels, but has been shown to preserve the blood–brain barrier as well.[84] There is also evidence that several of the flavonoids can inhibit platelet adhesiveness, thereby preventing strokes.[85] Finally, some of the flavonoids have the unique ability to inhibit certain enzymes, such as the COX-2 enzyme.[86]

FLAVONOIDS AND INFLAMMATION

Neuroinflammation plays a vital role in neurodegenerative diseases and its inhibition by neuroprotective herbs, the antioxidant activity of herbal extracts is certainly another important aspect of neuroprotection.[87,88] A variety of herbal extracts and their components have been demonstrated to exert neuroprotective effects associated with antioxidant activities, either by directly stimulating antioxidant response genes or by potentiating the bodies' own natural antioxidant defence systems. This is supported by the findings that many herbal extracts and their components with neuroprotective activities exert both anti-inflammatory and antioxidant effects at the same time.[89-92]

One of the more useful properties of flavonoids is their ability to prevent inflammation via their interaction, with various steps, along the eicosanoid pathway. For example, certain flavonoids, such as the flavones and hesperidin, in high concentrations, can directly inhibit the release of arachidonic acid from the cell membrane.[93] Others, such as quercetin, myricetin, kaempferol, naringenin, and hesperidin, can inhibit activation of phospholipase A2, which initiates the release of arachidonic acid from the cell membrane. Certain flavonoids have been shown to either inhibit lipo-oxygenase (LPO) (hesperidin) or the cyclo-oxygenase-2 [(COX)-2] enzymes, or even both (quercetin).[94] Pycnogenol is known to inhibit LPO, but not COX.[95] There is at least some evidence that prostaglandins can inhibit glutamate uptake, thereby increasing neurodegenerative excitotoxicity.[96] Knowing that Alzheimer's patients have elevated levels of eicosanoids prostaglandin D2 (PGD2) and thromboxane-B2 (TXB2), it seems reasonable that flavonoids that inhibit the enzymes known to contribute to this abnormal rise in inflammatory substrates, should at least slow the progress of neurodegeneration or even prevent it. This appears to be the case in the clinical studies cited earlier in the text.

FLAVONOIDS AS FREE RADICAL SCAVENGERS

The flavonoid compounds have two properties that make them especially useful as antioxidants. First, many are powerful, primary free radical scavengers against a wide variety of radicals, including singlet oxygen, superoxide, peroxyl, hydroxyl, and the peroxynitrite radicals.[81] Second, several are known to be very effective metal chelators.[97] Most flavonoids are present in plants as glycosides. In the intestines, this moiety is cleaved off, leaving the aglycone form of the flavonoid.[98] It is the aglycone form that is thought to have the highest antioxidant activity in biological systems. There is experimental evidence that hydrogen peroxide accumulation occurs during the process of catecholamine catabolism, making it especially important in PD.[99] Recent evidence also indicates that H_2O_2 plays an important role in the toxicity of Alzheimer's plaques. As we have seen, iron accumulation within neurons is characteristic of ageing of the nervous system, but is especially high in the case of neurodegeneration. A multitude of phytochemicals have specific properties that make them especially useful in combating neurodegeneration, and a list of nutrients that stimulate energy generation, primarily through the mitochondrial system, are shown in Tables 1 and 2.

Table 1: Known effects of flavonoids on living systems as related to neurodegeneration

Neuroprotective effects of phytochemicals and flavonoids
Powerful free radical scavengers for many radicals
Iron and copper chelation
Direct interaction with cell membranes (improves fluidity)
Increases glucose uptake by neurons
Regeneration of other antioxidants
Restoration of cellular glutathione
Reduced excitotoxicity (NMDA receptor blockade)
Inhibition of LOX and COX enzymes
Inhibition of phospholipase A2
Anti-inflammatory properties
Restoration of blood–brain barrier
Microvascular stability and improved blood flow

Table 2: Nutrients that stimulate energy generation, primarily through the mitochondrial system

Nutrients increasing neuronal metabolism
Coenzyme Q 10
Acetyl L-carnitine
α-lipoic acid
Vitamin K
Nicotinamide
Riboflavin
Pyridoxine
Folate/B 12
Thiamine
Magnesium

Of the flavonoids acting as iron and copper chelators, the most powerful, in order of decreasing potency, include: rutin, hesperidin, quercitin, and naringenin.[81] Rutin, interestingly, was found to reduce lipid peroxidation in normal liposomes only slightly, but reduced it by 75% in the same process in iron-overloaded macrophages.[100] This would mean that rutin would not interfere with normal macrophage function during infections, but would reduce the pathological states of macrophage activation. There is new evidence to indicate that persons with PD disease have a defect in iron metabolism.[101] In iron-induced oxidation only, they vary in order of potency. There is also evidence that other cellular antioxidants measured in the brains of persons having PD, HD, progressive supranuclear palsy, and multiple system atrophy, had total iron levels that were elevated in the brain in all of these disorders, but only in PD there was a generalized reduction of brain ferritin. Ferritin is the normal chelating protein of iron, which keeps it from catalyzing the hydroxyl ion production. There is also evidence of iron accumulation in the motor neurons of ALS patients.[102]

