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Invited Review

ScopeMed

Oxidative stress in neurodegenerative conditions and the protective potential of a natural antioxidant, dietary saffron

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ABSTRACT

Age-related disorders of the central nervous system such as Alzheimer's disease, Parkinson's disease and retinal degenerations are debilitating conditions that pose major health, economic and social problems. Current treatments for these conditions have failed to provide fully effective treatments for the condition.
Detrimental oxidative changes that are associated with metabolic activities affect the brain and cognitive function over time and tend to lead to the onset of dementia and several other neurodegenerative diseases. Over the past decade, extensive epidemiological studies have shown significant associations of regular phytochemical consumption of fruits, vegetables, tea leaves with health improvements as well as reduced risk for cardiovascular disease, stroke, diabetes, some cancers, and neurodegenerative diseases.
In this review, evidence is presented to show that increased levels of oxidative stress leads to the aging and neurodegenerative process, and dietary saffron, a potent antioxidant, could be beneficial to combat the debilitating disorders of the central nervous system. Beneficial actions of antioxidant saffron include anti-carcinogenic, anti-depressant, anti-inflammatory, anti-hypertensive, anti-diabetic and anxiolytic effects; and these beneficial effects have been demonstrated to contribute to improvements in cellular integrity and function in animal and human studies. However, the mechanism of action of saffron is yet to be completely understood and this needs to be further explored.

KEY WORDS: Neurodegenerative diseases, neuroprotection, saffron

INTRODUCTION

Oxidative stress is prominent in normal aging and has been proposed as a key factor in many neurological disorders [1, 2]. Typically, oxidative stress can be induced by a lack of oxygen (hypoxia or ischemia), or elevated oxygen levels (hyperoxia), leading to an increase in free radical formation. Mitochondria, the sites of oxidative phosphorylation, are the major organelles that produce free radicals during normal metabolic activities. Free radicals, also known as reactive oxygen species (ROS), mainly attack lipids and fatty acids of neurons, DNA and proteins [1].

The byproducts of lipid and protein breakdown form lipofuscin [3, 4], which slowly accumulates in the brain parenchyma. The brain is the biggest consumer of oxygen and glucose in the body, needed to generate adenosine-5'-triphosphate (ATP), and this makes the brain more susceptible to oxygen overload than any other organ [5]. Under physiological conditions, 1-2% of the oxygen consumed is converted to ROS but in the aged brain, this percentage rises, due to reduced surveillance activities of innate adaptive defense against ROS by endogenous enzymatic and non-enzymatic antioxidants such as superoxide dismutase enzymes, glutathione peroxidase, catalase, ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), carotenoids and flavonoids [5-8].

As a consequence, ROS cause mitochondrial dysfunction and neuronal death; both are elevated in the Alzheimer and parkinsonian brains [9-11], and in retinal diseases [12]. Oxidative stress and genetic flaws that accumulate gradually over time have been implicated in biological aging and in an array of diseases. Since oxidative stress and subsequent mitochondrial dysfunction are implicated in several diseases of the central nervous system (CNS), these processes have become the focus for new therapeutic targets.

THE ENDOGENOUS ANTIOXIDANT SYSTEM

Phagocytes are one of the major sources of free radicals and represent one of the functions of the immune system. Usually, phagocytes attack and eliminate invading pathogens and/or apoptotic cells, and organelles by engulfing and eliminating them. Phagocytes help protect the CNS against infectious organisms through the combined actions of lysosomal digestive enzymes and internally self-generated excessive amounts of oxidants such as superoxide, nitric oxide and hydrogen peroxide [13]. Nonetheless, excessive

activation of phagocytic cells, as in chronic inflammation, which is implicated in a number of neurological diseases such as Alzheimer's disease (AD) [14], can lead to free radical damage in cellular systems [15, 16].

