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An Evaluation of Sulforaphane as a Potential Agent for Disease Prevention.

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ABSTRACT

This research examines sulforaphane (SFN), a phytochemical element that is found in certain plants and vegetables. SFN has particular biological behaviours and roles to combat many diseases. It can be found mainly in vegetables such as Brussels sprouts, kale, cauliflower, and broccoli. SFN is a nutritional isothiocyanate compound and is known as a glucosinolate precursor. It is reputed to have valuable pharmacological properties, namely antioxidant, anti-inflammatory, and antitumor properties, and also functions as an agent of defence against diabetes, ocular disorders and cardiovascular diseases as well as for neurodegenerative disease. It also efficiently searches out and feeds on ROS (reactive oxygen species). Sulforaphane is a potential antioxidant for relieving oxidative stress and reducing tissue/cell damage in various *in vivo* and *in vitro* experimental models. The results of this research confirm that the natural compound, SFN, has several protective properties and that the nuclear factor, erythroid-2-related factor 2 system, which facilitates the expression of several antioxidant genes, plays a crucial role in the protective effect of such isothiocyanates against practically all the medical conditions mentioned above. The findings of this study serve to provide a more in-depth understanding of the nature of SFN and its functions, thus adding to the existing literature and research on the subject.

Keywords: Sulforaphane, Nrf2, Phase 2 detoxifying enzymes

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INTRODUCTION

Nowadays, food, and particularly food plants, is not only regarded as a basic source of nutrition, but also promises to be a source of wholesome and natural products [1-4]. Many wild edible plants, together with several cultivated plant species, can be classified as food–medicine as they contain different types of natural products [5, 6] or active compounds [7-9].

Isothiocyanates (ITCs), which go by the general formula R-NCS, are organic compounds with a low molecular weight. They are stored in plants as glucosinolate precursors. ITCs are formed when these glucosinolate precursors are hydrolysed by the plant enzyme, myrosinase, which is activated by tissue damage caused by chopping or mastication.

Sulforaphane (SFN) is an isothiocyanate that is produced from the enzymatic breakdown of glucoraphanin, which is present in substantial quantities in cruciferous vegetables such as cabbage, kale, and broccoli [10]. SFN is a powerful Nrf2 activator that is able to effectively stimulate the production of cyto-protective enzymes. It has been proven that sulforaphane prevents oxidative stress [11] through the activation of Nrf2 [12-15]. It is an indirect antioxidant that detaches Nrf2 from the Nrf2/Keap1 complex, hence allowing Nrf2 to be translocated into the nucleus, where it forms a heterodimer with other transcription factors such as the small Maf protein. This in turn attaches to the 5-upstream cis-acting regulatory sequence, known as the antioxidant response elements or electrophile response elements, which are found in the promoter region of genes encoding various antioxidant and phase 2 detoxifying enzymes such as glutathione reductase, glutathione peroxidase, glutathione-S-transferase, catalase, heme oxygenase-1, glutamyl cysteine ligase (GCL, once known as gamma-glutamyl cysteine synthetase) and NAD(P)H: quinone oxidoreductase 1 (NQO1) [16]. It has also been shown that SFN has powerful anti-inflammatory, anti-cancer and neuro-protective properties [17-19].

Protective effects against neurodegeneration:

Oxidative stress ensues when there is an imbalance between the production of free radicals and their destruction by the antioxidant defence system, thus resulting in a high accumulation of free radicals [20, 21]. ROS are classic examples of free radicals [21]. More than 90% of ROS is formed in the mitochondria “by accident” during the metabolism of oxygen when some of the electrons that are being passed ‘‘down’’ the electron transport chain escape from the main chain and head straight for the oxygen molecules to break them down and to produce the superoxide anions [20].

The damage to brain tissues after an ischemic insult is mainly due to oxidative stress [22] which also plays a role in the destruction of cells in chronic neurological diseases like Parkinson’s disease and Alzheimer’s disease [23] as well as in atherosclerosis [24] and cancer [25].