Quercetin and epicatechin gallate have been shown to be very effective scavengers of the superoxide radical.[103] Remember, it is the superoxide that is the basic substrate for the formation of two of the most powerful free radicals responsible for neurodegeneration, the hydroxyl and radicals. Flavonoids are also very efficient scavengers of singlet oxygen (comparable to α-tocopherol), an oxidant that can oxidize proteins, lipids, and DNA bases.[79] Both myricetin and quercetin strongly scavenge the hydroxyl radical. Recent evidence indicates that the peroxynitrite radical plays a pivotal part in neurodegenerative diseases. Flavonoids are known to powerfully scavenge this radical.[104] These phytochemicals can also inhibit free radical injury to neurons by inhibiting glutamate toxicity in a dose-dependent manner,[105] and they are also known to spare tocopherols, both of which significantly reduce excitotoxicity.[106]

It has been shown that kampferol and quercetin are excellent membrane antioxidants. In fact, quercetin, being more hydrophilic than vitamin E, blocks lipid peroxidation at the initiation stage, making it a more efficient membrane antioxidant than vitamin E.[107] Catechins can also inhibit lipid peroxidation. Flavonoids are known to interact at the polar regions of the phospholipid bilayers. As stated earlier, one of the by-products of lipid peroxidation is the production of a destructive compound called 4-hydroxynonenal, which can inhibit key metabolic enzymes, inhibit glutamate uptake, decrease membrane fluidity, interfere with neuronal G-receptor proteins, and inactivate glutathione reductase.[108] The only antioxidant system that inactivates this destructive compound is glutathione.[109] In PD, one of the earliest events is a drastic reduction in the glutathione levels in the neurons of the substantia nigra. This makes these neurons especially vulnerable to 4-hydroxynonenal as well as to other reactive oxygen and nitrogen species. By inhibiting lipid peroxidation, these flavonoids will prevent the accumulation of 4-hydroxynonenal.

In the case of autoxidation of cerebral membranes, that is, in the absence of an iron or copper catalyst, it has been demonstrated that several of the flavonoids are very protective. These include, in decreasing potency: quercitin, rutin, hesperidin, and naringinin.[79] You will note that these are the same flavonoids that are most protective against iron-induced oxidation, only they vary in order of potency. There is also evidence that other cellular antioxidants play a vital role in protecting the brain from neurodegeneration. In one study, it was found that recall, recognition, and vocabulary correlated significantly with vitamin C and β-carotene levels.[110] The study was controlled for education level and age. Those participants beyond age 65 demonstrated a close correlation between high ascorbic acid and β-carotene levels and better memory performance. This is consistent with the finding of significantly low levels of vitamins A, E, and β-carotene in a group of Alzheimer's patients. β-carotene and vitamin E levels were also significantly low in multi-infarct dementia.

Even as the antioxidant effects of flavonoids may override their pro-oxidant effects, there is some concern about DNA damage. For example, myricetin, querecitin, and kaempferol, when tested on isolated rat liver nuclei, have resulted in concentration-dependent DNA damage, concurrent with lipid peroxidation, stimulated by iron and copper.[111,112] This may indicate that the chelating activity of flavonoids can be toxic to DNA. Yet, experiments using whole biological systems indicate that albumin prevents this pro-oxidant action of phenolic compounds.[113] In fact, a recent study using human lymphocytes has found that quercetin and myricetin both protect DNA against strand breaks and oxidized pyrimidine bases induced by H_2O_2.[97] They have also found that none of the flavonoids tested, quercetin, myricetin or norsilymarin, are themselves

genotoxic. Interestingly, neither α-tocopherol nor β-carotene have decreased DNA breakage.

The standardized extract from the herb *Ginkgo biloba* (Egb-761) contains 24% flavonoids (ginkgo-flavone glycosides) and 6% terpenoids (ginkgoides). It also contains numerous other flavonoids, including rutin and quercetin. This extract has been shown to have some very useful medicinal properties, which include, clastogenic effects, powerful scavenging of superoxide, hydroxyl, peroxyl, and peroxynitrite radicals, chelation of iron and copper, inhibition of lipid peroxidation in a dose-dependent manner, preservation of vitamins E, C, and β-carotene, reduction of cardiac arrhythmia, anti-inflammatory and anti-allergy properties, antagonization of the platelet activating factor (PAF), prevention of cerebral edema, prevention of stroke-induced uncoupling of mitochondrial respiration, reduction of intraneuronal calcium, increase of cerebral blood flow, maintenance of neuronal ATP levels during ischaemia, prevention of diabetic retinal damage, potent inhibitory effects of the nitric oxide system, and protection of the heart and brain during ischaemia-reperfusion injury.[114]