Conversely, in non-phagocytic cells, oxidant production is a function of aging, evident from the numerous cellular by-products such as lipofuscin found in aged post-mortem brain and retinal tissues [4]. Lipofuscin accumulation is likely the consequence of failed cellular lysosomal degradation, usually elevated by an increased cellular oxidative stress environment [4, 17]. Metal ions such as iron are predominant in lipofuscin [4]. The redox-active iron, a catalyst of oxidative stress, may also increases lipofuscin accumulation in other type of cells such as cultured human glial cells and rat cardiac myocytes [17]. In parallel, the cerebral lipofuscin accumulation may eventually lead to dementia after prolonged and increased oxidative damage [3, 15]. Indeed, homogenates of the frontal cortex from AD brains obtained at autopsy revealed a 22% higher production of free radicals and, in the presence of iron, a 50% higher production of free radicals than those produced in age-matched normal control brains [18]. Consequently, iron chelators and antioxidants such as glutathione retard cellular lipofuscin accumulation and oxidative stress [15, 17].

In living healthy subjects, several enzymes and substances act co-operatively and synergistically to achieve adequate levels of antioxidant activity. Innate antioxidant enzymes include glutathione peroxidase, catalase and cytosolic superoxide dismutase, which play an important role in protecting cells against oxidative stress and ROS [6]. This system can neutralize ROS such as nitric oxide, peroxynitrite, hydrogen peroxide and lipid peroxide present in both cytosol and mitochondria. Some of these enzymes respond to increased stress by elevating their activities [13]. This response is referred to as an antioxidant adaptive response and an elevation in levels of these antioxidants is an indicator of the presence of high oxidative stress in the cell [7]. However, the activities of some of these antioxidant enzymes may decline as a function of aging [19, 20]. Changes in enzymatic activities, and in several major cellular and molecular activities that may occur in the CNS during normal aging include increased oxidative stress, ROS accumulation, impaired cellular energy metabolism, perturbed cellular calcium signaling and an abnormal accumulation of damaged proteins and organelles [7]. In some cases, these changes may also lead to cognitive and motor impairments with dementia in subsequent years during an individual's life.

Changes in Innate Antioxidant Activities in Aging and Neurodegenerative Diseases

Antioxidants are defined as any substance that can significantly delay or prevent the oxidation of a substrate [21]. The serum concentrations of innate antioxidants such as superoxide dismutase, glutathione peroxidase and albumin in older humans are lower than those found in younger individuals [19]. Moreover, the total antioxidant capacity of blood serum is decreased and the level of lipid peroxides increased in older subjects *vs* younger cohorts [19]. These levels are critically imbalanced, especially in the diseased states of the CNS such as macular degeneration and AD.

Age-related macular degeneration (AMD) is the major cause of vision impairment and most prevalent cause of irreversible blindness in developed countries [22, 23]. The retina of patients with AMD show an increase in oxidative stress and a decrease in superoxide dismutase and glutathione peroxidase [23, 24]. The combined effect of several phytochemicals (such as beta carotene, vitamins C and E, and zinc) substantially reduces the risk for AMD in elderly patients [22, 23], and this highlights that the innate adaptive response of antioxidant enzymes could be boosted with phytochemical supplementation.

In AD patients, it has been revealed that the activities of alpha-ketoglutarate dehydrogenase complex (a mitochondrial enzyme) [25], glutathione peroxidase [26] and glutamine synthetase (which converts the oxidizing agent glutamate to the less harmless glutamine) [27] are decreased compared to age-matched control subjects. Likewise, reduced levels of antioxidant enzymes were found in several cerebral regions from autopsied brains [28, 29] and serum samples [30] of patients with Parkinson's disease. Even well-nourished patients suffering from clinically diagnosed vascular and non-vascular dementias also consistently recorded low serum levels of the diet-derived free radicals scavengers vitamins A, B, C and E [31]. These studies suggest that the activities of certain antioxidant enzymes in the brain of patients with neurodegenerative diseases decline more rapidly than those found in age-matched control subjects. This has led to the suggestion that various antioxidant supplements and phytochemical components might be beneficial for preserving brain functions and forestalling age-related deficits [32, 33].