Isothiocyanate has neuro-protective effects that last for a long time. After SFN had been exposed to astrocytes for 4 hours, the levels of NQO1 and HO-1 mRNA remained high for 24

hours, while the protein levels were raised for at least 48 hours. More NQO1 was accumulated with repeated exposures and protection was maintained against oxidative stress [26]. The treatment of dorsal root ganglion neurons with SFN resulted in the nuclear translocation of Nrf2 and the up-regulation of GST and NQO1 activities [27].

SFN averted oxidative stress-induced cytotoxicity in rat striatal cultures by increasing the GSH content between cells through an increase in the γ-GCS expression brought about by the activation of the Nrf2-antioxidant reactive element pathway [28]. In rat organotypic nigrostriatal co-cultures, SFN displayed protective properties to combat the toxicity of 6-hydroxydopamine [29].

It was observed that when primary cortical neurons in mice were treated with endogenous neurotoxin S-S-cysteinyl dopamine, the sub-micromolar concentrations of SFN displayed neuro-protective properties, which were based on the stimulation of Nrf2-dependent genes and the activation of ERK1/2 and PI3K/Akt signalling [30]. The destruction of cells by kainite was reduced when wild-type hippocampal slices were treated with SFN, but not in Nrf2-knockout in mice [31].The protective effects of SFN were also clearly observed in C57BL/6 wild-type mice but not in Nrf2-knockout animals. Thus, the activation of Nrf2 is the primary mechanism that triggers the protective action of SFN. When SFN was administered to a sample of Sprague-Dawley rats with intra-cerebral haemorrhage, the Nrf2-dependent genes were stimulated while the indicators of oxidative damage in the perihematoma area were reduced [32].

In another study concerning injuries caused by trauma to the brain, treatment with SFN resulted in the activation of Nrf2 and the up-regulation of Nrf2-dependent genes, and a reduction in oxidative damage, neuronal death, contusion volume, and neurological deficits [33]. In an experimental rat suffering from neonatal hypoxic-ischemic injury caused by carotid artery ligation and hypoxia, the administration of SFN half-an-hour before the injury activated the Nrf2 and reduced the oxidative stress indicators and the extent of tissue death in the brain[34].

In general, SFN and the inducers of the Keap1/Nrf2/ARE pathway possess anti-inflammatory properties. Thus, in primary co-cultures of rat microglial and astroglial cells, SFN stimulates Nrf2-dependent genes and reduces the LPS-stimulated production of TNF-α, IL-1β, IL-6, and nitric oxide (NO [35].

SFN treatment decreased microglial activation and the up-regulation of inflammatory markers (iNOS, IL-6, and TNF-α) in C57BL/6 mice injected with endotoxin [18]. Unlike the wild-type mice, the Nrf2-knockout mice were more highly sensitive to LPS-induced neuro-inflammation caused by LPS. Thus, even a minimum expression of Nrf2-dependent genes is enough to have a protective effect. In the methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of Parkinson's disease, SFN prevented cell death in the nigral dopaminergic neurons, lowered astrogliosis and microgliosis, and discharged pro-inflammatory mediators into the

basal ganglia. It was observed that compared to the wild-type mice, the Nrf2-knockout animals were not protected by SFN [36-38].

As with the harmful effects of microgliosis in the brain, microglia activation in the spinal cord after a peripheral nerve injury also results in tissue damage. With the intrathecal administration of SFN activated Nrf2 into the spinal cord, there was a significant reduction in oxidative stress and pro-inflammatory cytokine expression due to spinal nerve transection, and the development of neuropathic pain was hampered [39]. Two studies have reported the neuroprotective effects of post-injury treatment with SFN in spinal cord injury models in Fischer rats and ICR mice. Both the induction of Nrf2-dependent genes and anti-inflammatory activity have critical functions in the protective mechanism(s) [40, 41].

SFN may step up the metabolism and removal of neurotoxic chemicals through the Nrf2-dependent up-regulation of cyto-protective proteins. SFN treatment in wild-type and Nrf2-knockout mice exposed to resulted in a decreased accumulation of mercury in the brain and liver of the former group of mice, but not in the latter [42].