Pycnogenol and grape seed extract are also a group of flavonoid compounds that hold promise in combating neurodegeneration and microvascular brain disease. Both are high in catechins, which are known to inhibit lipid peroxidation in various models and to powerfully scavenge the superoxide and hydroxyl radicals.[115] Pycnogenol contains various other flavonoids that can inhibit lipid peroxidation and scavenge powerful free radicals. These include taxifolin, caeffic acid, protocatechin, gallic acid, ferulic acid, and vanillin.[116] Pycnogenol added to cultures of rat brain cells has been shown to significantly protect them from amyloid β-protein toxicity.[117] This may be secondary to its scavenging of the hydroxyl radical, produced by amyloid plague-activated hydrogen peroxide. In mouse hippocampal cell cultures, pycnogenol's flavonoids were protective against glutamate excitotoxicity, in a dose-dependent manner.[118] Another useful property of flavonoids in preventing the changes seen with CNS ageing and neurodegeneration, is their ability to enhance the capillary strength, most likely as a result of their high affinity for collagen and elastin. As mentioned previously, catechin protects collagen against degradation by collagenase, and elastin from destruction by elastase.

This unique property would be important in protecting the cerebral microvessels from chronic free radical damage. This type of microvascular damage is known to occur with ageing, and is especially severe in the case of neurodegenerative disease, especially Alzheimer's dementia.[119] Protection of microvessels would prevent the breakdown of the blood–brain barrier and transport systems (glucose transporter), commonly seen with ageing and neurodegeneration. As stated earlier, pycnogenol has been shown to inhibit the -LPO enzyme, but not the COX enzyme. This would be important in preventing the accumulation of thromboxane and leukotrienes within the brain. In addition, pycnogenol inhibited in a dose-dependent manner, epinephrine-induced platelet aggregation that was seen with stress and smoking.[120] It has been shown that platelet reactivity was significantly reduced after a single dose of 100 mg of pycnogenol. This could be very important in preventing multi-infarct dementia.

Red wine flavonoids are known to be powerful inhibitors of copper-catalysed oxidation of human LDL-cholesterol, more so than vitamin E.[121] Inhibition of platelet aggregation by red wine is thought to be secondary to the high concentrations of the flavonoids, fisetin, kaempferol, morin, myricetin, and quercetin. These flavonoids have been shown to inhibit thromboxane formation and to antagonise the thromboxane receptor. Major phenolic compounds of *Ocimum sanctum* include phenolic di- and tri-terpenes; flavonoids and phenolic acids; and sterols, among which rosmarinic acid, an ester of caffeic acid and 3, 4-dihydroxyphenyllactic acid, and ursolic acid, an isomer of oleanolic acid, have been extensively studied for their neuroprotective properties, and quantified.[77,122,123]

SUMMARY AND CONCLUSIONS

The common thread of microglial activation across numerous neurodegenerative diseases, define the current perceptions of how microglia are damaging neurons, and explain how the microglial response to neuronal damage results in a self-propelling cycle of neuron death. As heightened microglial activation was shown to play a role in the pathogenesis of experimental inflammatory CNS disorders, understanding the molecular mechanisms of microglial activation might lead to new treatment strategies for neurodegenerative disorders, multiple sclerosis, and bacterial or viral infections of the nervous system.[124] Activation of microglia and astrocytes plays a pivotal role in the initiation and progression of various neurodegenerative diseases. Inhibition of the neuroglial activation may provide an effective therapeutic intervention that alleviates the progression of neurodegenerative diseases. Herbal medicine, especially their flavonoid constituents, may be useful candidates for such a therapeutic approach. Continual investigation of the mechanisms underlying neuroglial activation, regulation of neuroinflammation, and the modulator role of herbal medicine in these processes, would not only lead to the discovery of novel neuroprotective agents based on medicinal herbs, but also help to understand the complex

pathophysiology of neurodegenerative diseases. These compounds, sourced from natural products, and used with treatments preventing pathogenesis and neuronal death, are expected to play an important role, as new categorized drugs, in curing neurodegenerative diseases in the near future. Although we have shown the high potential of neuronal regeneration from compounds isolated from Ginseng drugs, *Ashwagandha*, and coffee beans, it is dangerous to simply imply that these herbal drugs are expected to be excellent anti-dementia drugs. When taking herbal drugs, the risk of side effects brought by other constituents, and sufficient efficacy compared with isolated compounds should be investigated and carefully considered. However, drugs used in traditional medicine may offer a treasury of new medicines to treat intractable diseases with the use of novel study concepts and the application of objective scientific analyses. We suspect that neurodegeneration, a pathological state beyond normal ageing, is compounded by repeated episodes of ischaemia, hypoxia, and hypoglycemia throughout the latter years of life. The key is early intervention, before irreversible changes have taken place. We know that, in general, the clinical onset of most neurodegenerative diseases does not occur until 70 to 80% of a particular set of neurons are destroyed. This means intervention before a child is even born becomes important. How the brain develops is tied closely to early nutrition, first in the pregnant mother, and then immediately following birth. The field of research involved in preventing excitotoxic injury has been directed towards three basic areas: a reduction of free radical injury, reducing glutamate release, and blocking excitatory amino-acid receptors.[125,126] Our understanding of protection against neurodegeneration has increased significantly over the past decade. However, there are still many unknowns that must be explored and understood before an answer to these devastating diseases can be found.