NEUROPROTECTIVE STRATEGIES

A major challenge in medical research is the development of effective therapies for the treatment of neurodegenerative disorders, many of which are age-related. An ideal treatment should have a neuroprotective action with the ability to reduce the processes of natural aging and progression into neurodegenerative disease. It should be cheap, non-invasive and safe to administer, with minimal side-effects. Currently, however, no established treatment meets these criteria [34]. Recent reports from experimental and large-cohort studies have provided evidence that micronutrient or phytochemical supplementation may provide neuroprotection in age-related neurodegenerative disorders in the CNS [35-41].

Diet-induced interventions for neuroprotection

Is there a way to enjoy the benefits of extrinsic antioxidants without the need to rely on commercially isolated and

manufactured bioenergetics compounds? Certainly, dietary components contain several vitamins and minerals which are beneficial to the body's defense mechanism. The ideal phytochemical compounds should have two characteristics: (1) neuroprotective action and (2) anti-aging effect.

Indeed, evidence from epidemiological studies suggests that several food compounds can enhance longevity in humans. Green tea, certain green vegetables, fruits, nuts, olive oil, red wine, ginkgo extracts, and spices have strong antioxidant properties [42, 43]. For example, red wine and olive oil contain the beneficial flavonoids such as phenols and polyphenols that are cardioprotective with strong antioxidant properties [42, 44]. This is proposed as the reason why Mediterranean people live longer and have lower prevalence of cardiovascular and brain pathology than their neighbours in Scandinavia [42, 44].

Similarly, epidemiological studies provided evidence that Indians (patients aged 70-79 from the Indian subcontinent) produced better MMSE (mini-mental state examination) cognitive test scores and recorded a 4.4-fold lower prevalence of dementia than a cohort of similarly aged adults from the United states [45]. Typical Indian food contains several spices that are rich in antioxidants. In fact, spices were one of the first commodities traded frequently between the East and the West during the middle ages (1400s-1900s) for their strong medicinal properties and the ability to prevent oxidation of food products, hence, allowing food to remain viable while remaining several months in transportation [42].

These natural food compounds are candidates for the purpose of neuroprotection and anti-aging due to their low toxicity and antioxidant properties. They should also be able to penetrate the blood-brain barrier, and preserve brain function and/or forestall age-related deficits, even at low doses. Consequently, any treatment capable of interrupting the initiation or execution, or both, of mitochondrial dysfunction and apoptosis may be of value in neurodegenerative diseases. As such, in this review, we are interested in the neuroprotective properties of an ancient spice – 'saffron'.

Saffron as a neuroprotectant

Saffron is derived from the plant *Crocus sativus*, and has been used in traditional Indian medicine for centuries as an analgesic and to treat cognitive dysfunction and mental illness [46, 47]. Several rigorous modern studies have highlighted its potent antioxidant [48, 49], anti-inflammatory [50, 51], anxiolytic [52], antidepressant [53-55], anti-carcinogenic [56-58], antihypertensive [59, 60], anti-obesity [61-63] and anti-diabetic [64-66] effects. For example, oral saffron was found to have a similar effect as the known antidepressant drug fluoxetine, in patients with mild to moderate depression [67].

The *Crocus sativus* flower has three deep-red colored stigmas, which are harvested by hand as spice [68]. Chemical studies on *Crocus sativus* stigmas report that it contains many active substances of which the most-studied for their neuroprotective capacity are crocetin, crocin and safranal (Figure 1) [69]. Crocin is the water-soluble component of saffron that confers its golden yellowish-red colour, picrocrocin is responsible for the bitter taste, whereas safranal is mainly responsible for the aroma of saffron which is the distinct hay-like smell that appears after the drying and storage of saffron [47, 69]. Crocetin shows the significant potential use as an antitumor agent that occurs in both the petals and stigmas. Using several *in vitro* biochemical assays to assess the total antioxidant contents of several herbs, spices, fruits and nuts, it was revealed that saffron contained the strongest antioxidant content of all foods tested [48].