Protective effects of SFN against diabetes:

Diabetes, which is one of the most incapacitating ailments in sufferers, affects a considerable number of people globally. A person with diabetes is more likely to experience metabolic, cardiovascular disorders and obesity, and these physiological manifestations come together with vascular complications. [43]. High blood sugar damages the cells in the inner lining of blood vessels, giving rise to micro-vascular complications of the disease such as diabetic neuropathy, nephropathy and retinopathy, and macro-vascular complications such as cardiomyopathy [44].

Diabetic nephropathy is marked by early oxidative stress, inflammatory reactions, thickening of basement membranes, expansion of mesangial matrix and interstitial fibrosis, podocytes and destruction of kidney cells, excessive protein in the urine, and kidney impairment [45, 46].

A recent study revealed that following prolonged treatment with sulforaphane, diabetic mice were significantly protected from kidney disease through the stimulation of the NRF2-mediated antioxidant pathway[47].

Cui and colleagues investigated whether sulforaphane can inhibit diabetic nephropathy in a type 1 diabetic mouse model induced by several low doses of streptozotocin [48]. Sulforaphane at 0.5 mg/kg body weight was administered to diabetic and age-matched control mice every day for 3 months [48]. At the end of the study, the harmful effects of diabetes, which are often indicated by kidney dysfunction, oxidative parameters and fibrosis, were mostly avoided together with a significant rise in renal NRF2 expression and transcription in the diabetes-sulforaphane group.

In order to figure out the effect of blocking the NRF2 expression, human renal tubular kallikrein-11 cells were infected with NRF2 small interfering RNA. This procedure completely eliminated the sulforaphane prevention of the pro-fibrotic effect that is brought about by high glucose levels. These results confirm the findings that renal NRF2 expression and its transcription play vital roles in the prevention by sulforaphane of kidney damage due to diabetes [48].

Heart failure has been proven to be one of the major causes of deaths in diabetic patients, while diabetic cardiomyopathy (DCM), an apparent complication due to diabetes, is highly likely to end in congestive heart failure [49]. Diabetic cardiomyopathy is defined as defects in the structure and function of the heart muscle due to diabetes and not to other coronary artery diseases. There is substantial proof to suggest that overproduction of ROS due to high blood sugar is a major cause of diabetic cardiomyopathy (DCM) [50, 51].

Bai, Cui [52] explored whether this compound can prevent diabetic cardiomyopathy, and discovered that this condition can be prevented by SFN, which was marked by an up-regulation in the Nrf2 expression and transcription function.

Xue, Qian [53] administered sulforaphane (SFN) to bring about the nuclear translocation of Nrf2 with considerable increases in its downstream antioxidant genes such as a three- to five-fold increase in the expression of transketolase and glutathione reductase. The treatment with SFN significantly prevented HG: increased formation of ROS and activation of the hexosamine and PKC pathways, both of which have been clearly described as important cellular changes due to the effects of diabetes on the target organs.

Protective effects of SFN against cardiovascular disease:

Cardiovascular diseases, such as myocardial ischemia-reperfusion damage, coronary heart disease and atherosclerosis, are one of the major causes of death globally [54]. Hypertension, hypercholesterolemia, smoking, obesity, diabetes, and aging are recognised as cardiovascular risk factors. Oxidative stress is linked to all the known cardiovascular risk factors and is responsible for the formation of atherosclerotic plaques. These observations emphasize that oxidative stress is the connection between cardiovascular risk factors and vascular diseases; thus, it is critical that oxidative stress be alleviated in order to prevent cardiovascular disease [55]. It has been proven through both in vitro and in vivo experiments that the administration of antioxidants is able to prevent the effects of oxidative cardiovascular disorders [56, 57].

It was shown by Zhu, Jia [58] that the incubation of rat aortic smooth muscle A10 cells with various concentrations of SFN triggered the level and activity of antioxidants and phase II enzymes such as catalase, super oxide dismutase (SOD), GPx, GR, GST, NQO1, and GSH. SFN is also able to stimulate the expression and activity of catalase, GSH, SOD, and GST in detached mitochondria of aortic smooth muscle cells. The same study also showed that early treatment

with SFN prevented cell death, ROS production, and oxidative cytotoxicity brought about by xanthine oxidase/xanthine, H2O2, 4-hydroxy-2-nonenal, and acrolein.