REFERENCES

1. Blaylock RL. Neurodegeneration and aging of the central nervous system: prevention and treatment by phytochemicals and metabolic nutrients. Integr Med 1998;1:117-33.
2. Singh RP, Sharad S, Kapur S. Free radicals and oxidative stress in neurodegenerative diseases: Relevance of dietary antioxidants. J Indian Acad Clin Med 2004;5:218-25.
3. Standaert DG, Young AB. Treatment of central nervous degenerative disorders. In: Brunton LL, Lazo P, Parker KL, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th ed. New Delhi: McGraw-Hill; 2006. p. 527-46.
4. Block ML, Hong JS. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. Progr Neurobiol 2005:76;77-98.
5. Beal MF. Aging, energy and oxidative stress in neurodegenerative diseases. Ann Neurol 1995;38:357-66.
6. Gao HM, Jiang J, Wilson B, Zhang W, Hong JS, Liu B. Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. J Neurochem 2002;81:1285-97.
7. Block ML, Wu X, Pei Z, Li G, Wang T, Qin L, *et al*. Nanometer size diesel exhaust particles are selectively toxic to dopaminergic neurons: the role of microglia, phagocytosis, and NADPH oxidase. FASEB J 2004;18:1618-20.
8. Qin L, Liu Y, Cooper C, Liu B, Wilson B, Hong JS. Microglias enhance beta-amyloid peptide-induced toxicity in cortical and mesencephalic neurons by producing reactive oxygen species. J Neurochem 2002;83:973-83.
9. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neueurosci 2000;3:1301-6.
10. Greenamyre JT, MacKenzie G, Peng TI, Stephans SE. Mitochondrial dysfunction in Parkinson's disease. Biochem Soc Symp 1999;66:85-97.
11. Campbell A, Oldham M, Becaria A, Bondy SC, Meacher D, Sioutas C, *et al*. Particulate matter in polluted air may increase biomarkers of inflammation in mouse brain. Neurotoxicology 2005;26:133-40.
12. Zhang W, Wang T, Pei Z, Miller DS, Wu X, Block ML, *et al*. Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson's disease. FASEB J 2005;19:533-42.
13. Streit WJ, Walter SA, Pennell NA. Reactive microgliosis. Prog Neurobiol 1999;57:563-81.
14. Eikelenboom P, Bate C, Van Gool WA, Hoozemans JJ, Rozemuller JM, Veerhuis R, *et al*. Neuroinflammation in Alzheimer's disease and prion disease. Glia 2002;40:232-9.
15. Sánchez-Moreno C, Dashe JF, Scott T, Thaler D, Folstein MF, Martin A. Decreased levels of plasma vitamin C and increased concentrations of inflammatory and oxidative stress markers after stroke. Stroke 2003;35:163-8.
16. Wenk GL. Neuropathologic changes in Alzheimer's disease. J Clin Psychiatry 1995;64Suppl 9:7-10.
17. Gehrmann J, Matsumoto Y, Kreutzberg GW. Microglia: Intrinsic immuneffector cell of the brain. Brain Res Brain Res Rev 1995;20:269-87.
18. Gremo F, Sogos V, Ennas MG, Meloni A, Persichini T, Colasanti M, *et al*. Features and functions of human microglia cells. Adv Exp Med Biol 1997;429:79-97.
19. Stoll G, Jander S. The role of microglia and macrophages in the pathophysiology of the CNS. Prog Neurobiol 1999;58:233-47.
20. Kreutzberg GW. Microglia: A sensor for pathological events in the CNS. Trends Neurosci 1996;19:312-18.
21. Minghetti L, Levi G. Microglia as effector cells in brain damage and repair: Focus on prostanoids and nitric oxide. Prog Neurobiol 1998;54:99-125.
22. Gonzalez-Scarano F, Baltuch G. Microglia as mediators of inflammatory and degenerative diseases. Annu Rev Neurosci 1999;22:219-40.
23. Araque A, Perea G. Glial modulation of synaptic transmission in culture. Glia 2004;47:241-8.
24. Vesce S, Bezzi P, Volterra A. The active role of astrocytes in synaptic transmission. Cell Mol Life Sci 1999;56:991-1000.
25. Aschner M. Astrocytes as mediators of immune and inflammatory responses in the CNS. Neurotoxicology 1998;19:269-81.
26. Galea E, Feinstein DL, Reis DJ. Induction of calcium-independent nitric oxide synthase activity in primary rat glial cultures. Proc Natl Acad Sci U S A 1992;89:10945-9.
27. Sawada M, Kondo N, Suzumura A, Marunouchi T. Production of tumor necrosis factor-alpha by microglia and astrocytes in culture. Brain Res 1989;491:394-7.
28. Simmons ML, Murphy S. Induction of nitric oxide synthase in glial cells. J Neurochem 1992;59:897-905.