Recent evidence has identified that cardiovascular risk factors such as high blood pressure, uncontrolled diabetes, cholesterolemia, atherosclerosis overlap considerably with AD [70]. There is now a growing consensus that AD is delayed by factors that improve cardiovascular health [70]. Recent evidence has gathered that saffron can improve cardiovascular function by attenuating atherosclerosis, reducing hypertension and diabetes, and significantly decreasing blood cholesterol levels [66, 68, 71-73]. Since oxygen diffusibility in plasma decreases as a function of age and atherosclerosis may be due to the hypoxia of the vascular walls, and treatment with saffron (crocin and crocetin) appeared to reduce oxidation of endothelial cells, potentially preventing atherosclerosis [74, 75]. A small clinical trial study also confirmed that saffron supplementation improved lipid metabolism in both healthy individuals and patients with coronary heart disease [76].

Similarly, researchers began to investigate compounds that could facilitate the transfer of oxygen between blood erythrocytes, vascular walls and the body tissues [77]. *In vivo* studies showed that a low dosage of crocetin increases oxygen diffusibility by about 80% [78] and suggesting that it could potentially attenuate atherosclerosis. Confirming this property, the oral administration of crocetin in human subjects was shown to improve muscle function and fatigue after vigorous exercise [79]. These authors concluded that the improvement in physical function may be due to the

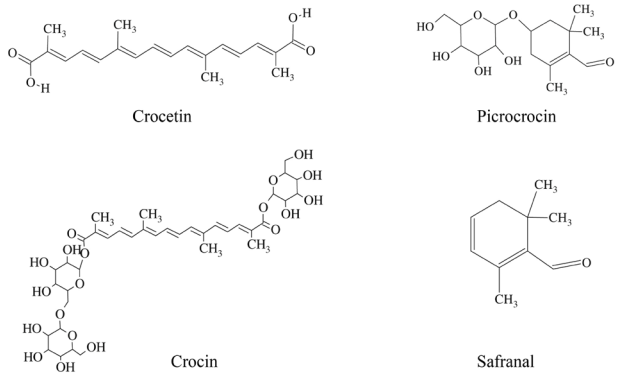

Figure 1. The characteristic compounds of saffron stigma (*Crocus sativus*) - crocin, crocetin, picrocrocin and safranal.

strong ability of saffron to remove free radicals from muscle tissue and its ability to increase alveolar oxygen transport, pulmonary oxygenation and oxygen diffusibility after exercise. Others have speculated that these properties may explain why the people in Spain, a major saffron-growing country, have a low incidence of cardiovascular disease [68].

The highly investigated ingredients in saffron - crocin, crocetin and safranal - also have been shown to improve memory and learning skills, to lower total oxidation products and increase total brain antioxidant activities in mice and rats [46, 66, 80]. It is thought that saffron attenuates reductions in cholinergic markers, such as acetylcholine, choline acetyltransferase and acetylecholinesterase during normal aging, so improving memory and learning [46]. Saffron was also shown to improve spatial learning and memory in humans [81] and in brain damaged rats following cerebral ischemia [82]. Moreover, saffron was found to be neuroprotective in the retina and brains in rodents infused with toxins that induced parkinsonism such as 6-OHDA [83] and MPTP [35]. Crocin also inhibited amyloid aggregation *in vitro* [84], while saffron was shown to be similarly effective in preserving cognition in patients with mild-to-moderate AD [85, 86].

Crocin analogs isolated from saffron also increased blood flow and oxygen to the retina and choroid, and this facilitated recovery of retinal function and probably prevents ischemic retinopathy and AMD in animal models [87]. Oxygen tension in the inner segments of the photoreceptors is low as it is an area of high metabolic demand for oxygen, met by diffusion from the choroidal circulation [88, 89]. Saffron and its constituents may increase the diffusibility of oxygen [78], and thereby accelerate retinal recovery after stress [90]. In a rat model of retinal degeneration, dietary saffron preserved the photoreceptor layer after the rats were exposed to bright continuous light for 24 h [36, 91]. This may be due to the suppression of inflammation, oxidative stress and subsequent inhibition of apoptotic pathways in the light damaged retina [91, 92]. Similarly, in a rat P23H model of retinitis pigmentosa, saffron protected photoreceptor morphology and restored retinal function [93]. Human patients with early AMD also responded positively to oral saffron medication, showing partial recovery of macular function [94, 95], followed by extended stability of function [87].