Similar outcomes were obtained by Angeloni, Leoncini [57], who showed in their research that SFN increased the gene transcription, protein expression, and enzyme activity of phase II enzymes comprising GR, GST, NQO1, and thioredoxin reductase in cultured rat neonatal cardiomyocyte models. These increases happened in a time-concentration manner. They also discovered that early treatment with SFN prevented cell death, ROS production, and DNA fragmentation induced by H_2O_2 in cultured rat neonatal cardiomyocytes. On the other hand, SFN prevented ischemia-reperfusion damage in hearts [59].

The protective effect prevented any increase in post-ischemic left ventricular end-diastolic pressure, enhanced the post-ischemic left ventricular developed-pressure coronary flow, diminished the infarcted area, and lowered the lactate dehydrogenase levels during reperfusion. SFN prevented a reduction in the protein expression of several antioxidant enzymes including catalase, Mn-SOD, and HO-1.

SFN possesses anti-inflammatory properties with regard to vascular endothelial cells [60, 61] and with macrophages stimulated with LPS-induced inflammation through the activation of Nrf2 [62]. Pro-inflammatory mediators affect atherosclerosis by stimulating adhesion molecules such as vascular cell adhesion molecule 1 (VCAM-1) on endothelial cells through signalling intermediaries including p38-MAP kinase.

Zakkar, Van der Heiden [60] verified this by showing that sulforaphane curbs the activation of endothelial cells by blocking the signalling of p38-VCAM-1 by way of the transcription factor Nrf2 in wild-type, but not in $Nrf2^{(-/-)}$ animals. Moreover in rats, SFN protects the heart against ischemia–reperfusion injury by raising the antioxidant enzyme levels of Mn-superoxide dismutase, catalase and heme oxygenase-1 obstructed by early treatment with 5-hydroxydecanoic acid, a mitochondrial K(ATP) channel blocker [59]. In another study, SFN, through the activation of Nrf2, prevented the biochemical dysfunction of endothelial cells caused by hyperglycemia [53].

Protective effects of SFN against renal damage:

Renal ischemia reperfusion (I/R) injury is a complicated pathophysiological process, which occurs in many different types of clinical situations, such as trauma, shock, sepsis, and various surgical procedures. Renal I/R injury is one of the main causes of acute kidney damage, a potentially fatal condition that is related to high mortality and morbidity [63]**.**

It has been proven through clinical and experimental studies that the tissue damage that takes place after ischemia-reperfusion, particularly during reperfusion, is partially due to the reactive oxygen species (ROS) [64], whose role in the pathophysiology of ischemia-reperfusion injury is backed by an increase in the formation of lipid hydroperoxides and other toxic products that are produced following such an injury [65].

SFN is able to effectively lessen the impact of renal dysfunction or damage due to ischemia–reperfusion of the kidney. The sulforaphane mechanism that protects the kidneys is believed to be triggered by the preconditioning of the kidney through the activation of Nrf2 and the resultant stimulation of phase 2 enzymes such as heme oxygenase-1, NADPH: quinone oxidoreductase 1, GSH reductase and GSH peroxidase [16]. It was indicated in a recent study that after prolonged treatment with SFN for 4 months, diabetic mice displayed significant resistance to diabetes induced renal damage most probably because of the induction of the Nrf2-mediated antioxidant pathway [47].

Cisplatin or cis-diamminedichloroplatinum II is a platinum chemotherapeutic agent that triggers nephrotoxicity, which is partially linked to oxidative stress. Furthermore, cisplatin generates various toxicities in cells, including cytotoxicity through the generation of ROS, thereby initiating mitogen-activated protein kinases, leading to cell death and causing inflammation and fibrosis [66].