29. Becher B, Prat A, Antel JP. Brain-immune connection: Immunoregulatory properties of CNS resident cells. Glia 2000;29:293-304.
30. Nakamura Y. Regulating factors for microglial activation. Biol Pharm Bull 2002;25:945-53.
31. Ho LJ, Lai JH. Chinese herbs as immunomodulators and potential disease-modifying antirheumatic drugs in autoimmune disorders. Curr Drug Metab 2004;5:181-92.
32. Li FQ, Lu XZ, Liang XB, Zhou HF, Xue B, Liu XY, *et al*. Triptolide, a Chinese herbal extract, protects dopaminergic neurons from inflammation-mediated damage through inhibition of microglial activation. J Neuroimmunol 2004;148:24-31.
33. Ward PA, Warren JS, Johnson KJ. Oxygen radicals, inflammation, and tissue injury. Free Radic Biol Med 1988;5:403-8.
34. Henson PM, Johnston RB Jr. Tissue injury in inflammation. Oxidants, proteinases, and cationic proteins. J Clin Invest 1987;79:669-74.
35. Rankin JA. Biological mediators of acute inflammation. AACN Clin Issues 2004;15:3-17.
36. Halliwell B. Oxygen radicals, nitric oxide and human inflammatory joint disease. Ann Rheum Dis 1995;54:505-10.
37. Nakajima K, Kohsaka S. Microglia: Activation and their significance in the central nervous system. J Biochem 2001;130:169-75.
38. Liu B, Hong JS. Role of microglia in inflammation-mediated neurodegenerative diseases: Mechanisms and strategies for therapeutic intervention. J Pharmacol Exp Ther 2003;304:1-7.
39. Chavarria A, Alcocer-Varela J. Is damage in central nervous system due to inflammation? Autoimmun Rev 2004;3:251-60.
40. Popovich PG, Jones TB. Manipulating neuroinflammatory reactions in the injured spinal cord: Back to basics. Trends Pharmacol Sci 2003;24:13-7.
41. von Knethen A, Lotero A, Brune B. Etoposide and cisplatin induced apoptosis in activated RAW 264.7 macrophages is attenuated by cAMP-induced gene expression. Oncogene 1998;17:387-94.
42. Albina JE, Cui S, Mateo RB, Reichner JS. Nitric oxide-mediated apoptosis in murine peritoneal macrophages. J Immunol 1993;150:5080-5.
43. Adler B, Adler H, Jungi TW, Peterhans E. Interferon-alpha primes macrophages for lipopolysaccharide-induced apoptosis. Biochem Biophys Res Commun 1995;215:921-7.
44. Liu B, Wang K, Gao HM, Mandavilli B, Wang JY, Hong JS. Molecular consequences of activated microglia in the brain: Overactivation induces apoptosis. J Neurochem 2001;77:182-9.
45. Lee P, Lee J, Kim S, Lee MS, Yagita H, Kim SY, *et al*. NO as an autocrine mediator in the apoptosis of activated microglial cells: Correlation between activation and apoptosis of microglial cells. Brain Res 2001;892:380-5.
46. Suk K, Lee J, Hur J, Kim YS, Lee M, Cha S, *et al*. Activation-induced cell death of rat astrocytes. Brain Res 2001;900:342-7.
47. Donjerkovic D, Scott DW. Activation-induced cell death in B lymphocytes. Cell Res 2000;10:179-92.
48. Crispe IN. Death and destruction of activated T lymphocytes. Immunol Res 1999;19:143-57.
49. Kingham PJ, Cuzner ML, Pocock JM. Apoptotic pathways mobilized in microglia and neurons as a consequence of chromogranin A-induced microglial activation. J Neurochem 1999;73:538-47.
50. Kingham PJ, Pocock JM. Microglial apoptosis induced by chromogranin A is mediated by mitochondrial depolarisation and the permeability transition but not by cytochrome c release. J Neurochem 2000;74:1452-62.
51. Yang MS, Park EJ, Sohn S, Kwon HJ, Shin WH, Pyo HK, *et al*. Interleukin-13 and -14 induce death of activated microglia. Glia 2002;38:273-80.
52. Takano K, Nakamura Y, Yoneda Y. Microglialcell death induced by a low concentration of polyamines. Neurosci 2003;120:961-67.
53. Lee J, Hur J, Lee P, Kim JY, Cho N, Kim SY, *et al*. Dual role of inflammatory stimuli in activation-induced cell death of mouse microglial cells: initiation of two separate apoptotic pathways via induction of interferon regulatory factor-1 and caspase-11. J Biol Chem 2001;276:32956-65.
54. Lee H, Cha S, Lee MS, Cho GJ, Choi WS, Suk K. Role of antiproliferative B cell translocation gene-1 as an apoptotic sensitizer in activation induced cell death of brain microglia. J Immunol 2003;171:5802-11.
55. Suk K, Kim SY, Kim H. Essential role of caspase-11 in activation-induced cell death of rat astrocytes. J Neurochem 2002;80:230-8.
56. Drache B, Diehl GE, Beyreuther K, Perlmutter LS, Konig G. bcl-xl-specific antibody labels activated microglia associated with Alzheimer's disease and other pathological states. J Neurosci Res 1997;47:98-108.
57. Migheli A, Cavalla P, Piva R, Giordana MT, Schiffer D. bcl-2 protein expression in aged brain and neurodegenerative diseases. Neuroreport 1994;5:1906-8.
58. Streit WJ. Microglia and Alzheimer's disease pathogenesis. J Neurosci Res 2004;77:1-8.
59. Giulian D. Amoeboid microglia as effectors of inflammation in the central nervous system. J Neurosci Res 1987;18:155-71, 132-3.
60. Giulian D, Haverkamp LJ, Li J, Karshin WL, Yu J, Tom D, *et al*. Senile plaques stimulate microglia to release a neurotoxin found in Alzheimer brain. Neurochem Int 1995;27:119-37.
61. Nagata K, Takei N, Nakajima K, Saito H, Kohsaka S. Microglial conditioned medium promotes survival and development of cultured mesencephalic neurons from embryonic rat brain. J Neurosci Res 1993;34:357-63.
62. McGeer PL, McGeer EG. The inflammatory response system of brain: Implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Brain Res Rev 1995;21:195-218.
63. McGeer PL, Kawamata T, Walker DG, Akiyama H, Tooyama I, McGeer EG. Microglia in degenerative neurological disease. Glia 1993;7:84-92.
64. Hanisch UK. Microglia as a source and target of cytokines. Glia 2002;40:140-55.
65. Rice-Evans CA, Packer L, editors. Flavonoids in health and disease. New York: Marcel Dekker Inc; 1998.
66. Hirano T, Oka K, Akibam M. Antiproliferative effect of synthetic and naturally occurring flavonoids on tumor cells of the human breast carcinoma cell line, ZR-75-1. Res Comm Chem Pathol Pharmacol 1989;64:69-79.
67. Arnold A, Carughi M, Farina G, Merlini L, Parrino MG. Synthetic analogs of phytoalexins, synthesis and antifungal activity of potential free radical scavengers. J Agric Food Chem 1989;37:508-12.
68. Wacker A, Hilbig W. Virus inhibition by *Echinacea purpurea*. Planta Med 1978;33:89-102.
69. Stimpel M, Proksch A, Wagner H, Lohmann-Matthes ML. Macrophage activation and induction of macrophage cytotoxicity by purified polysaccharide fractions from the plant *Echinacea purpura*. Infect Immun 1984;46:845-9.
70. Hertog MGL, Feskens EJ, Hollaman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. Lancet 1993;342:1007-11.
71. Krieglstein J, Beck T, Seibert A. Influence of an extract of *Ginkgo biloba* on cerebral blood flow and metabolism. Life Sci 1986;39:2327-34.
72. De Smet PA. Herbal remedies. N Engl J Med 2002;347:2046-56.
73. Harvey AL. Medicines from nature: are natural products still relevant to drug discovery? Trends Pharmacol Sci 1999;20:196-8.
74. Ashok AB. The status and scope of Indian medicinal plants acting on central nervous system. Ind J Pharmacol 1997;29:S340-3.
75. Carlini EA. Plants and the central nervous system. Pharmacol Biochem Behav 2003;75:501-12.