Despite the fact that the mechanisms underlying the protective effects of saffron and its constituents are still yet to be completely elucidated [49], and more pharmacological and neurochemical research is required to establish the therapeutic advantage, saffron seems to be effective in the prevention and/or treatment of age-related diseases of the CNS. Figure 2 summarizes the potential beneficial effects of saffron supplementation for human health, as evident from studies derived from the laboratory and clinical settings.

CONCLUDING REMARKS

The capacity to scavenge free radicals is proposed to be the main mechanism by which antioxidants such as saffron may protect against diseases of the CNS. The functional characteristics of saffron, in conjunction with its low-toxicity, commercial availability and ability to cross the blood-retina and -brain barriers, make these naturally derived substances an attractive option for combating various diseases. In parallel, the exact bioavailability of orally consumed saffron after ingestion is still unknown, and this will be of particular interest for saffron intake recommendations and for new product development [96].

The protective mechanisms of saffron is still relatively unknown, though, it is possible that it may act by upregulating the endogenous protective pathways. An emerging idea known as adaptive stress response has emerged- by varying the dose of phytochemical exposure and to mediate the local protective pathways. Lee and colleagues revealed that intermittent instead of continuous

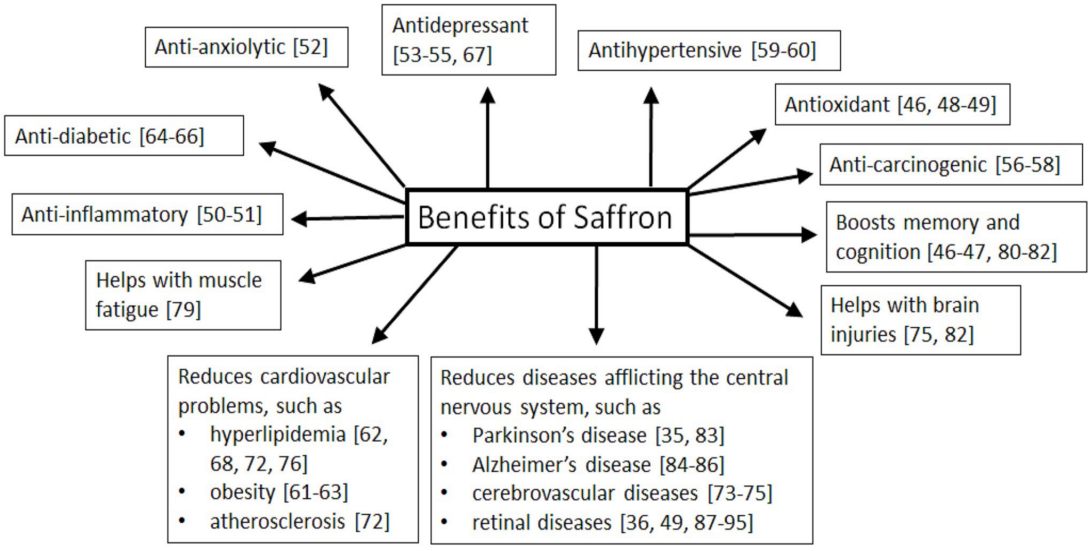

Figure 2. A schematic diagram summarizing the potential benefits of saffron (with published data) towards health and disease.

dosage with phytochemicals may provide health benefits to allow the cycles of stress (recovery, stress and then recovery) to stimulate adaptive stress response pathways during the stress period, and then allowing the cells to repair during the resting period [20].

Overall, reducing the risk of degenerative diseases by modifying consumption of dietary factors seems to be a practical preventative or interventional strategy. More studies are warranted to fully understand the beneficial effects of saffron antioxidant supplementation to combat the insidious onset of neurodegenerative diseases.

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