SFN mitigated renal dysfunction induced by CIS, structural damage, oxidative/nitrosative stress, the rate of GSH depletion as well as enhanced H2O2 excretion in urine and reduced antioxidant enzymes (catalase, GSH, peroxidase, and GSH-S-transferase). The renoprotective effects of SFN on CIS-induced nephrotoxicity was linked to the mitigation of oxidative/nitrosative stress and the conservation of antioxidant enzymes [67].

SFN was able to avert CIS-induced mitochondrial changes both in LLC-PK1 cells (loss of membrane potential) and in solitary mitochondria (preventing the intake of calcium by the mitochondria, the discharge of cytochromes, as well as decreasing the GSH content, aconitase activity, adenosine triphosphate content, and oxygen consumption). The protection provided by SFN with regard to mitochondrial changes and the NQO1 and YGCL enzymes may explain the reno-protective properties of SFN against CIS [68].

Shin, Park [69] showed that the epithelial-mesenchymal transition, which is an underlying tool of tissue fibrosis in the production of myofibroblasts, is the main method of extracellular matrix production in tissue epithelial cells and has been associated with renal fibrosis caused by the immunosuppressive cyclosporin A (CsA). They studied the possible role of Nrf2 in CsA-induced epithelial-mesenchymal transition renal fibrosis. The prior treatment with SFN of tubular epithelial NRK-52E cells in rats inhibited the expression changes in the markers related to the epithelial-mesenchymal transition (reduction in E-cadherin expression and increase in α-smooth muscle actin and fibronectin-1 expressions).

On the other hand, the inhibition of Nrf2 in these cells aggravated CsA-induced changes in the epithelial-mesenchymal transition markers.[69] also verified these observations in Nrf2-deficient mice, in which treatment with CsA produced higher renal damage and fibrosis. Moreover, the Nrf2-deficient mice displayed an increase in the expression of α-smooth muscle actin in contrast to the wild-type mice. The authors also indicated that HO-1 may be the protein that controls the restorative effects of SFN on the cyclosporin-induced renal fibrosis and epithelial–mesenchymal transition.

Protective effects of SFN against respiratory disorders:

It has been proven that ROS generation and oxidative stress are crucial in triggering a strong inflammatory response and are the cause of inflammation of the respiratory tract due to an allergy or asthma [70, 71]. In a placebo-controlled dose escalation experiment, it was concluded that the oral administration of SFN safely and effectively stimulated the mucosal phase 2 enzymes (GST, QR, NADPH quinine reductase) expression in the upper respiratory tracts of human subjects [72]. Bearing in mind that phase 2 enzymes (e.g., GST and NQO1) are up-regulated in the epithelial cells of the respiratory tract, it was found that SFN lessened the impact of airborne particulate contaminants such as diesel extracts by way of the epithelial cells lining the respiratory tract[73]. These observations indicate the possible use of SFN as a unique therapeutic approach for oxidant-induced breathing disorders.

Protective effects of SFN against hepatic disease:

It is vital that blood flow be restored to an ischemic organ in order to prevent permanent tissue damage. However, reperfusion may trigger a local and systemic inflammatory response that may further damage the tissue [74]. One of the main causes of severe tissue damage during an ischemia-reperfusion (I/R) injury is the over-production of reactive oxygen species. However, efficient endogenous antioxidant systems, including glutathione reductase, superoxide dismutase (SOD), and catalase, shield tissues against the destructive effects of reactive oxygen species [75, 76].

Zhao, Zhang [77] examined the effects of sulforaphane on the control of the Nrf2/ARE pathway in liver injury caused by ischemia/reperfusion in the intestines. They showed that early treatment with sulforaphane reduces the damage to the intestines and liver due to ischemia–reperfusion (rise in aspartate aminotransferase and alanine aminotransferase in the blood serum, decrease in SOD and GPx activities and in GSH content and increase in myeloperoxidase in the liver). This protection was linked to the increase in the expressions of Nrf2 and HO-1 in the liver. SFN up-regulates the expression of the π class of GST through the Nrf2 pathway in the liver cells of Clone 9 rats [78].