76. Howes MJ, Houghton PJ. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. Pharmacol Biochem Behav 2003;75:513-27.
77. Shanmuga Sundaram R, Ramanathan M, Gowtham L, Kumar JP, Bihari CG, Manikandan P, *et al*. Investigation of standardized ethanol extract of *Ocimum sanctum* Linn. (Holy Basil) leaves for its *in vitro* antioxidant potential and phenolic composition. Asian J Chem 2012;24:1819-24.
78. Gertz HJ, Kiefer M. Review about *Ginkgo biloba* special extract EGb 761 (Ginkgo). Curr Pharm Des 2004;10:261-4.
79. Jovanovic SV, Steenken S, Simic MG, Hara Y. Antioxidant properties of flavonoids: reduction potentials and electron transfer reactions of flavonoid radicals. In: Rice-Evans CA, Packer L, editors. Flavonoids in health and disease. New York: Marcel Dekker, Inc; 1998. p. 137-61.
80. Morel I, Cillard P, Cillard J. Flavonoid-metal interactions in biological systems. In: Rice-Evans CA, Packer L, editors. Flavonoids in health and disease. New York: Marcel Dekker Inc; 1998. p. 163-77.
81. Saija A, Scalese M, Lanza M, Marzullo D, Bonina F, Castelli F. Flavonoids as antioxidant agents: importance of their interactions with biomembranes. Free Radic Biol Med 1995;19:481-6.
82. Ratty AK, Das NP. Effects of flavonoids on nonenzymatic lipid peroxidation: structure-activity relationship. Biochem Med Metab Biol 1988;39:69-79.
83. Kuttan R, Donnely PV, DiFerrante N. Collagen treated with (1)-catechin becomes resistant to the action of mammalian collagenase. Expermentia 1981;37:221-5.
84. Robert AM, Godeau G, Moati F, Miskulin M. Action of anthrocyanosides of *vaccinium myrtillis* on the permeability of the blood-brain barrier. J Med 1977;8:321-32.
85. Tzeng SH, Ko WC, Ko FN, Teng CM. Inhibition of platelet aggregation by some flavonoids. Thromb Res 1991;64:91-100.
86. Kim HP, Mani I, Iversen L, Ziboh VA. Effects of naturally occurring flavonoids and biflavonoids on epidermal cyclooxygenase and lipooxygenase from guinea pigs. Prostaglandins Leukot Essent Fatty Acids 1998;58:17-24.
87. Fahn S, Cohen G. The oxidant stress hypothesis in Parkinson's disease: Evidence supporting it. Ann Neurol 1992;32:804-12.
88. Metodiewa D, Koska C. Reactive oxygen species and reactive nitrogen species: Relevance to cyto (neuro) toxic events and neurologic disorders: an overview. Neurotox Res 2000;1:197-233.
89. Ahlemeyer B, Krieglstein J. Neuroprotective effects of *Ginkgo biloba* extract. Cell Mol Life Sci 2003;60:1779-92.
90. Kim YO, Leem K, Park J, Lee P, Ahn DK, Lee BC, *et al*. Cytoprotective effect of *Scutellaria baicalensis* in CA1 hippocampal neurons of rats after global cerebral ischaemia. J Ethnopharmacol 2001;77:183-8.
91. Oyama Y, Chikahisa L, Ueha T, Kanemaru K, Noda K. *Ginkgo biloba* extract protects brain neurons against oxidative stress induced by hydrogen peroxide. Brain Res 1996;712:349-52.
92. Rahman K. Garlic and aging: new insights into an old remedy. Ageing Res Rev 2003;2:39-56.
93. Lindahl M, Tagesson C. Flavonoids as phospholipase A2 inhibitors: importance of their structure for selective inhibition of group II phospholipase A2. Inflammation 1997;21:347-56.
94. Pietta P. Flavonoids in medicinal plants. In: Rice-Evans CA, Packer L, editors. Flavonoids in health and disease. New York: Marcel Dekker Inc; 1998. p. 61-110.
95. Rohdewald P. Pycnogenol. In: Rice-Evans CA, Packer L, editors. Flavonoids in health and disease. New York: Marcel Dekker Inc; 1998. p. 405-19.
96. Ben-Ari Y, Aniksztejn L, Bregestovski P. Protein kinase C modulation for NMDA currents: an important link for LTP induction. Trends Neurosci 1992;15:333-8.
97. Duthie SJ, Collins AR, Duthie GG, Dobsoin VL. Quercetin and myricetin protect against hydrogen peroxide-induced DNA damage (strand breaks and oxidized pyrimidines) in human lymphocytes. Mutat Res 1997;393:223-31.
98. Griffiths LA. Mammalian metabolism of flavonoids. In: Harborne JB, Mabry IJ, editors. The flavonoids: advances in research. London: Chapman and Hall; 1982. p. 681.
99. Li CL, Werner P, Cohen G. Lipid peroxidation in brain: interaction of L-DOPA/dopamine with ascorbate and iron. Neurodegeneration 1995;4:147-53.
100. Afanas'ev BI, Ostrachovitch AE, Abramova EN, Korkina GL. Different antioxidant activities of bioflavonoid rutin in normal and iron-overloading rats. Biochem Pharmacol 1995;50:627-35.
101. Dexter DT, Carayon A, Javoy-Agid F, Agid Y, Wells FR, Daniel SE, *et al*. Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglion. Brain 1991;114:1953-75.
102. Kasarskis EJ, Tandon L, Lovell MA, Ehmann WD. Aluminum, calcium, and iron in the spinal cord of patients with sporadic amyotrophic lateral sclerosis using laser microprobe mass spectroscopy: a preliminary study. J Neurol Sci 1995;130:203-8.
103. Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Van Poel B, *et al*. Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. J Nat Prod 1998;61:71-6.
104. Van Acker SA, Tromp MN, Haenen GR, van der Vijgh WJ, Bast A. Flavonoids as scavengers of nitric oxide radical. Biochem Biophys Res Comm 1995;214:755-9.
105. Schubert D, Kimura H, Maher P. Growth factors and vitamin E modify neuronal glutamate toxicity. Proc Natl Acad Sci U S A 1992;89:8264-8.
106. Hertog MGL, Katan MB. Quercetin in foods, cardiovascular disease and cancer. In: Rice-Evans CA, Packer L, editors. Flavonoids in health and disease. New York: Marcel Dekker, Inc; 1998. p. 447-67.
107. Tero J, Piskula MK. Flavanoids as inhibitors of lipid peroxidation in membranes. In: Rice-Evans CA, Packer L, editors. Flavonoids in health and disease. New York: Marcel Dekker, Inc; 1998. p. 277-93.
108. Blanc EM, Kelly JF, Mark RJ, Waeg G, Mattson MP. 4-Hydroxynonenal, an aldehyde product of lipid peroxidation, impairs signal transduction associated with muscarinic acetylcholine and metabotropic glutamate receptors: possible actions on G alpha (q/II). J Neurochem 1997;69:570-80.
109. Blanc EM, Keller JN, Fernandez S, Mattson MP. 4-Hydroxynonenal, a lipid peroxidation product, impairs glutamate transport in cortical astrocytes. Glia 1998;22:149-60.
110. Chromé J, Paul T, Pudel V, Bleyl H, Heseker H, Hppe R, *et al*. Effect of suboptimal vitamin status on behavior. In: Somogyi JC, Hotzel D, editors. Nutrition and neurobiology. New York: Kaeger; 1986. p. 94-103.
111. Sahu SC, Gray GC. Interactions of flavonoids, trace metals and oxygen: nuclear DNA damage and lipid peroxidation induced by myricetin. Cancer Lett 1993;70:73-9.
112. Sahu SG, Gray GC. Kaempferol-induced nuclear DNA damage and lipid peroxidation. Cancer Lett 1994;85:159-64.
113. Smith S, Halliwell B, Aruma OI. Protection by albumin against prooxidant actions of phenolic dietary components. Food Chem Toxicol 1992;30:483-9.
114. Packer L, Saliou C, Droy-Lefaix MT, Christen Y. *Ginkgo biloba* extract Egb 761: biological actions, antioxidant activity, and regulation of nitric oxide synthetase. In: Rice-Evans CA, Packer L, editors. Flavonoids in health and disease. New York: Marcel Dekker, Inc; 1998. p. 303-41.