Liver fibrosis is a fundamental clinicopathological condition of prolonged liver disease that renders the patient susceptible to cirrhosis and malignant hepatoma (HCC) [79]. It is marked by excessive production of extracellular matrix (ECM), essentially type I and III collagens [80, 81]. The net accumulation of ECM alters the architecture of the liver and results in high blood pressure in the portal vein system [79, 82]. Oh, Kim [83] indicated that SFN brings about an anti-fibrotic effect on liver fibrosis when Nrf2 obstructs the TGF-β/Smad signalling and subsequently represses the activation of HSC and the expression of fibrogenic genes.

Studies conducted on predominant liver cells have shown that exposure to SFN resulted in the activation of Nrf2 and the up-regulation of γ-glutamatecysteine ligase (and increased levels of GSH accordingly), GSTA1, and MRP2, hence lowering the level of mercury accumulation and cytotoxicity[42].

Protective effects of SFN against eye disorders and skin diseases:

Retinitis pigmentosa (RP) is a group of genetic diseases in which the gradual impairment of photoreceptors is accompanied by the destruction of cells and ultimately retinal atrophy [84]. RP is a disease that affects approximately 1.5 million people throughout the world, and just like other retinal degenerative diseases such as macular degeneration, which is the main cause of blindness in the elderly, there are not many effective medical treatments available for it [85]. A few original observations have suggested that a reduction in the expression of thioredoxin (Trx), thioredoxin reductase (TrxR), and Nrf2 is the cause of retinal degeneration in this disease. The early treatment of human adult RPE 19 cells with SF resulted in a potent and prolonged protection against the harmful effects of various oxidants and photo-oxidative impairment by the up-regulation of the expression of antioxidant and detoxification enzymes and the inhibition of inflammatory reactions [86].

It has been proven that the photo-oxidative protection by SFN is consistent with the quantitative stimulation of phase 2 response enzymes such as NAD(P)H : quinone oxidoreductase and increased levels of reduced glutathione [87]. The administration of SFN into the peritoneum or by mouth increased the expression of Trx in the tissues of the retina and up-regulated those genes having cyto-protective properties against damage brought about by the effects of light on photoreceptors and RPE in mice [88]. Kong et ai.,2007 [89] showed that the administration of SF into the circulatory system could defer the deterioration of photoreceptors by triggering the activity of ERKs and up-regulating the Trx/TrxR/Nrf2 system in the retinas of tub/tub mice.

Another common eye disease is cataracts, which has been linked by many studies to the destruction of free radicals. Liu et al.,2013 [90] discovered that through the early treatment of cells or whole lenses with SFN, anti-oxidant defence mechanisms can be adapted by the stimulation of the Keap1-Nrf2- ARE pathway, thus enabling the cells in the lens to suppress the gradual destruction of tissues due to oxidative stress. The consumption of an SFN-rich diet or the intake of supplements could be a unique way to impede cataracts from forming in the human lens.

Prolonged exposure to UV rays may weaken the stress response and the antioxidant defence mechanisms in the human skin, thus negatively affecting the role and soundness of the skin. Kleszczyński, Ernst [91] conducted tests on sulforaphane (SFN) and phenylethyl isothiocyanate (PEITC) to discover their ability to offset oxidative stress brought about by exposure to UVR and programmed cell death in ex vivo human full-thickness skin combined with in vitro HaCaT keratinocytes. It was discovered that the induction of Nrf2-dependent antioxidant pathways appeared to be a possible process by which UVR-induced oxidative stress and programmed cell death in human skin are obstructed by SFN and PEITC.

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CONCLUSION

More researches are being carried out on sulforaphane because of the increasing evidence pointing to its many health benefits. At present, sulforaphane displays a variety of healing properties making it a popular candidate for the treatment of human medical conditions. These health benefits may be ascribed to a range of possible mechanisms. Most of the reports highlighted the Nrf-2 mediated induction of phase 2 detoxification enzymes that prevent oxidative damage to cells. Further studies need to be carried out to discover all the protective effects of sulforaphane, the players involved and how it acts on various human disease representations.

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