115. Yoneda T, Hiramatsu M, Sakamoto M, Togasaki K, Komatsu M, Yamaguchi K. Antioxidant effects of beta catechins. Biochem Mol Biol Int 1995;35:995-1008.
116. Rohdewald P. Pycnogenol. In: Rice-Evans CA, Packer L, editors. Flavonoids in health and disease. New York: Marcel Dekker, Inc; 1998. p. 405-19.
117. Behl C, Davis JB, Lesley R, Schubert D. Hydrogen peroxide mediates amyloidal beta-protein toxicity. Cell 1994;77:817-27.
118. Schubert D, Kimurah H, Maher P. Growth factors and vitamin E modify neuronal glutamate toxicity. Proc Natl Acad Sci U S A 1992;89:8264-8.
119. Scheibel AB, Duong T. On the possible relationship of cortical microvascular pathology to blood-brain barrier changes in Alzheimer's disease. Neurobiol Aging 1988;9:41-2.
120. Grotemeyer KH. The platelet-reactivity test- a useful by-product of blood sampling procedure. Thromb Res 1991;61:423-31.
121. Demrow HS, Slane PR, Folts JD. Administration of wine and grape juice inhibits *in vivo* platelet activity and thrombosis in stenosed cainine coronary arteries. Circulation 1995;91:1182-8.
122. Shanmuga Sundaram R, Ramanathan M, Rajesh R, Satheesh B, Saravanan D. LC-MS quantification of rosmarinic acid and ursolic acid in the *Ocimum sanctum* Linn. leaf extract (Holy basil, Tulsi). J Liq Chromatogr Relat Technol 2012;35:634-50.
123. Shanmuga Sundaram R, Gowtham L, Manikandan P, Venugopal V, Kamalakannan D. The role of reactive microglia in neurodegenerative disease: Multiple triggers with a common mechanism. Int J Recent Adv Pharm Res 2012;2:29-40.
124. Shanmuga Sundaram R, Gowtham L, Bhabani SN. The role of excitatory neurotransmitter glutamate in brain physiology and pathology. Asian J Pharm Clin Res 2012;5:1-7.
125. Shanmuga Sundaram R, Gowtham L, Manikandan P, Venugopal V, Kamalakannan D. Neuronal apoptosis and necrosis: role of excitotoxins, calcium, oxidative stress. Int J Res Pharm Biomed Sci 2012;3:567-75.
126. Lolic MM, Fiskum G, Rosenthal RE. Neuroprotective effects of acetyl-L-carnitine after stroke in rats. Ann Emerg Med 1997; 29:758–65.

How to cite this article: Sundaram RS, Gowtham L. Microglia and regulation of inflammation-mediated neurodegeneration: Prevention and treatment by phytochemicals and metabolic nutrients. Int J Green Pharm 2012;6:81-92.

Source of Support: Nil, **Conflict of Interest:** None declared.